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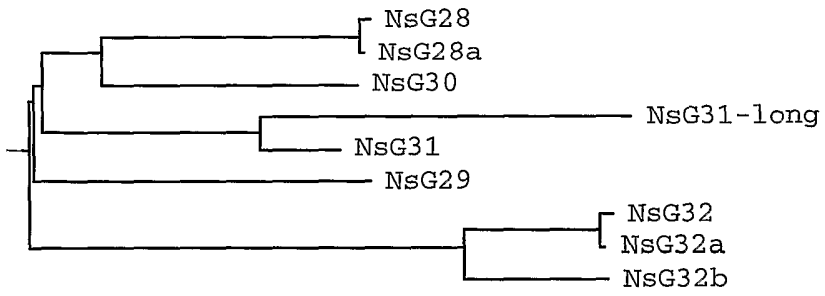
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(54) Title: GROWTH FACTORS NSG28, NSG30, AND NSG32



(57) Abstract: Disclosed are NsG28, NsG30, NsG32 polypeptides, nucleic acids encoding NsG28, NsG30, NsG32 polypeptides, and antibodies that bind to NsG28, NsG30, NsG32 polypeptides as well as methods of making and using the same.

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Growth Factors NsG28, NsG30, and NsG32

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from Danish Application No. PA 2004 01162, filed July 30, 2004, Danish Application No. PA 2005 00034, filed January 7, 2005, Danish Application No. PA 2004 01164, filed July 30, 2004, Danish Application No. PA 2005 00036, filed January 7, 2005, Danish Application No. PA 2004 01166, filed July 30, 2004, and Danish Application No. PA 2005 00038, filed January 7, 2005. The entire content of each of these prior applications is incorporated herein by reference in its entirety.

TECHNICAL FIELD

The present invention relates to the field of therapeutic use of proteins, genes and cells, in particular to the therapy based on the biological function of secreted therapeutic proteins, NsG28, NsG30, and NsG32 in particular for the treatment of disorders of the nervous system. The invention also relates to bioactive NsG28, NsG30, and NsG32 polypeptide fragments and the corresponding encoding DNA sequences.

BACKGROUND ART

Extracellular proteins play important roles in, among other things, the formation, differentiation and maintenance of multicellular organisms. The fate of many individual cells, e.g., growth, proliferation, migration, differentiation, or interaction with other cells, is typically governed by information received from other cells and/or the immediate environment. This information is often transmitted by secreted polypeptides (for instance, mitogenic factors, survival factors, cytotoxic factors, differentiation factors, neuropeptides, and hormones) which are, in turn, received and interpreted by diverse cell receptors or membrane-bound proteins. These secreted polypeptides or signalling molecules normally pass through the cellular secretory pathway to reach their site of action in the extracellular environment.

Disorders such as Parkinson's disease, Alzheimer's disease, Huntington's disease, multiple and amyotrophic lateral sclerosis, stroke, schizophrenia, epilepsy and peripheral neuropathy and associated pain affect millions of people. It is the loss of normal neuronal function, which produces the behavioral and physical deficits which are characteristic of each of

the different neurological disorders. In addition to chronic and acute neurodegenerative disorders, the aging process, physical trauma to the nervous system, and metabolic disorders may result in the loss, dysfunction, or degeneration of neural cells accompanied by the associated behavioral and physical deficits. Many of these diseases are today incurable, highly debilitating, and traditional drug therapies often fail. There is thus a great medical need for new therapeutic proteins that are disease modifying and not only for symptomatic use.

Several secreted factors with expression in the nervous system or associated target areas have important therapeutic uses in various neurological indications associated with reduction or loss of neuronal functions. E.g. NGF is a candidate for treatment of Alzheimer's disease, Neublastin (Artemin) a candidate for treatment of peripheral neuropathy, and GDNF is a candidate for treatment of Parkinson's Disease.

SUMMARY OF THE INVENTION

The present invention relates to various aspects and uses of Nerve Survival and Growth factor 28 (NsG28), Nerve Survival and Growth Factor 30 (NsG30), and Nerve Survival and Growth factor 32 (NsG32).

In a first aspect the invention relates to an isolated polypeptide for medical use, said polypeptide comprising an amino acid sequence selected from the group consisting of:

a) the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, and 16 (or a subgroup thereof);

b) a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, and 16 (or a subgroup thereof), wherein the variant has at least 70% sequence identity to said SEQ ID No.; and

c) a biologically active fragment of at least 50 contiguous amino acids of any of a) through b).

The invention also relates to an isolated polypeptide for medical use, said polypeptide comprising an amino acid sequence selected from the group consisting of:

a) the amino acid sequence selected from the group consisting of SEQ ID No. 18, 19, 20, 21, 23, 24, and 25 (or a subgroup thereof);

b) a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 18, 19, 20, 21, 23, 24, and 25 (or a subgroup thereof), wherein the variant has at least 70% sequence identity to said SEQ ID No.; and

c) a biologically active fragment of at least 50 contiguous amino acids of any of a) through b).

The invention also relates to an isolated polypeptide for medical use, said polypeptide comprising an amino acid sequence selected from the group consisting of:

a) the amino acid sequence selected from the group consisting of SEQ ID No. 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41 (or a subgroup thereof);

b) a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41 (or a subgroup thereof), wherein the variant has at least 70% sequence identity to said SEQ ID No.; and

c) a biologically active fragment of at least 50 contiguous amino acids of any of a) through b).

In a further aspect, the invention relates to an isolated nucleic acid molecule for medical use comprising a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence coding for a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, and 16 (or a subgroup thereof);

b) a nucleotide sequence coding for a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, and 16 (or a subgroup thereof), wherein the variant has at least 70% sequence identity to said SEQ ID No.;

c) a nucleotide sequence coding for a biologically active fragment of at least 50 contiguous amino acids of any of a) through b);

d) a nucleotide sequence selected from the group consisting of SEQ ID No. 2 and 8;

e) a nucleotide sequence having at least 70% sequence identity to a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 2 and 8;

f) a nucleic acid sequence of at least 150 contiguous nucleotides of a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 2 and 8;

g) the complement of a nucleic acid capable of hybridising with a nucleic acid molecule having the sequence of the coding sequence of SEQ ID No.: 2 and 8 under conditions of high stringency;

h) the nucleic acid sequence of the complement of any of the above.

The invention also relates to an isolated nucleic acid molecule for medical use comprising a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence coding for a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID No. 18, 19, 20, 21, 23, 24, and 25 (or a subgroup thereof);

b) a nucleotide sequence coding for a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 18, 19, 20, 21, 23, 24, and 25 (or a subgroup thereof), wherein the variant has at least 70% sequence identity to said SEQ ID No.;

c) a nucleotide sequence coding for a biologically active fragment of at least 50 contiguous amino acids of any of a) through b);

d) a nucleotide sequence selected from the group consisting of SEQ ID No. 17 and 22;

e) a nucleotide sequence having at least 70% sequence identity to a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 17 and 22;

f) a nucleic acid sequence of at least 150 contiguous nucleotides of a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 17 and 22;

g) the complement of a nucleic acid capable of hybridising with a nucleic acid molecule having the sequence of the coding sequence of SEQ ID No.: 17 and 22 under conditions of high stringency;

h) the nucleic acid sequence of the complement of any of the above.

The invention also relates to an isolated nucleic acid molecule for medical use comprising a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence coding for a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID No. 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41 (or a subgroup thereof);

b) a nucleotide sequence coding for a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41

(or a subgroup thereof), wherein the variant has at least 70% sequence identity to said SEQ ID No.;

c) a nucleotide sequence coding for a biologically active fragment of at least 50 contiguous amino acids of any of a) through b);

d) a nucleotide sequence selected from the group consisting of SEQ ID No. 26, 30, 35, 37, and 39 (or a subgroup thereof);

e) a nucleotide sequence having at least 70% sequence identity to a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 26, 30, 35, 37, and 39 (or a subgroup thereof);

f) a nucleic acid sequence of at least 150 contiguous nucleotides of a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 26, 30, 35, 37, and 39 (or a subgroup thereof);

g) the complement of a nucleic acid capable of hybridising with a nucleic acid molecule having the sequence of the coding sequence of SEQ ID No.: 26, 30, 35, 37, or 39 under conditions of high stringency; and

h) the nucleic acid sequence of the complement of any of the above.

In a further aspect, the invention relates to a vector comprising a nucleic acid molecule of any of the invention.

In a further aspect the invention relates to an isolated host cell transformed or transduced with a vector of the invention.

In a further aspect the invention relates to a packaging cell line capable of producing an infective virus particle, said virus particle comprising a Retroviridae derived genome comprising a 5' retroviral LTR, a tRNA binding site, a packaging signal, a promoter operably linked to a polynucleotide sequence encoding a polypeptide of the invention, an origin of second strand DNA synthesis, and a 3' retroviral LTR.

In a further aspect the invention relates to an implantable biocompatible cell device, the device comprising:

i) a semipermeable membrane permitting the diffusion of a polypeptide or a virus vector of the invention; and

ii) a composition of cells according to the invention or a packaging cell line according to the invention.

In a further aspect, there is provided a pharmaceutical composition comprising

i) a polypeptide of the invention; or

ii) an isolated nucleic acid sequence of the invention; or

iii) an expression vector of the invention; or

iv) a composition of host cells according to the invention; or

v) a packaging cell line according to the invention; or

vi) an implantable biocompatible cell device according to the invention; and

vii) a pharmaceutically acceptable carrier.

In a further aspect, the invention relates to the use of

i) a polypeptide of the invention; or

ii) an isolated nucleic acid sequence of the invention; or

iii) an expression vector of the invention; or

iv) a composition of host cells according to the invention;

v) an implantable biocompatible cell device according to the invention; or

vi) a packaging cell line according of the invention;

for the manufacture of a medicament.

Furthermore, the invention relates to a method of treatment of a pathological condition in a subject comprising administering to an individual in need thereof a therapeutically effective amount of:

i) a polypeptide of the invention; or

ii) an isolated nucleic acid sequence of the invention; or

iii) an expression vector of the invention; or

iv) a composition of host cells according to the invention; or

v) an implantable biocompatible cell device according to the invention; or

vi) a packaging cell line according to the invention.

In a further aspect, the invention relates to the use of

i) a polypeptide of the invention; or

ii) an isolated nucleic acid sequence of the invention; or

iii) an expression vector of the invention; or

iv) a composition of host cells according to the invention; or

v) an implantable biocompatible cell device according to the invention;

as a male contraceptive.

In a further aspect the invention relates to a method of expanding a composition of mammalian cells, comprising administering to said composition the polypeptide of the invention; or transducing/transfecting the cells with the expression vector of the invention.

In a still further aspect, the invention relates to a method of differentiating a composition of mammalian cells, comprising administering to said composition the polypeptide of the invention; or transducing/transfecting the cells with the expression vector of the invention.

The invention also relates to an antibody capable of binding to a polypeptide of the invention.

Furthermore, the invention relates to an immunoconjugate comprising the antibody of the invention and a conjugate selected from the group consisting of: a cytotoxic agent such as a chemotherapeutic agent, a toxin, or a radioactive isotope; a member of a specific binding pair, such as avidin or streptavidin or an antigen; an enzyme capable of producing a detectable product.

The invention also provides an isolated polypeptide having an amino acid sequence selected from the group consisting of SEQ ID No. 6, 11, and 15 (or a subgroup thereof), and variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15 of the amino acid residues in the sequence are so changed.

The invention also provides an isolated polypeptide having an amino acid sequence selected from the group consisting of SEQ ID No 7, 12, and 16 (or a subgroup thereof), and variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15 of the amino acid residues in the sequence are so changed.

Furthermore, the invention relates to an isolated polypeptide having the amino acid sequence of SEQ ID No. 20, and variants of said polypeptide, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15 of the amino acid residues in the sequence are so changed.

The invention, in a further aspect relates to an isolated polypeptide having the amino acid sequence of SEQ ID No 21, and variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15 of the amino acid residues in the sequence are so changed.

In a further aspect, the invention relates to an isolated polypeptide having an amino acid sequence of SEQ ID No. 33 and variants of said polypeptide, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed.

In a further aspect, the invention relates to an isolated polypeptide having an amino acid sequence of SEQ ID No 34, and variants of said polypeptide, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed.

Furthermore, the invention provides an isolated polynucleotide coding for a polypeptide described herein.

In a further aspect, the invention relates to a method of preventing apoptosis in a neuronal cell by contacting a neuronal cell with an affective amount of a polypeptide, nucleic acid, or vector described herein.

The invention relates to a method of treating a disorder characterized by neuronal apoptosis by administering to a subject (e.g., a human) having a disorder characterized by neuronal apoptosis a therapeutically affective amount of a polypeptide, nucleic acid, or vector described herein.

FIGURES

Figs 1A-1B. Clustal W (1.82) alignment of the mature human and mouse (_m) peptides of the growth factor family, Cys10. In the alignments, conserved regions are shown in bold and variable regions in grey. Fig. 1A shows an alignment based on the four most similar members of

the family. Fig. 1B shows an alignment based on all five members of the family of growth factors.

Fig. 1C shows a phylogenetic tree of the human protein family. The tree was generated by using Multi-Way Alignment (Align Plus 5 version 5.03, Scientific and Educational Software) and BLOSUM62 as scoring Matrix. The branch length is proportional to the difference between the sequences. The corresponding full length protein sequences are shown in Table 1.

Figs. 2A-2B. Output from signal peptide prediction server SignalP v.2.0 for human, NsG28 (SEQ ID No. 4). Graphs shown for neural network based method (panel A) and hidden Markov model method (panel B).

Fig. 3. Prediction output from the ProtFun 2.1 protein function prediction algorithm for human, mouse, and rat NsG28 (SEQ ID No. 4, 9, and 13).

Figs. 4A-4B. Output for human NsG30 (SEQ ID No. 18) from signal peptide prediction server SignalP v.2.0. Graphs shown for neural network based method (panel A) and hidden Markov model method (panel B).

Fig. 5. Prediction output from the ProtFun 2.1 protein function prediction algorithm for human and mouse NsG30 (SEQ ID No 18 and 23). Odds above 1 are shown in **bold**.

Fig 6. Prediction of start codon using Human NsG32a cDNA. In the lines below the sequence, the predicted start codon is indicated by the letter "i" (initiation), other instances of "ATG" by the letter "N" (non-start). The dots (".") are placeholders for all the other sequence elements.

Fig 7. Prediction of start codon using Mouse NsG32a cDNA. In the lines below the sequence, the predicted start codon is indicated by the letter "i" (initiation), other instances of "ATG" by the letter "N" (non-start). The dots (".") are placeholders for all the other sequence elements.

Figs. 8A-8B. Output for human NsG32a (SEQ ID NO:27) from signal peptide prediction server SignalP v.2.0. Graphs shown for neural network based method (panel A) and hidden Markov model method (panel B).

Figs. 9A-9B. Output for human NsG32b (SEQ ID No. 31) from signal peptide prediction server SignalP v.2.0. Graphs shown for neural network based method (panel A) and hidden Markov model method (panel B).

Fig. 10. Prediction output from the ProtFun 2.1 protein function prediction algorithm for human NsG32a (SEQ ID No 27 and 28) and NsG32b (SEQ ID No 31).

Fig. 11. Clustal W (1.82) alignment of human NsG28a (SEQ ID No 4) to mouse (SEQ ID No 9) and rat (SEQ ID No 13) full length polypeptides.

Fig. 12. Clustal W (1.82) alignment of human NsG30 (SEQ ID No. 18) to mouse NsG30 (SEQ ID No 23) and partial rat NsG30 (SEQ ID No. 25). The signal sequence is shown in **bold**.

Fig. 13A. Clustal W (1.82) alignment of human NsG32a (SEQ ID No. 27) to mouse NsG32a (SEQ ID No 36), and rat NsG32a (SEQ ID No. 41). The signal sequence is shown in **bold**.

Fig. 13B. Clustal W (1.82) alignment of human NsG32b (SEQ ID No. 31) to mouse NsG32b (SEQ ID No 39). The signal sequence is shown in **bold**.

Fig. 14. Upper panel shows relative expression of NsG28, as measured by quantitative RT-PCR, (relative to tissue with the lowest expression) assuming same amounts of cDNA were synthesized from equal amounts of total RNA used for the cDNA step. Lower panel shows the relative expression of NsG28, as measured by quantitative RT-PCR, normalised to $ß_2$-microglobulin (relative to tissue with the lowest normalized expression). Results should be interpreted with caution as $ß_2$-microglobulin expression levels vary between some tissues.

Fig. 15. Upper panel shows relative expression of NsG28, as measured by quantitative RT-PCR, (relative to tissue with the lowest expression) assuming same amounts of cDNA were synthesized from equal amounts of poly (A) RNA used for the cDNA step. Lower panel shows relative expression of NsG28, as measured by quantitative RT-PCR, normalised to $ß_2$-microglobulin (relative to tissue with the lowest normalized expression).

Fig.16 in the upper panel shows the relative expression of NsG30, as measured by quantitative RT-PCR, (relative to tissue with the lowest expression) assuming same amounts of cDNA were synthesised from equal amounts of total RNA used for the cDNA step. Fig. 16 shows in the lower panel the relative expression of NsG30, as measured by quantitative RT-PCR, normalised to $ß_2$-microglobulin (relative to tissue with the lowest normalized expression). Results should be interpreted with caution as $ß_2$-microglobulin expression levels vary between some tissues.

Fig. 17 in the upper panel shows the relative expression of NsG30, as measured by quantitative RT-PCR, (relative to tissue with the lowest expression) assuming same amounts of

cDNA were synthesised from equal amounts of poly (A) RNA used for the cDNA step. Fig. 17 in the lower panel shows the relative expression of NsG30, as measured by quantitative RT-PCR, normalised to $ß_2$-microglobulin (relative to tissue with the lowest normalized expression).

Fig. 18 shows in the upper panel the relative expression of human NsG32, as measured by quantitative RT-PCR, (relative to tissue with the lowest expression) assuming same amounts of cDNA were synthesized from equal amounts of total RNA used for the cDNA step. Fig. 18 shows in the lower panel, the relative expression of human NsG32 normalised to $ß_2$-microglobulin (relative to tissue with the lowest normalized expression). Results should be interpreted with caution as $ß_2$-microglobulin expression levels vary between some tissues.

Fig. 19 shows in the upper panel the relative expression of human NsG32, as measured by quantitative RT-PCR, (relative to tissue with the lowest expression) assuming same amounts of cDNA were synthesized from equal amounts of poly (A) RNA used for the cDNA step. Fig. 19 shows in the lower panel the relative expression of human NsG32 normalised to $ß_2$-microglobulin (relative to tissue with the lowest normalized expression).

Fig. 20. Human NsG28 cDNA (SEQ ID No. 2) and encoded polypeptide (NsG28a, SEQ ID No. 4). The start codon for NsG28a and the alternative start codon for NsG28 (SEQ ID No. 3) are shown in bold.

Fig. 21. Mouse NsG28 cDNA (SEQ ID No. 8) and encoded polypeptide (SEQ ID No. 9).

Figs. 22A-22C show the relative expression of mGAPDH (panel A) as measured by quantitative RT-PCR (relative to tissue with the lowest expression) assuming same amounts of cDNA were synthesized from equal amounts of total RNA used for the cDNA step. In panel B and C is shown the expression of mALDH1A1 (panel B) and mOTX2 (panel C) has been normalised to the expression of mGADPH and is shown relative to the tissue with the lowest normalised expression. The expression is shown for regions of the developing mouse brain. For details, see example 13. Legend: dorsal forebrain (DFB), ventral forebrain (VFB), ventral mesencephalon (VM), dorsal mesencephalon (DM), spinal cord (SC), cortex (CTX), medial and lateral ganglionic eminences (MGE/LGE), cerebellum (Cb). E10.5, E11.5, and E13.5: embryo days (or days post conception) 10.5, 11.5 and 13.5 respectively. P1: one day post-natum. Ad: adult.

Fig. 23 shows the relative expression of mGDNF in regions of the developing mouse brain. The expression is measured by quantitative RT-PCR normalised to the expression of

mGAPDH relative to tissue with the lowest normalised expression. For details, see Example 13. Legend as in Figs. 22A-22C.

Fig. 24 shows the relative expression of mNsG28 in regions of the developing mouse brain. The expression is measured by quantitative RT-PCR normalised to the expression of mGAPDH relative to tissue with the lowest normalised expression. For details, see example 13. Legend as in Figs. 22A-22C.

Fig. 25. Results of the PC12 viability assay described in Example 5. Data shown are means ± SEM (n=6) from a representative experiment, and * indicates a significant difference from cells transduced with cDNA for EGFP ($P<0.05$, one way ANOVA, Dunnett's Method). LV-EGFP: lentivirus EGFP transduced PC12 cells. LV-NsG30: PC12 cells transduced with human full length NsG30.

Fig. 26. Human NsG30 cDNA (SEQ ID No.17) and encoded protein (SEQ ID No. 18).

Fig. 27. Mouse NsG30 cDNA (SEQ ID No 22) and encoded protein (SEQ ID No. 23).

Fig. 28 shows the relative expression of mNsG30 in regions of the developing mouse brain. The expression is measured by quantitative RT-PCR normalised to the expression of mGAPDH relative to tissue with the lowest normalised expression. For details, see Example 13. Legend as in Figs. 22A-22C.

Fig. 29. Gene structure of human NsG32 leading to two splice variants called NsG32a, and NsG32b.

Fig. 30A. Human NsG32a cDNA (SEQ ID No. 26) and encoded polypeptide (SEQ ID No. 27).

Fig. 30B. Human NsG32a cDNA (SEQ ID No. 26) and encoded polypeptide (SEQ ID No. 28).

Fig. 31. Mouse NsG32a cDNA (SEQ ID No. 35) and encoded polypeptide (SEQ ID No 36).

Fig. 32. Human NsG32b cDNA (SEQ ID No.30) and encoded polypeptide (SEQ ID No. 31).

Fig. 33. Mouse NsG32b cDNA (SEQ ID No. 37) and encoded polypeptide (SEQ ID No. 38).

Fig. 34. Effect of human NsG32a and b on PC12 survival in serum-free medium. For additional details see Example 5. Cells were seeded in collagen-coated 48-well plates, $2x10^4$ cells/well in growth medium. The following day, cells were transduced by incubation overnight with 10^5 transducing units virus/well (MOI=5) in the presence of 5 µg/ml polybrene. After transduction, medium was changed to serum-free DMEM (Invitrogen) and cell survival was then assayed after four days using the MTS assay. Data shown are means ± SEM (n=6) from a representative experiment, and * indicates a significant difference from cells transduced with cDNA for EGFP ($P<0.05$, one way ANOVA, Dunnett's Method). EGFP: lentivirus EGFP transduced PC12 cells. NsG32a: PC12 cells transduced with human full length NsG32a coding for a protein with the sequence of SEQ ID No. 28. NsG32b: PC12 cells transduced with human full length NsG32b.

Fig. 35 shows the relative expression of mNsG32a in regions of the developing mouse brain. The expression is measured by quantitative RT-PCR normalised to the expression of mGAPDH relative to tissue with the lowest normalised expression. For details, see Example 13. Legend as in Figs. 22A-22C.

Fig. 36. MTS measurements in CGN cultures from p9 mouse (DIV6) 2 days after change of medium to serum-free medium with high potassium ("25-S") or a depolarizing level of potassium, 5 mM KCl with or without 100 ng/ml IGF1 ("5-S+I" and "5-S", respectively). Control wells are left unchanged ("U") in medium with serum and 25 mM potassium. MTS reduction for each condition is compared between non-transduced control cells and cells transduced with rLV-NsG28, rLV-NsG32a and rLV-EGFP, respectively.

DEFINITIONS

NsG28, as used herein, refers to polypeptides having the amino acid sequences of substantially purified NsG28 obtained from any species, particularly mammalian, including chimpanzee, bovine, ovine, porcine, murine, equine, and preferably human, from any source whether natural, synthetic, semi-synthetic, or recombinant. The term also refers to biologically active fragments of NsG28 obtained from any of these species, as well as to biologically active sequence variants of these and to proteins subject to posttranslational modifications.

NsG30, as used herein, refers to polypeptides having the amino acid sequences of substantially purified NsG30 obtained from any species, particularly mammalian, including chimpanzee, bovine, ovine, porcine, murine, equine, and preferably human, from any source

whether natural, synthetic, semi-synthetic, or recombinant. The term also refers to biologically active fragments of NsG30 obtained from any of these species, as well as to biologically active sequence variants of these and to proteins subject to posttranslational modifications.

NsG32, as used herein, refers to polypeptides having the amino acid sequences of substantially purified NsG32 obtained from any species, particularly mammalian, including chimpanzee, bovine, ovine, porcine, murine, equine, and preferably human, from any source whether natural, synthetic, semi-synthetic, or recombinant. The term also refers to biologically active fragments of NsG32 obtained from any of these species, as well as to biologically active sequence variants of these and to proteins subject to posttranslational modifications. The term NsG32 refers to both splice variants of NsG32 unless one of the variants is specified.

Growth factor characteristics as used herein define sequence-related features similar to those of classical growth factors, which are secreted proteins acting on a target cell through a receptor to cause one or more of the following responses in the target cell: growth, proliferation, differentiation, survival, regeneration, migration, regain of function, improvement of function.

According to one embodiment of the invention, "treatment", "therapy", and "medical use" is intended to cover prophylaxis. "Treatment", "therapy" and "medical use" may also cover inhibition of a disease or disorder, protection against a disease or disorder, and/or prevention (not absolute) of a disease or disorder. "Treatment", "therapy" and "medical use" may also comprise curative, ameliorative, and/or symptomatic treatment, therapy and medical use.

An "allele" or "allelic sequence", as used herein, is an alternative form of the gene encoding NsG28, NsG30, or NsG32. Alleles may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

A "deletion", as used herein, refers to a change in the amino acid or nucleotide sequence and results in the absence of one or more amino acid residues or nucleotides.

An "insertion" or "addition", as used herein, refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, as compared to the naturally occurring molecule.

The terms "specific binding" or "specifically binding", as used herein, refers to the high affinity interaction between a protein or peptide and a binding molecule such as an antibody and a receptor or fragments thereof. The interaction is dependent upon the presence of a particular structure (i.e., the antigenic determinant or epitope) of the protein recognized by the binding molecule. For example, if an antibody is specific for epitope "A", the presence of a protein containing epitope A (or free, unlabeled A) in a reaction containing labelled "A" and the antibody will reduce the amount of labeled A bound to the antibody.

The term "substantially purified", as used herein, refers to nucleic or amino acid sequences that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated.

A "substitution", as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Sequence identity": The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-2268, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5877. Such an algorithm is incorporated into the BLASTN and BLASTP programs of Altschul, et al. (1990) J. Mol. Biol. 215:403-410.

In order to characterize the identity, subject sequences are aligned so that the highest order homology (match) is obtained. Based on these general principles the "percent identity" of two amino acid sequences may be determined using the BLASTP algorithm [*Tatiana A. Tatusova, Thomas L. Madden*: Blast 2 sequences - a new tool for comparing protein and nucleotide sequences; FEMS Microbiol. Lett. 1999 174 247-250], which is available from the National Center for Biotechnology Information (NCBI) web site (http://www.ncbi.nlm.nih.gov), and using the default settings suggested here (i.e. Matrix = Blosum62; Open gap = 11; Extension gap = 1; Penalties gap x_dropoff = 50; Expect = 10; Word size = 3; Filter on). The BLAST algorithm performs a two-step operation by first aligning two sequences based on the settings and then determining the % sequence identity in a range of overlap between two aligned sequences. In addition to % sequence identity, BLASTP also determines the % sequence similarity based on the settings.

In order to characterize the identity, subject sequences are aligned so that the highest order homology (match) is obtained. Based on these general principles, the "percent identity" of two nucleic acid sequences may be determined using the BLASTN algorithm [*Tatiana A. Tatusova, Thomas L. Madden*: Blast 2 sequences - a new tool for comparing protein and nucleotide sequences; FEMS Microbiol. Lett. 1999 174 247-250], which is available from the National Center for Biotechnology Information (NCBI) web site (http://www.ncbi.nlm.nih.gov), and using the default settings suggested here (i.e. Reward for a match = 1; Penalty for a match = -2; Strand option = both strands; Open gap = 5; Extension gap = 2; Penalties gap x_dropoff = 50; Expect = 10; Word size = 11; Filter on). The BLASTN algorithm determines the % sequence identity in a range of overlap between two aligned nucleotide sequences.

Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the FASTA sequence alignment software package (Pearson WR, Methods Mol Biol, 2000, 132:185-219). Align calculates sequence identities based on a global alignment. Align0 does not penalise to gaps in the end of the sequences. When utilizing the ALIGN or Align0 program for comparing amino acid sequences, a BLOSUM50 substitution matrix with gap opening/extension penalties of –12/-2 is preferably used.

DETAILED DESCRIPTION OF THE INVENTION

I. The Cys10 Family of Growth Factors

The present invention in one aspect relates to medical use of NsG28, NsG30, and NsG32 and to bioactive fragments of NsG28, NsG30, and NsG32. NsG28, NsG30, and NsG32 are distinct members of a family of growth factors in the following referred to as the Cys10 family (referring to the 10 cysteines conserved among four of the five family members).

The five members of the family are referred to as NsG28, NsG29, NsG30, NsG31, and NsG32 (See Table 1). For NsG28, there are two alternative start codons in human beings, referred to as NsG28 and NsG28a. NsG31 exists as two different splice variants with different length. The long form NsG31-long (IPI00174927.1 REFSEQ_XP:XP_209222 Tax_Id=9606 similar to hypothetical protein) differs from the other members of the family from position 89 to the C-terminal as a result of a frameshift. NsG32 also exists as two different splice variants,

called NsG32a and NsG32b. For the NsG32a splice variant there are two possible start codons. The difference between NsG32a and NsG32b results in different signal peptides and also in different mature proteins, both of which are shown in Fig. 1B.

Table 1. Full length Cys10 proteins. The signal peptides are shown in bold. The IPI reference numbers (IPI - The International Protein Index. The European Bioinformatics Institute) refer to the accession numbers of the sequences. The database version is shown in parentheses. The IPI databases were downloaded from ftp://ftp.ebi.ac.uk/pub/databases/IPI/.

Human NsG28, >IPI00065186.1 (ver. 2.24) **MRSPRMRVCA KSVLLSHWLF LAYVLMVCCK LMSA**SSQHLR GHAGHHQIKQ GTCEVVAVHR CCNKNRIEER SQTVKCSCFP GQVAGTTRAQ PSCVEASIVI QKWWCHMNPC LEGEDCKVLP DYSGWSCSSG NKVKTTKVTR
Human NsG28a, >IPI00065186.1.modi N-terminal predicted using NetStart (ver. 2.24) **MRVCAKSVLL SHWLFLAYVL MVCCKLMSA**S SQHLRGHAGH HQIKQGTCEV VAVHRCCNKN RIEERSQTVK CSCFPGQVAG TTRAQPSCVE ASIVIQKWWC HMNPCLEGED CKVLPDYSGW SCSSGNKVKT TKVTR
Mouse NsG28a, >IPI00311118.3 (ver. 1.23) **MRVCAKWVLL SRWLVLTYVL MVCCKLMSA**S SQHLRGHAGH HLIKPGTCEV VAVHRCCNKN RIEERSQTVK CSCFPGQVAG TTRAQPSCVE AAIVIEKWWC HMNPCLEGED CKVLPDSSGW SCSSGNKVKT TKVTR
Human NsG29, >IPI00376089.1 (ver. 2.29) **MAMVSAMSWV LYLWISACAM LLCHG**SLQHT FQQHHLHRPE GGTCEVIAAH RCCNKNRIEE RSQTVKCSCL PGKVAGTTRN RPSCVDASIV IGKWWCEMEP CLEGEECKTL PDNSGWMCAT GNKIKTTRIH PRT
Mouse NsG29, >IPI00380407.1 (ver. 1.20) **MAMVSAMSWA LYLWISACAM LLCHG**SLQHT FQQHHLHRPE GGTCEVIAAH RCCNKNRIEE RSQTVKCSCL PGKVAGTTRN RPSCVDASIV IGKWWCEMEP CLEGEECKTL PDNSGWMCAT GNKIKTTRIH PRT
Human NsG30, >IPI00166553.1 (ver. 2.24) **MSKRYLQKAT KGKLLIIIFI VTLWGKVVSS** ANHHKAHHVK TGTCEVVALH RCCNKNKIEE RSQTVKCSCF PGQVAGTTRA APSCVDASIV EQKWWCHMQP CLEGEECKVL PDRKGWSCSS GNKVKTTRVT H
Mouse NsG30, >IPI00338844.1 (ver. 1.22) **MNKRYLQKAT QGKLLIIIFI VTLWGKAVSS** ANHHKAHHVR TGTCEVVALH RCCNKNKIEE RSQTVKCSCF PGQVAGTTRA APSCVDASIV EQKWWCHMQP CLEGEECKVL PDRKGWSCSS GNKVKTTRVT H
Human NsG31, >IPI00334480.1 (ver. 2.24) **MSERVERNWS TGGWLLALCL AWLWTHLTLA** ALQPPTATVL VQQGTCEVIA AHRCCNRNRI EERSQTVKCS CFSGQVAGTT RAKPSCVDAS IVLQRWWCQM EPCLPGEECK VLPDLSGWSC SSGHKVKTTK VTR
Mouse NsG31, >IPI00380405.1 (ver. 1.22) **MERPTSNWSA GSWVLALCLA WLWTCPASA**S LQPPTSAVLV KQGTCEVIAA HRCCNRNRIE ERSQTVKCSC LSGQVAGTTR AKPSCVDASI VLQKWWCQME PCLLGEECKV LPDLSGWSCS SGHKVKTTKV TR

Human NsG31-long, >IPI00174927.1 (ver. 2.24) **MSERVERNWS TGGWLLALCL AWLWTHLTLA** ALQPPTATVL VQQGTCEVIA AHRCCNRNRI EERSQTVKCS CFSGQVAGTT RAKPSCVDDL LLAAHCARRD PRAALRLLLP QPPSSCRDGG VRWSPACRGR SVRCSRTCRD GAAAVDTKSK PPRSHDSSWG SRPGQERLD
Human NsG32a, > IPI00239265.1.modi, N-terminal predicted using NetStart (ver. 2.24) **MSSTFWAFMI LASLLIAYCS QLAAG**TCEIV TLDRDSSQPR RTIARQTARC ACRKGQIAGT TRARPACVDA RIIKTKQWCD MLPCLEGEGC DLLINRSGWT CTQPGGRIKT TTVS
Human NsG32b, >IPI00385234.1 (ver. 2.29) **MQLLKALWAL AGAALCCFLV LVIHA**QFLKE GQLAAGTCEI VTLDRDSSQP RRTIARQTAR CACRKGQIAG TTRARPACVD ARIIKTKQWC DMLPCLEGEG CDLLINRSGW TCTQPGGRIK TTTVS
Human NsG32a alternative start codon, > IPI00239265.1. (ver. 2.24) **MAPSPRTGSR QDATALPSMS STFWAFMILA SLLIAYCSQL AAG**TCEIVTL DRDSSQPRRT IARQTARCAC RKGQIAGTTR ARPACVDARI IKTKQWCDML PCLEGEGCDL LINRSGWTCT QPGGRIKTTT VS
Mouse NsG32a, >IPI00224493.1.modi N-terminal predicted using NetStart (ver. 1.19) **MSSTFWAFMI LASLLIAYCS QLAAG**TCEIV TLDRDSSQPR RTIARQTARC ACRKGQIAGT TRARPACVDA RIIKTKQWCD MLPCLEGEGC DLLINRSGWT CTQPGGRIKT TTVS
Mouse NsG32b, >IPI00128075.1 (ver. 1.20) **MQLLKALWAL AGAALCCFLV LVIHA**QFLKE GQLAAGTCEI VTLDRDSSQP RRTIARQTAR CACRKGQIAG TTRARPACVD ARIIKTKQWC DMLPCLEGEG CDLLINRSGW TCTQPGGRIK TTTVS
Mouse NsG32a alternative start codon, >IPI00224493.1. (ver. 1.19) **MAPSPRTSSR QDATALPSMS STFWAFMILA SLLIAYCSQL AAG**TCEIVTL DRDSSQPRRT IARQTARCAC RKGQIAGTTR ARPACVDARI IKTKQWCDML PCLEGEGCDL LINRSGWTCT QPGGRIKTTT VS

As can be seen from Figs. 1A and 1B, the proteins are highly conserved between man and mouse. Corresponding proteins with very high conservation are found in rat, and there is also evidence of expression of proteins with similar conserved cysteine-pattern in distantly related species such as Drosophila melanogaster, Danio rerio, Caenorhabditis elegans, Xenopus laevis, and Caenorhabditis briggsae. In these species, predicted protein fragments can be found, which fragments over lengths of 50-110 amino acids have 80-90% sequence identity to Cys10 proteins. The extremely high degree of evolutionary conservation among these species points to important functions in maintaining normal cell function in the tissues where the genes are expressed. In Xenopus laevis and in C. briggsae only two members of the Cys10 family are found. These are most similar to NsG28 and NsG32 respectively. A calculation of sequence identity and construction of a phylogenetic tree also indicate that NsG28-NsG31 have similar features and are distinct from NsG32. As predicted from the high degree of identity among NsG28, NsG29, NsG30, and NsG31, these genes may have a common ancestor gene and some functional redundancy among NsG28, NsG29, NsG30 and NsG31 may therefore be possible.

Together, this indicates that there are at least two distinct functions related to the Cys10 family and that NsG28 in higher animals has evolved into four distinct members.

The percent sequence identity calculated on the basis of the mature part of the human growth factors is shown in Table 2. NsG28-NsG31 share sequence identities above 60%. NsG32 only has 8 of the 10 conserved cysteines. NsG32 is approximately 40% identical to the other members of the family. None of the members of the Cys10 family have a significant sequence identity to any characterised proteins outside this family. NsG31-long only shows partial homology to the other family members due to the frameshift located after the 6th cysteine.

Table 2: Percent sequence identity between human mature protein sequences using Align0 (global alignment). Scoring matrix Blosum50. Gap penalty –12/-2.

	NsG29	NsG30	NsG31-long	NsG31	NsG32a	NsG32b
NsG28a	67.6	77.4	33.8	73.6	41.1	44.9
NsG29	-	66.1	31.4	61.4	39.4	40.5
NsG30		-	36.1	72.8	44.1	46.1
NsG31-long			-	48.2	19.9	20.5
NsG31				-	40.4	41.3
NsG32a					-	89.0

The Clustal W (1.82) multiple sequence alignment of the five human and mouse sequences (Fig. 1B) allows for the identification of one family fingerprint: G-T-**C**-E-[V/I]-[V/I]-x(3)-R-x(5)-[R/K]-x(5)-Q-T-[V/A]-[K/R]-**C**-x-**C**-x(2)-G-x-[V/I]-A-G-T-T-R-x(2)-P-x-**C**-V-[D/E]-A-x-I-[V/I]-x(2)-[K/R]-x-W-**C**-x-M-x-P-**C**-L-x-G-E-x-**C**-x(2)-L-x(4)-G-W-x-**C**-x(2-3)-G-x-[K/R]-[V/I]-K-T-T (SEQ ID NO:1).

It is believed that amino acids, which are fully or partly conserved in this family fingerprint, are important for biological activity, mainly because they are believed to be important for the secondary and/or tertiary structure of the proteins. Non- conserved residues (marked as x) can probably be substituted without affecting biological activity, in particular if the substitution is a conservative substitution or to a residue found at the same or corresponding position in another Cys10 protein. Only NgG32a does not have the initial glycine residue. The same core sequence with minor modifications can be found in Drosophila melanogaster, X.

laevis, C. briggsae, and Danio rerio. The present inventors therefore in one embodiment predict that the core sequence from GTCE to KTT is the bioactive core sequence, which can be used to elicit the same biological effects as mature Cys10 proteins.

From the alignment it can be seen, that NsG32 only has 8 of the 10 cysteines, that are conserved in the other members of the family and that the mature NsG32 protein in the region, where the other members have a double cysteine, has a stretch of amino acids, which differs from all the other members of the family. Another Clustal W (1.82) alignment of only the most similar members of the family, NsG28-NsG31 (Fig. 1A) gives another family fingerprint with a higher degree of conservation across the sub-family: G-T-**C**-E-V-[V/I]-A-x-H-R-**C**-**C**-N-[K/R]-N-[R/K]-I-E-E-R-S-Q-T-V-K-**C**-S-**C**-x(2)-G-x-V-A-G-T-T-R-x(2)-P-S-**C**-V-[D/E]-A-x-I-V-x(2)-[K/R]-W-W-**C**-x-M-x-P-**C**-L-x-G-E-[E/D]-**C**-K-x-L-P-D-x(2)-G-W-x-**C**-x-[S/T]-G-x-K-[V/I]-K-T-T-[R/K] (SEQ ID NO:42).

In another embodiment, the present inventors predict that a NsG28-NsG31 polypeptide comprising this conserved (GTCEV---KTT[RK]) region can elicit the biological effects of mature Cys10 proteins. It is believed that amino acids, which are fully or partly conserved in this family fingerprint, are important for biological activity, mainly because they are believed to be important for the secondary and/or tertiary structure of the proteins. Non- conserved residues (marked as x) can probably be substituted without affecting biological activity, in particular if the substitution is a conservative substitution or to a residue found at the same or corresponding position in another Cys10 protein.

The conserved cysteines may participate in forming intra- and inter-molecular cysteine bridges and may thus play an important role in the secondary and tertiary structure of the proteins, just as is the case for other growth factors with a conserved cysteine pattern (e.g. the TGF-beta family). It is possible that Cys10 proteins may form both homo- and heterodimers.

The ClustalW alignments in Figures 1, 11, 12, 13A, and 13B can be used to identify those parts of the growth factors in which mutations can be made without substantially altering the biological function. In particular it is expected that a residue, which is not fully conserved among mouse, rat, and man (Figs. 11, 12, 13A, and 13B) in the same Cys10 member can be replaced with a residue found at the same position in another species. Furthermore, it is expected that a residues that are non-conserved among the Cys10 members (Figs. 1A and 1B) can be substituted with a residue found in another Cys10 member. In particular, it is possible that

residues at positions that are non-conserved within the NsG28-NsG31 group can be substituted with each other.

Apart from sharing a high degree of sequence identity, all members and variants of the Cys10 family are predicted to belong to the gene ontology class, growth factor and/or hormone (ProtFun prediction, see Example 2). Expression of all five members seems to be restricted to the central and peripheral nervous system including the eye, but each individual member shows differential expression in sub-regions.

II. NsG28, NsG30, and NsG32

NsG28

NsG28a is a 135 amino acid secreted growth factor protein or hormone. The mouse (IPI00311118.3 version 1.23) and rat (IPI00214302.2 version 1.11) have full lengths of 135 and 134 amino acids and the % identities are 93.3 and 91.9, respectively. In the databases a longer version of NsG28 with a different start codon is also found (SEQ ID No 3). An analysis of the probability of start codons however indicates that the most likely start codon is the start codon leading to NsG28a (SEQ ID No 4). Furthermore, a corresponding start codon leading to a longer protein is not found in mouse. SEQ ID No 3 and 4 lead to the same mature protein after cleavage of the signal peptide.

Human NsG28a contains an N-terminal signal peptide sequence, which is predicted to be 29 amino acids long, and which is predicted to be cleaved at the sequence motif MSA-SS. This signal peptide cleavage site is predicted by the SignalP method (Nielsen et al., 1997) and the output graph shown in Figs. 2A-2B. A signal peptide cleavage site is found at a similar location in the mouse and rat NsG28 polypeptide sequences at position 29. As it is known in the art, signal peptide processing is not always exactly as predicted and actual cleavage may vary from case to case. Thus, it is expected that the N-terminal of mature NsG28 may vary by one to two or three amino acids from the predicted cleavage site. The actual N-terminal of mature NsG28 can be verified experimentally by C-terminal tagging with e.g. a his-tag, subsequent purification using a poly-his specific antibody or purification on a Ni column, and finally N-terminal sequencing of the purified mature peptide.

Human NsG28 belongs to the category of proteins acting as hormones or growth factors. This notion is supported by predictions by the ProtFun protein function prediction server (Jensen *et al.*, 2002 & 2003), which provides odds scores above 1 for these categories for both human, mouse and rat NsG28 as shown in Fig. 3.

Results of the quantitative expression analysis in human tissues are shown in Figs. 14 and 15. The expression analyses show:

High to moderate expression in: Substantia Nigra, Retina, Spinal Cord, Thalamus, Corpus Callosum, and Brain.

Low expression in: Putamen, testis, cerebellum, Dorsal Root Ganglion, fetal liver, Hippocampus, Amygdala, and Caudate Nucleus.

Very low or no expression in: Pancreas, heart, muscle, and Pituitary gland.

Results of the quantitative expression analysis in the developing mouse brain (Fig. 24) shows that the expression peaks in P1 ventral mesencephalon (from which the substantia nigra develops) and P1 lateral and medial ganglionic eminences (from which the striatum develops), i.e., at around the time of terminal differentiation in these tissues. Expression in cerebellum also peaks at P1 and is much lower in the adult mouse cerebellum. This means that NsG28 is expressed during development, primarily in the ventral mesencephalon and the striatum during the time of terminal differentiation, strongly suggesting a survival and/or differentiation effect on dopaminergic neurons and striatal medium spiny neurons.

Unlike structural proteins, growth factors are involved in cell signalling and in various functions such as growth, proliferation, differentiation, survival, regeneration, migration, regain of function and improvement of function. Therefore, growth factors can be administered and be used to exert a therapeutic effect. Based on the tissue specific expression, and the fact that NsG28 is predicted to be a secreted growth factor, NsG28 is contemplated for use in treating disorders of the nervous system in general (based on the nervous system specific expression and the neuroprotective and antiapoptotic effect mediated by NsG28), in particular cerebellar disorders (based on the neuroprotective effect of NsG28 in cerebellar granule cells), Parkinson's disease (based on the expression in substantia nigra and thalamus; and the expression in mouse P1 ventral mesencephalon and striatum), Huntington's disease (based on expression in putamen and substantia nigra; and the expression in mouse P1 ventral mesencephalon and striatum), Spinal Cord injury (based on expression in the spinal cord), ALS (based on expression in the spinal cord), peripheral neuropathies (based on expression in dorsal root ganglion and spinal

cord), multiple sclerosis (based on expression in corpus callosum), thalamic pain and essential tremor (based on expression in the thalamus), testicular disorders and male contraception (based on expression in the testicles), and retinopathies (based on expression in retina). The function for the various indications can be verified in in vitro and in vivo assays as described in the examples.

Likewise, expression of therapeutically relevant secreted growth factors including GDNF, NGF, and Neublastin (Artemin) is found in target areas of the neurological disorder they may be used to treat. Expression of GDNF (Fig. 23) is also found in target areas of Parkinson's Disease at around the time of terminal differentiation in this target area.

The therapeutic effect of NsG28 may be mediated through an effect on growth, proliferation, regeneration, regain of function, improvement of function, survival, migration, and/or differentiation of targeted cells.

One verified biological function of NsG28 is a neuroprotective effect against potassium deprivation-induced apoptosis in cerebellar granule cells (Fig. 36). Similarly, other members of the Cys10 family, NsG30, NsG32a, and NsG32b, have also demonstrated a survival enhancing effect in a cell line with neuronal potential (PC12 assay described in Example 5 herein) and in cerebellar granule cells (NsG32a; Example 7). These assays test the ability of the factors to protect cerebellar granule cells (Example 7) or a neuronal cell line (Example 5) against apoptotic cell death. Apoptotic cell death contributes to neuronal cell loss in the adult nervous system causing various neurological disorders like ischemic stroke, neurodegenerative diseases or brain traumata (Becker & Bonni, Prog Neurobiol, 2004 Jan; 72(1):1-25). A secreted growth factor capable of protecting neuronal cells (e.g., cerebellar cells) against apoptotic cell death is therefore a candidate for treating neurological disorders (e.g., disorders associated with the Cerebellum). The ability of a secreted growth factor to promote survival under conditions leading to apoptosis is an indication that this factor has a similar effect in other neuronal cell types of the central and/or peripheral nervous system.

NsG30

NsG30 is a 131 amino acid secreted growth factor protein or hormone. The mouse (IPI00338844.1 version 1.22) and rat (IPI00205786.1 version 1.3) homologues have are 131 and 95 (partial sequence) amino acids long and the % identities are 96.9 and 71.0, respectively.

Human NsG30 contains a N-terminal signal peptide sequence of 30 amino acids which is cleaved at the sequence motif VSS-AN. This signal peptide cleavage site is predicted by the

SignalP method (Nielsen *et al.*,1997) and the output graph shown in Figs. 4A-4B. A signal peptide cleavage site is found at a similar location in the mouse NsG30 at position 30.

As it is known in the art, signal peptide processing is not always exactly as predicted and actual cleavage may vary from case to case. Thus, it is expected that the N-terminal of mature NsG30 may vary by one to two or three amino acids from the predicted cleavage site. The actual N-terminal of mature NsG30 can be verified experimentally by C-terminal tagging with e.g. a his-tag, subsequent purification using a poly-his specific antibody or purification on a Ni column, and finally N-terminal sequencing of the purified mature peptide.

Human NsG30 belongs to the category of proteins acting as hormones or growth factors. This notion is supported by predictions by the ProtFun protein function prediction server (Jensen *et al.*, 2002 & 2003), which provides scores above 1 for both these categories as shown in Fig. 5.

Results of the quantitative expression analysis in human tissues (Example 4a) are shown in Figs. 16 and 17. The expression analyses show:

High to moderate expression in: Brain, Substantia Nigra, Hippocampus, Amygdala, and Thalamus

Intermediate and low expression in: Putamen, Retina, Spinal Cord, Dorsal Root Ganglion, testis, cerebellum, Corpus Callosum, Caudate Nucleus, and Pituitary

Very low or no expression in: Foetal liver, pancreas, muscle, Heart

The expression pattern of NsG30 in the developing mouse CNS (Fig. 28) resembles that of NsG28. There is high expression in the ventral mesencephalon and the striatum during the latter part of the development during the early postnatal period. This relatively restricted regional and temporal expression pattern indicates a role in the differentiation and termination of the projections between the VM and the striatum (LGE/MGE). Similar to GDNF (and NsG28) a therapeutic effect on neurons involved in Huntingdon's and Parkinson's diseases is strongly indicated. Expression in the cortex, cerebellum, and dorsal mesencephalon also peaks in the developing mouse CNS during the early postnatal period (P1).

Furthermore, gene chip experiments (Example 4B) have shown that human NsG30 is expressed at significant levels in the developing human mesencephalon. This expression is indicative of a potential therapeutic use in the treatment of Parkinson's Disease.

Unlike structural proteins, growth factors are involved in cell signalling and in various functions such as growth, proliferation, differentiation, survival, regeneration, migration, regain

of function and improvement of function. Therefore, growth factors can be administered and be used to exert a therapeutic effect. Based on the tissue specific expression, and the fact that NsG30 is predicted to be a secreted growth factor, NsG30 is contemplated for use in treating disorders of the nervous system in general (based on the nervous system specific expression), in particular Parkinson's disease (based on the expression in substantia nigra, thalamus, and the developing human mesencephalon; and the developing mouse mesencephalon and striatum), Huntington's disease (based on expression in putamen, substantia nigra, and thalamus; and the developing mouse striatum), Alzheimer's disease (based on expression in hippocampus and amygdala), tremor including central tremor (based on expression in thalamus), thalamic pain (based on expression in the thalamus), cerebellar disorders (based on expression in adult human cerebellum and the developing mouse cerebellum), Spinal Cord injury (based on expression in the adult human spinal cord and the developing mouse spinal cord), ALS (based on expression in the adult human spinal cord and the developing mouse spinal cord), peripheral neuropathies (based on expression in dorsal root ganglion), retinopathies (based on expression in retina), testicular disorders (based on expression in testes). The function for the various indications can be verified in in vitro and in vivo assays as described in the examples.

Likewise, expression of therapeutically relevant secreted growth factors including GDNF, NGF, and Neublastin (Artemin) is found in target areas of the neurological disorder they may be used to treat.

The therapeutic effect of NsG30 may be mediated through an effect on growth, proliferation, regeneration, regain of function, improvement of function, survival, migration, and/or differentiation of targeted cells.

One verified biological function of NsG30 is a neuroprotective effect against starvation induced apoptosis in PC12 (pheochromocytoma) cells. Pheochromocytomas are tumors with characteristics of immature and adult chromaffin cells of the adrenal medulla. Chromaffin cells, sensory and sympathetic neurons in addition to pigment cells (melanocytes) are derived from a common precursor cell in the neural crest. Its differentiation into the specific lineages is highly dependent on external signals including secreted factors.

PC12 is a clonal cell line, which was originally established from a transplantable rat adrenal medullary pheochromocytoma (Greene and Tischler, 1976 Proc. Natl. Acad. Sci. U.S.A. 73, 2424). PC-12 cells are available from ATCC (American Type Culture Collection; accession number CRL-1721). PC12 cells are considered to be the pluripotent chromaffin precursor cell as

it possesses the ability to differentiate to mature chromaffin cells, sympathetic neurons, as well as melanocytes depending on the culture conditions. PC12 cells have been widely used as a model system for studies of neuronal differentiation and survival. In serum-containing medium PC12 cells proliferate, whereas addition of certain neurotrophic factors including NGF induces differentiation of PC12 cells into a neuronal phenotype very similar to sympathetic neurons. In serum-free medium, PC12 cells will become apoptotic and die unless supplied with certain growth factors, hormones or small molecules that can act as survival factors.

The factors capable inducing differentiation and survival in PC12 cells including one of the neurotrophins (NGF) and a member of the secretin/glucagon/VIP family (PACAP) also display a similar activity in both the peripheral and central nervous system indicating that receptors and response systems expressed in PC12 cells are shared with many other neuronal cells.

NGF is an important differentiation and survival factor for responsive sympathetic and sensory neurons in addition to cholinergic neurons in the basal forebrain. PACAP promotes the differentiation of nascent dorsal root ganglion (DRG) neurons in that it increases both the number of neural-marker-positive cells and axonogenesis without affecting the proliferation of neural progenitor cells (Nielsen et al., Mol Cell Neurosci. 2004 Apr;25(4):629-41). PACAP also show similar activities in neuronal populations in the CNS (Vaudry et al., Proc Natl Acad Sci U S A. 2002 Apr 30;99(9):6398-403; Dicicco-Bloom et al., Ann N Y Acad Sci. 1998 Dec 11;865:274-89).

Apoptotic cell death contributes to the neuronal cell loss in the adult nervous system causing various neurological disorders like ischemic stroke, neurodegenerative diseases or brain traumata (Becker and Bonni, Prog Neurobiol. 2004 Jan;72(1):1-25). A secreted growth factor capable of protecting neuronal cells against apoptotic cell death is therefore a candidate for treating disorders of the nervous system in general and neurodegenerative disorders in particular. Thus, the ability of a secreted factor to induce neurite outgrowth and/or to promote survival in under conditions leading to apoptosis is an indication that this factor has a similar effect in other neuronal cell types of the central and/or peripheral nervous system and that this factor is a candidate for treating nervous system disorders, in particular neurodegenerative disorders.

NsG32

Two splice variants of NsG32 are found (Fig. 29). One is referred to as NsG32a, and the other splice variant is referred to as NsG32b. The amino acid sequence for human NsG32b is set forth in SEQ ID No 31. The identical amino acid sequence for mouse NsG32b is set forth in SEQ ID No 38. The difference between NsG32a and b on the polypeptide level is found in the signal sequence. From amino acid no 32 of human NsG32b, it is identical to human NsG32a. The difference in signal sequence also results in a difference in predicted cleavage, so that the mature NsG32b proteins have a longer N-terminal than NsG32a. Because of the longer N-terminal, mature NsG32b is more similar to the other members of the Cys10 protein family than mature NsG32a (see Fig. 1B) and thus appears the most probable splice variant.

NsG32a (SEQ ID No 27) is a 114 amino acid secreted protein. The mouse and rat homologues both have full lengths of 114 amino acids and 100 % sequence identity to human NsG32a (Fig. 13A). Two alternative start codons can be found for both mouse and human NsG32a. These, however result in the same mature protein after cleavage of the signal peptide. The start codons for human and mouse NsG32a have been predicted using the program NetStart (A.G. Pedersen and H. Nielsen, 1997) and these predictions are shown in Figs. 6 and 7, respectively. The prediction shows that the start codon corresponding to the short version of NsG32a is the most likely start codon.

Human and mouse NsG32b are identical (Fig. 13B).

Human NsG32a contains an N-terminal signal peptide sequence of 25 amino acids, which is cleaved at the sequence motif AAG-TC. This signal peptide cleavage site is predicted by the SignalP method (Nielsen *et al.*, 1997) and the output graph shown in Figs. 8A-8B.

Human NsG32b also contains an N-terminal signal peptide sequence of 25 amino acids, which is cleaved at the sequence motif IHA-QF. This signal peptide cleavage site is predicted by the SignalP method (Nielsen *et al.*, 1997) and the output graph shown in Figs. 9A-9B.

As it is known in the art, signal peptide processing is not always exactly as predicted and actual cleavage may vary from case to case. Thus, it is expected that the N-terminal of mature NsG32 may vary by one to two or three amino acids from the predicted cleavage site. The actual N-terminal of mature NsG32 can be verified experimentally by C-terminal tagging with e.g. a his-tag, subsequent purification using a poly-his specific antibody or purification on a Ni column, and finally N-terminal sequencing of the purified mature peptide.

NsG32a belongs to the category of proteins acting as hormones or growth factors. This notion is supported by predictions by the ProtFun protein function prediction server (Jensen *et*

al., 2002 & 2003), which provides odds scores above 1 for the hormone as well as growth factor category as shown in Fig. 10. Human NsG32b also provides odds scores above 1 for the hormone ontology class.

The results of the quantitative RT-PCR expression analyses in human tissues are shown in Figs. 18 and 19,

High expression was found in: Cerebellum, Substantia Nigra, Brain, Drosal Root Ganglion, Spinal Cord, Putamen, Foetal Brain, Hippocampus, Amygdala, Thalamus, Corpus Callosum, and Caudate Nucleus

Low expression was found in: Prostate, Placenta, Small Intestines, Foetal liver, Stomach, Lung, Heart, Uterus, Skeletal muscle, Colon, Pancreas, Trachea, Thymus, Spleen, and pituitary.

Expression has also been found in the developing human mesencephalon (Example 4b).

The results of the quantitative RT-PCR in the developing mouse CNS are shown in Fig. 35. The expression peaks in the early postnatal ventral mesencephalon and the early postnatal medium and lateral ganglionic eminences (from which the striatum develops). This corresponds to the period of differentiation and terminal differentiation of the projections between the VM and the striatum.

Unlike structural proteins, growth factors are involved in cell signalling and in various functions such as growth, proliferation, differentiation, survival, regeneration, migration, regain of function and improvement of function. Therefore, growth factors can be administered and be used to exert a therapeutic effect. Based on the tissue specific expression, and the fact that NsG32 is predicted to be a secreted growth factor, NsG32 is contemplated for use in treating disorders of the nervous system in general (based on the nervous system-specific expression), in particular Parkinson's disease (based on the expression in human substantia nigra and thalamus, and the differential expression in the developing mouse VM and striatum), Alzheimer's disease (based on expression in amygdala and hippocampus), Huntington's disease (based on expression in human putamen, substantia nigra, and thalamus, and the differential expression in the developing mouse striatum and VM), cerebellar disorders including ataxia (based on expression in cerebellum), peripheral neuropathies (based on expression in dorsal root ganglion), thalamic pain and essential tremor (based on expression in the thalamus), multiple sclerosis (based on expression in corpus callosum and spinal cord), and amyotrophic lateral sclerosis and spinal cord injury (based on expression in the spinal cord). The function for the various indications can be verified in in vitro and in vivo assays as described in the examples.

Likewise, expression of therapeutically relevant secreted growth factors including GDNF, NGF, and Neublastin (Artemin) is found in target areas of the neurological and/or testicular disorder they may be used to treat.

The therapeutic effect of NsG32 may be mediated through an effect on growth, proliferation, regeneration, regain of function, improvement of function, survival, migration, and/or differentiation of targeted cells.

One verified biological function of both splice variants of NsG32 is a neuroprotective effect against starvation-induced apoptosis in PC12 (pheochromocytoma) cells (Fig. 34). Pheochromocytomas are tumors with characteristics of immature and adult chromaffin cells of the adrenal medulla. Chromaffin cells, sensory and sympathetic neurons in addition to pigment cells (melanocytes) are derived from a common precursor cell in the neural crest. Its differentiation into the specific lineages is highly dependent on external signals including secreted factors.

An additional verified biological function of NsG32a is a neuroprotective effect against potassium deprivation-induced apoptosis in cerebellar granule cells (Fig. 36). A secreted growth factor capable of protecting neuronal cells against apoptotic cell death is therefore a candidate for treating neurological disorders (e.g., disorders associated with the Cerebellum). Thus, the ability of a secreted factor to induce neurite outgrowth and/or to promote survival in under conditions leading to apoptosis is an indication that this factor has a similar effect in other neuronal cell types of the central and/or peripheral nervous system and that this factor is a candidate for treating nervous system disorders, in particular neurodegenerative disorders.

III. NsG28, NsG30, and NsG32 Polypeptides

NsG28 Polypeptides

In addition to full-length NsG28, substantially full-length NsG28, and to truncated forms of NsG28, the present invention provides for biologically active variants of the polypeptides. An NsG28 polypeptide or fragment is biologically active if it exhibits a biological activity of naturally occurring NsG28. It is to be understood that the invention relates to substantially purified NsG28 as herein defined.

One biological activity is the ability to compete with naturally occurring NsG28 in a receptor-binding assay. Another biological activity is the ability to bind to an antibody, which is directed at an epitope, which is present on naturally occurring NsG28.

Biologically active variants may also be defined with reference to one or more of the other in vitro and/or in vivo biological assays described in the examples.

A preferred biological activity is the ability to elicit substantially the same response as in the PC12 assay described in the Examples. In this assay PC12 cells are transduced with full length human NsG28 coding sequence (Fig. 20). By substantially the same response in the PC12 assay is intended that the number of neurite bearing cells is at least 10% of the number obtained in Example 5 (transduction with full length human NsG28), more preferably at least 20%, more preferably at least 30%, more preferably at least 40%, more preferably at least 50%, more preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%. The PC12 assay may also be used to document the percentage improvement in survival over a control treatment. Substantially the same response in this context means an activity resulting in at least 10% of the improvement obtained in Example 5, more preferably at least 20%, more preferably at least 30%, more preferably at least 40%, more preferably at least 50%, more preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 90%. The biological activity of a fragment or variant of NsG28 may also be higher than that of the naturally occurring NsG28.

Specific fragments of NsG28 include polypeptides having a sequence selected from the group consisting of SEQ ID No. 6, 11, and 15 (or a subgroup thereof), and sequence variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed. These isolated polypeptides constitute NsG28 polypeptides spanning from the first to the last of the 10 conserved cysteine residues of the Cys10 family. It is believed that the biological activity mainly resides in this part of the protein, and that the sequence with all the ten cysteines is required for correct folding of the protein. Preferably any changes in these peptides are to residues marked in the alignment of Fig. 1A as unconserved, weakly conserved, or strongly conserved. More preferably any changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in Fig. 11. Preferably any changed amino acid is changed to a residue found at the same or corresponding position in another Cys10

protein (see Figs. 1A and 1B), more preferably to a residue found at the same or corresponding position in an NsG28 sequence from another species, such as the species shown in Fig. 11. In a preferred embodiment, less than 8 amino acids have been changed, more preferably less than 5 amino acids, more preferably 1 or 2 amino acids, more preferably no amino acids have been changed. These truncated NsG28 polypeptides may have up to 5 additional C and/or N-terminal amino acids selected from those of the mature NsG28 polypeptides, i.e. fragments up to AA_{14}-AA_{98} of SEQ ID No 5, fragments up to AA_{14}-AA_{98} of SEQ ID No. 10, and fragments up to AA_{14}-AA_{98} of SEQ ID No. 14.

Further specific polypeptides are selected from the group consisting of SEQ ID No 7, 12, and 16 (or a subgroup thereof), and sequence variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed. These isolated polypeptides constitute the NsG28 core sequence, which is conserved in the Cys10 subfamily shown in Fig. 1A. Preferably any changes in these peptides are to residues marked in the alignment of Fig. 1A as unconserved, weakly conserved, or strongly conserved. More preferably any changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in Fig. 11. Preferably any changed amino acid is changed to a residue found at the same or corresponding position in another Cys10 protein (see Figs. 1A and 1B), more preferably to a residue found at the same or corresponding position in an NsG28 sequence from another species, such as the species shown in Fig. 11. In a preferred embodiment, less than 8 amino acids have been changed, more preferably less than 5 amino acids, more preferably 1 or 2 amino acids, more preferably no amino acids have been changed. These truncated NsG28 polypeptides may have up to 5 additional C and/or N-terminal amino acids selected from those of the mature NsG28 polypeptides, i.e. fragments up to AA_{12}-AA_{106} of SEQ ID No 5, fragments up to AA_{12}-AA_{106} of SEQ ID No. 10, and fragments up to AA_{12}-AA_{105} of SEQ ID No. 14.

Variants can differ from naturally occurring NsG28 in amino acid sequence or in ways that do not involve sequence, or in both ways. Variants in amino acid sequence ("sequence variants") are produced when one or more amino acids in naturally occurring NsG28 is substituted with a different natural amino acid, an amino acid derivative or non-native amino acid. Particularly preferred variants include naturally occurring NsG28 or biologically active fragments of naturally occurring NsG28 whose sequences differ from the wild type sequence by one or more conservative and/or semi-conservative amino acid substitutions, which typically have minimal influence on the secondary and tertiary structure and hydrophobic nature of the

protein or peptide. Variants may also have sequences, which differ by one or more non-conservative amino acid substitutions, deletions or insertions, which do not abolish the NsG28 biological activity. The Clustal W alignments in Figs. 1A, 1B, and 11 can be used to predict which amino acid residues can be substituted without substantially affecting the biological activity of the protein.

Substitutions within the following group (Clustal W, 'strong' conservation group) are to be regarded as conservative substitutions within the meaning of the present invention

-STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

Substitutions within the following group (Clustal W, 'weak' conservation group) are to be regarded as semi-conservative substitutions within the meaning of the present invention

-CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY.

Other variants within the invention are those with modifications which increase peptide stability. Such variants may contain, for example, one or more nonpeptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are: variants that include residues other than naturally occurring L-amino acids, such as D-amino acids or non-naturally occurring or synthetic amino acids such as beta or gamma amino acids and cyclic variants. Incorporation of D-instead of L-amino acids into the polypeptide may increase its resistance to proteases. See, e. g., U. S. Patent 5,219,990. Splice variants are specifically included in the invention.

When the result of a given substitution cannot be predicted with certainty, the derivatives may be readily assayed according to the methods disclosed herein to determine the presence or absence of biological activity.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with human, murine or rat NsG28 (SEQ ID NO: 3, 4, 9, and 13). More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with a polypeptide having a sequence selected from the group consisting of SEQ ID NO: 5, 10, and 14 (or a subgroup

thereof). More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with a polypeptide having the sequence of SEQ ID NO: 6, 11, or 15. More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with a polypeptide having the sequence of SEQ ID NO: 7, 12, or 16, More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %.

In a preferred embodiment the sequence identity of the variant NsG28 is determined with reference to a human NsG28 polypeptide (SEQ ID No 3, 4, 5, 10, and 14).

For the purposes of determining homology the minimum length of comparison sequences will generally be at least 8 amino acid residues, usually at least 12 amino acid residues. For the purposes of the present invention, the percent sequence identity is preferably calculated in a range of overlap of at least 25 amino acids, more preferably at least 30 amino acids, more preferably at least 35, more preferably at least 40, more preferably at least 45, more preferably at least 50, more preferably at least 55, more preferably at least 60, such as at least 70, for example at least 80, such as at least 90, the range being determined by BLASTP under default settings.

In one embodiment the percent sequence identity is calculated using global alignment (GAP or Align), so that the variant and SEQ ID sequences are aligned, the total number of identical amino acid residues calculated and divided by the length of the SEQ ID NO.

In one embodiment, a variant NsG28 comprises a naturally occurring allelic variant of the sequence selected from the group consisting of SEQ ID No 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, and 16 (or a subgroup thereof). Said allelic variant sequence may be an amino acid sequence that is the translation of a nucleic acid sequence differing by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID No 2 and 8.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 3, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 4, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 5, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 6, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 7, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 9, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 10, more preferably at least 65%, more

preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 11, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 12, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 13, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 14, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 15, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 16, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more

preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, a variant NsG28 at corresponding positions comprises the residues marked in Fig. 11 as fully conserved (*), more preferably a variant NsG28 also comprises at corresponding positions the residues marked in Figs. 11, 12, 13A, and 13B as strongly conserved (: strongly conserved groups include: STA, NEQK, NHQK, NEDQ, QHRK, MILV, MILF, HY FYW), more preferably a variant NsG28 also comprises at corresponding positions the residues marked in Figs. 11, 12, 13A, and 13B as less conserved (less conserved groups include: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHK, NEQHRK, VLIM, HFY). In particular, it is contemplated that the conserved cysteines (Fig. 1B) preferably are located at corresponding positions in a variant NsG28.

NsG30 Polypeptides

In addition to full-length NsG30, substantially full-length NsG30, and to truncated forms of NsG30, the present invention provides for biologically active variants of the polypeptides. An NsG30 polypeptide or fragment is biologically active if it exhibits a biological activity of naturally occurring NsG30. It is to be understood that the invention relates to substantially purified NsG30 as herein defined.

One biological activity is the ability to compete with naturally occurring NsG30 in a receptor-binding assay. Another biological activity is the ability to bind to an antibody, which is directed at an epitope, which is present on naturally occurring NsG30.

Biologically active variants may also be defined with reference to one or more of the other in vitro and/or in vivo biological assays described in the examples.

A preferred biological activity is the ability to elicit substantially the same response as in the PC12 assay described in the Examples. In this assay, PC12 cells are transduced with full length human NsG30 coding sequence (Fig. 26). By substantially the same response in the PC12 assay is intended that the number of neurite bearing cells is at least 10% of the number obtained in Example 5 (transduction with full length human NsG30), more preferably at least 20%, more preferably at least 30%, more preferably at least 40%, more preferably at least 50%, more preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%. The PC12 assay may also be used to document the percentage improvement in survival over a control

treatment. Substantially the same response in this context means an activity resulting in at least 10% of the improvement obtained in Example 5, more preferably at least 20%, more preferably at least 30%, more preferably at least 40%, more preferably at least 50%, more preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 90%. The biological activity of a fragment or variant of NsG30 may also be higher than that of the naturally occurring NsG30.

Specific fragments of NsG30 include polypeptides having the sequence of SEQ ID No. 20, and sequence variants of said polypeptide, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed. These isolated polypeptides constitute NsG30 polypeptides spanning from the first to the last of the 10 conserved cysteine residues of the Cys10 family. It is believed that the biological activity mainly resides in this part of the protein, and that the sequence with all the ten cysteines is required for correct folding of the protein. Preferably any changes in these peptides are to residues marked in the alignment of Fig. 1A as unconserved, weakly conserved, or strongly conserved. More preferably any changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in Fig. 12. Preferably any changed amino acid is changed to a residue found at the same or corresponding position in another Cys10 protein (see Figs. 1A and 1B), more preferably to a residue found at the same or corresponding position in an NsG30 sequence from another species, such as the species shown in Fig. 12. In a preferred embodiment, less than 8 amino acids have been changed, more preferably less than 5 amino acids, more preferably 1 or 2 amino acids, more preferably no amino acids have been changed. These truncated NsG30 polypeptides may have up to 5 additional C and/or N-terminal amino acids selected from those of the mature NsG30 polypeptides, i.e. fragments up to AA_9-AA_{93} of SEQ ID No 19 or 24.

Further specific fragments are polypeptides having the sequence of SEQ ID No 21, and sequence variants of said polypeptide, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed. These isolated polypeptides constitute the NsG30 core sequence, which is conserved in the Cys10 subfamily shown in Fig. 1A. Preferably any changes in these peptides are to residues marked in the alignment of Fig. 1A as unconserved, weakly conserved, or strongly conserved. More preferably any changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in Fig. 12. Preferably any changed amino acid is changed to a residue found at the same or corresponding position in

another Cys10 protein (see Figs. 1A and 1B), more preferably to a residue found at the same or corresponding position in an NsG30 sequence from another species, such as the species shown in Fig. 12. In a preferred embodiment, less than 8 amino acids have been changed, more preferably less than 5 amino acids, more preferably 1 or 2 amino acids, more preferably no amino acids have been changed. These truncated NsG30 polypeptides may have up to 5 additional C and/or N-terminal amino acids selected from those of the mature NsG30 polypeptides, i.e. fragments up to AA_7-AA_{101} of SEQ ID No 19 or 24.

Variants can differ from naturally occurring NsG30 in amino acid sequence or in ways that do not involve sequence, or in both ways. Variants in amino acid sequence ("sequence variants") are produced when one or more amino acids in naturally occurring NsG30 is substituted with a different natural amino acid, an amino acid derivative or non-native amino acid. Particularly preferred variants include naturally occurring NsG30, or biologically active fragments of naturally occurring NsG30, whose sequences differ from the wild type sequence by one or more conservative and/or semi-conservative amino acid substitutions, which typically have minimal influence on the secondary and tertiary structure and hydrophobic nature of the protein or peptide. Variants may also have sequences, which differ by one or more non-conservative amino acid substitutions, deletions or insertions, which do not abolish the NsG30 biological activity. The Clustal W alignments in Figs. 1A, 1B, and 12 can be used to predict which amino acid residues can be substituted without substantially affecting the biological activity of the protein.

Substitutions within the following group (Clustal W, 'strong' conservation group) are to be regarded as conservative substitutions within the meaning of the present invention

-STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

Substitutions within the following group (Clustal W, 'weak' conservation group) are to be regarded as semi-conservative substitutions within the meaning of the present invention

-CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY.

Other variants within the invention are those with modifications which increase peptide stability. Such variants may contain, for example, one or more nonpeptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are: variants that include residues other than naturally occurring L-amino acids, such as D-amino acids or non-naturally occurring or synthetic amino acids such as beta or gamma amino acids and cyclic variants. Incorporation of

D-instead of L-amino acids into the polypeptide may increase its resistance to proteases. See, e. g., U. S. Patent 5,219,990. Splice variants are specifically included in the invention.

When the result of a given substitution cannot be predicted with certainty, the derivatives may be readily assayed according to the methods disclosed herein to determine the presence or absence of biological activity.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with human, murine or rat NsG30 (SEQ ID NO: 18, 23, and 25). More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with a polypeptide having a sequence selected from the group consisting of SEQ ID NO: 19 and 24. More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %.

In a preferred embodiment the sequence identity of the variant NsG30 is determined with reference to a human NsG30 polypeptide (SEQ ID No 18, 19, 20, and 21).

For the purposes of determining homology the minimum length of comparison sequences will generally be at least 8 amino acid residues, usually at least 12 amino acid residues. For the purposes of the present invention, the percent sequence identity is preferably calculated in a range of overlap of at least 25 amino acids, more preferably at least 30 amino acids, more preferably at least 35, more preferably at least 40, more preferably at least 45, more preferably at least 50, more preferably at least 55, more preferably at least 60, such as at least 70, for example at least 80, such as at least 90, the range being determined by BLASTP under default settings.

In one embodiment the percent sequence identity is calculated using global alignment (GAP or Align), so that the variant and SEQ ID sequences are aligned, the total number of identical amino acid residues calculated and divided by the length of the SEQ ID NO.

In one embodiment, a variant NsG30 comprises a naturally occurring allelic variant of the sequence selected from the group consisting of SEQ ID No 18, 19, 20, 21, 23, 24, and 25 (or a subgroup thereof). Said allelic variant sequence may be an amino acid sequence that is the

translation of a nucleic acid sequence differing by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID No 17 and 22.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 18, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 19, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 20, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 21, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 23, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 24, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 25, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, a variant NsG30 at corresponding positions comprises the residues marked in Fig. 12 as fully conserved (*), more preferably a variant NsG30 also comprises at corresponding positions the residues marked in Fig. 12 as strongly conserved (strongly conserved groups include: STA, NEQK, NHQK, NEDQ, QHRK, MILV, MILF, HY FYW), more preferably a variant NsG30 also comprises at corresponding positions the residues marked in Fig. 12 as less conserved (less conserved groups include: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHK, NEQHRK, VLIM, HFY). In particular, it is contemplated that the conserved cysteines (Fig. 1B) preferably are located at corresponding positions in a variant NsG30.

NsG32 Polypeptides

In addition to full-length NsG32, substantially full-length NsG32, mature NsG32, and to truncated forms of NsG32, the present invention provides for biologically active variants of the polypeptides. An NsG32 polypeptide or fragment is biologically active if it exhibits a biological activity of naturally occurring NsG32. It is to be understood that the invention relates to substantially purified NsG32 as herein defined.

One biological activity is the ability to compete with naturally occurring NsG32 in a receptor-binding assay. Another biological activity is the ability to bind to an antibody, which is directed at an epitope, which is present on naturally occurring NsG32.

Biologically active variants may also be defined with reference to one or more of the other in vitro and/or in vivo biological assays described in the examples.

Preferably, biological activity of naturally occurring NsG32 is the activity of human or mouse NsG32b having the amino acid sequence of SEQ ID No. 30 and 37, respectively.

A preferred biological activity is the ability to elicit substantially the same response as in the PC12 assay described in the Examples. In this assay PC12 cells are transduced with full-length human NsG32a or NsG32b coding sequence. By substantially the same response in the

PC12 assay is intended that the number of neurite bearing cells is at least 10% of the number obtained in Example 5 (transduction with full length human NsG32a or NsG32b CDS), more preferably at least 20%, more preferably at least 30%, more preferably at least 40%, more preferably at least 50%, more preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%. The PC12 assay may also be used to document the percentage improvement in survival over a control treatment. Substantially the same response in this context means an activity resulting in at least 10% of the improvement obtained in Example 5, more preferably at least 20%, more preferably at least 30%, more preferably at least 40%, more preferably at least 50%, more preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 90%. The biological activity of a fragment or variant of NsG32 may also be higher than that of the naturally occurring NsG32.

Specific fragments of NsG32 include polypeptides having the of SEQ ID No. 34, and sequence variants of said polypeptide, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed. These isolated polypeptides constitute NsG32 polypeptides spanning from the first to the last of the 8 Cys10 conserved cysteine residues found in NsG32. It is believed that the biological activity mainly resides in this part of the protein, and that the sequence with all the eight cysteines is required for correct folding of the protein. Preferably any changes in these peptides are to residues marked in the alignment of Fig. 1A as unconserved, weakly conserved, or strongly conserved. Preferably any changed amino acid is changed to a residue found at the same or corresponding position in another Cys10 protein (see Figs. 1A and 1B). In a preferred embodiment, less than 8 amino acids have been changed, more preferably less than 5 amino acids, more preferably 1 or 2 amino acids, more preferably no amino acids have been changed. These truncated NsG32 polypeptides may have up to 5 additional C and/or N-terminal amino acids selected from those of the mature NsG32 polypeptides, i.e. fragments up to AA_8-AA_{92} of SEQ ID No 32.

Further specific fragments include polypeptides having the sequence of SEQ ID No 33, and sequence variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed. These isolated polypeptides constitute a core fragment of NsG32b corresponding to the core sequence, which is conserved in the Cys10 subfamily

shown in Fig. 1A. Preferably any changes in these peptides are to residues marked in the alignment of Fig. 1A as unconserved, weakly conserved, or strongly conserved. Preferably any changed amino acid is changed to a residue found at the same or corresponding position in another Cys10 protein (see Fig. 1B). In a preferred embodiment, less than 8 amino acids have been changed, more preferably less than 5 amino acids, more preferably 1 or 2 amino acids, more preferably no amino acids have been changed. These truncated NsG32b polypeptides may have up to 5 additional C and/or N-terminal amino acids selected from those of the mature NsG32b polypeptides, i.e. fragments up to AA_6-AA_{100} of SEQ ID No 32.

Variants can differ from naturally occurring NsG32 in amino acid sequence or in ways that do not involve sequence, or in both ways. Variants in amino acid sequence ("sequence variants") are produced when one or more amino acids in naturally occurring NsG32 is substituted with a different natural amino acid, an amino acid derivative or non-native amino acid. Particularly preferred variants include naturally occurring NsG32, or biologically active fragments of naturally occurring NsG32, whose sequences differ from the wild type sequence by one or more conservative and/or semi-conservative amino acid substitutions, which typically have minimal influence on the secondary and tertiary structure and hydrophobic nature of the protein or peptide. Variants may also have sequences, which differ by one or more non-conservative amino acid substitutions, deletions or insertions, which do not abolish the NsG32 biological activity. The Clustal W alignment in Fig. 1B can be used to predict which amino acid residues can be substituted without substantially affecting the biological activity of the protein.

Substitutions within the following group (Clustal W, 'strong' conservation group) are to be regarded as conservative substitutions within the meaning of the present invention

-STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

Substitutions within the following group (Clustal W, 'weak' conservation group) are to be regarded as semi-conservative substitutions within the meaning of the present invention

-CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY.

Other variants within the invention are those with modifications, which increase peptide stability. Such variants may contain, for example, one or more nonpeptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are: variants that include residues other than naturally occurring L-amino acids, such as D-amino acids or non-naturally occurring or synthetic amino acids such as beta or gamma amino acids and cyclic variants. Incorporation of

D-instead of L-amino acids into the polypeptide may increase its resistance to proteases. See, e. g., U. S. Patent 5,219,990. Splice variants are specifically included in the invention.

When the result of a given substitution cannot be predicted with certainty, the derivatives may be readily assayed according to the methods disclosed herein to determine the presence or absence of biological activity.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with human or mouse NsG32a (SEQ ID NO: 27, 28, and 36). More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with a polypeptide having a sequence selected from the group consisting of SEQ ID NO: 27, 28, and 31 (or a subgroup thereof). More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %. These polypeptides constitute full-length NsG32 polypeptides.

Preferred variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with a polypeptide having a sequence selected from the group consisting of SEQ ID NO: 31 and 32. More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %. These polypeptides constitute full length NsG32b and mature NsG32b.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with a polypeptide having the sequence of SEQ ID NO: 29 and 32. More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %. These polypeptides constitute mature human NsG32 proteins.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with a polypeptide having the sequence of SEQ ID NO: 27, 28, 29, 31, 32, 33, and 34. More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %. These polypeptides constitute human NsG32 proteins.

For the purposes of determining homology the minimum length of comparison sequences will generally be at least 8 amino acid residues, usually at least 12 amino acid residues. For the purposes of the present invention, the percent sequence identity is preferably calculated in a range of overlap of at least 25 amino acids, more preferably at least 30 amino acids, more preferably at least 35, more preferably at least 40, more preferably at least 45, more preferably at least 50, more preferably at least 55, more preferably at least 60, such as at least 70, for example at least 80, such as at least 90, the range being determined by BLASTP under default settings.

In one embodiment the percent sequence identity is calculated using global alignment (GAP or Align), so that the variant and SEQ ID sequences are aligned, the total number of identical amino acid residues calculated and divided by the length of the SEQ ID NO.

In one embodiment, a variant NsG32 comprises a naturally occurring allelic variant of the sequence selected from the group consisting of SEQ ID No 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41 (or a subgroup thereof). Said allelic variant sequence may be an amino acid sequence that is the translation of a nucleic acid sequence differing by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID No 26, 30, 35, 37, and 39 (or a subgroup thereof).

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 27, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 28, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more

preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 29, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 31, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 32, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 33, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 34, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 41, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In particular, it is contemplated that the eight conserved cysteines (Fig. 1B) preferably are located at corresponding positions in a variant NsG32.

Non-sequence modifications may include, for example, in vivo or in vitro chemical derivatisation of portions of naturally occurring NsG28, NsG30, or NsG32, as well as acetylation, methylation, phosphorylation, carboxylation, PEG-ylation, or glycosylation. Just as it is possible to replace substituents of the protein, it is also possible to substitute functional groups, which are bound to the protein with groups characterized by similar features. Such modifications do not alter primary sequence. These will initially be conservative, i.e., the replacement group will have approximately the same size, shape, hydrophobicity and charge as the original group.

Many amino acids, including the terminal amino acids, may be modified in a given polypeptide, either by natural processes such as glycosylation and other post-translational modifications, or by chemical modification techniques which are well known in the art. Among the known modifications which may be present in polypeptides of the present invention are, to name an illustrative few, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a polynucleotide or polynucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

Such modifications are well known to those of skill and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, for instance, are described in most basic texts, such as, for instance, I. E. Creighton, Proteins-Structure and Molecular Properties, 2nd Ed., W. H. Freeman and Company, New York, 1993. Many detailed reviews are available on this subject, such as, for example, those provided by Wold, F., in Posttranslational Covalent Modification of Proteins, B. C. Johnson, Ed., Academic Press, New York, pp 1-12, 1983; Seifter et al., Meth. Enzymol. 182: 626-646, 1990

and Rattan et al., Protein Synthesis: Posttranslational Modifications and Aging, Ann. N.Y. Acad. Sci. 663: 48-62, 1992.

In addition, the protein may comprise a protein tag to allow subsequent purification and optionally removal of the tag using an endopeptidase. The tag may also comprise a protease cleavage site to facilitate subsequent removal of the tag. Non-limiting examples of affinity tags include a polyhis tag, a GST tag, a HA tag, a Flag tag, a C-myc tag, a HSV tag, a V5 tag, a maltose binding protein tag, a cellulose binding domain tag. Preferably for production and purification, the tag is a polyhistag. Preferably, the tag is in the C-terminal portion of the protein.

The native signal sequence of NsG28, NsG30, or NsG32 may also be replaced in order to increase secretion of the protein in recombinant production in other mammalian cell types.

It will be appreciated, as is well known and as noted above, that polypeptides are not always entirely linear. For instance, polypeptides may be branched as a result of ubiquitination, and they may be circular, with or without branching, generally as a result of posttranslational events, including natural processing events and events brought about by human manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by non-translational natural processes and by entirely synthetic methods, as well and are all within the scope of the present invention.

Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention, as well. For instance, the amino terminal residue of polypeptides made in E. coli, prior to proteolytic processing, almost invariably will be N-formylmethionine.

The modifications that occur in a polypeptide often will be a function of how it is made. For polypeptides made by expressing a cloned gene in a host, for instance, the nature and extent of the modifications in large part will be determined by the host cell's posttranslational modification capacity and the modification signals present in the polypeptide amino acid sequence. For instance, glycosylation often does not occur in bacterial hosts such as E. coli. Accordingly, when glycosylation is desired, a polypeptide should be expressed in a glycosylating host, generally a eukaryotic cell. Insect cells often carry out the same posttranslational glycosylations as mammalian cells and, for this reason, insect cell expression systems have been

developed to efficiently express mammalian proteins having native patterns of glycosylation, inter alia. Similar considerations apply to other modifications.

It will be appreciated that the same type of modification may be present to the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications.

In general, as used herein, the term polypeptide encompasses all such modifications, particularly those that are present in polypeptides synthesized by expressing a polynucleotide in a host cell.

Also included within the invention are agents, which specifically bind to a protein of the invention, or a fragment of such a protein. These agents include Ig fusion proteins and antibodies (including single chain, double chain, F_{ab} fragments, and others, whether native, humanized, primatized, or chimeric). Additional descriptions of these categories of agents are in WO 95/16709, the disclosure of which is herein incorporated by reference.

Antibodies refer to intact molecules as well as fragments thereof, such as F_{ab}, $F_{(ab')}$, and F_v, which are capable of binding the epitopic determinant. Antibodies that bind NsG28, NsG30, or NsG32 polypeptides can be prepared using intact polypeptides or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal can be derived from the translation of RNA or synthesized chemically and can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin and thyroglobulin, keyhole limpet hemocyanin. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

Humanised antibodies, as used herein, refer to antibody molecules in which amino acids have been replaced in the non-antigen binding regions in order to more closely resemble a human antibody, while still retaining the original binding ability. Humanised antibodies may be used therapeutically to treat conditions, where it is desirable to limit or block the action of NsG28, NsG30, or NsG32.

Also included within the scope of the present invention are immunoconjugates of antibodies and conjugates selected from the group consisting of: a cytotoxic agent such as a chemotherapeutic agent, a toxin, or a radioactive isotope; a member of a specific binding pair, such as avidin, or streptavidin, or an antigen; an enzyme capable of producing a detectable

product. These immunoconjugates can be used to target the conjugates to cells expressing a NsG28, NsG30, or NsG32 receptor.

Specific antibodies to any NsG28, NsG30, or NsG32 are also useful in immunoassays to quantify the substance for which a given antibody has specificity. Specific antibodies to an NsG28, NsG30, or NsG32 may also be bound to solid supports, such as beads or dishes, and used to remove the ligand from a solution, either for use in purifying the protein or in clearing it from the solution. Each of these techniques is routine to those of skill in the immunological arts.

Also with the scope of the present invention are NsG28, NsG30, or NsG32 fusion proteins. An NsG28, NsG30, or NsG32 fusion protein can be used to allow imaging of tissues which express a receptor for NsG28, NsG30, or NsG32, or in the immunohistological or preparative methods described above for antibodies to an NsG28, NsG30, or NsG32. Fusion proteins encompassing an NsG28, NsG30, or NsG32 can be used to specifically target medical therapies against cells, which express an NsG28, NsG30, or NsG32 receptor.

IV. NsG28, NsG30, or NsG32 Nucleotide Sequences

NsG28 Nucleotide Sequences

The invention provides medical use of genomic DNA and cDNA coding for NsG28, including for example the nucleotide sequence of human, and mouse NsG28 cDNA (SEQ ID NO 2 and 8).

Variants of these sequences are also included within the scope of the present invention.

The invention relates to an isolated nucleic acid molecule for medical use comprising a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence coding for a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, and 16 (or a subgroup thereof);

b) a nucleotide sequence coding for a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, and 16 (or a subgroup thereof), wherein the variant has at least 70% sequence identity to said SEQ ID No.;

c) a nucleotide sequence coding for a biologically active fragment of at least 50 contiguous amino acids of any of a) through b);

d) a nucleotide sequence selected from the group consisting of SEQ ID No. 2 and 8;

e) a nucleotide sequence having at least 70% sequence identity to a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 2 and 8;

f) a nucleic acid sequence of at least 150 contiguous nucleotides of a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 2 and 8;

g) the complement of a nucleic acid capable of hybridising with a nucleic acid molecule having the sequence of the coding sequence of SEQ ID No.: 2 and 8 under conditions of high stringency;

h) the nucleic acid sequence of the complement of any of the above.

The nucleic acid molecule may comprise the nucleotide sequence of a naturally occurring allelic nucleic acid variant.

The nucleic acid molecule of the invention may encode a variant polypeptide, wherein the variant polypeptide has the polypeptide sequence of a naturally occurring polypeptide variant.

In one embodiment the nucleic acid molecule differs by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID No. 2 and 8.

Preferably the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 3, 4, 9, and 13 (or a subgroup thereof) preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID Nos.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, and 7 (or a subgroup thereof), preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence

identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID Nos. Said sequences constitute human NsG28.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 5, 10, and 14 (or a subgroup thereof), preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID Nos. Said sequences constitute mature NsG28.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 6, 11, and 15 (or a subgroup thereof), preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID Nos. Said sequences constitute NsG28 fragments.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 7, 12, and 16 (or a subgroup thereof), preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID Nos. Said sequences constitute NsG28 fragments.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 3, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least

98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 4, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 5, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 6, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 7, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

SEQ ID No 6, 7, 11, 12, 15, and 16 represent the polypeptide sequences of NsG28 fragment polypeptides. For recombinant expression in a eukaryotic expression system, these are

preferably ligated to an appropriate signal sequence to ensure that the NsG28 polypeptide is secreted from the cells.

In one aspect the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of

a) the nucleotide sequence selected from the group consisting of SEQ ID No. 2 and 8;

b) a nucleotide sequence having at least 70% sequence identity to a nucleotide sequence selected from the group consisting of the coding sequence of SEQ ID No. 2 and 8;

c) a nucleic acid sequence of at least 150 contiguous nucleotides of a sequence selected from the group consisting of the coding sequence of SEQ ID No. 2 and 8;

d) the complement of a nucleic acid capable of hybridising with nucleic acid having the sequence selected from the group consisting of the coding sequence of SEQ ID No. 2 and 8 under conditions of high stringency; and

e) the nucleic acid sequence of the complement of any of the above.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence selected from the group consisting of the coding sequence of SEQ ID NO: 2 and 8.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence presented as the coding sequence of SEQ ID NO: 2.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence presented as the coding sequence of SEQ ID NO: 8.

The nucleotide sequence for nucleic acids coding for full length NsG28, for mature NsG28 and for fragments of NsG28 can be derived from Figs. 20 and 21, which show the cDNA sequence and the translated peptides of human NsG28 (Fig. 20) and mouse NsG28 (Fig. 21).

Specific fragments of these cDNA sequences include those coding for mature NsG28 and fragments of NsG28, including the nucleic acid molecule having the nucleotide sequence of nucleotides 466-888 of SEQ ID No. 2 (long human NsG28 CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 481-888 of SEQ ID No. 2 (human NsG28 CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 568-885 of SEQ ID No. 2 (human mature NsG28 CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 622-846 of SEQ ID No. 2 (human NsG28 cys1-cys10 fragment CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 616-876 of SEQ ID No. 2 (human NsG28 core fragment CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 206-613 of SEQ ID No. 8 (mouse NsG28 CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 293-610 of SEQ ID No. 8 (mouse mature NsG28 CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 347-571 of SEQ ID No. 8 (mouse NsG28 cys1-cys10 fragment CDS); and the nucleic acid molecule having the nucleotide sequence of nucleotides 341-601 of SEQ ID No. 8 (mouse NsG28 core fragment CDS).

NsG30 Nucleotide Sequences

The invention provides medical use of nucleic acids coding for NsG30, including for example the nucleotide sequence of human, and mouse NsG30 cDNA (SEQ ID NO 17 and 22).

Variants of these sequences are also included within the scope of the present invention.

The invention relates to an isolated nucleic acid molecule for medical use comprising a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence coding for a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID No. 18, 19, 20, 21, 23, 24, and 25 (or a subgroup thereof);

b) a nucleotide sequence coding for a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 18, 19, 20, 21, 23, 24, and 25 (or a subgroup thereof), wherein the variant has at least 70% sequence identity to said SEQ ID No.;

c) a nucleotide sequence coding for a biologically active fragment of at least 50 contiguous amino acids of any of a) through b);

d) a nucleotide sequence selected from the group consisting of SEQ ID No. 17 and 22;

e) a nucleotide sequence having at least 70% sequence identity to a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 17 and 22;

f) a nucleic acid sequence of at least 150 contiguous nucleotides of a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 17 and 22;

g) the complement of a nucleic acid capable of hybridising with a nucleic acid molecule having the sequence of the coding sequence of SEQ ID No.: 17 and 22 under conditions of high stringency;

h) the nucleic acid sequence of the complement of any of the above.

The nucleic acid molecule may comprise the nucleotide sequence of a naturally occurring allelic nucleic acid variant.

The nucleic acid molecule of the invention may encode a variant polypeptide, wherein the variant polypeptide has the polypeptide sequence of a naturally occurring polypeptide variant.

In one embodiment the nucleic acid molecule differs by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID No. 17 and 22.

Preferably the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 18, 19, 20, and 21 (or a subgroup thereof) preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID Nos. Said sequences constitute human NsG30.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 18, 23, and 25 (or a subgroup thereof) preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID Nos.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 19 and 24, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID Nos. Said sequences constitute mature NsG30.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 18, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 19, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 20, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 21, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least

80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 23, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 24, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 25, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

SEQ ID No 19, 20, 21, and 24 represent the polypeptide sequences of NsG30 fragments. For recombinant expression in a eukaryotic expression system, these are preferably ligated to an appropriate signal sequence to ensure that the NsG30 polypeptide is secreted from the cells.

In one aspect the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of

a) the nucleotide sequence selected from the group consisting of SEQ ID No. 17 and 22;

b) a nucleotide sequence having at least 70% sequence identity to a nucleotide sequence selected from the group consisting of the coding sequence of SEQ ID No. 17 and 22;

c) a nucleic acid sequence of at least 150 contiguous nucleotides of a sequence selected from the group consisting of the coding sequence of SEQ ID No. 17 and 22;

d) the complement of a nucleic acid capable of hybridising with nucleic acid having the sequence selected from the group consisting of the coding sequence of SEQ ID No.: 17 and 22 under conditions of high stringency; and

e) the nucleic acid sequence of the complement of any of the above.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence selected from the group consisting of the coding sequence of SEQ ID NO: 17 and 22.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 60, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to the polynucleotide sequence presented as the coding sequence of SEQ ID NO: 17.

In one embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence presented as the coding sequence of SEQ ID NO: 22.

The nucleotide sequence of nucleic acids coding for full length NsG30, for mature NsG30 and for fragments of NsG30 can be derived from Figs. 26 and 27, which show the cDNA sequence and the translated peptides of human NsG30 (Fig. 26) and mouse NsG30 (Fig. 27). Specific fragments of these cDNA sequences include those coding for mature NsG30, and fragments of NsG30, including the nucleic acid molecule having the nucleotide sequence of having sequence of nucleotides 156-551 of SEQ ID No. 17 (human NsG30 CDS); the nucleic acid molecule having sequence of nucleotides 246-548 of SEQ ID No. 17 (human mature NsG30 CDS); the nucleic acid molecule having sequence of nucleotides 285-509 of SEQ ID No. 17 (human Cys1-Cys10 fragment CDS); the nucleic acid molecule having sequence of nucleotides

279-539 of SEQ ID No. 17 (human core fragment CDS); the nucleic acid molecule having sequence of nucleotides 367-762 of SEQ ID No. 22 (mouse NsG30 CDS); the nucleic acid molecule having sequence of nucleotides 457-759 of SEQ ID No. 22 (mouse mature NsG30 CDS); the nucleic acid molecule having sequence of nucleotides 496-720 of SEQ ID No. 22 (mouse cys1-cys10 fragment CDS); and the nucleic acid molecule having sequence of nucleotides 490-750 of SEQ ID No. 22 (mouse core fragment CDS).

NsG32 Nucleotide Sequences

The invention provides medical use of DNA coding for NsG32 including the nucleotide sequence of human, mouse, and rat NsG32 cDNA (SEQ ID NO 26, 30, 35, 37, and 39) and fragments thereof.

Variants of these sequences are also included within the scope of the present invention.

The invention relates to an isolated nucleic acid molecule for medical use comprising a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence coding for a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID No. 27, 28, 29, 31, 32, 33, 34, 36, 38, and 40 (or a subgroup thereof);

b) a nucleotide sequence coding for a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 27, 28, 29, 31, 32, 33, 34, 36, 38, and 40 (or a subgroup thereof), wherein the variant has at least 70% sequence identity to said SEQ ID No.;

c) a nucleotide sequence coding for a biologically active fragment of at least 50 contiguous amino acids of any of a) through b);

d) a nucleotide sequence selected from the group consisting of SEQ ID No. 26, 30, 35, 37, and 39 (or a subgroup thereof);

e) a nucleotide sequence having at least 70% sequence identity to a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 26, 30, 35, 37, and 39 (or a subgroup thereof);

f) a nucleic acid sequence of at least 150 contiguous nucleotides of a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 26, 30, 35, 37, and 39 (or a subgroup thereof);

g) the complement of a nucleic acid capable of hybridising with a nucleic acid molecule having the sequence of the coding sequence of SEQ ID No.: 26, 30, 35, 37, and 39 under conditions of high stringency; and

h) the nucleic acid sequence of the complement of any of the above.

The nucleic acid molecule may comprise the nucleotide sequence of a naturally occurring allelic nucleic acid variant.

The nucleic acid molecule of the invention may encode a variant polypeptide, wherein the variant polypeptide has the polypeptide sequence of a naturally occurring polypeptide variant.

In one embodiment the nucleic acid molecule differs by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID No. 26, 30, 35, 37, and 39 (or a subgroup thereof).

Preferably the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 27, 28, 29, 31, 32, 33, and 34 (or a subgroup thereof), preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID Nos. Said sequences constitute human NsG32.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 27, 28, and 31 (or a subgroup thereof), preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID Nos. Said sequences constitute full length NsG32 polypeptide sequences.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 29 and 32, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75%

sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID Nos. Said sequences constitute mature NsG32 sequences.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 31, 32, 33, and 34 (or a subgroup thereof), preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID Nos. Said sequences constitute NsG32b sequences.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 27, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 28, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 29, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least

98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 31, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 32, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 33, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 34, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 41, preferably at least 65% sequence identity, more preferably at

least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 98% sequence identity, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.

SEQ ID No 29, 32, 33, and 34 represent the polypeptide sequences of mature NsG32 and NsG32 fragments. For recombinant expression in a eukaryotic expression system, these are preferably ligated to an appropriate signal sequence to ensure that the NsG32 polypeptide is secreted from the cells.

In one aspect the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of

a) the nucleotide sequence selected from the group consisting of SEQ ID No. 26, 30, 35, 37, and 39 (or a subgroup thereof);

b) a nucleotide sequence having at least 70% sequence identity to a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 26, 30, 35, 37, and 39 (or a subgroup thereof);

c) a nucleic acid sequence of at least 150 contiguous nucleotides of a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 26, 30, 35, 37, and 39 (or a subgroup thereof);

d) the complement of a nucleic acid capable of hybridising with a nucleic acid having a sequence selected from the group consisting of the coding sequence of SEQ ID No.: 26, 30, 35, 37, and 39 (or a subgroup thereof) under conditions of high stringency; and

e) the nucleic acid sequence of the complement of any of the above.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence selected from the group consisting of the coding sequence of SEQ ID NO: 26 and 30.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at

least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence selected from the group consisting of the coding sequence of SEQ ID NO: 26, 35, and 39 (or a subgroup thereof).

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence selected from the group consisting of the coding sequence of SEQ ID NO: 30 and 37.

In one embodiment, the isolated polynucleotide of the invention has at least 60, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to the polynucleotide sequence presented as SEQ ID NO: 26.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to the coding sequence of SEQ ID NO: 30.

In one embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence presented as SEQ ID NO: 35.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to the coding sequence of SEQ ID NO: 37.

In one embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at

least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence presented as SEQ ID NO: 39.

The nucleotide sequence of nucleic acids coding for full length NsG32, for mature NsG32 and for fragments of NsG32 can be derived from Figs. 30A-30B, 31, 32, and 33, which show the cDNA sequence and the translated peptides of human NsG32a (Figs. 30A-30B), mouse NsG32a (Fig. 31), human NsG32b (Fig. 32), and mouse NsG32b (Fig. 33). Specific fragments of these cDNA sequences include those coding for mature NsG32a, mature NsG32b and fragments of NsG32b, including the nucleic acid molecule having the nucleotide sequence of nucleotides 113-511 of SEQ ID No. 26 (long human NsG32a CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 167-511 of SEQ ID No. 26 (human NsG32a CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 242-508 of SEQ ID No. 26 (human mature NsG32a CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 74-418 of SEQ ID No. 35 (mouse NsG32a CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 20-418 of SEQ ID No. 35 (long mouse NsG32a CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 149-415 of SEQ ID No. 35 (mouse mature NsG32a CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 95-472 of SEQ ID No. 30 (human NsG32b CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 170-469 of SEQ ID No. 30 (human mature NsG32b CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 206-430 of SEQ ID No. 30 (human cys1-cys8 fragment CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 200-463 of SEQ ID No. 30 (human NsG32b core fragment CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 168-545 of SEQ ID No. 37 (mouse NsG32b CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 243-542 of SEQ ID No. 37 (mouse mature NsG32b CDS); the nucleic acid molecule having the nucleotide sequence of nucleotides 279-503 of SEQ ID No. 37 (mouse cys1-cys8 fragment CDS); and the nucleic acid molecule having the nucleotide sequence of nucleotides 273-536 of SEQ ID No. 37 (mouse NsG32b core fragment CDS).

In addition, the nucleotide sequences of the invention include sequences, which are derivatives of these sequences. The invention also includes vectors, liposomes and other carrier vehicles, which encompass one of these sequences or a derivative of one of these sequences. The invention also includes proteins transcribed and translated from NsG28, NsG30, or NsG32

cDNA, preferably human NsG28, NsG30, or NsG32 cDNA, including but not limited to human NsG28, NsG30, or NsG32 and derivatives and variants.

In another embodiment, the invention relates to the use of the nucleic acids and proteins of the present invention to design probes to isolate other genes, which encode proteins with structural or functional properties of the NsG28, NsG30, or NsG32 proteins of the invention. The probes can be a variety of base pairs in length. For example, a nucleic acid probe can be between about 10 base pairs in length to about 150 base pairs in length.

Alternatively, the nucleic acid probe can be greater than about 150 base pairs in length. Experimental methods are provided in Ausubel et al., "Current Protocols in Molecular Biology", J. Wiley (ed.) (1999), the entire teachings of which are herein incorporated by reference in their entirety.

The design of the oligonucleotide (also referred to herein as nucleic acid) probe should preferably follow these parameters:

i) it should be designed to an area of the sequence which has the fewest ambiguous bases, if any and

ii) it should be designed to have a calculated T_m of about 80°C (assuming 2°C for each A or T and 4°C for each G or C).

The oligonucleotide should preferably be labelled to facilitate detection of hybridisation. Labelling may be with γ-^{32}P ATP (specific activity 6000 Ci/mmole) and T4 polynucleotide kinase using commonly employed techniques for labelling oligonucleotides. Other labelling techniques can also be used. Unincorporated label should preferably be removed by gel filtration chromatography or other established methods. The amount of radioactivity incorporated into the probe should be quantitated by measurement in a scintillation counter. Preferably, specific activity of the resulting probe should be approximately 4 x 10^6 dpm/pmole. The bacterial culture containing the pool of full-length clones should preferably be thawed and 100 µL of the stock used to inoculate a sterile culture flask containing 25 ml of sterile L-broth containing ampicillin at 100 µg/ml.

The culture should preferably be grown to saturation at about 37°C, and the saturated culture should preferably be diluted in fresh L-broth. Aliquots of these dilutions should preferably be plated to determine the dilution and volume which will yield approximately 5000 distinct and well-separated colonies on solid bacteriological media containing L-broth containing

ampicillin at 100 µg/ml and agar at 1.5% in a 150 mm petri dish when grown overnight at about 37°C. Other known methods of obtaining distinct, well-separated colonies can also be employed.

Standard colony hybridization procedures should then be used to transfer the colonies to nitrocellulose filters and lyse, denature and bake them. Highly stringent (also referred to herein as "high stringency") conditions are those that are at least as stringent as, for example, 1xSSC at about 65°C, or 1xSSC and 50% formamide at about 42°C. "Moderate stringency" conditions are those that are at least as stringent as 4xSSC at about 65°C, or 4x SSC and 50% formamide at about 42°C. "Reduced stringency" conditions are those that are at least as stringent as 4x SSC at about 50°C, or 6x SSC and 50% formamide at 40°C.

The filter is then preferably incubated at about 65°C for 1 hour with gentle agitation in 6X SSC (20x stock is 175.3 g NaCl/liter, 88.2 g Na citrate/liter, adjusted to pH 7.0 with NaOH) containing 0.5% SDS, 100 g/ml of yeast RNA, and 10 mM EDTA (approximately 10 mL per 150 mm filter). Preferably, the probe is then added to the hybridization mix at a concentration greater than or equal to 1 x 10^6 dpm/mL. The filter is then preferably incubated at about 65°C with gentle agitation overnight. The filter is then preferably washed in 500 mL of 2x SSC/0.5% SDS at room temperature without agitation, preferably followed by 500 mL of 2x SSC/0.1% SDS at room temperature with gentle shaking for 15 minutes. A third wash with 0.1x SSC/0.5% SDS at about 65°C for 30 minutes to 1 hour is optional. The filter is then preferably dried and subjected to autoradiography for sufficient time to visualize the positives on the X-ray film. Other known hybridization methods can also be employed. The positive colonies are then picked, grown in culture, and plasmid DNA isolated using standard procedures. The clones can then be verified by restriction analysis, hybridisation analysis, or DNA sequencing.

Alternatively, suitable experimental conditions for determining hybridization between a nucleotide probe and a homologous DNA or RNA sequence, involves pre-soaking of the filter containing the DNA fragments or RNA to hybridize in 5 x SSC [Sodium chloride/Sodium citrate; cf. *Sambrook et al.*; Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Lab., Cold Spring Harbor, NY 1989] for 10 minutes, and pre-hybridization of the filter in a solution of 5 x SSC, 5 x Denhardt's solution [cf. *Sambrook et al.; Op cit.*], 0.5 % SDS and 100 µg/ml of denatured sonicated salmon sperm DNA [cf. *Sambrook et al.; Op cit.*], followed by hybridization in the same solution containing a concentration of 10 ng/ml of a random-primed [*Feinberg A P & Vogelstein B*; Anal. Biochem. 1983 **132** 6-13], ^{32}P-dCTP-labeled (specific activity > 1 x 10^9 cpm/µg) probe for 12 hours at approximately 45°C. The filter is then washed twice for 30

minutes in 0.1 x SSC, 0.5 % SDS at a temperature of at least at least 60°C (medium stringency conditions), preferably of at least 65°C (medium/high stringency conditions), more preferred of at least 70°C (high stringency conditions), and even more preferred of at least 75°C (very high stringency conditions). Molecules to which the oligonucleotide probe hybridizes under these conditions may be detected using a x-ray film.

In yet another embodiment, the invention relates to nucleic acid sequences (e. g., DNA, RNA) that hybridise to nucleic acids of NsG28. In particular, nucleic acids which hybridise to SEQ ID NO: 1, SEQ ID NO: 2, and/or SEQ ID NO:8 under high, moderate or reduced stringency conditions as described above.

In still another embodiment, the invention relates to a complement of nucleic acid of NsG28. In particular, it relates to complements of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID No 8.

In another embodiment, the invention relates to an RNA counterpart of the DNA nucleic acid of NsG28. In particular, it relates to RNA counterparts of SEQ ID NO: 2 and SEQ ID No 8.

In yet another embodiment, the invention relates to nucleic acid sequences (e. g., DNA, RNA) that hybridise to nucleic acids of NsG30. In particular, nucleic acids which hybridise to SEQ ID NO: 17 and/or SEQ ID NO:22 under high, moderate or reduced stringency conditions as described above.

In still another embodiment, the invention relates to a complement of nucleic acid of NsG30. In particular, it relates to complements of SEQ ID NO: 17 and SEQ ID No 22.

In another embodiment, the invention relates to an RNA counterpart of the DNA nucleic acid of NsG30. In particular, it relates to RNA counterparts of SEQ ID NO: 17 and SEQ ID No 22.

In yet another embodiment, the invention relates to nucleic acid sequences (e. g., DNA, RNA) that hybridise to nucleic acids of NsG32. In particular, nucleic acids which hybridise to a molecule having a sequence selected from the group consisting of the coding sequence of SEQ ID NO: 26, SEQ ID NO: 30, SEQ ID No. 35, SEQ ID NO:37, and SEQ ID No. 39 (or a subgroup thereof) under high, moderate or reduced stringency conditions as described above.

In still another embodiment, the invention relates to a complement of nucleic acid of NsG32. In particular, it relates to complements of the coding sequence of SEQ ID NO: 26, SEQ ID NO: 30, SEQ ID No. 35, SEQ ID NO:37, and SEQ ID No. 39.

In another embodiment, the invention relates to an RNA counterpart of the DNA nucleic acid of NsG32. In particular, it relates to RNA counterparts comprising the coding sequence of SEQ ID NO: 26, SEQ ID NO: 30, SEQ ID No. 35, SEQ ID NO:37, and SEQ ID No. 39.

Codon optimised nucleic acid molecules for enhanced expression in selected host cells, including but not limited to E. coli, yeast species, Chinese Hamster, Baby Hamster, insect, and fungus are also contemplated.

Variant nucleic acids can be made by state of the art mutagenesis methods.

Methods for shuffling coding sequences from human with those of mouse, rat or chimpanzee are also contemplated.

A shuffled variant may be between SEQ ID No 2 on one hand and 8 on the other hand. Also included are shuffled variants between SEQ ID No. 2 or 8 on one hand and a sequence coding for another Cys10 protein on the other hand.

A shuffled variant may be between SEQ ID No 17 on one hand and 22 on the other hand. Also included are shuffled variants between SEQ ID No. 17 or 22 on one hand and a sequence coding for another Cys10 protein on the other hand.

Also included are shuffled variants between the CDS of SEQ ID No. 26 or 35 on one hand and a sequence coding for another Cys10 protein on the other hand. Further included are shuffled variants between the CDS of SEQ ID No. 30 or 37 on one hand and a sequence coding for another Cys10 protein on the other hand.

V. Use of NsG28, NsG30, and NsG32 Polypeptides, Polynucleotides, and NsG28, NsG30, and NsG32 Secreting Cells for Treatment of Disorders of the Nervous System

NsG28

In one embodiment, native, variant NsG28, and fragments thereof and/or fusion proteins comprising NsG28 are provided for the treatment of disorders of the mammalian nervous system. NsG28 may be used to stimulate neural cell growth, proliferation, neural function, neural regeneration, neural differentiation, neural migration, and/or neural survival in disease situations where these cells are lost or damaged.

In one embodiment, NsG28 polynucleotides and/or polypeptides of the invention may be used to treat conditions or diseases where neural growth, proliferation, differentiation, function, survival, and/or regeneration is desirable. The NsG28 polypeptides of the present invention may be used directly via, e.g., injected, implanted or ingested pharmaceutical compositions to treat a pathological process responsive to the NsG28 polypeptides. This is supported by: bioinformatics analyses showing that NsG28 is a secreted growth factor and the fact that NsG28 is preferentially expressed in the human nervous system, including the eye (Figs. 14 and 15); the fact that mNgS28 is expressed differentially in the developing mouse brain and specifically at the time of terminal differentiation in the striatum and substantia nigra (Fig. 24); the fact that NsG28 protects cerebellar granule cells from apoptosis (Fig. 36); and the fact that the closely related growth factors, NsG30, NsG32a, and NsG32b, have shown an effect on survival in a cell line with neuronal potential (PC12 cells).

NsG28 may act on a range of different cell types, which are present in the nervous system. In the context of the present invention, the nervous system is intended to encompass the central nervous system, the peripheral nervous system, the eye, and the cochleovestibular complex.

In one embodiment, NsG28 polypeptides may act on neurons, including but not limited to motor neurons, sensory neurons, relay cells, Purkinje cells, and interneurons.

In another embodiment, the therapeutic effect of NsG28 polypeptides may be through action on glial cells, such as oligodendrocytes and/or astrocytes. Through their action on glial cells, NsG28 polypeptides may be involved in myelination, and in the maintenance of neuron function and survival.

In another embodiment, NsG28 polypeptides may act on sensory cells, including but not limited to retinal ganglion cells, photoreceptor cells, supportive tissue such as retinal epithelial cells, and hair cells of the ear.

In a further embodiment, NsG28 polypeptides may act on stem cells, and downstream precursor cells including but not limited to neuronal precursors and glial precursors. NsG28 polypeptides may act on stem cells and/or neuronal or glial precursors to cause growth, proliferation, enhance survival, to cause differentiation, and/or migration. Stem cell therapy may be done through in vivo or ex vivo gene therapy, or in vitro treatment of isolated stem cells, or the protein may be administered to a location with stem cells. The effect of NsG28 on stem cells may be tested using the Neurosphere assay described herein (Example 12).

The disorder or disease or damage may be damages of the nervous system caused by trauma, surgery, ischaemia, infection, metabolic diseases, nutritional deficiency, malignancy or toxic agents, and genetic or idiopathic processes.

In one embodiment of the method of the invention, the disease or disorder or damage involves injury to the brain, brain stem, the spinal cord, and/or peripheral nerves, resulting in conditions such as stroke, traumatic brain injury (TBI), spinal cord injury (SCI), diffuse axonal injury (DAI), epilepsy, neuropathy, peripheral neuropathy, and associated pain and other symptoms that these syndromes may cause.

In another embodiment, the disease, disorder, or damage involves the degeneration of neurons and their processes in the brain, brain stem, the spinal cord, and/or peripheral nerves, such as neurodegenerative disorders including but not limited to Parkinson's Disease, Alzheimer's Disease, senile dementia, Huntington's Disease, amyotrophic lateral sclerosis (ALS), neuronal/axonal injury associated with Multiple Sclerosis (MS), and associated symptoms.

In another embodiment, the disease, disorder, or damage involves dysfunction, and/or loss of neurons in the brain, brain stem, the spinal cord, and/or peripheral nerves, such as dysfunction and/or loss caused by metabolic diseases, nutritional deficiency, toxic injury, malignancy, and/or genetic or idiopathic conditions, including but not limited to diabetes, renal dysfunction, alcoholism, chemotherapy, chemical agents, drug abuse, vitamin deficiencies, infection, and associated symptoms.

In another embodiment, the disease, disorder, or damage involves the degeneration or sclerosis of glia such as oligodendrocytes, astrocytes, and Schwann cells in the brain, brain stem, the spinal cord, and peripheral nervous system, including but not limited to Multiple Sclerosis (MS), optic neuritis, cerebral sclerosis, post-infectious encephalomyelitis, and epilepsy, and associated symptoms.

In another embodiment, the disease, disorder, or damage involves the retina, photoreceptors, and associated nerves including but not limited to retinitis pigmentosa, macular degeneration, glaucoma, and associated symptoms.

In another embodiment, the disease, disorder, or damage involves the sensory epithelium and associated ganglia of the vestibuloacoustic complex, including but not limited to noise induced hearing loss, deafness, tinnitus, otitis, labyrintitis, hereditary and cochleovestibular atrophies, Meniere's Disease, and associated symptoms.

In a preferred embodiment, the NsG28 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of Parkinson's Disease. This function is based on the finding of high levels of NsG28 expression in the central midbrain in substantia nigra, and in the thalamus, and expression in the putamen (see Example 4). This function is strongly supported by the finding of high levels of expression of the NsG28 mouse orthologue in P1 mice in the ventral mesencephalon and the lateral and medial ganglionic eminences (Example 13). The function can be verified using the Bioassay for dopaminergic neurotrophic activities (example 10) and in vivo through the instrastriatal 6-OHDA lesion model (Example 11).

Huntington's disease (HD) is an autosomal dominant disorder that results in the progressive degeneration of various neuronal populations within the brain, particularly the GABA-ergic medium spiny neurons located in the caudate nucleus. Associated with this degeneration, the cortical glutaminergic input neurons also degenerate and the combined degeneration account for most of the characteristic symptoms of progressive dyskinetic motor movements as well as dementia.

In a preferred embodiment, the NsG28 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of Huntington's disease. This is based on the finding of NsG28 expression in the putamen and high NsG28 expression in substantia nigra, as well as thalamus, combined with the results of the bioinformatics analyses. This function is also strongly supported by the finding of high levels of expression of the NsG28 mouse orthologue in P1 mice in the ventral mesencephalon and the lateral and medial ganglionic eminences (Example 13). Huntington's disease is an excitotoxic disease. An excitotoxic bioassay is the assay described in Example 6 of the present invention. Another exemplary bioassay for verification of this neuroprotective effect of NsG28 include e.g. the bioassay on protection of primary hippocampal slice cultures against the excitoxic effects of NMDA (WO 03/004527, example 5). Another relevant assay is an in vitro assay with cortical neurons.

In another preferred embodiment, the NsG28 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of peripheral neuropathies. This is based on the finding of NsG28 expression in the dorsal root ganglion and high NsG28 expression in the spinal cord, combined with the results of the bioinformatics analyses. Verification of this function can be done with the dorsal root ganglion

culture assay described in Example 9. Among the peripheral neuropathies contemplated for treatment with the molecules of this invention are trauma-induced neuropathies, e.g., those caused by physical injury or disease state, physical damage to the peripheral nerves such as hermited discs, and the brain, physical damage to the spinal cord, stroke associated with brain damage, and neurological disorders related to neurodegeneration. We also contemplate treatment of chemotherapy-induced neuropathies (such as those caused by delivery of chemotherapeutic agents, e.g., taxol or cisplatin); toxin-induced neuropathies, drug-induced neuropathies, vitamin-deficiency-induced neuropathies; idiopathic neuropathies; and diabetic neuropathies.

In another embodiment, the NsG28 polypeptides, nucleic acids, expression vectors, capsules, and compositions of the invention are used in the treatment of disorders, diseases, or damages associated with the Cerebellum, including but not limited to sensory ataxia, multiple sclerosis, neurodegenerative spinocerebellar disorders, hereditary ataxia, cerebellar atrophies (such as Olivopontocerebellar Atrophy (OPCA), Shy-Drager Syndrome (multiple systems atrophy)), and alcoholism. This function is supported by: the high NsG28 expression levels in the human cerebellum and the developing mouse cerebellum combined with the bioinformatics analyses; and experimental analyses showing that NsG28 protects cerebellar granule cells from apoptosis (Fig. 36). Verification of this function may be done with the assays described in Examples 6 and 7 (Protection of cerebellar granule cells from glutamate toxicity and potassium deprivation).

In another preferred embodiment, the NsG28 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of amyotrophic lateral sclerosis, spinal muscular atrophy, and spinal cord injury. This is based on the finding of high NsG28 expression levels in the human spinal cord and expression in the developing mouse spinal cord combined with the results of the bioinformatics analyses. Verification of this specific therapeutic function may be done with the motorneuron assay described in Example 9. Another relevant assay for ALS is an in vitro assay with cortical neurons.

In another preferred embodiment, the NsG28 polypeptides, nucleic acids, expression vectors, capsules, and compositions of the invention are used in the treatment of multiple sclerosis. This function is based on the finding of high NsG28 expression levels in corpus callosum combined with the results of the bioinformatics analyses. The corpus callosum consists

primarily of glial cells. Expression in the corpus callosum thus indicates a strong association with glia.

In a preferred embodiment, the polypeptides, nucleic acids, vectors, capsules, and compositions of the invention are used in the treatment of diseases, disorders, or damages involving the retina, including but not limited to retinitis pigmentosa, macular degeneration and glaucoma. This specific therapeutic use is supported by the bioinformatics and experimental analyses showing that NsG28 is a secreted growth factor highly expressed in the retina (Fig. 14).

Confirmation of such use can be obtained by using various state of the art in vitro assays (retinal explant assays, corneal cultures). Verification of function may also be performed in state of the art animal models for corneal wounds (corneal lesion in rabbits) and retina (retinitis pigmentosa mutant models available for mouse and rat).

Other growth factors have important therapeutic uses in both the central and peripheral nervous system and in various eye indications associated with loss of cells in retina and/or cornea. E.g. NGF, is a candidate for both Alzheimer's disease, corneal ulcer (US 6,063,757 and EP 0 973 872), and retinopathies. Neublastin (Artemin) is a candidate for both peripheral neuropathy (WO 02/078730) and corneal wound healing (EP 1 223 966). GDNF is a candidate for Parkinson's Disease, ALS, spinal cord injury, and for wound healing, in particular in cornea (EP 1 223 966).

In a preferred embodiment, the polypeptides, nucleic acids, vectors, capsules, and compositions of the invention are used in the treatment of thalamic pain. This function is based on the finding of high NsG28 expression levels in the thalamus combined with the results of the bioinformatics analyses. Thalamic pain is a syndrome caused by stroke involving the thalamus.

In a preferred embodiment, the polypeptides, nucleic acids, vectors, capsules, and compositions of the invention are used in the treatment of essential tremor. This function is based on the finding of high NsG28 expression levels in the thalamus combined with the results of the bioinformatics analyses. Essential tremor is an idiopathic syndrome, which can be treated by deep brain/thalamic stimulation in the thalamus and by thalamotomy.

In another preferred embodiment the invention relates to a pathological condition related to testis. This embodiment is based on the present inventor's finding of high NsG28 expression in testis combined with the results of the bioinformatics analyses. Examples of diseases included within the scope of this embodiment include male sterility, impotence, erectile dysfunction, cancer, and germ cell tumours. NsG28 may also possess potential as a male contraceptive. Other

growth factors with expression in both the central nervous system and testis have been shown to possess therapeutic potential in treating testes-related disorders, including use as a male contraceptive (WO 00/10594).

In another embodiment the neurodegenerative disease is an excitotoxic disease selected from the group consisting of ischaemia, epilepsy, and trauma due to injury, cardiac arrest or stroke. The above-mentioned hippocampal slice culture assay and the assay of Example 6 of the present invention are non-limiting examples of an assay, which can be used to demonstrate a biological effect, indicative of therapeutic use for the treatment of excitotoxic diseases.

The term "subject" used herein is taken to mean any mammal to which NsG28 polypeptide or polynucleotide, therapeutic cells or biocompatible capsules may be administered. Subjects specifically intended for treatment with the method of the invention include humans, as well as nonhuman primates, sheep, horses, cattle, goats, pigs, dogs, cats, rabbits, guinea pigs, hamsters, gerbils, rats and mice, as well as the organs, tumors, and cells derived or originating from these hosts.

NsG30

In one embodiment, native, variant NsG30, and fragments thereof and/or fusion proteins comprising NsG30 are provided for the treatment of disorders of the mammalian nervous system. NsG30 may be used to stimulate neural cell growth, proliferation, neural function, neural regeneration, neural differentiation, neural migration, and/or neural survival in disease situations where these cells are lost or damaged.

In one embodiment, NsG30 polynucleotides and/or polypeptides of the invention may be used to treat conditions or diseases where neural growth, proliferation, differentiation, function, survival, and/or regeneration is desirable. The NsG30 polypeptides of the present invention may be used directly via, e.g., injected, implanted or ingested pharmaceutical compositions to treat a pathological process responsive to the NsG30 polypeptides. This is supported by the bioinformatics analyses showing that NsG30 is a secreted growth factor and the fact that NsG30 is preferentially expressed in the nervous system, including the eye (Figs. 16, 17, and 28). Furthermore, when expressed in a cell line with neuronal potential (PC12 cells, Example 5), NsG30 is able to mediate a survival effect under suboptimal culture conditions.

NsG30 may act on a range of different cell types, which are present in the nervous system. In the context of the present invention, the nervous system is intended to encompass the

central nervous system, the peripheral nervous system, the eye, and the cochleovestibular complex.

In one embodiment, NsG30 polypeptides may act on neurons, including but not limited to motor neurons, sensory neurons, relay cells, Purkinje cells, and interneurons.

In another embodiment, the therapeutic effect of NsG30 polypeptides may be through action on glial cells, such as oligodendrocytes and/or astrocytes. Through their action on glial cells, NsG30 polypeptides may be involved in myelination, and in the maintenance of neuron function and survival.

In another embodiment, NsG30 polypeptides may act on sensory cells, including but not limited to retinal ganglion cells, photoreceptor cells, supportive tissue such as retinal epithelial cells, and hair cells of the ear.

In a further embodiment, NsG30 polypeptides may act on stem cells, and downstream precursor cells including but not limited to neuronal precursors and glial precursors. NsG30 polypeptides may act on stem cells and/or neuronal or glial precursors to cause growth, proliferation, enhance survival, to cause differentiation, and/or migration. Stem cell therapy may be done through in vivo or ex vivo gene therapy, or in vitro treatment of isolated stem cells, or the protein may be administered to a location with stem cells. The effect of NsG30 on stem cells may be tested using the Neurosphere assay described herein (Example 12).

The disorder or disease or damage may be damages of the nervous system caused by trauma, surgery, ischaemia, infection, metabolic diseases, nutritional deficiency, malignancy or toxic agents, and genetic or idiopathic processes.

In one embodiment of the method of the invention, the disease or disorder or damage involves injury to the brain, brain stem, the spinal cord, and/or peripheral nerves, resulting in conditions such as stroke, traumatic brain injury (TBI), spinal cord injury (SCI), diffuse axonal injury (DAI), epilepsy, neuropathy, peripheral neuropathy, and associated pain and other symptoms that these syndromes may cause.

In another embodiment, the disease, disorder, or damage involves the degeneration of neurons and their processes in the brain, brain stem, the spinal cord, and/or peripheral nerves, such as neurodegenerative disorders including but not limited to Parkinson's Disease, Alzheimer's Disease, senile dementia, Huntington's Disease, amyotrophic lateral sclerosis (ALS), neuronal/axonal injury associated with Multiple Sclerosis (MS), and associated symptoms.

In another embodiment, the disease, disorder, or damage involves dysfunction, and/or loss of neurons in the brain, brain stem, the spinal cord, and/or peripheral nerves, such as dysfunction and/or loss caused by metabolic diseases, nutritional deficiency, toxic injury, malignancy, and/or genetic or idiopathic conditions, including but not limited to diabetes, renal dysfunction, alcoholism, chemotherapy, chemical agents, drug abuse, vitamin deficiencies, infection, and associated symptoms.

In another embodiment, the disease, disorder, or damage involves the degeneration or sclerosis of glia such as oligodendrocytes, astrocytes, and Schwann cells in the brain, brain stem, the spinal cord, and peripheral nervous system, including but not limited to Multiple Sclerosis (MS), optic neuritis, cerebral sclerosis, post-infectious encephalomyelitis, and epilepsy, and associated symptoms.

In another embodiment, the disease, disorder, or damage involves the retina, photoreceptors, and associated nerves including but not limited to retinitis pigmentosa, macular degeneration, glaucoma, and associated symptoms.

In another embodiment, the disease, disorder, or damage involves the sensory epithelium and associated ganglia of the vestibuloacoustic complex, including but not limited to noise induced hearing loss, deafness, tinnitus, otitis, labyrintitis, hereditary and cochleovestibular atrophies, Meniere's Disease, and associated symptoms.

In a preferred embodiment, the NsG30 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of Parkinson's Disease. This function is based on the finding of high levels of NsG30 expression in the central midbrain in substantia nigra and thalamus and intermediate expression in putamen (see Example 4A), high expression in the developing human mesencephalon (Example 4B), and high expression in the developing mouse mesencephalon and striatum at around the time of termination of projections between these two regions (Example 13). The function can be verified using the Bioassay for dopaminergic neurotrophic activities (Example 10) and in vivo through the instrastriatal 6-OHDA lesion model (Example 11).

Huntington's disease (HD) is an autosomal dominant disorder that results in the progressive degeneration of various neuronal populations within the brain, particularly the GABA-ergic medium spiny neurons located in the caudate nucleus. Associated with this degeneration, the cortical glutaminergic input neurons also degenerate and the combined

degeneration account for most of the characteristic symptoms of progressive dyskinetic motor movements as well as dementia.

In a preferred embodiment, the NsG30 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of Huntington's disease. This is based on the finding of high expression in thalamus and intermediate expression in putamen, and the finding of high expression in the developing mouse striatum, combined with the results of the bioinformatics analyses. Huntington's disease is an excitotoxic disease. An excitotoxic bioassay is the assay described in Example 6 of the present invention. Another exemplary bioassay for verification of this neuroprotective effect of NsG30 include e.g. the bioassay on protection of primary hippocampal slice cultures against the excitoxic effects of NMDA (WO 03/004527, Example 5).

The most consistent abnormality for Alzheimer's disease, as well as for vascular dementia and cognitive impairment due to organic brain disease related to alcoholism, is the degeneration of the cholinergic system arising from the basal forebrain (BF) to both the codex and hippocampus (Bigl et al. in Brain Cholinergic Systems, M. Steriade and D. Biesold, eds., Oxford University Press, Oxford, pp.364-386 (1990)). In chronic alcoholism the resultant organic brain disease, like Alzheimer's disease and normal aging, is also characterized by diffuse reductions in cortical cerebral blood flow in those brain regions where cholinergic neurons arise (basal forebrain) and to which they project (cerebral cortex) (Lofti et al., Cerebrovasc. and Brain Metab. Rev 1:2 (1989)). Therefore, in a preferred embodiment, the NsG30 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of Alzheimer's Disease. This function is based on the finding of high levels of NsG30 expression in hippocampus and amygdala combined with the results of the bioinformatics analyses. The therapeutic potential can be tested in an in vitro assay with basal cholinergic forebrain neurons, which are subjected to conditioned medium as described in the examples. An increase in ChAT (choline acetyltransferase) activity in basal cholinergic forebrain neurons is an indication of therapeutic effect in the treatment of Alzheimer's disease.

In another preferred embodiment, the NsG30 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of thalamic pain and tremor, including central tremor. This function is based on the high NsG30 expression in thalamus combined with the bioinformatics analyses.

In another preferred embodiment, the NsG30 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of peripheral neuropathies. This is based on the finding of NsG30 expression in the dorsal root ganglion combined with the results of the bioinformatics analyses. Verification of this function can be done with the dorsal root ganglion culture assay described in Example 9. Among the peripheral neuropathies contemplated for treatment with the molecules of this invention are trauma-induced neuropathies, e.g., those caused by physical injury or disease state, physical damage to the peripheral nerves such as hermited discs, and the brain, physical damage to the spinal cord, stroke associated with brain damage, and neurological disorders related to neurodegeneration. We also contemplate treatment of chemotherapy-induced neuropathies (such as those caused by delivery of chemotherapeutic agents, e.g., taxol or cisplatin); toxin-induced neuropathies, drug-induced neuropathies, vitamin-deficiency-induced neuropathies; idiopathic neuropathies; and diabetic neuropathies.

In another embodiment, the NsG30 polypeptides, nucleic acids, expression vectors, capsules, and compositions of the invention are used in the treatment of disorders, diseases, or damages associated with the Cerebellum, including but not limited to sensory ataxia, multiple sclerosis, neurodegenerative spinocerebellar disorders, hereditary ataxia, cerebellar atrophies (such as Olivopontocerebellar Atrophy (OPCA), Shy-Drager Syndrome (multiple systems atrophy)), and alcoholism. This function is supported by NsG30 expression in the adult human cerebellum and the developing mouse cerebellum, combined with the bioinformatics analyses. Verification of this function may be done with the assays described in Examples 6 and 7 (Protection of cerebellar granule cells from glutamate toxicity and potassium deprivation).

In another embodiment, the NsG30 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of amyotrophic lateral sclerosis, spinal muscular atrophy, and spinal cord injury. This is based on the finding of NsG30 expression in the adult human spinal cord and the developing mouse spinal cord combined with the results of the bioinformatics analyses. Verification of this specific therapeutic function may be done with the motorneuron assay described in Example 9.

In one preferred embodiment, the NsG30 polypeptides, nucleic acids, expression vectors, capsules, and compositions of the invention are used in the treatment of multiple sclerosis. This function is based on the finding of NsG30 expression in corpus callosum combined with the

results of the bioinformatics analyses. The corpus callosum consists primarily of glial cells. Expression in the corpus callosum thus indicates a strong association with glia.

In one preferred embodiment, the NsG30 polypeptides, nucleic acids, vectors, capsules, and compositions of the invention are used in the treatment of thalamic pain. This function is based on the finding of high NsG30 expression levels in the thalamus combined with the results of the bioinformatics analyses. Thalamic pain is a syndrome caused by stroke involving the thalamus.

In one embodiment, the NsG30 polypeptides, nucleic acids, vectors, capsules, and compositions of the invention are used in the treatment of essential tremor. This function is based on the finding of high NsG30 expression levels in the thalamus combined with the results of the bioinformatics analyses. Essential tremor is an idiopathic syndrome, which can be treated by deep brain/thalamic stimulation in the thalamus and by thalamotomy.

In an embodiment, the NsG30 polypeptides, nucleic acids, vectors, capsules, and compositions of the invention are used in the treatment of diseases, disorders, or damages involving the retina, including but not limited to retinitis pigmentosa, macular degeneration and glaucoma. This specific therapeutic use is supported by the bioinformatics and experimental analyses showing that NsG30 is a secreted growth factor expressed in the retina (Fig. 16).

Other growth factors have important therapeutic uses in both the central and peripheral nervous system and in various eye indications associated with loss of cells in retina and/or cornea. E.g. NGF, is a candidate for both Alzheimer's disease, corneal ulcer (US 6,063,757 and EP 0 973 872), and retinopathies. Neublastin (Artemin) is a candidate for both peripheral neuropathy (WO 02/078730) and corneal wound healing (EP 1 223 966). GDNF is a candidate for Parkinson's Disease, ALS, spinal cord injury, and for wound healing, in particular in cornea (EP 1 223 966).

Confirmation of such use can be obtained by using various state of the art in vitro assays (retinal explant assays, corneal cultures). Verification of function may also be performed in state of the art animal models for corneal wounds (corneal lesion in rabbits) and retina (retinitis pigmentosa mutant models available for mouse and rat).

In another embodiment the neurodegenerative disease is an excitotoxic disease selected from the group consisting of ischaemia, epilepsy, and trauma due to injury, cardiac arrest or stroke. The above-mentioned hippocampal slice culture assay and the assay of Example 6 of the

present invention are non-limiting examples of an assay, which can be used to demonstrate a biological effect, indicative of therapeutic use for the treatment of excitotoxic diseases.

In another preferred embodiment the invention relates to a pathological condition related to testis. This embodiment is based on the present inventor's finding of NsG30 expression in testis combined with the results of the bioinformatics analyses. Examples of diseases included within the scope of this embodiment include male sterility, impotence, erectile dysfunction, cancer, and germ cell tumours. NsG30 may also possess potential as a male contraceptive. Other growth factors with expression in both the central nervous system and testis have been shown to possess therapeutic potential in treating testes-related disorders, including use as a male contraceptive (WO 00/10594).

The term "subject" used herein is taken to mean any mammal to which NsG30 polypeptide or polynucleotide, therapeutic cells or biocompatible capsules may be administered. Subjects specifically intended for treatment with the method of the invention include humans, as well as nonhuman primates, sheep, horses, cattle, goats, pigs, dogs, cats, rabbits, guinea pigs, hamsters, gerbils, rats and mice, as well as the organs, tumors, and cells derived or originating from these hosts.

NsG32

In one embodiment, native NsG32, variant NsG32, and fragments thereof and/or fusion proteins comprising NsG32 are provided for the treatment of disorders of the mammalian nervous system. NsG32 may be used to stimulate neural cell growth, proliferation, neural function, neural regeneration, neural differentiation, neural migration, and/or neural survival in disease situations where these cells are lost or damaged.

In one embodiment, NsG32 polynucleotides and/or polypeptides of the invention may be used to treat conditions or diseases where neural growth, proliferation, differentiation, function, survival, and/or regeneration is desirable. The NsG32 polypeptides of the present invention may be used directly via, e.g., injected, implanted or ingested pharmaceutical compositions to treat a pathological process responsive to the NsG32 polypeptides. This is supported by the bioinformatics analyses showing that both NsG32a and NsG32b are secreted growth factors/hormones and the fact that NsG32 is preferentially expressed in the nervous system, including the eye (Figs. 18 and 19), as well as the fact that mouse NsG32a is differentially expressed in the developing mouse CNS (Fig. 35). Furthermore, when expressed in a cell line

with neuronal potential (PC12 cells, Example 5), NsG32a and NsG32b are capable of mediating a survival-enhancing effect under sub-optimal conditions. In addition, NsG32a protects cerebellar granule cells from apoptosis (Fig. 36).

NsG32 may act on a range of different cell types, which are present in the nervous system. In the context of the present invention, the nervous system is intended to encompass the central nervous system, the peripheral nervous system, the eye, and the cochleovestibular complex.

In one embodiment, NsG32 polypeptides may act on neurons, including but not limited to motor neurons and sensory neurons.

In another embodiment, the therapeutic effect of NsG32 polypeptides may be through action on glial cells, such as oligodendrocytes and/or astrocytes. Through their action on glial cells, NsG32 polypeptides may be involved in myelination, and in the maintenance of neuron function and survival.

In another embodiment, NsG32 polypeptides may act on sensory cells, including but not limited to retinal ganglion cells, photoreceptor cells, supportive tissue such as retinal epithelial cells, and hair cells of the ear.

In a further embodiment, NsG32 polypeptides may act on stem cells, and downstream precursor cells including but not limited to neuronal precursors and glial precursors. NsG32 polypeptides may act on stem cells and/or neuronal or glial precursors to cause growth, proliferation, enhance survival, to cause differentiation, and/or migration. Stem cell therapy may be done through in vivo or ex vivo gene therapy, or in vitro treatment of isolated stem cells, or the protein may be administered to a location with stem cells. The effect of NsG32 on stem cells may be tested using the Neurosphere assay described herein (Example 12).

The disorder or disease or damage may be damages of the nervous system caused by trauma, surgery, ischaemia, infection, metabolic diseases, nutritional deficiency, malignancy or toxic agents, and genetic or idiopathic processes.

In one embodiment of the method of the invention, the disease or disorder or damage involves injury to the brain, brain stem, the spinal cord, and/or peripheral nerves, resulting in conditions such as stroke, traumatic brain injury (TBI), spinal cord injury (SCI), diffuse axonal injury (DAI), epilepsy, neuropathy, peripheral neuropathy, and associated pain and other symptoms that these syndromes may cause.

In another embodiment, the disease, disorder, or damage involves the degeneration of neurons and their processes in the brain, brain stem, the spinal cord, and/or peripheral nerves, such as neurodegenerative disorders including but not limited to Parkinson's Disease, Alzheimer's Disease, senile dementia, Huntington's Disease, amyotrophic lateral sclerosis (ALS), neuronal/axonal injury associated with Multiple Sclerosis (MS), and associated symptoms.

In another embodiment, the disease, disorder, or damage involves dysfunction, and/or loss of neurons in the brain, brain stem, the spinal cord, and/or peripheral nerves, such as dysfunction and/or loss caused by metabolic diseases, nutritional deficiency, toxic injury, malignancy, and/or genetic or idiopathic conditions, including but not limited to diabetes, renal dysfunction, alcoholism, chemotherapy, chemical agents, drug abuse, vitamin deficiencies, infection, and associated symptoms.

In another embodiment, the disease, disorder, or damage involves the degeneration or sclerosis of glia such as oligodendrocytes, astrocytes, and Schwann cells in the brain, brain stem, the spinal cord, and peripheral nervous system, including but not limited to Multiple Sclerosis (MS), optic neuritis, cerebral sclerosis, post-infectious encephalomyelitis, and epilepsy, and associated symptoms.

In another embodiment, the disease, disorder, or damage involves the retina, photoreceptors, and associated nerves including but not limited to retinitis pigmentosa, macular degeneration, glaucoma, and associated symptoms.

In another embodiment, the disease, disorder, or damage involves the sensory epithelium and associated ganglia of the vestibuloacoustic complex, including but not limited to noise induced hearing loss, deafness, tinnitus, otitis, labyrintitis, hereditary and cochleovestibular atrophies, Meniere's Disease, and associated symptoms.

In a preferred embodiment, the NsG32 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of Parkinson's Disease. This function is based on the finding of high levels of NsG32 expression in the central midbrain in substantia nigra and thalamus and in the developing human midbrain (see Examples 4A and 4B), as well as the finding of high levels of expression in the early postnatal mouse VM and striatum (Example 13) combined with the bioinformatics analyses. The function can be verified using the Bioassay for dopaminergic neurotrophic activities (Example 10) and in vivo through the instrastriatal 6-OHDA lesion model (Example 11).

Huntington's disease (HD) is an autosomal dominant disorder that results in the progressive degeneration of various neuronal populations within the brain, particularly the GABA-ergic medium spiny neurons located in the caudate nucleus. Associated with this degeneration, the cortical glutaminergic input neurons also degenerate and the combined degeneration account for most of the characteristic symptoms of progressive dyskinetic motor movements as well as dementia.

In one embodiment, the NsG32 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of Huntington's disease. This is based on the finding of expression in the human putamen, substantia nigra, caudate nucleus, and thalamus as well as the finding of expression in the early postnatal mouse striatum and VM, combined with the results of the bioinformatics analyses. Huntington's disease is an excitotoxic disease. An excitotoxic bioassay is the assay described in Example 6 of the present invention. Another exemplary bioassay for verification of this neuroprotective effect of NsG32 include e.g. the bioassay on protection of primary hippocampal slice cultures against the excitoxic effects of NMDA (WO 03/004527, example 5).

The most consistent abnormality for Alzheimer's disease, as well as for vascular dementia and cognitive impairment due to organic brain disease related to alcoholism, is the degeneration of the cholinergic system arising from the basal forebrain (BF) to both the codex and hippocampus (Bigl et al. in Brain Cholinergic Systems, M. Steriade and D. Biesold, eds., Oxford University Press, Oxford, pp.364-386 (1990)). In chronic alcoholism the resultant organic brain disease, like Alzheimer's disease and normal aging, is also characterized by diffuse reductions in cortical cerebral blood flow in those brain regions where cholinergic neurons arise (basal forebrain) and to which they project (cerebral cortex) (Lofti et al., Cerebrovasc. and Brain Metab. Rev 1:2 (1989)).

Therefore, in a preferred embodiment, the NsG32 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of Alzheimer's Disease. This function is based on the finding of high levels of NsG32 expression in hippocampus and amygdala combined with the results of the bioinformatics analyses. The therapeutic potential can be tested in an in vitro assay with basal cholinergic forebrain neurons, which are subjected to conditioned medium as described in the examples. An increase in ChAT (choline acetyltransferase) activity in basal cholinergic forebrain neurons is an indication of therapeutic effect in the treatment of Alzheimer's disease.

In another preferred embodiment, the NsG32 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of peripheral neuropathies. This is based on the finding of high NsG32 expression in the dorsal root ganglion combined with the results of the bioinformatics analyses. Verification of this function can be done with the dorsal root ganglion culture assay described in Example 9. Among the peripheral neuropathies contemplated for treatment with the molecules of this invention are trauma-induced neuropathies, e.g., those caused by physical injury or disease state, physical damage to the peripheral nerves such as hermited discs, and the brain, physical damage to the spinal cord, stroke associated with brain damage, and neurological disorders related to neurodegeneration. We also contemplate treatment of chemotherapy-induced neuropathies (such as those caused by delivery of chemotherapeutic agents, e.g., taxol or cisplatin); toxin-induced neuropathies, drug-induced neuropathies, vitamin-deficiency-induced neuropathies; idiopathic neuropathies; and diabetic neuropathies.

In another preferred embodiment, the NsG32 polypeptides, nucleic acids, expression vectors, capsules, and compositions of the invention are used in the treatment of disorders, diseases, or damages associated with the Cerebellum, including but not limited to sensory ataxia, multiple sclerosis, neurodegenerative spinocerebellar disorders, hereditary ataxia, cerebellar atrophies (such as Olivopontocerebellar Atrophy (OPCA), Shy-Drager Syndrome (multiple systems atrophy)), and alcoholism. This function is supported by the high expression levels in the cerebellum combined with the bioinformatics analyses. This function is also supported by experimental analyses showing that NsG32a protects cerebellar granule cells from apoptosis (Fig. 36). Verification of this function may be done with the assays described in Examples 6 and 7 (Protection of cerebellar granule cells from glutamate toxicity and potassium deprivation).

In another preferred embodiment, the NsG32 polypeptides, nucleic acids, expression vectors, capsules, and compositions of the invention are used in the treatment of multiple sclerosis. This function is based on the finding of high NsG32 expression levels in corpus callosum combined with the results of the bioinformatics analyses. The corpus callosum consists primarily of glial cells. Expression in the corpus callosum thus indicates a strong association with glia.

In one preferred embodiment, the NsG32 polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of amyotrophic lateral sclerosis, spinal muscular atrophy, and spinal cord injury. This is based on

the finding of NsG32 expression in the spinal cord combined with the results of the bioinformatics analyses. Verification of this specific therapeutic function may be done with the motorneuron assay described in Example 9.

In a preferred embodiment, the NsG32 polypeptides, nucleic acids, vectors, capsules, and compositions of the invention are used in the treatment of thalamic pain. This function is based on the finding of high NsG32 expression levels in the thalamus combined with the results of the bioinformatics analyses. Thalamic pain is a syndrome caused by stroke involving the thalamus.

In a preferred embodiment, the NsG32 polypeptides, nucleic acids, vectors, capsules, and compositions of the invention are used in the treatment of essential tremor. This function is based on the finding of high NsG32 expression levels in the thalamus combined with the results of the bioinformatics analyses. Essential tremor is an idiopathic syndrome, which can be treated by deep brain/thalamic stimulation in the thalamus and by thalamotomy.

In one embodiment, the NsG32 polypeptides, nucleic acids, vectors, capsules, and compositions of the invention are used in the treatment of diseases, disorders, or damages involving the retina, including but not limited to retinitis pigmentosa, macular degeneration and glaucoma. This specific therapeutic use is supported by the bioinformatics and experimental analyses showing that NsG32 is a secreted growth factor expressed in the retina (Fig. 18).

Confirmation of such use can be obtained by using various state of the art in vitro assays (retinal explant assays, corneal cultures). Verification of function may also be performed in state of the art animal models for corneal wounds (corneal lesion in rabbits) and retina (retinitis pigmentosa mutant models available for mouse and rat).

Other growth factors have important therapeutic uses in both the central and peripheral nervous system and in various eye indications associated with loss of cells in retina and/or cornea. E.g. NGF, is a candidate for both Alzheimer's disease, corneal ulcer (US 6,063,757 and EP 0 973 872), and retinopathies. Neublastin (Artemin) is a candidate for both peripheral neuropathy (WO 02/078730) and corneal wound healing (EP 1 223 966). GDNF is a candidate for Parkinson's Disease, ALS, spinal cord injury, and for wound healing, in particular in cornea (EP 1 223 966).

In another embodiment the neurodegenerative disease is an excitotoxic disease selected from the group consisting of ischaemia, epilepsy, and trauma due to injury, cardiac arrest or stroke. The above-mentioned hippocampal slice culture assay and the assay of Example 6 of the

present invention are non-limiting examples of an assay, which can be used to demonstrate a biological effect, indicative of therapeutic use for the treatment of excitotoxic diseases.

The term "subject" used herein is taken to mean any mammal to which NsG32 polypeptide or polynucleotide, therapeutic cells or biocompatible capsules may be administered. Subjects specifically intended for treatment with the method of the invention include humans, as well as nonhuman primates, sheep, horses, cattle, goats, pigs, dogs, cats, rabbits, guinea pigs, hamsters, gerbils, rats and mice, as well as the organs, tumors, and cells derived or originating from these hosts.

VI. Polypeptide Administration and Formulations

A target tissue for NsG28, NsG30, or NsG32 therapy is a region selected for its retained responsiveness to NsG28, NsG30, or NsG32. In humans, neurons, which retain responsiveness to growth factors into adulthood include the cholinergic basal forebrain neurons, the entorhinal cortical neurons, the thalamic neurons, the locus coeruleus neurons, the spinal sensory neurons, the spinal motor neurons, neurons of substantia nigra, sympathetic neurons, dorsal root ganglia, retina neurons, otic neurons, cerebellar neurons, and ciliary ganglia. Stem cells, such as stem cells of the subventricular zone, and neural and glial progenitor cells also retain responsiveness to growth factors into adulthood. Also myelinating oligodendrocytes retain responsiveness to growth factors into adulthood.

NsG28, NsG30, or NsG32 polypeptides may be administered in any manner, which is medically acceptable. This may include injections, by parenteral routes such as intravenous, intravascular, intraarterial, subcutaneous, intramuscular, intratumor, intraperitoneal, intraventricular, intraepidural, intertracheal, intrathecal, intracerebroventricular, intercerebral, interpulmonary, or others as well as nasal, ophthalmic, rectal, or topical. Sustained release administration is also specifically included in the invention, by such means as depot injections or erodible implants. Peroral administration is also conceivable provided the protein is protected against degradation in the stomach.

Administration of an NsG28, NsG30, or NsG32 according to this invention may be achieved using any suitable delivery means, including: pump (see, e.g., Annals of Pharmacotherapy, 27:912 (1993); Cancer, 41:1270 (1993); Cancer Research, 44:1698 (1984), incorporated herein by reference); microencapsulation (see, e.g., United States patents 4,352,883; 4,353,888; and 5,084,350, herein incorporated by reference); continuous release polymer implants (see, e.g., Sabel, United States patent 4,883,666, incorporated herein by reference);

encapsulated cells (see, Section X); naked or unencapsulated cell grafts to the CNS (see, e.g., United States patents 5,082,670 and 5,618,531, each incorporated herein by reference); injection, either subcutaneously, intravenously, intra-arterially, intramuscularly, or to other suitable site; inhalation; and oral administration, in capsule, liquid, tablet, pill, or prolonged release formulation.

Administration may be by periodic injections of a bolus of the preparation, or may be made more continuous by intravenous or intraperitoneal administration from a reservoir which is external (*e.g.*, an IV bag) or internal (*e.g.*, a bioerodable implant, a bioartificial organ, a biocompatible capsule of NsG28, NsG30, or NsG32 production cells, or a colony of implanted NsG28, NsG30, or NsG32 production cells). See, *e.g.*, U.S. Patents 4,407,957, 5,798,113, and 5,800,828, each incorporated herein by reference. Intrapulmonary delivery methods and apparatus are described, for example, in U.S. Patents 5,654,007, 5,780,014, and 5,814,607, each incorporated herein by reference.

Apart from systemic delivery, delivery directly to the CNS or the eye behind the blood-brain or blood-retina barriers is also contemplated.

Localised delivery may be by such means as delivery via a catheter to one or more arteries, such as the ophthalmic artery to the eye, and the cerebral artery to the CNS. Methods for local pump-based delivery of protein formulations to the CNS are described in US 6,042,579 (Medtronic). Another type of localised delivery comprises delivery using encapsulated cells (see Section XI). A further type of localised delivery comprises local delivery of gene therapy vectors, which are normally injected.

For the treatment of eye disorders, delivery may be systemic, or local such as delivery via the ophthalmic artery. In another embodiment, delivery is via Encapsulated Cell Therapy, where the encapsulated cells are implanted intravitreally. Delivery of protein formulations or gene therapy vector may be done using subretinal injections, intravitreal injection, or transcleral injection.

For the treatment of Parkinson's Disease, various delivery routes can be taken. Protein formulations can be administered with pumps intracerbroventricularly or intraparenchymally, preferably to the striatum and/or substantia nigra, more preferably to the intraputamen. However, a more preferred delivery method comprises encapsulated cell therapy, where the capsulses are implanted intracerebroventricularly, or intraparenchymally, preferably into the striatum, and/or substantia nigra, and more preferably into the putamen. In one embodiment relating to treatment of Parkinson's Disease, gene therapy vector is administered to the striatum of the brain. Injection

into the striatum can label target sites located in various distant regions of the brain, for example, the globus pallidus, amygdala, subthalamic nucleus or the substantia nigra. Transduction of cells in the pallidus commonly causes retrograde labelling of cells in the thalamus. In a preferred embodiment the (or one of the) target site(s) is the substantia nigra.

In an embodiment to treat HD, NsG28, NsG30, or NsG32 is applied to the striatum, preferably the caudate nucleus in order to protect the neurons from degeneration, resulting in both protection of the caudate neurons and the cortical input neurons. In a preferred embodiment, the application should occur before the onset of major degenerative changes. The treatment would involve the genetic diagnosis of the disease through family history and DNA analysis of the blood followed by the local application of NsG28, NsG30, or NsG32. This would be accomplished by delivering the NsG28, NsG30, or NsG32 to the striatum via pumping of the protein with the use of medically applicable infusion pumps and catheters, e.g. Medtronic Synchrotron pump. In a second strategy, direct gene therapy using viral or non-viral vectors could be utilized to modify the host cells in the striatum or other affected neurons to secrete NsG28, NsG30, or NsG32. In a third strategy, naked or encapsulated cells genetically modified to make and secrete NsG28, NsG30, or NsG32 can be applied locally to deliver NsG28, NsG30, or NsG32 behind the blood-brain-barrier and within the diseased region, preferably the striatum, even more preferred, the caudate nucleus.

In ALS, both upper and lower motor neurons degenerate, causing progressive paralyses, eventually leading to death, most commonly through respiratory complications. To treat ALS, NsG28, NsG30, or NsG32 would be delivered to the CNS including the spinal cord through the infusion of NsG28, NsG30, or NsG32 into the lumbar intrathecal space thereby mixing with the cerebrospinal fluid (CSF), which bathes the spinal cord and brain. The delivery could be accomplished through the implantation of pump and catheters, e.g. Medtronic Synchrotron pump or through the use of encapsulated cell devices implanted into the lumbar inthrathecal space. Direct gene therapy could also be used by injecting DNA carrying vectors into the CSF, thereby transferring the gene to cells lining the CSF space. In addition, gene transfer vectors can be injected into the cervical or lumbar spinal cord or intracerebral, thereby secreting NsG28, NsG30, or NsG32 in the anatomical regions containing the majority of the motor neurons involved in motor paralyses and respiratory function. These injections would occur under surgical navigation and could be performed relatively safely.

In subjects with neurodegenerative diseases such as AD, neurons in the Ch4 region (nucleus basalis of Meynert) which have nerve growth factor (NGF) receptors undergo marked

atrophy as compared to normal controls (see, e. g., Kobayashi, et al., Mol. Chem. Neuropathol., 15: 193-206 (1991)). In normal subjects, neurotrophins prevent sympathetic and sensory neuronal death during development and prevents cholinergic neuronal degeneration in adult rats and primates (Tuszynski, et al., Gene Therapy, 3 : 305314 (1996)). The resulting loss of functioning neurons in this region of the basal forebrain is believed to be causatively linked to the cognitive decline experienced by subjects suffering from neurodegenerative conditions such as AD (Tuszynski, et al., supra and, Lehericy, et al., J. Comp. Neurol., 330: 15-31 (1993)). In general it is contemplated, that AD can be treated with NsG28, NsG30, or NsG32 protein formulations delivered intracerebroventricularly, or intraparenchymally. Within the intraparenchymal area, delivery is preferably to the basal forebrain, and to the hippocampus. Gene therapy vector, encapsulated or naked cells secreting NsG28, NsG30, or NsG32 can also be administered to the basal forebrain or the hippocampus.

For the treatment of spinal cord injury, protein, gene therapy vector or encapsulated or naked cells secreting NsG28, NsG30, or NsG32 can be delivered intrathecally at the position of the injury as described above for the treatment of ALS.

For the treatment of peripheral neuropathy, delivery is either systemic (using protein formulations), intrathecally using protein formulations, gene therapy vectors, or encapsulated or naked cells secreting NsG28, NsG30, or NsG32, or intramuscularly depending on retrograde transport to the spinal cord.

For the treatment of multiple sclerosis, delivery is either systemic (using protein formulations), or intraventricular, intrathecal or intralessional using protein formulations, gene therapy vectors, or encapsulated or naked cells secreting NsG28, NsG30, or NsG32.

For the treatment of epilepsy NsG28, NsG30, or NsG32 protein could be delivered intraparenchymally in the epilepsy focus. This may be done with encapsulated or naked cells, with protein formulation administered with catheter or pump or with gene therapy vector delivered to this site.

For the treatment of stroke or trauma, delivery is intrathecal, intracerbroventricular, or preferably intralessionar.

The term "pharmaceutically acceptable carrier" means one or more organic or inorganic ingredients, natural or synthetic, with which NsG28, NsG30, or NsG32 polypeptide is combined to facilitate its application. A suitable carrier includes sterile saline although other aqueous and non-aqueous isotonic sterile solutions and sterile suspensions known to be pharmaceutically acceptable are known to those of ordinary skill in the art. An "effective amount" refers to that

amount which is capable of ameliorating or delaying progression of the diseased, degenerative or damaged condition. An effective amount can be determined on an individual basis and will be based, in part, on consideration of the symptoms to be treated and results sought. An effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

A liposome delivery system may be any variety of unilamellar vesicles, multilamellar vesicles, or stable plurilamellar vesicles, and may be prepared and administered according to methods well known to those of skill in the art, for example in accordance with the teachings of United States Patents 5,169,637,4,762,915, 5,000,958 or 5,185,154. In addition, it may be desirable to express the novel polypeptides of this invention, as well as other selected polypeptides, as lipoproteins, in order to enhance their binding to liposomes. A recombinant NsG28, NsG30, or NsG32 protein is purified, for example, from CHO cells by immunoaffinity chromatography or any other convenient method, then mixed with liposomes and incorporated into them at high efficiency. The liposome-encapsulated protein may be tested in vitro for any effect on stimulating cell growth.

Any of the NsG28, NsG30, or NsG32 polypeptides of this invention may be used in the form of a pharmaceutically acceptable salt. Suitable acids and bases which are capable of forming salts with an NsG28, NsG30, or NsG32 polypeptide are well known to those of skill in the art, and include inorganic and organic acids and bases.

In addition to the active ingredients, the pharmaceutical compositions may comprise suitable ingredients. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

Various dosing regimes for systemic administration are contemplated. In one embodiment, methods of administering to a subject a formulation comprising an NsG28, NsG30, or NsG32 polypeptide include administering NsG28, NsG30, or NsG32 at a dosage of between 1 μg/kg to 30,000 μg/kg body weight of the subject, per dose. In another embodiment, the dosage is between 10 μg/kg to 30,000 μg/kg body weight of the subject, per dose. In a further embodiment, the dosage is between 10 μg/kg to 10,000 μg/kg body weight of the subject, per dose. In a different embodiment, the dosage is between 25 μg/kg to 10,000 μg/kg body weight of the subject, per dose. In yet another embodiment, the dosage is between 25 μg/kg to 3,000 μg/kg body weight of the subject, per dose. In a most preferable embodiment, the dosage is between 50 μg/kg to 3,000 μg/kg body weight of the subject, per dose.

Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. Nos. 4,657,760; 5,206,344; or 5,225,212. It is anticipated that different formulations will be effective for different treatment compounds and different disorders, that administration targeting one organ or tissue, for example, may necessitate delivery in a manner different from that to another organ or tissue.

Where sustained-release administration of an NsG28, NsG30, or NsG32 polypeptide is desired in a formulation with release characteristics suitable for the treatment of any disease or disorder requiring administration of an NsG28, NsG30, or NsG32 polypeptide, microencapsulation of an NsG28, NsG30, or NsG32 polypeptide is contemplated. Microencapsulation of recombinant proteins for sustained release has been successfully performed with human growth hormone (rhGH), interferon-(rhIFN-), interleukin-2, and MN rgp120. Johnson et al., Nat. Med., 2:795-799 (1996); Yasuda, Biomed. Ther., 27:1221-1223 (1993); Hora et al., Bio/Technology, 8:755-758 (1990); Cleland, "Design and Production of Single Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems," in Vaccine Design: The Subunit and Adjuvant Approach, Powell and Newman, eds, (Plenum Press: New York, 1995), pp. 439-462; WO 97/03692, WO 96/40072, WO 96/07399; and U.S. Pat. No. 5,654,010.

The sustained-release formulations of these proteins were developed using poly-lactic-coglycolic acid (PLGA) polymer due to its biocompatibility and wide range of biodegradable properties. The degradation products of PLGA, lactic and glycolic acids, can be cleared quickly within the human body. Moreover, the degradability of this polymer can be adjusted from months to years depending on its molecular weight and composition. Lewis, "Controlled release of bioactive agents from lactide/glycolide polymer," in: M. Chasin and R. Langer (Eds.), Biodegradable Polymers as Drug Delivery Systems (Marcel Dekker: New York, 1990), pp. 1-41.

The dose administered should be carefully adjusted to the age, weight and condition of the individual being treated, as well as the route of administration, dosage form and regimen, and the result desired, and the exact dosage should be determined by the practitioner.

VII. Pharmaceutical Preparations for Gene Therapy

To form an NsG28, NsG30, or NsG32 composition for gene therapy use in the invention, NsG28, NsG30, or NsG32 encoding expression viral vectors may be placed into a pharmaceutically acceptable suspension, solution or emulsion. Suitable mediums include saline and liposomal preparations.

More specifically, pharmaceutically acceptable carriers may include sterile aqueous of non-aqueous solutions, suspensions, and emulsions. Examples of nonaqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils.

Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like.

Preservatives and other additives may also be present such as, for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like. Further, a composition of NsG28, NsG30, or NsG32 transgenes may be lyophilized using means well known in the art, for subsequent reconstitution and use according to the invention.

A colloidal dispersion system may also be used for targeted gene delivery. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. Liposomes are artificial membrane vesicles which are useful as delivery vehicles in vitro and in vivo. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 μm can encapsulate a substantial percentage of an aqueous buffer containing large macro molecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., Trends Biochem. Sci., 6: 77,1981). In addition to mammalian cells, liposomes have been used for delivery of operatively encoding transgenes in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes encoding the NsG28, NsG30, or NsG32 at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, et al., Biotechniques, 6: 682,1988).

The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries.

Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted gene delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

A further example of a delivery system includes transplantation into the therapeutic area of a composition of packaging cells capable of producing vector particles as described in the present invention. Methods for encapsulation and transplantation of such cells are known in the art, in particular from WO 97/44065 (Cytotherapeutics). By selecting a packaging cell line capable of producing lentiviral particles, transduction of non-dividing cells in the therapeutic area is obtained. By using retroviral particles capable of transducing only dividing cells, transduction is restricted to de-novo differentiated cells in the therapeutic area.

VIII. Dosing Requirements and Delivery Protocol for Gene Therapy

An important parameter is the dosage of NsG28, NsG30, or NsG32 gene therapy vector to be delivered into the target tissue. For viral vectors, the concentration may be defined by the number of transducing units/ml. Optimally, for delivery using a viral expression vector, each unit dosage will comprise 2.5 to 25 μL of a composition, wherein the composition includes a viral

expression vector in a pharmaceutically acceptable fluid and provides from 10^8 up to 10^{10} NsG28, NsG30, or NsG32 transducing units per ml.

Importantly, specific in vivo gene delivery sites are selected so as to cluster in an area of loss, damage, or dysfunction of neural cells, glial cells, retinal cells, sensory cells, or stem cells. Such areas may be identified clinically using a number of known techniques, including magnetic resonance imaging (MRI) and biopsy. In humans, non-invasive, in vivo imaging methods such as MRI will be preferred. Once areas of neuronal loss are identified, delivery sites are selected for stereotaxic distribution so each unit dosage of NsG28, NsG30, or NsG32 is delivered into the brain at, or within 500 μm from, a targeted cell, and no more than about 10 mm from another delivery site.

Within a given target site, the vector system may transduce a target cell. The target cell may be a cell found in nervous tissue, such as a neuron, astrocyte, oligodendrocyte, microglia, stem cells, neural precursor cells, or ependymal cell.

The vector system is preferably administered by direct injection. Methods for injection into the brain are well known in the art (Bilang-Bleuel et al (1997) Proc. Acad. Nat. Sci. USA 94:8818-8823; Choi-Lundberg et al (1998) Exp. Neurol.154:261-275; Choi-Lundberg et al (1997) Science 275:838-841; and Mandel et al (1997)) Proc. Acad. Natl. Sci. USA 94:14083-14088). Stereotaxic injections may be given.

As mentioned above, for transduction in tissues such as the brain, it is necessary to use very small volumes, so the viral preparation is concentrated by ultracentrifugation. The resulting preparation should have at least 10^8 t.u./ml, preferably from 10^8 to 10^{10} t.u./ml, more preferably at least 10^9 t.u./ml. (The titer is expressed in transducing units per ml (t.u./ml) as described in example 6). It has been found that improved dispersion of transgene expression can be obtained by increasing the number of injection sites and decreasing the rate of injection (Horellou and Mallet (1997) as above). Usually between 1 and 10 injection sites are used, more commonly between 2 and 6. For a dose comprising $1\text{-}5\times10^9$ t.u./ml, the rate of injection is commonly between 0.1 and 10 μl/min, usually about 1 μl/min.

The virus composition is delivered to each delivery cell site in the target tissue by microinjection, infusion, scrape loading, electroporation or other means suitable to directly deliver the composition directly into the delivery site tissue through a surgical incision. The delivery is accomplished slowly, such as over a period of about 5-10 minutes (depending on the total volume of virus composition to be delivered).

IX. Viral Vectors

Broadly, gene therapy seeks to transfer new genetic material to the cells of a patient with resulting therapeutic benefit to the patient. Such benefits include treatment or prophylaxis of a broad range of diseases, disorders and other conditions.

Ex vivo gene therapy approaches involve modification of isolated cells (including but not limited to stem cells, neural and glial precursor cells, and foetal stem cells), which are then infused, grafted or otherwise transplanted into the patient. See, e.g., U.S. Pat. Nos. 4,868,116, 5,399,346 and 5,460,959. In vivo gene therapy seeks to directly target host patient tissue in vivo.

Viruses useful as gene transfer vectors include papovavirus, adenovirus, vaccinia virus, adeno-associated virus, herpesvirus, and retroviruses. Suitable retroviruses include the group consisting of HIV, SIV, FIV, EIAV, MoMLV.

Preferred viruses for treatment of disorders of the nervous system are lentiviruses and adeno-associated viruses. Both types of viruses can integrate into the genome without cell divisions, and both types have been tested in pre-clinical animal studies for indiations of the nervous system, in particular the central nervous system.

Methods for preparation of AAV are described in the art, e.g. US 5,677,158. US 6,309,634 and US 6,683,058 describe examples of delivery of AAV to the central nervous system.

Preferably, a lentivirus vector is a replication-defective lentivirus particle. Such a lentivirus particle can be produced from a lentiviral vector comprising a 5' lentiviral LTR, a tRNA binding site, a packaging signal, a promoter operably linked to a polynucleotide signal encoding said fusion protein, an origin of second strand DNA synthesis and a 3' lentiviral LTR. Methods for preparation and in vivo administration of lentivirus to neural cells are described in US 20020037281 (Methods for transducing neural cells using lentiviral vectors).

Retroviral vectors are the vectors most commonly used in human clinical trials, since they carry 7-8 kb and since they have the ability to infect cells and have their genetic material stably integrated into the host cell with high efficiency. See, e.g., WO 95/30761; WO 95/24929. Oncovirinae require at least one round of target cell proliferation for transfer and integration of exogenous nucleic acid sequences into the patient. Retroviral vectors integrate randomly into the patient's genome. Retroviruses can be used to target stem cells of the nervous system as very few cell divisions take place in other cells of the nervous system (in particular the CNS).

Three classes of retroviral particles have been described; ecotropic, which can infect murine cells efficiently, and amphotropic, which can infect cells of many species. The third class

includes xenotrophic retrovirus which can infect cells of another species than the species which produced the virus. Their ability to integrate only into the genome of dividing cells has made retroviruses attractive for marking cell lineages in developmental studies and for delivering therapeutic or suicide genes to cancers or tumors.

For use in human patients, the retroviral vectors should be replication defective. This prevents further generation of infectious retroviral particles in the target tissue--instead the replication defective vector becomes a "captive" transgene stable incorporated into the target cell genome. Typically in replication defective vectors, the gag, env, and pol genes have been deleted (along with most of the rest of the viral genome). Heterologous DNA is inserted in place of the deleted viral genes. The heterologous genes may be under the control of the endogenous heterologous promoter, another heterologous promoter active in the target cell, or the retroviral 5' LTR (the viral LTR is active in diverse tissues). Typically, retroviral vectors have a transgene capacity of about 7-8 kb.

Replication defective retroviral vectors require provision of the viral proteins necessary for replication and assembly in trans, from, e.g., engineered packaging cell lines. It is important that the packaging cells do not release replication competent virus and/or helper virus. This has been achieved by expressing viral proteins from RNAs lacking the ψ signal, and expressing the gag/pol genes and the env gene from separate transcriptional units. In addition, in some 2. and 3. generation retriviruses, the 5' LTR's have been replaced with non-viral promoters controlling the expression of these genes, and the 3' promoter has been minimised to contain only the proximal promoter. These designs minimize the possibility of recombination leading to production of replication competent vectors, or helper viruses.

X. Expression Vectors

Construction of vectors for recombinant expression of NsG28, NsG30, or NsG32 polypeptides for use in the invention may be accomplished using conventional techniques which do not require detailed explanation to one of ordinary skill in the art. For review, however, those of ordinary skill may wish to consult Maniatis et al., in Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, (NY 1982). Expression vectors may be used for generating producer cells for recombinant production of NsG28, NsG30, or NsG32 polypeptides for medical use, and for generating therapeutic cells secreting NsG28, NsG30, or NsG32 polypeptides for naked or encapsulated therapy.

Briefly, construction of recombinant expression vectors employs standard ligation techniques. For analysis to confirm correct sequences in vectors constructed, the genes are sequenced using, for example, the method of Messing, et al., (Nucleic Acids Res., 9: 309-, 1981), the method of Maxam, et al., (Methods in Enzymology, 65: 499, 1980), or other suitable methods which will be known to those skilled in the art.

Size separation of cleaved fragments is performed using conventional gel electrophoresis as described, for example, by Maniatis, et al., (Molecular Cloning, pp. 133-134,1982).

For generation of efficient expression vectors, these should contain regulatory sequences necessary for expression of the encoded gene in the correct reading frame. Expression of a gene is controlled at the transcription, translation or post-translation levels. Transcription initiation is an early and critical event in gene expression. This depends on the promoter and enhancer sequences and is influenced by specific cellular factors that interact with these sequences. The transcriptional unit of many genes consists of the promoter and in some cases enhancer or regulator elements (Banerji et al., Cell 27: 299 (1981); Corden et al., Science 209: 1406 (1980); and Breathnach and Chambon, Ann. Rev. Biochem. 50: 349 (1981)). For retroviruses, control elements involved in the replication of the retroviral genome reside in the long terminal repeat (LTR) (Weiss et al., eds., The molecular biology of tumor viruses: RNA tumor viruses, Cold Spring Harbor Laboratory, (NY 1982)). Moloney murine leukemia virus (MLV) and Rous sarcoma virus (RSV) LTRs contain promoter and enhancer sequences (Jolly et al., Nucleic Acids Res. 11: 1855 (1983); Capecchi et al., In : Enhancer and eukaryotic gene expression, Gulzman and Shenk, eds., pp. 101-102, Cold Spring Harbor Laboratories (NY 1991). Other potent promoters include those derived from cytomegalovirus (CMV) and other wild-type viral promoters.

Promoter and enhancer regions of a number of non-viral promoters have also been described (Schmidt et al., Nature 314: 285 (1985); Rossi and deCrombrugghe, Proc. Natl. Acad. Sci. USA 84: 5590-5594 (1987)). Methods for maintaining and increasing expression of transgenes in quiescent cells include the use of promoters including collagen type I (1 and 2) (Prockop and Kivirikko, N. Eng. J. Med. 311: 376 (1984) ; Smith and Niles, Biochem. 19: 1820 (1980) ; de Wet et al., J. Biol. Chem., 258: 14385 (1983)), SV40 and LTR promoters.

According to one embodiment of the invention, the promoter is a constitutive promoter selected from the group consisting of: ubiquitin promoter, CMV promoter, JeT promoter (US 6,555,674), SV40 promoter, and Elongation Factor 1 alpha promoter (EF1-alpha).

Examples of inducible/repressible promoters include: Tet-On, Tet-Off, Rapamycin-inducible promoter, Mx1.

In addition to using viral and non-viral promoters to drive transgene expression, an enhancer sequence may be used to increase the level of transgene expression. Enhancers can increase the transcriptional activity not only of their native gene but also of some foreign genes (Armelor, Proc. Natl. Acad. Sci. USA 70 : 2702 (1973)). For example, in the present invention collagen enhancer sequences may be used with the collagen promoter 2 (I) to increase transgene expression. In addition, the enhancer element found in SV40 viruses may be used to increase transgene expression. This enhancer sequence consists of a 72 base pair repeat as described by Gruss et al., Proc. Natl. Acad. Sci. USA 78: 943 (1981); Benoist and Chambon, Nature 290: 304 (1981), and Fromm and Berg, J. Mol. Appl. Genetics, 1 : 457 (1982), all of which are incorporated by reference herein. This repeat sequence can increase the transcription of many different viral and cellular genes when it is present in series with various promoters (Moreau et al., Nucleic Acids Res. 9 : 6047 (1981).

Further expression enhancing sequences include but are not limited to Woodchuck hepatitis virus post-transcriptional regulation element, WPRE, SP163, CMV enhancer, and Chicken [beta]-globin insulator or other insulators.

Transgene expression may also be increased for long term stable expression using cytokines to modulate promoter activity. Several cytokines have been reported to modulate the expression of transgene from collagen 2 (I) and LTR promoters (Chua et al., connective Tissue Res., 25: 161-170 (1990); Elias et al., Annals N. Y. Acad. Sci., 580 : 233-244 (1990)); Seliger et al., J. Immunol. 141: 2138-2144 (1988) and Seliger et al., J. Virology 62: 619-621 (1988)). For example, transforming growth factor (TGF), interleukin (IL)-l, and interferon (INF) down regulate the expression of transgenes driven by various promoters such as LTR. Tumor necrosis factor (TNF) and TGF 1 up regulate, and may be used to control, expression of transgenes driven by a promoter. Other cytokines that may prove useful include basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF).

Collagen promoter with the collagen enhancer sequence (Coll (E)) may also be used to increase transgene expression by suppressing further any immune response to the vector which may be generated in a treated brain notwithstanding its immune-protected status. In addition, anti-inflammatory agents including steroids, for example dexamethasone, may be administered to the treated host immediately after vector composition delivery and continued, preferably, until any cytokine-mediated inflammatory response subsides. An immunosuppression agent such as

cyclosporin may also be administered to reduce the production of interferons, which downregulates LTR promoter and Coll (E) promoter-enhancer, and reduces transgene expression.

The vector may comprise further sequences such as a sequence coding for the Cre-recombinase protein, and LoxP sequences. A further way of ensuring temporary expression of the NsG28, NsG30, or NsG32 is through the use of the Cre-LoxP system which results in the excision of part of the inserted DNA sequence either upon administration of Cre-recombinase to the cells (Daewoong et al, Nature Biotechnology 19:929-933) or by incorporating a gene coding for the recombinase into the virus construct (Plück, Int J Exp Path, 77:269-278). Incorporating a gene for the recombinase in the virus construct together with the LoxP sites and a structural gene (an NsG28, NsG30, or NsG32 in the present case) often results in expression of the structural gene for a period of approximately five days.

XI. Biocompatible Capsules

Encapsulated cell therapy is based on the concept of isolating cells from the recipient host's immune system by surrounding the cells with a semipermeable biocompatible material before implantation within the host. The invention includes a device in which cells capable of expressing and secreting NsG28, NsG30, or NsG32 are encapsulated in an immunoisolatory capsule. An "immunoisolatory capsule" means that the capsule, upon implantation into a recipient host, minimizes the deleterious effects of the host's immune system on the cells in the core of the device. Cells are immunoisolated from the host by enclosing them within implantable polymeric capsules formed by a microporous membrane. This approach prevents the cell-to cell contact between host and implanted tissues, eliminating antigen recognition through direct presentation. The membranes used can also be tailored to control the diffusion of molecules, such as antibody and complement, based on their molecular weight (Lysaght et al., 56 J. Cell Biochem. 196 (1996), Colton, 14 Trends Biotechnol. 158 (1996)). Using encapsulation techniques cells can be transplanted into a host without immune rejection, either with or without use of immunosuppressive drugs. Useful biocompatible polymer capsules usually contain a core that contains cells, either suspended in a liquid medium or immobilized within an immobilizing matrix, and a surrounding or peripheral region of permselective matrix or membrane ("jacket") that does not contain isolated cells, that is biocompatible, and that is sufficient to protect cells in the core from detrimental immunological attack. Encapsulation hinders elements of the immune system from entering the capsule, thereby protecting the encapsulated cells from immune

destruction. The semipermeable nature of the capsule membrane also permits the biologically active molecule of interest to easily diffuse from the capsule into the surrounding host tissue.

The capsule can be made from a biocompatible material. A "biocompatible material" is a material that, after implantation in a host, does not elicit a detrimental host response sufficient to result in the rejection of the capsule or to render it inoperable, for example through degradation. The biocompatible material is relatively impermeable to large molecules, such as components of the host's immune system, but is permeable to small molecules, such as insulin, growth factors such as NsG28, NsG30, or NsG32 polypeptides, and nutrients, while allowing metabolic waste to be removed. A variety of biocompatible materials are suitable for delivery of growth factors by the composition of the invention. Numerous biocompatible materials are known, having various outer surface morphologies and other mechanical and structural characteristics. Preferably the capsule of this invention will be similar to those described in WO 92/19195 or WO 95/05452, incorporated by reference; or U.S. Pat. Nos. 5,639,275; 5,653,975; 4,892,538; 5,156,844; 5,283,187; or U.S. Pat. No. 5,550,050, incorporated by reference. Such capsules allow for the passage of metabolites, nutrients and therapeutic substances while minimizing the detrimental effects of the host immune system. Components of the biocompatible material may include a surrounding semipermeable membrane and the internal cell-supporting scaffolding. Preferably, the genetically altered cells are seeded onto the scaffolding, which is encapsulated by the permselective membrane. The filamentous cell-supporting scaffold may be made from any biocompatible material selected from the group consisting of acrylic, polyester, polyethylene, polypropylene polyacetonitrile, polyethylene teraphthalate, nylon, polyamides, polyurethanes, polybutester, silk, cotton, chitin, carbon, or biocompatible metals. Also, bonded fiber structures can be used for cell implantation (U.S. Pat. No. 5,512,600, incorporated by reference). Biodegradable polymers include those comprised of poly(lactic acid) PLA, poly(lactic-coglycolic acid) PLGA, and poly(glycolic acid) PGA and their equivalents. Foam scaffolds have been used to provide surfaces onto which transplanted cells may adhere (WO 98/05304, incorporated by reference). Woven mesh tubes have been used as vascular grafts (WO 99/52573, incorporated by reference). Additionally, the core can be composed of an immobilizing matrix formed from a hydrogel, which stabilizes the position of the cells. A hydrogel is a 3-dimensional network of cross-linked hydrophilic polymers in the form of a gel, substantially composed of water.

Various polymers and polymer blends can be used to manufacture the surrounding semipermeable membrane, including polyacrylates (including acrylic copolymers),

polyvinylidenes, polyvinyl chloride copolymers, polyurethanes, polystyrenes, polyamides, cellulose acetates, cellulose nitrates, polysulfones (including polyether sulfones), polyphosphazenes, polyacrylonitriles, poly(acrylonitrile/covinyl chloride), as well as derivatives, copolymers and mixtures thereof. Preferably, the surrounding semipermeable membrane is a biocompatible semipermeable hollow fiber membrane. Such membranes, and methods of making them are disclosed by U.S. Pat. Nos. 5,284,761 and 5,158,881, incorporated by reference. The surrounding semipermeable membrane is formed from a polyether sulfone hollow fiber, such as those described by U.S. Pat. No. 4,976,859 or U.S. Pat. No. 4,968,733, incorporated by reference. An alternate surrounding semipermeable membrane material is poly(acrylonitrile/covinyl chloride).

The capsule can be any configuration appropriate for maintaining biological activity and providing access for delivery of the product or function, including for example, cylindrical, rectangular, disk-shaped, patch-shaped, ovoid, stellate, or spherical. Moreover, the capsule can be coiled or wrapped into a mesh-like or nested structure. If the capsule is to be retrieved after it is implanted, configurations which tend to lead to migration of the capsules from the site of implantation, such as spherical capsules small enough to travel in the recipient host's blood vessels, are not preferred. Certain shapes, such as rectangles, patches, disks, cylinders, and flat sheets offer greater structural integrity and are preferable where retrieval is desired.

When macrocapsules are used, preferably between 10^3 and 10^8 cells are encapsulated, most preferably 10^5 to 10^7 cells are encapsulated in each device. Dosage may be controlled by implanting a fewer or greater number of capsules, preferably between 1 and 10 capsules per patient.

The scaffolding may be coated with extracellular matrix (ECM) molecules. Suitable examples of extracellular matrix molecules include, for example, collagen, laminin, and fibronectin. The surface of the scaffolding may also be modified by treating with plasma irradiation to impart charge to enhance adhesion of cells.

Any suitable method of sealing the capsules may be used, including the use of polymer adhesives or crimping, knotting and heat sealing. In addition, any suitable "dry" sealing method can also be used, as described, e.g., in U.S. Pat. No. 5,653,687, incorporated by reference.

The encapsulated cell devices are implanted according to known techniques. Many implantation sites are contemplated for the devices and methods of this invention. These implantation sites include, but are not limited to, the central nervous system, including the brain,

spinal cord (see, U.S. Pat. Nos. 5,106,627, 5,156,844, and 5,554,148, incorporated by reference), and the aqueous and vitreous humors of the eye (see, WO 97/34586, incorporated by reference).

Methods and apparatus for implantation of capsules into the CNS are described in US 5,487,739. Methods and apparatus for implantation of capsules into the eye are described in US 5,904,144, US 6,299,895, US 6,439,427, and US 20030031700.

In one aspect the invention relates to a biocompatible capsule comprising: a core comprising living packaging cells that secrete a viral vector for infection of a target cell, wherein the viral vector is a vector according to the invention; and an external jacket surrounding said core, said jacket comprising a permeable biocompatible material, said material having a porosity selected to permit passage of retroviral vectors of approximately 100 nm diameter thereacross, permitting release of said viral vector from said capsule.

Preferably, the core additionally comprises a matrix, the packaging cells being immobilized by the matrix. According to one embodiment, the jacket comprises a hydrogel or thermoplastic material.

Examples of suitable cells for packaging cell lines include HEK293, NIH3T3, PG13, and ARPE-19 cells. Preferred cells include PG13 and 3T3 cells.

Packaging cell lines may be encapsulated and administered using the methods and compositions disclosed in US 6,027,721 and WO 97/01357 hereby incorporated by reference in their entirety.

XII. Support Matrix for NsG28, NsG30, or NsG32 Producing Cells

The present invention further comprises culturing NsG28, NsG30, or NsG32 producing cells in vitro on a support matrix prior to implantation into the mammalian nervous system. The preadhesion of cells to microcarriers prior to implantation is designed to enhance the long-term viability of the transplanted cells and provide long term functional benefit.

To increase the long term viability of the transplanted cells, i.e., transplanted NsG28, NsG30, or NsG32 secreting cells, the cells to be transplanted can be attached in vitro to a support matrix prior to transplantation. Materials of which the support matrix can be comprised include those materials to which cells adhere following in vitro incubation, and on which cells can grow, and which can be implanted into the mammalian body without producing a toxic reaction, or an inflammatory reaction which would destroy the implanted cells or otherwise interfere with their biological or therapeutic activity. Such materials may be synthetic or natural chemical substances, or substances having a biological origin.

The matrix materials include, but are not limited to, glass and other silicon oxides, polystyrene, polypropylene, polyethylene, polyvinylidene fluoride, polyurethane, polyalginate, polysulphone, polyvinyl alcohol, acrylonitrile polymers, polyacrylamide, polycarbonate, polypentent, nylon, amylases, natural and modified gelatin and natural and codified collagen, natural and modified polysaccharides, including dextrans and celluloses (e.g., nitrocellulose), agar, and magnetite. Either resorbable or non-resorbable materials may be used. Also intended are extracellular matrix materials, which are well-known in the art. Extracellular matrix materials may be obtained commercially or prepared by growing cells which secrete such a matrix, removing the secreting cells, and allowing the cells which are to be transplanted to interact with and adhere to the matrix. The matrix material on which the cells to be implanted grow, or with which the cells are mixed, may be an indigenous product of RPE cells. Thus, for example, the matrix material may be extracellular matrix or basement membrane material, which is produced and secreted by RPE cells to be implanted.

To improve cell adhesion, survival and function, the solid matrix may optionally be coated on its external surface with factors known in the art to promote cell adhesion, growth or survival. Such factors include cell adhesion molecules, extracellular matrix, such as, for example, fibronectin, laminin, collagen, elastin, glycosaminoglycans, or proteoglycans or growth factors.

Alternatively, if the solid matrix to which the implanted cells are attached is constructed of porous material, the growth- or survival promoting factor or factors may be incorporated into the matrix material, from which they would be slowly released after implantation in vivo.

When attached to the support according to the present invention, the cells used for transplantation are generally on the "outer surface" of the support. The support may be solid or porous. However, even in a porous support, the cells are in direct contact with the external milieu without an intervening membrane or other barrier. Thus, according to the present invention, the cells are considered to be on the "outer surface" of the support even though the surface to which they adhere may be in the form of internal folds or convolutions of the porous support material which are not at the exterior of the particle or bead itself.

The configuration of the support is preferably spherical, as in a bead, but may be cylindrical, elliptical, a flat sheet or strip, a needle or pin shape, and the like. A preferred form of support matrix is a glass bead. Another preferred bead is a polystyrene bead.

Bead sizes may range from about 10 μm to 1 mm in diameter, preferably from about 90 μm to about 150 μm. For a description of various microcarrier beads, see, for example, isher

Biotech Source 87-88, Fisher Scientific Co., 1987, pp. 72-75; Sigma Cell Culture Catalog, Sigma Chemical Co., St, Louis, 1991, pp. 162-163; Ventrex Product Catalog, Ventrex Laboratories, 1989; these references are hereby incorporated by reference. The upper limit of the bead's size may be dictated by the bead's stimulation of undesired host reactions, which may interfere with the function of the transplanted cells or cause damage to the surrounding tissue. The upper limit of the bead's size may also be dictated by the method of administration. Such limitations are readily determinable by one of skill in the art.

XIII. Host Cells

In one aspect the invention relates to isolated host cells genetically modified with the vector according to the invention.

According to one embodiment, the host cells are prokaryotic cells such as E. coli which are capable producing recombinant protein in high quantities and which can easily be scaled up to industrial scale. The use of prokaryotic producer cells may require refolding and glycosylation of the NsG28, NsG30, or NsG32 in order to obtain a biologically active protein. In another embodiment, the host cells are eukaryotic producer cells from non-mammals, including but not limited to known producer cells such as yeast (Saccharomyces cerevisiae), filamentous fungi such as aspergillus, and insect cells, such as Sf9.

According to another embodiment, the cells preferably are mammalian host cells because these are capable of secreting and processing the encoded NsG28, NsG30, or NsG32 correctly. Preferred species include the group consisting of human, feline, porcine, simian, canina, murine, rat, rabbit, mouse, and hamster.

Examples of primary cultures and cell lines that are good candidates for transduction or transfection with the vectors of the present invention include the group consisting of CHO, CHO-K1, HEI193T, HEK293, COS, PC12, HiB5, RN33b, neuronal cells, foetal cells, ARPE-19, C2C12, HeLa, HepG2, striatal cells, neurons, astrocytes, and interneurons. Preferred cell lines for mammalian recombinant production include CHO, CHO-1, HEI193T, HEK293, COS, PC12, HiB5, RN33b, and BHK cells.

For ex vivo gene therapy, the preferred group of cells include neuronal cells, neuronal precursor cells, neuronal progenitor cells, stem cells and foetal cells.

The invention also relates to cells suitable for biodelivery of NsG28, NsG30, or NsG32 via naked or encapsulated cells, which are genetically modified to overexpress NsG28, NsG30,

or NsG32, and which can be transplanted to the patient to deliver bioactive NsG28, NsG30, or NsG32 polypeptide locally. Such cells may broadly be referred to as therapeutic cells.

In a preferred embodiment of the invention, a therapeutic cell line has not been immortalised with the insertion of a heterologous immortalisation gene. As the invention relates to cells which are particularly suited for cell transplantation, whether as naked cells or – preferably as encapsulated cells, such immortalised cell lines are less preferred as there is an inherent risk that they start proliferating in an uncontrolled manner inside the human body and potentially form tumours.

Preferably, the cell line is a contact inhibited cell line. By a contact inhibited cell line is intended a cell line which when cultured in Petridishes grow to confluency and then substantially stop dividing. This does not exclude the possibility that a limited number of cells escape the mono-layer. Contact inhibited cells may also be grown in 3D, e.g. inside a capsule. Also inside the capsules, the cells grow to confluency and then significantly slow down proliferation rate or completely stop dividing. A particularly preferred type of cells include epithelial cells which are by their nature contact-inhibited and which form stable monolayers in culture.

Even more preferred are retinal pigment epithelial cells (RPE cells). The source of RPE cells is by primary cell isolation from the mammalian retina. Protocols for harvesting RPE cells are well-defined (Li and Turner, 1988, Exp. Eye Res. 47:911-917; Lopez et al., 1989, Invest. Ophthalmol. Vis. Sci. 30:586-588) and considered a routine methodology. In most of the published reports of RPE cell cotransplantation, cells are derived from the rat (Li and Turner, 1988; Lopez et al., 1989). According to the present invention RPE cells are derived from humans. In addition to isolated primary RPE cells, cultured human RPE cell lines may be used in the practice of the invention.

For encapsulation, the cells need to be able to survive and maintain a functional NsG28, NsG30, or NsG32 secretion at the low oxygen tension levels of the CNS. Preferably the cell line of the invention is capable of surviving at an oxygen tension below 5%, more preferably below 2%, more preferably below 1%. 1% oxygen tension corresponds approximately to the oxygen level in the brain.

To be a platform cell line for an encapsulated cell based delivery system, the cell line should have as many of the following characteristics as possible: (1) The cells should be hardy under stringent conditions (the encapsulated cells should be functional in the vascular and avascular tissue cavities such as in the central nervous system intraparenchymally or within the ventricular or intrathecal fluid spaces or the eye, especially in the intra-ocular environment). (2)

The cells should be able to be genetically modified to express NsG28, NsG30, or NsG32. (3) The cells should have a relatively long life span (the cells should produce sufficient progenies to be banked, characterised, engineered, safety tested and clinical lot manufactured). (4) The cells should be of human origin (which increases compatibility between the encapsulated cells and the host). (5) The cells should exhibit greater than 80% viability for a period of more than one month in vivo in device (which ensures long-term delivery). (6) The encapsulated cells should deliver an efficacious quantity of NsG28, NsG30, or NsG32 (which ensures effectiveness of the treatment). (7) when encapsulated the cells should not cause a significant host immune reaction (which ensures the longevity of the graft). (8) The cells should be non-tumorigenic (to provide added safety to the host, in case of device leakage).

For encapsulation the preferred cells include retinal pigmented epithelial cells, including ARPE-19 cells; human immortalised fibroblasts; and human immortalised astrocytes.

The ARPE-19 cell line is a superior platform cell line for encapsulated cell based delivery technology and is also useful for unencapsulated cell based delivery technology. The ARPE-19 cell line is hardy (i.e., the cell line is viable under stringent conditions, such as implantation in the central nervous system or the intra-ocular environment). ARPE-19 cells can be genetically modified to secrete a substance of therapeutic interest. ARPE-19 cells have a relatively long life span. ARPE-19 cells are of human origin. Furthermore, encapsulated ARPE-19 cells have good in vivo device viability. ARPE-19 cells can deliver an efficacious quantity of growth factor. ARPE-19 cells elicit a negligible host immune reaction. Moreover, ARPE-19 cells are non-tumorigenic. Methods for culture and encapsulation of ARPE-19 cells are described in US 6,361,771.

In another embodiment the therapeutic cell line is selected from the group consisting of: human fibroblast cell lines, human astrocyte cell lines, human mesencephalic cell lines, and human endothelial cell lines, preferably immortalised with TERT, SV40T or vmyc.

The method for generating an immortalised human astrocyte cell lines has previously been described (Price TN, Burke JF, Mayne LV. A novel human astrocyte cell line (A735) with astrocyte-specific neurotransmitter function. In Vitro Cell Dev Biol Anim. 1999 May;35(5):279-88.). This protocol may be used to generate astrocyte cell lines.

The following three modifications of that protocol are preferably made to generate additional human astrocyte cell lines.

Human foetal brain tissue dissected from 5-12 weeks old foetuses may be used instead of 12-16 weeks old tissue.

The immortalisation gene *v-myc*, or TERT (telomerase) may be used instead of the *SV40 T antigen.*

Retroviral gene transfer may be used instead of transfection with plasmids by the calcium phosphate precipitation technique.

EXAMPLES

The following examples are offered by way of illustration and not by way of limitation. It will be understood that the specific conditions of the examples can be varied without departing from the purpose of the examples.

Example 1: NsG28, Nsg30, and NsG32 Sequences

NsG28 Sequences

SEQ ID NO:2, human NsG28 cDNA
SEQ ID NO:3, human NsG28 full length amino acid sequence
SEQ ID NO:4, human NsG28a full length amino acid sequence
SEQ ID NO:5, human NsG28 mature protein
SEQ ID NO:6, human NsG28 Cys1-Cys10 polypeptide fragment
SEQ ID NO:7, human NsG28 core polypeptide fragment
SEQ ID NO:8, mouse NsG28 cDNA
SEQ ID NO:9, mouse NsG28 full length amino acid sequence
SEQ ID NO:10, mouse NsG28 mature protein
SEQ ID NO:11, mouse Cys1-Cys10 fragment
SEQ ID NO:12, mouse core polypeptide fragment
SEQ ID NO:13, rat NsG28 full length amino acid sequence
SEQ ID NO:14, rat NsG28, mature protein
SEQ ID NO:15, rat Cys1-Cys10 polypeptide fragment
SEQ ID NO:16, rat core polypeptide fragment

Human NsG28 (2274 bp; CDS=466 – 888; CDS 481-888) (SEQ ID NO:2)

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>gi|32967232|gb|AY325117.1| Homo sapiens TAFA4 mRNA, complete cds
AATTTTCGGGTCGACGATTTCGTGGGCTCGGCTCCGCACTGCTAGCTGCGCGCCGCCCTGGACGGGGCGC
ACCGACTGCGCGCGCGGCTGCGGGCAAACATCGGGAGTCCTGCCTCAGCTGCCGCTTCTCCAGCAGCAGC
```

TTCAGGCTTCTCCCGCAGGAGCTTCGGGCTTCTCCTGGTAGAGACGTGGGAACTTTTCTTCTCCTGGCGA
GGCTGCAGAGGTGATGGGCCGCTCCCGGGGCTCCCGCGGGGAGGCGGCACGGTGAGCGTCCTCGGGCTCC
GGTGCGGCGATCAGTACCTAGTTCCGGACGCGCCGGTCCGACTTGGATGCCGGCTCTAGTCGAGTCTTCT
GAGCTACGATAATTTTTTGGAACGGCAGAAATGATTGGTTCTAGCAACAGATGGGAATTTGGAGTCACTC
TGAAATATATCCTGGAATAAGTGTGTTTGACTAGAACCACATCTTATGAGGTCCCCAAGGATGAGAGTCT
GTGCTAAGTCAGTGTTGCTGTCGCACTGGCTCTTTCTAGCCTACGTGTTAATGGTGTGCTGTAAGCTGAT
GTCCGCCTCAAGCCAGCACCTCCGGGGACATGCAGGTCACCACCAAATCAAGCAAGGGACCTGTGAGGTG
GTCGCCGTGCACAGGTGCTGCAATAAGAACCGCATAGAAGAGCGGTCACAAACGGTCAAGTGCTCTTGCT
TCCCGGGACAGGTGGCGGGCACAACTCGGGCTCAACCTTCTTGTGTTGAAGCTTCCATTGTGATTCAGAA
ATGGTGGTGTCACATGAATCCGTGTTTGGAAGGAGAGGATTGTAAAGTGCTGCCAGATTACTCAGGTTGG
TCCTGTAGCAGTGGCAATAAAGTCAAAACTACGAAGGTAACGCGGTAGCGAAGAGAGAGGTGTGCTTCAA
TCCTGGAGGGGCAGCAGGAGGCGGAGCTCTTTTGCTTGGATTCCCATACATGGCCCCTCTGCAGAAAATT
GTCTAGGATTTCAGCAACTTCATATTTGTATATTGTGAGCTGTGAGAAGGTGGCATTCACTTAACTGGCC
CAGCCCTCTCTGCTTCGTGATTTTATTTCATTGAATTATAACCACAAGCCACAACCCATTTGACATCCTC
TCTCGGATTCCCAAGGAGCATACCTCCAAAATCCGAGAAGAGCAAATCAGAGTCTTCAAAATGGATCACC
ACTAAGGGCATGTTCATTCTTCACTTTCTTTCTGCTTTTACAAAAGAACTTGGATGTATGTTCCAAAGGG
TCCTCATTCTGTTCCTCTTTTGAACTTTTCCTTTTGTCCTTGTATTAAAGTGGTTTTAAAGGGGTCTAAA
AAGATTTTGGCAAAACATATTTGCAGATGTAGATTAGCTGGTGAAGAAAATTACTGCTAGAGATCAACTG
ATTAACTGGTAAAGAACGTTTATTTTATAACCCTTGAAGAATAGAAGGACATAGTTGGATTATTGTGTGT
GCATTGTATTTTTACTTCTATTTTTTTTTTGCTTTCCATTTTCCAGTTAGCAGAGATAAAATGAGAGCGT
TTTAACTTCAATGTACCATTTTACTGAGTGCTAAGGAAGCATATCAATTCCAATATTTTATAACCAAAGC
TCTATCAGAACATATTTATAAAACTTGTTGGAATTTTTACGGCTTTGGTGTAGTCATGTAGGTAAATCAT
TTAAAATATAAAACAATCTCAATTTAGATCAAGGGTTATTTCTTAGATCAAATTTATGCCAATTATATGA
AAAGATTTTAACTCCGAGACAGGAGTCTTTCAGTGCTGAATTTTTAGACTGTAAATGAGTTCTTCTTAAC
TTAGCTGTTTCCCTACTTCTGTGACTTCTGTGTTAGCCATCTTATTTCTTTAAAATCTGAGTCCTGATTG
GCTTAATGATTTTGCAGCAGACATGTCTCCACATATTCTCAAATGCTGTCATGCGGAAACGTATGAAACA
GATGAAGAATGACTGACCCAGATTTTAGATGTATAATGTTGTTAAAGTACATACTACTGTAAAAATATGG
GATGAATTTTATATATTAAGAAATGCCAAAAACATAGTTTCTGCACCAAGTTAATTATCCCTGTCCTTTC
ACATTTATAGGGGGAAAATAAATACTTTATAGCCTAACAGTTATTTGATGTTATTCTTGCAGAGGGAATG
GAAAGGAATGGAAAGATTTGTTGGCGTAATTTTTGAATATTTGTTATGATCATATGAATAAGTAAAAAAA
TTCATCCTGCTGATGGCAAAAAAAAAAAAAAAAA

Human NsG28 full length amino acid sequence (SEQ ID NO:3)

>IPI00065186.1 REFSEQ_NP:NP_872328 TREMBL:Q96LR4 REFSEQ_XP:XP_087261
ENSEMBL:ENSP00000295569 Tax_Id=9606 Hypothetical protein FLJ25161
MRSPRMRVCA KSVLLSHWLF LAYVLMVCCK LMSASSQHLR GHAGHHQIKQ GTCEVVAVHR
CCNKNRIEER SQTVKCSCFP GQVAGTTRAQ PSCVEASIVI QKWWCHMNPC LEGEDCKVLP
DYSGWSCSSG NKVKTTKVTR

Human NsG28a full length amino acid sequence (SEQ ID NO:4)

MRVCAKSVLL SHWLFLAYVL MVCCKLMSAS SQHLRGHAGH HQIKQGTCEV VAVHRCCNKN
RIEERSQTVK CSCFPGQVAG TTRAQPSCVE ASIVIQKWWC HMNPCLEGED CKVLPDYSGW
SCSSGNKVKT TKVTR

Human NsG28, mature protein (SEQ ID NO:5)

SSQHLRGHAG HHQIKQGTCE VVAVHRCCNK NRIEERSQTV KCSCFPGQVA GTTRAQPSCV
EASIVIQKWW CHMNPCLEGE DCKVLPDYSG WSCSSGNKVK TTKVTR

Human NsG28, cys1-cys10 polypeptide fragment (SEQ ID NO:6)

CEVVAVHRCC NKNRIEERSQ TVKCSCFPGQ VAGTTRAQPS CVEASIVIQK WWCHMNPCLE
GEDCKVLPDY SGWSC

Human NsG28, core polypeptide fragment (SEQ ID NO:7)

GTCEVVAVHR CCNKNRIEER SQTVKCSCFP GQVAGTTRAQ PSCVEASIVI
QKWWCHMNPC LEGEDCKVLP DYSGWSCSSG NKVKTTK

Mouse NsG28 (901 bp; CDS= 206 - 613) (SEQ ID NO:8)

>gi|32967244|gb|AY325123.1| Mus musculus TAFA4 mRNA, complete cds
CAGTGCTGTGATCGGCGCTTCACTCTGGACTCCCGGATCTGACTCGGCTGCTCTTTCTAGTCTAACAGGT
CCTCCAAGCCCAAGAAGTTTTGGGGGGCTGCAGAGATGATTGGTCCCAGTTACAAAGGGACATTCACGGT
CACTCTCCAGGACCTTCTTGAGTAACAGTGTTTGACTGGAACCATATACTGTGAACTCTCCAAGGATGAG
AGTCTGTGCTAAGTGGGTGCTGCTGTCACGCTGGCTGGTTCTGACCTATGTGTTAATGGTCTGCTGTAAG
CTGATGTCCGCCTCGAGCCAGCACCTCCGGGGACATGCAGGTCACCACCTGATCAAGCCAGGTACCTGCG
AGGTGGTCGCCGTGCACCGCTGCTGCAATAAGAACCGCATAGAGGAGCGCTCTCAGACGGTCAAATGTTC
CTGCTTCCCAGGCCAGGTGGCTGGCACAACTCGGGCACAGCCTTCTTGTGTTGAAGCTGCCATTGTGATT
GAGAAGTGGTGGTGCCACATGAATCCTTGCTTGGAAGGAGAGGACTGTAAAGTGCTTCCAGACTCTTCGG
GTTGGTCCTGTAGCAGTGGCAATAAAGTCAAAACCACCAAGGTGACCCGGTAGCAGACACAAAAGTGTTT
TGACCCCGAGGGGCATCGGGACGCAGAGCTGTGCTGTGTGGATTACCAGACTAGTGAGAACTCACTCACC
TTGGAACTTGCTCAGAAGTTCATAGCCAAAATATGTGCAGTGTGGAAGGTGGCATTTACGTGGCTGTTCC
CGTTTGTCTGCTTTGTGATCTCATCACACTGAACTACAGCTTAGGTGGCCACTCAACTGAGGTGTTACCT
GGATCTCAAAAGGAGCCTGCCTAGCACAAGAAATGCTGGAGAGGACATCGGAGGTGCTAAT

Mouse NsG28a full length amino acid (SEQ ID NO:9)

>IPI00311118.3 REFSEQ_NP:NP_796207 TREMBL:Q8BV02;Q7TPG5
ENSEMBL:ENSMUSP00000053456 Tax_Id=10090 Ensembl_locations(Chr-bp):6-
98501359 TAFA4
MRVCAKWVLL SRWLVLTYVL MVCCKLMSAS SQHLRGHAGH HLIKPGTCEV VAVHRCCNKN
RIEERSQTVK CSCFPGQVAG TTRAQPSCVE AAIVIEKWWC HMNPCLEGED CKVLPDSSGW
SCSSGNKVKT TKVTR

Mouse mature NsG28 (SEQ ID NO:10)

SSQHLRGHAG HHLIKPGTCE VVAVHRCCNK NRIEERSQTV KCSCFPGQVA GTTRAQPSCV
EAAIVIEKWW CHMNPCLEGE DCKVLPDSSG WSCSSGNKVK TTKVTR

Mouse NsG28, Cys1-Cys10 fragment (SEQ ID NO:11)

CEVVAVHRCC NKNRIEERSQ TVKCSCFPGQ VAGTTRAQPS CVEAAIVIEK WWCHMNPCLE
GEDCKVLPDS SGWSC

Mouse NsG28, core polypeptide fragment (SEQ ID NO:12)

GTCEVVAVHR CCNKNRIEER SQTVKCSCFP GQVAGTTRAQ PSCVEAAIVI
EKWWCHMNPC LEGEDCKVLP DSSGWSCSSG NKVKTTK

Rat NsG28 full length amino acid sequence (SEQ ID NO:13)

>IPI00214302.2 |ENSEMBL:ENSRNOP00000007650
MRVCTKWVLL SHWLVLAYML MVCCKLMSAS SQHLRGHAGH HVIKQGTCEV VAVHRCCNKN
RIEERSQTVK CSCFPGQVAG TTRAQPSCVE ASIVIEKWWC HMDPCLEGED CKVLPDSSGW
SCSSGNKVKT TKAS

Rat NsG28 mature protein (SEQ ID NO:14)

SSQHLRGHAG HHVIKQGTCE VVAVHRCCNK NRIEERSQTV KCSCFPGQVA
GTTRAQPSCV EASIVIEKWW CHMDPCLEGE DCKVLPDSSG WSCSSGNKVK
TTKAS

Rat NsG28, Cys1-Cys10 polypeptide fragment (SEQ ID NO:15)

CEVVAVHRCC NKNRIEERSQ TVKCSCFPGQ VAGTTRAQPS CVEASIVIEK WWCHMDPCLE
GEDCKVLPDS SGWSC

Rat NsG28, core polypeptide fragment (SEQ ID NO:16)

GTCEVVAVHR CCNKNRIEER SQTVKCSCFP GQVAGTTRAQ PSCVEASIVI
EKWWCHMDPC LEGEDCKVLP DSSGWSCSSG NKVKTTK

NsG30 Sequences

SEQ ID NO:17, human NsG30 cDNA

SEQ ID NO:18, human NsG30 full length amino acid sequence

SEQ ID NO:19, human NsG30, mature protein

SEQ ID NO:20, human NsG30, Cys1-Cys10 peptide fragment

SEQ ID NO:21, human NsG30 core polypeptide fragment

SEQ ID NO:22, mouse NsG30 cDNA

SEQ ID NO:23, mouse NsG30 full length amino acid sequence

SEQ ID NO:24, mouse NsG30 mature protein

SEQ ID NO:25, rat partial NsG30 polypeptide sequence

Human NsG30 (671 bp; CDS=156- 551) (SEQ ID NO:17)

>gi|32967228|gb|AY325115.1| Homo sapiens TAFA2 mRNA, complete cds
TCACACAGCACCAGACTTCGGTTCCCTATGCCCCTGGATGGTGACGGCGGATTGGCATCTTGGAAGCGAT
GCGAGAGCGATAAGGCTGGCGCCGGCCCGCAAAAGCTGCAGGAGCATCGCTAGGTGTTGCCGCCACCGGG
AAGCGGGGCTGCAGGATGAGTAAGAGATACTTACAGAAAGCAACAAAAGGAAAACTGCTAATAATAATAT
TTATTGTAACCTTGTGGGGGAAAGTTGTATCCAGTGCAAACCATCATAAAGCTCACCATGTTAAAACGGG
AACTTGTGAGGTGGTGGCACTCCACAGATGCTGTAATAAGAACAAGATAGAAGAACGGTCACAAACAGTC
AAGTGCTCCTGCTTCCCTGGGCAGGTGGCAGGCACCACGCGAGCTGCTCCATCATGTGTGGATGCTTCAA
TAGTGGAACAGAAATGGTGGTGCCATATGCAGCCATGTCTAGAGGGAGAAGAATGTAAAGTTCTTCCGGA
TCGGAAAGGATGGAGCTGTTCCTCTGGGAATAAAGTCAAAACAACTAGGGTAACCCATTAACCCAGGAGA
AATCAAGTGATCCTCAAGGCTGATGACATTGAACATGCGCATAGAAACTTAACTCAACTCCTGAGGTGAT
CTTGAAGATTTTTATACCACTTGAAAGAGGCGCTCAATAGT

Human NsG30 full length amino acid sequence (SEQ ID NO:18)

>IPI00166553.1 REFSEQ_NP:NP_848634 TREMBL:Q8N3H0 REFSEQ_XP:XP_290586
ENSEMBL:ENSP00000331094 Tax_Id=9606 Hypothetical protein
MSKRYLQKAT KGKLLIIIFI VTLWGKVVSS ANHHKAHHVK TGTCEVVALH RCCNKNKIEE
RSQTVKCSCF PGQVAGTTRA APSCVDASIV EQKWWCHMQP CLEGEECKVL PDRKGWSCSS
GNKVKTTRVT H

Human NsG30 mature protein (SEQ ID NO:19)

ANHHKAHHVK TGTCEVVALH RCCNKNKIEE RSQTVKCSCF PGQVAGTTRA APSCVDASIV EQKWWCHMQP CLEGEECKVL PDRKGWSCSS GNKVKTTRVT H

Human NsG30 Cys1-Cys10 peptide fragment (SEQ ID NO:20)

CEVVALHRCC NKNKIEERSQ TVKCSCFPGQ VAGTTRAAPS CVDASIVEQK WWCHMQPCLE GEECKVLPDR KGWSC

Human NsG30 core polypeptide fragment (SEQ ID NO:21)

GTCEVVALHR CCNKNKIEER SQTVKCSCFP GQVAGTTRAA PSCVDASIVE QKWWCHMQPC LEGEECKVLP DRKGWSCSSG NKVKTTR

Mouse NsG30 (1101 bp; CDS= 367 - 762) (SEQ ID NO:22)

>gi|33413407|ref|NM_182807.1| Mus musculus expressed sequence AI851790 (AI851790), mRNA

CACACACCGGCGGACTTGAGGTCCCTATGCCCCTGGATGGTGCCAGCGGACCGGCATCTCGGAAGCGATG
CAGGAGCGGTGAGGCTGGCGTCGGCCCGCGGAAGCTACCGGACCATCGCTAGGTGCTGCCGCCCCCGGGA
CGCCCGGCTGCAGGGTCTCTCACTGGACGTGGAAATAAGCTGGTGACCAGCAAGCCCTAGCGTCTCTGTC
CAGTGACTGTTACAGGAGCACAGGACCATGCTGCCCATCAGTTTTATGTGGACACTGGGACTGAACTGGT
ACCTGTGCTTGTGCAGCGAGGGCTCTTACCCACTGCGCTGTCTCTTCAGCTTGCAATGAGTATCTATTCA
GTGAATGATCACCAAGATGAATAAGAGATACTTGCAGAAAGCAACACAAGGAAAGCTTCTGATAATTATT
TTTATAGTGACCTTGTGGGGGAAAGCCGTTTCCAGCGCCAACCATCACAAAGCTCACCATGTTAGAACTG
GGACTTGCGAGGTTGTGGCGCTGCACAGATGCTGTAATAAGAACAAGATAGAAGAACGGTCCCAAACGGT
CAAGTGCTCCTGCTTCCCTGGGCAGGTGGCAGGCACTACCCGAGCTGCTCCGTCTTGTGTGGATGCATCC
ATAGTGGAACAAAAGTGGTGGTGTCATATGCAGCCATGCCTGGAGGGAGAGGAATGTAAAGTCCTTCCAG
ATCGCAAAGGATGGAGCTGTTCCTCTGGAAACAAAGTAAAAACAACTAGGGTAACCCATTAACCACTCCT
AAATCAAATAATACTGATGGCTGGGTTCATGGAGACACAAGAATAGAAACTGGACTCCTGCCGTGACCTT
GAAGATTTTTATACTGCTTAGAAGAGACATTTAAAATCCATTTCCAAGGAATTCTATGGCTTTTCATCTA
CTTCTTAGTGAAACTAAGACTTTACAGAAGTCTACAGTGAACGTTGGGTCCTGAAGACTTCATCCGCTGT
GAACTAACGCTTGGCTCACAACACTTCTGAGGGAAGGGGGCGCAGTTCTCCACGGAGCTGGGATAGAGTT
GGTTTTCTGGGGTGACACCAGAGACTGTCACCTTTAAGGTTCCTTGCTTCA

Mouse NsG30 full length amino acid (SEQ ID NO:23)

>IPI00338844.1 REFSEQ_NP:NP_877959 TREMBL:Q7TPG7 ENSEMBL:ENSMUSP00000050199 Tax_Id=10090 Ensembl_locations(Chr-bp):10-123230431 TAFA2

MNKRYLQKAT QGKLLIIIFI VTLWGKAVSS ANHHKAHHVR TGTCEVVALH RCCNKNKIEE RSQTVKCSCF PGQVAGTTRA APSCVDASIV EQKWWCHMQP CLEGEECKVL PDRKGWSCSS GNKVKTTRVT H

Mouse NsG30 mature protein (SEQ ID NO:24)

ANHHKAHHVR TGTCEVVALH RCCNKNKIEE RSQTVKCSCF PGQVAGTTRA APSCVDASIV EQKWWCHMQP CLEGEECKVL PDRKGWSCSS GNKVKTTRVT H

Rat NsG30 partial amino acid sequence (SEQ ID NO:25)

>IPI00205786.1 ENSEMBL:ENSRNOP00000005537 Tax_Id=10116 Ensembl_locations(Chr-bp):7-55659676

AHHVRTGTCE VVALHRCCNK NKIEERSQTV KCSCFPGQVA GTTRAAPSCV DASIVEQKWW CHMQPCLEGE ECKVLPDRKG WSCSSGNKVK TTRVS

NsG32 Sequences

SEQ ID NO:26, human NsG32a cDNA

SEQ ID NO:27, human NsG32a full length amino acid sequence

SEQ ID NO:28, human NsG32a, alternative start methionine

SEQ ID NO:29, human NsG32a mature protein

SEQ ID NO:30, human NsG32b, cDNA

SEQ ID NO:31, human NsG32b, full length amino acid sequence

SEQ ID NO:32, human NsG32b, mature protein

SEQ ID NO:33, human NsG32b, core polypeptide fragment

SEQ ID NO:34, human NsG32, Cys1-Cys8 polypeptide fragment

SEQ ID NO:35, mouse NsG32a cDNA

SEQ ID NO:36, mouse NsG32a full length amino acid sequence

SEQ ID NO:37, mouse NsG32b, cDNA

SEQ ID NO:38, mouse NsG32b full length amino acid sequence

SEQ ID NO:39, rat NsG32a cDNA

SEQ ID NO:40, rat NsG32a full length amino acid sequence

SEQ ID NO:41, rat NsG32b partial amino acid sequence

Human NsG32a (1501 bp; CDS=167 – 511 or 113-511) (SEQ ID NO:26)

>gi|29789067|ref|NM_015381.1| Homo sapiens hypothetical protein CLONE25003 (CLONE25003), mRNA

GGCGGCGGAGGATGGCGCGCGCGGGGCCCGCACGTGGAGGCCGGCGCGGGGGCGCGGGCAGGGCCGGCTG
CTGAGACGCGCTGCTGCCCCCCGCGCGGGCGCCGCGGCTTCAATGGCGCCATCGCCCAGGACCGGCAGCC
GGCAAGATGCGACCGCCCTGCCCAGCATGTCCTCAACTTTCTGGGCGTTCATGATCCTGGCCAGCCTGCT
CATCGCCTACTGCAGTCAGCTGGCCGCCGGCACCTGTGAGATTGTGACCTTGGACCGGGACAGCAGCCAG
CCTCGGAGGACGATCGCCCGGCAGACCGCCCGCTGTGCGTGTAGAAAGGGGCAGATCGCCGGCACCACGA
GAGCCCGGCCCGCCTGTGTGGACGCAAGAATCATCAAGACCAAGCAGTGGTGTGACATGCTTCCGTGTCT
GGAGGGGGAAGGCTGCGACTTGTTAATCAACCGGTCAGGCTGGACGTGCACGCAGCCCGGCGGGAGGATA
AAGACCACCACGGTCTCCTGACAAACACAGCCCCTGAGGGGGCCCCGGGAGTGGCCTTGGCTCCCTGGAG
AGCCCACGTCTCAGCCACAGTTCTCCACTCGCCTCGGACTTCACCCGTTCTCTGCCGCCCGCCCACTCCG
TTTCCCTGTGGTCCGTGAAGGACGGCCTCAGGCCTTGGCATCCTGAGCTTCGGTCTGTCCAGCCGACCCG
AGGAGGCCGGACTCAGACACATAGGCGGGGGGCGGCACCTGGCATCAGCAATACGCAGTCTGTGGGAGCC
CGGCCGCGCCCAGCCCCCGCCGACCGTGGCGTTGGCCCTGCTGTCCTCAGAGGAGGAGGAGGAGGAGGCA
GCTCCGGCAGCCACAGAAGGCTGCAGCCCAGCCCGCCTGAGACACGACGCCTGCCCCAGGGGACTGTCAG
GCACAGAAGCGGCCTCCTCCCGTGCCCCAGACTGTCCGAATTGCTTTTATTTTCTTATACTTTCAGTATA
CTCCATAGACCAAAGAGCAAAATCTATCTGAACCTGGACGCACCCTCACTGTCAGGGTCCCTGGGGTCGC
TTGTGCGGGCGGGAGGGCAATGGTGGCAGAGACATGCTGGTGGCCCCGGCGGAGCGGAGAGGGCGGCCGT
GGTGGAGGCCTCCACCCCAGGAGCACCCCGCACACCCTCGGAGGACGGGCTTCGGCTGCGCGGAGGCCGT
GGCACACCTGCGGGAGGCAGCGACGGCCCCCACGCAGACGCCGGGAACGCAGGCCGCTTTATTCCTCTGT
ACTTAGATCAACTTGACCGTACTAAAATCCCTTTCTGTTTTAACCAGTTAAACATGCCTCTTCTACAGCT
CCATTTTTGATAGTTGGATAATCCAGTATCTGCCAAGAGCATGTTGGGTCTCCCGTGACTGCTGCCTCAT

CGATACCCCATTTAGCTCCAGAAAGCAAAGAAAACTCGAGTAACACTTGTTTGAAAGAGATCATTAAATG
TATTTTGCAAAGCCCAAAAAAAAAAAAAAAA

Human NsG32a full length amino acid sequence (SEQ ID NO:27)

>Human NsG32 The N-terminal is 18 residues shorter compared to IPI00239265.1.
MSSTFWAFMI LASLLIAYCS QLAAGTCEIV TLDRDSSQPR RTIARQTARC
ACRKGQIAGT TRARPACVDA RIIKTKQWCD MLPCLEGEGC DLLINRSGWT
CTQPGGRIKT TTVS

Human NsG32a full length amino acid sequence, alternative start methionine (SEQ ID NO:28)

MAPSPRTGSR QDATALPSMS STFWAFMILA SLLIAYCSQL AAGTCEIVTL DRDSSQPRRT
IARQTARCAC RKGQIAGTTR ARPACVDARI IKTKQWCDML PCLEGEGCDL LINRSGWTCT
QPGGRIKTTT VS

Human NsG32a mature protein (SEQ ID NO:29)

TCEIVTLDRD SSQPRRTIAR QTARCACRKG QIAGTTRARP ACVDARIIKT
KQWCDMLPCL EGEGCDLLIN RSGWTCTQPG GRIKTTTVS

Human NsG32b (575 bp; CDS=95 – 472) (SEQ ID NO:30)

>gi|5451563|gb|AI830892.1|AI830892 wj61e07.x1 NCI_CGAP_Lu19 Homo sapiens cDNA clone IMAGE:2407332 3', mRNA sequence
GCGGCCGCGGAGGCTGCACCCGGCGGGGCGCTGATGCGGCGCCTGGACCTTCGCTGCGCGACTTCGGGGG
CGTCGGCCGAGTTGGGACTCCGCGATGCAGCTCCTGAAGGCGCTCTGGGCACTGGCAGGGGCCGCGCTCT
GCTGCTTCCTCGTCCTAGTGATCCACGCGCAGTTCCTCAAAGAAGGTCAGCTGGCCGCCGGCACCTGTGA
GATTGTGACCTTGGACCGGGACAGCAGCCAGCCTCGGAGGACGATCGCCCGGCAGACCGCCCGCTGTGCG
TGTAGAAAGGGGCAGATCGCCGGCACCACGAGAGCCCGGCCCGCCTGTGTGGACGCAAGAATCATCAAGA
CCAAGCAGTGGTGTGACATGCTTCCGTGTCTGGAGGGGGAAGGCTGCGACTTGTTAATCAACCGGTCAGG
CTGGACGTGCACGCAGCCCGGCGGGAGGATAAAGACCACCACGGTCTCCTGACAAACACAGCCCCTGAGG
GGCCCCGGGAGTGGCCTTGGCTCCCTGGAGAGCCCACGTCTCAGCCACAGTTCTCCACTCGCCTCGGACT
TCACCCGTTCTCTGC

Human NsG32b full length amino acid sequence, splice variant (SEQ ID NO:31)

MQLLKALWAL AGAALCCFLV LVIHAQFLKE GQLAAGTCEI VTLDRDSSQP RRTIARQTAR
CACRKGQIAG TTRARPACVD ARIIKTKQWC DMLPCLEGEG CDLLINRSGW TCTQPGGRIK
TTTVS

Human NsG32b mature protein, splice variant (SEQ ID NO:32)

QFLKEGQLAA GTCEIVTLDR DSSQPRRTIA RQTARCACRK GQIAGTTRAR PACVDARIIK
TKQWCDMLPC LEGEGCDLLI NRSGWTCTQP GGRIKTTTVS

Human NsG32b, core polypeptide (SEQ ID NO:33)

GTCEIVTLDR DSSQPRRTIA RQTARCACRK GQIAGTTRAR PACVDARIIK TKQWCDMLPC
LEGEGCDLLI NRSGWTCTQP GGRIKTTT

Human NsG32 Cys1-Cys8 peptide fragment (SEQ ID NO:34)

CEIVTLDRDS SQPRRTIARQ TARCACRKGQ IAGTTRARPA CVDARIIKTK
QWCDMLPCLE GEGCDLLINR SGWTC

Mouse NsG32a (1343 bp; CDS=74 - 418) (SEQ ID NO:35)

>gi|26355279|dbj|AK090194.1| Mus musculus 16 days neonate male medulla oblongata cDNA, RIKEN full-length enriched library, clone:G630016A22 product:hypothetical protein, full insert sequence
GGCGGGCACCGCGGCTTCAATGGCGCCATCGCCCAGGACCAGCAGCCGGCAAGATGCGACCGCCCTGCCC
AGCATGTCCTCAACTTTTTGGGCATTCATGATCCTGGCCAGCCTGCTCATCGCCTACTGCAGTCAGCTGG
CCGCTGGAACCTGTGAGATTGTGACCCTAGACCGGGACAGCAGCCAGCCACGGAGGACGATCGCCCGGCA
GACAGCACGCTGTGCATGCAGAAAGGGGCAGATAGCAGGCACCACTCGAGCCCGGCCTGCTTGTGTGGAC
GCTCGAATTATCAAGACAAAGCAGTGGTGTGACATGCTTCCTTGCCTGGAGGGGGAAGGCTGTGACTTGT
TAATCAACCGGTCAGGCTGGACTTGCACACAGCCCGGAGGGCGGATAAAGACCACCACGGTCTCCTGACA
ATCGCAGCCCCTGCGGTGCCCCCTGGGAGTGGCTGTGGCTCCCTGGAAATTCTACACCTCAGCCACAGTC
CTGCATCCTATGTGGACACCGCTCATTCTCCCCCATCCACCCACTGTTTCCCAGAGGCCTAACAAAGACA
GCCCAATTCCTCGGAGCTCTGAGCTCAAGTCTGTCCAGCTGAACCCAGGAGGCTGGGACTCAGACACACA
TGTCAAGGGTGGTACCTGGAATCAGCAATACCTGGTGTGTGGAGCCTGGCTGCATCCATCCTCACTAGCC
GCTTGCTCCCTGGAGTTGGAGGACCGGAACAAGGAAATTGTGGCCCGGCCAGCTGGGCCACAACGCAAGG
TCCGGGGACTGTCAGGTGCAGAAGCCACCACCCCCTCTGTCCCAGACTGTCTGAATCGCTTTTATTTTCT
TATACTTTCAGTATACTCAATAGACCAAAGAGCAAATCTATTTTAACGTGGACACATCCCTGCTGCCAAG
AGTTCCCTGGGTTTGATTGTGGGGCAGGGGAGGGCGCAGTGGCAGACATGCTGGTGGCCCAGGCAGCATG
GAGAGGGCATCTGAGGCCAAGACCCCCGCCCCACGAACACTGCAGCGTGCCCTAAGAGGATGGCTTTGCC
CAGAAGGACACCACCGTGGTGCACCTGCAGGACCCAGCAGGGGCATGGCCGGCGACGGCCCCCAGCGGAC
AGCAAGGGCCCCCGGACACAGACCCTTTGCCCCTGTGCTTAGACCAACTCTATCGTACTAAAATCACTTT
CTGTTTTAACCAGTTAGACATGCCTCTTCCACAGCTCCATTTTTGATAGTTGGATAATCCACCATTTGCC
AAGAGCATGCTGGGTCTCCTGTGACTGCTATCTCATCTGATACACCATTTAGCTCCAGAAAGCAAATTAA
AGAAAACTGAAGC

Mouse NsG32a full length amino acid (SEQ ID NO:36)

MSSTFWAFMI LASLLIAYCS QLAAGTCEIV TLDRDSSQPR RTIARQTARC
ACRKGQIAGT TRARPACVDA RIIKTKQWCD MLPCLEGEGC DLLINRSGWT
CTQPGGRIKT TTVS

Mouse NsG32b (2618 bp; CDS= 168 - 545) (SEQ ID NO:37)

>gi|15929769|gb|BC015306.1| Mus musculus expressed sequence AW049604, mRNA (cDNA clone MGC:19343 IMAGE:4223181), complete cds
GGATCGCCTCCCGGAGTGGCGCCTCCAGTCGCGGCGGAGCGCGGCGTTGGCGGCGGATGGAGGGCGCGAT
CGGGCGGCCAAAGCGGCTGCACCCAGCGGGGCGCTGATGCGGCTCCTGGACCTTCGCTGCGCGACTTCGG
GGGCGTCGGCCGAGTTGGCACTCCGCGATGCAGCTCCTGAAGGCGCTCTGGGCACTCGCAGGGGCCGCGC
TCTGCTGCTTCCTCGTCCTGGTGATTCACGCGCAGTTCCTCAAAGAAGGTCAGCTGGCCGCTGGAACCTG
TGAGATTGTGACCCTAGACCGGGACAGCAGCCAGCCACGGAGGACGATCGCCCGGCAGACAGCACGCTGT
GCATGCAGAAAGGGGCAGATAGCAGGCACCACTCGAGCCCGGCCTGCTTGTGTGGACGCTCGAATTATCA
AGACAAAGCAGTGGTGTGACATGCTTCCTTGCCTGGAGGGGGAAGGCTGTGACTTGTTAATCAACCGGTC
AGGCTGGACTTGCACACAGCCCGGAGGGCGGATAAAGACCACCACGGTCTCCTGACAATCGCAGCCCCTG
CGGTGCCCCCTGGGAGTGGCTGTGGCTCCCTGGAAATTCTACACCTCAGCCACAGTCCTGCATCCTATGT
GGACACCGCTCATTCTCCCCCATCCACCCACTGTTTCCTAGAGGCCTAACAAAGACAGCCCAATTCCTCG
GAGCTCTGAGCTCAAGTCTGTCCAGCTGAACCCAGGAGGCTGGGACTCAGACACACATGTCAAGGGTGGT
ACCTGGAATCAGCAATACCTGGTGTGTGGAGCCTGGCTGCATCCATCCTCACTAGCCGCTTGCTCCCTGG
AGTTGGAGGACCGGAACAAGGAAATTGTGGCCCGGCCAGCTGGGCCACAACGCAAGGTCCGGGGACTGTC
AGGTGCAGAAGCCACCACCCCCTCTGTCCCAGACTGTCTGAATCGCTTTTATTTTCTTATACTTTCAGTA
TACTCAATAGACCAAAGAGCAAATCTATTTTAACGTGGACACATCCCTGCTGCCAAGAGTTCCCTGGGTT
TGATTGTGGGGCAGGGGAGGGCGCAGTGGCAGACATGCTGGTGGCCCAGGCAGCATGGAGAGGGCATCTG

AGGCCAAGACCCCCGCCCCACGAACACTGCAGCGTGCCCTAAGAGGATGGCTTTGCCCAGAAGGACACCA
CCGTGGTGCACCTGCAGGACCCAGCAGGGGCATGGCCGGCGACGGCCCCCAGCGGACAGCAAGGGCCCCG
GACACAGACCCTTTGCCCCTGTGCTTAGACCAACTCTATCGTACTAAAATCACTTTCTGTTTTAACCAGT
TAGACATGCCTCTTCCACAGCTCCATTTTTGATAGTTGGATAATCCACCATTTGCCAAGAGCATGCTGGG
TCTCCTGTGACTGCTATCTCATCTGATACACCATTTAGCTCCAGAAAGCAAATTAAAGAAAACTGAAGTA
ACCCTGTTTGAAAGAGATCATTAAATGTATTTTGCAAAAGCCTAAAGTTATATATTTAACAGTTTTTATA
TGTTGTATATTTGTAGAAAATCCTATTTAACATTTGACGGCAGGTCTGACCATCCTGAGAGTTGTGGCCA
GGCCTCCCTGTGATCCTGGAGACTCTGGGGTGGCCATGGAGGACCTCAGAGCAGACATGGACGGGCAGAG
CTGTGGGTGAGGTGCAGAGGACCCTGCCCATTCTAGGAAGCTCTGTCTCTCTTTGCTTTGGTTTGGTCTG
ATAGTTCACATCCCCCTGTGAGTCTGTGTGTTGGTAAACATTGCGTATACAATATTTTGGTCATGGATTT
TGGTCCCTCTCTCTCTTAACCCCTTTTGCCATCCATAGATCTTGAGTCCATGTACGGCCAACTTGCCCTT
CCCAGGTGAATGACCCCCTTCGTCTTCAAAGGTCCTTGTTCTCCACATTGGGAGGCAAAGACAGCAGGGA
TGGAGGGGGTGGAGAAGCTGGGGAGCAGAGTCACTGTCCTTGCTTGCCCTACACTGTGGGGTCAGAGGCT
ATCAGATAAGGGACACAGTCCCCATTCATTTTCCTGAGTTCTGCTACCAGCATACTACCTACATGTCCAA
TTAACATGACTATCATGCTAGTAATATACTACTAAAGGCAAACATTTAAAAAAATGTATTTAACTCCAGT
ATATAGATGCCGTTCCAGCAACCATAGGCTAAAGATAGGAAAGAAAGTCCATTTCTGTGAGACCCATTCC
GGTACCCATGTGTCCTCCCTTCACTCATGCAGAGATTAGTATCCTGGGCAGGGAGATGAAGCTGACATGA
GGGGCTCTCATCAAAGGAGGTGGTCAAACAAGGCCACTGTCTCCGAAGTGTCAGGCTCCAGTGGCCTGTG
TTGCAGGGGGTCAACAGGGCAGGAGGTCTCATTTCACCACTGAACCTCAACATCCGCCCCACCTCCCCTT
GGCTGTTTCTTTTGAAAGCTAGCATAGAGACCTCCGAGGGCAGAGACCAGGAGACTTAAGATGTTACAGC
ATATTTCCCCCTTGTTTTACAGTATCCAATTTTTGTGTTGATTCAGCTAAATTATGAAAATAAAGAAAAA
AAGTCCTTTGATAAAAAAAAAAAAAAA

Mouse, NsG32b, full length amino acid sequence, splice variant (SEQ ID NO:38)

MQLLKALWAL AGAALCCFLV LVIHAQFLKE GQLAAGTCEI VTLDRDSSQP RRTIARQTAR
CACRKGQIAG TTRARPACVD ARIIKTKQWC DMLPCLEGEG CDLLINRSGW TCTQPGGRIK
TTTVS

Rat NsG32a (360 bp; CDS 1 - 360) (SEQ ID NO:39)

>Rat CDS (predicted from mouse NsG32 and Rattus norvegicus cDNA clone
nrdg1-00059-f11 5'
atgtcctcaacttttgggcattcatgatcctggccagcctgctcatcgcctactgcagt
cagctggccgctggaacctgtgagattgtgaccctagaccgggacagcagccagccacgg
aggacgatcgcccggcagacagcacgctgtgcatgcagaaaggggcagatagcaggcacc
acgcgagcccggcctgcttgtgtggacgctcgaattatcaagacaaagcagtggtgtgac
atgcttccttgcctggagggggaaggctgtgacttgttaatcaaccggtcaggctggact
tgcacacagccaggagggcggataaagaccaccacggtctcctga

Rat NsG32a full length amino acid sequence (SEQ ID NO:40)

MSSTFWAFMI LASLLIAYCS QLAAGTCEIV TLDRDSSQPR RTIARQTARC
ACRKGQIAGT TRARPACVDA RIIKTKQWCD MLPCLEGEGC DLLINRSGWT
CTQPGGRIKT TTVS

Rat NsG32b, partial amino acid sequence (SEQ ID NO:41)

LGAVWASGCG SLGCGHLACG YFFFAGQLAA GTCEIVTLDR DSSQPRRTIA RQTARCACRK
GQIAGTTRAR PACVDARIIK TKQWCDMLPC LEGEGCDLLI NRSGWTCTQP GGRIKTTTV

Example 2: Bioinformatics

NsG28

NsG28a is a 135 amino acid secreted growth factor protein or hormone. The mouse (IPI00311118.3 version 1.23) and rat (IPI00214302.2 version 1.11) have full lengths of 135 and 134 amino acids and the % identities are 93.3 and 91.9, respectively. In the databases a longer version of NsG28 with a different start codon is also found (SEQ ID No 3). An analysis of the probability of start codons however indicates that the most likely start codon is the start codon leading to NsG28a (SEQ ID No 4). Furthermore, a corresponding start codon leading to a longer protein is not found in mouse. SEQ ID No 3 and 4 lead to the same mature protein after cleavage of the signal peptide.

The sequence identities in Tables 3 and 4 below have been calculated with the align0 program, using a BLOSUM50 matrix and gap penalties -12/-2.

Table 3 shows the % sequence identity between full length human NsG28a (SEQ ID No 4) versus mouse and rat full length amino acid sequences.

Sequence	% id
human	-
mouse	93.3
rat	91.9

Table 4 shows the % sequence identity between human and mouse NsG28 sequences after removal of N-terminal signal peptide.

Sequence	% id
human	-
mouse	95.3
rat	93.4

Protein processing: Human NsG28a contains a N-terminal signal peptide sequence of 29 amino acids which is cleaved at the sequence motif MSA-SS. This signal peptide cleavage site is predicted by the SignalP method (Nielsen et al., 1997) and the output graph shown in Figs. 2A and 2B. A signal peptide cleavage site is found at a similar location in the mouse and rat NsG28 polypeptide sequences at position 29.

Protein function: Human NsG28 belongs to the category of proteins acting as hormones or growth factors. This notion is supported by predictions by the ProtFun protein function prediction server (Jensen *et al.*, 2002 & 2003), which provides odds scores above 1 for these categories for both human, mouse and rat NsG28 as shown in Fig. 3. In general, an odds score of 1 indicates that the score is similar to "background" score, and no preference for a particular class is therefore predicted. Odds above 1 indicate that there is a significant prediction indicating that the protein indeed does belong to the predicted ontology class. The higher the odds score, the more certain the prediction.

The ProtFun method predicts protein function based on sequence-derived features as opposed to sequence similarity. Features which are important for discriminating between the 'growth factor/hormone' classes versus all other classes are: protein sorting potential, protein targeting potential, signal peptide potential, low complexity regions, secondary protein structure, number of negative residues and number of atoms (Jensen *et al.*, 2003).

NsG30

NsG30 is a 131 amino acid secreted growth factor protein or hormone. The mouse (IPI00338844.1 version 1.22) and rat (IPI00205786.1 version 1.3) homologues have are 131 and 95 (partial sequence) amino acids long and the % identities are 96.9 and 71.0, respectively. The low degree of identity to rat NsG30 is due to lack of the N-terminal.

Human NsG30 contains a N-terminal signal peptide sequence of 30 amino acids which is cleaved at the sequence motif VSS-AN. This signal peptide cleavage site is predicted by the SignalP method (Nielsen *et al.*,1997) and the output graph shown in Figs. 4A and 4B. A signal peptide cleavage site is found at a similar location in the mouse NsG30 at position 30.

Human NsG30 belongs to the category of proteins acting as hormones or growth factors. This notion is supported by predictions by the ProtFun protein function prediction server (Jensen *et al.*, 2002 & 2003), which provides scores above 1 for both these categories as shown in Fig. 5.

In general, an odds score of 1 indicates that the score is similar to "background" score, and no preference for a particular class is therefore predicted. Odds above 1 indicate that there is a significant prediction indicating that the protein indeed does belong to the predicted ontology class. The higher the odds score, the more certain the prediction.

The ProtFun method predicts protein function based on sequence-derived features as opposed to sequence similarity. Features which are important for discriminating between the 'growth factor/hormone' classes versus all other classes are: protein sorting potential, protein targeting potential, signal peptide potential, low complexity regions, secondary protein structure, number of negative residues and number of atoms (Jensen *et al.*, 2003).

The calculations of sequence identity below (Tables 5 and 6) have been made with the align0 program, using a BLOSUM50 matrix and gap penalties -12/-2.

Table 5 shows the % sequence identity between full length human NsG30 versus mouse and rat sequences.

Sequence	% id
human	-
mouse	96.9
rat	71.0

Table 6 shows the % sequence identity between human and mouse NsG30 sequences after removal of N-terminal signal peptide.

Sequence	% id
human	-
mouse	99.0

NsG32

Two splice variants of NsG32 are found (Fig. 29). The other splice variant is referred to as NsG32b. The amino acid sequence for human NsG32b is set forth in SEQ ID No 31. The identical amino acid sequence for mouse NsG32b is set forth in SEQ ID No 38. The difference between NsG32a and b on the polypeptide level is found in the signal sequence. From amino acid no 32 of human NsG32b, it is identical to human NsG32a. The difference in signal sequence also results in a difference in predicted cleavage, so that the mature NsG32b proteins have a longer N-terminal than NsG32a.

NsG32a (SEQ ID No 27) is a 114 amino acid secreted protein. The mouse and rat homologues both have full lengths of 114 amino acids and 100 % sequence identity. Two alternative start codons can be found for both mouse and human NsG32a. These, however result

in the same mature protein after cleavage of the signal peptide. The start codon for human and mouse NsG32a have been predicted using the program NetStart (A.G. Pedersen and H. Nielsen, 1997) and these predictions are shown in Figs. 6 and 7, respectively. The prediction shows that the start codon corresponding to the short version of NsG32a is the most likely start codon.

Human and mouse NsG32b are identical. The partial rat NsG32b sequence available (IPI00209104.2 |ENSEMBL:ENSRNOP00000005889) is also 100% identical in a range of overlap, but differs in both the C and N-terminal. Considering the high degree of conservation between man and mouse, it is probable that the rat sequence is not correct, in particular in the N-terminal.

Human NsG32a contains an N-terminal signal peptide sequence of 25 amino acids, which is cleaved at the sequence motif AAG-TC. This signal peptide cleavage site is predicted by the SignalP method (Nielsen *et al.*, 1997) and the output graph shown in Figs. 8A and 8B.

Human NsG32b contains an N-terminal signal peptide sequence of 25 amino acids, which is cleaved at the sequence motif IHA-QF. This signal peptide cleavage site is predicted by the SignalP method (Nielsen *et al.*, 1997) and the output graph shown in Figs. 9A and 9B.

NsG32a belongs to the category of proteins acting as hormones or growth factors. This notion is supported by predictions by the ProtFun protein function prediction server (Jensen *et al.*, 2002 & 2003), which provides high odds scores for the hormone as well as growth factor category as shown in Fig. 10. Human NsG32b also provides high odds scores for the hormone ontology class.

References:

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SignalP: Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Henrik Nielsen, Jacob Engelbrecht, Søren Brunak and Gunnar von Heijne, Protein Engineering 10, 1-6 (1997).

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Prediction of human protein function according to Gene Ontology categories, L.J. Jensen, R. Gupta, H.H. Stærfeldt, S. Brunak, Bioinformatics, 19, 635-642 (2003).

align0 Optimal alignments in linear space. Myers, E.W. and Miller,W. Comput. Appl. Biosci., 4, 11-17 (1998).

Example 3: Obtaining a Full Length Coding Sequence for NsG28, NsG30, and NsG32

NsG28

NsG28 cDNA was PCR amplified from an IMAGE clone (The I.M.A.G.E. Consortium: "An integrated molecular analysis of genomes and their expression", Lennon, Auffray, Polymeropoulos, and Soares, [1996], Genomics 33: 151-152) obtained from RZPD, Berlin, Germany (RZPD clone ID: IRAKp961M2050) using the following primers:

5' primer: 5'- CGGGATCCGGCACGGTCTTCTGAGCTAC-3'

3' primer: 5'- TATACTCGAGTCGCTACCGCGTTACCTTCG-3'

Three identical PCR reactions were set up with 50 ng/µl of the RZPD clone as DNA template in a 50 µl reaction volume. A proofreading polymerase (pfu-turbo polymerase, Stratagene) was applied for the PCR amplification, with the following amplification profile: pre-denaturation step: 95°C, 1' followed by 35 3-step cycles: denaturation step: 95°C, 30''; annealing step: 55°C, 30''; elongation step: 72°C, 90''. Then an elongation step: 72°C, 2' followed by cooling to 4°C.

PCR reactions were pooled and the 572 bp NsG28 PCR fragment was agarose gel-purified and cut with BamHI and XhoI. The now 560 bp BamHI/XhoI-restricted NsG28 PCR fragment was gel-purified. Five µg of a lentiviral transfer vector, pHsCXW, (GenBank accession #: AY468486) was digested with BamHI and XhoI and the vector backbone was gel purified.

The BamHI/XhoI NsG28 PCR fragment was ligated into the BamHI and XhoI sites of the pHsCXW lentiviral transfer vector followed by transformation into XL1-B electrocompetent cells.

NsG30

NsG30 cDNA was PCR amplified from an IMAGE clone (The I.M.A.G.E. Consortium: "An integrated molecular analysis of genomes and their expression", Lennon, Auffray, Polymeropoulos, and Soares, [1996], Genomics 33: 151-152) obtained from RZPD, Berlin, Germany (RZPD clone ID: IRAKp961G0746) using the following primers:

5' primer: 5'-CGGGATCCCGGATTGGCATCTTGGAAGC-3'

3' primer: 5'-TATACTCGAGTGAGCGCCTCTTTCAAGTGG-3'

Three identical PCR reactions were set up with 50 ng/µl of the RZPD clone as DNA template in a 50 µl reaction volume. A proofreading polymerase (pfu-turbo polymerase, Stratagene) was applied for the PCR amplification, with the following amplification profile: pre-denaturation step: 95°C, 1' followed by 35 3-step cycles: denaturation step: 95°C, 30''; annealing step: 55°C, 30''; elongation step: 72°C, 90''. Then an elongation step: 72°C, 2' followed by cooling to 4°C.

PCR reactions were pooled and the 637 bp NsG30 PCR fragment was agarose gel-purified and cut with BamHI and XhoI. The now 625 bp BamHI/XhoI-restricted NsG30 PCR fragment was gel-purified. Five µg of a lentiviral transfer vector, pHsCXW, (GenBank accession #: AY468486) was digested with BamHI and XhoI and the vector backbone was gel purified.

The BamHI/XhoI NsG30 PCR fragment was ligated into the BamHI and XhoI sites of the pHsCXW lentiviral transfer vector followed by transformation into XL1-B electrocompetent cells.

NsG32b

NsG32b was PCR amplified from an IMAGE clone (The I.M.A.G.E. Consortium: "An integrated molecular analysis of genomes and their expression", Lennon, Auffray, Polymeropoulos, and Soares, [1996], Genomics 33: 151-152) obtained from RZPD, Berlin, Germany (RZPD clone ID: IMAGp998G135981.1) using the following primers:

5' primer: 5'- CGGGATCCACTCCGCGATGCAGCTCCTGAAG-3'

3' primer: 5'- TATACTCGAGTCCGAGGCGAGTGGAGAACTGTG-3'

Three identical PCR reactions were set up with 50 ng/µl of the RZPD clone as DNA template in a 50 µl reaction volume. A proofreading polymerase (pfu-turbo polymerase, Stratagene) was applied for the PCR amplification, with the following amplification profile: pre-denaturation step: 95°C, 1' followed by 35 3-step cycles: denaturation step: 95°C, 30''; annealing

step: 55°C, 30''; elongation step: 72°C, 90''. Then an elongation step: 72°C, 2' followed by cooling to 4°C.

PCR reactions were pooled and the 490 bp NsG32b PCR fragment was agarose gel-purified and cut with BamHI and XhoI. The now 478 bp BamHI/XhoI-restricted NsG32b PCR fragment was gel-purified. Five µg of a lentiviral transfer vector, pHsCXW, (GenBank accession #: AY468486) was digested with BamHI and XhoI and the vector backbone was gel purified.

The BamHI/XhoI NsG32b PCR fragment was ligated into the BamHI and XhoI sites of the pHsCXW lentiviral transfer vector followed by transformation into XL1-B electrocompetent cells.

NsG32a

NsG32a was PCR amplified from human adult whole brain cDNA reverse transcribed from human adult brain total RNA (included in Master Panel II from Becton Dickinson, cat # 636643) using the following primers:

5' primer: 5'- CGGGATCCGCCACCATGGCGCCATCGCCCAGGACC-3'

3' primer: 5'- TATACTCGAGTCCGAGGCGAGTGGAGAACTGTG-3'

Three identical PCR reactions were set up with 20 ng/µl of human adult brain cDNA as DNA template in a 50 µl reaction volume. A proofreading polymerase (pfu-turbo polymerase, Stratagene) was applied for the PCR amplification, with the following amplification profile: pre-denaturation step: 95°C, 1' followed by 35 3-step cycles: denaturation step: 95°C, 30''; annealing step: 60°C, 30''; elongation step: 72°C, 90''. Then an elongation step: 72°C, 2' followed by cooling to 4°C.

PCR reactions were pooled and the 510 bp NsG32a PCR fragment was agarose gel-purified and cut with BamHI and XhoI. The now 498 bp BamHI/XhoI-restricted NsG32a PCR fragment was gel-purified. Five µg of a lentiviral transfer vector, pHsCXW, (GenBank accession #: AY468486) was digested with BamHI and XhoI and the vector backbone was gel purified.

The BamHI/XhoI NsG32a PCR fragment was ligated into the BamHI and XhoI sites of the pHsCXW lentiviral transfer vector followed by transformation into XL1-B electrocompetent cells.

The cloned PCR fragment contains the sequence coding for NsG32a (SEQ ID No. 28) and consequently also the sequence coding for the shorter NsG32a, SEQ ID No. 27. Due to the

presence of a Kosak sequence upstream of the first start codon of the cloned fragment, expression of this sequence is expected to result in the expression of the protein corresponding to SEQ ID No. 28. Expression of a protein corresponding to SEQ ID No. 27 and 28 will result in the same secreted mature protein (SEQ ID No. 29).

Example 4: Expression Analysis

NsG28

Method

Primers amplifying cDNA fragments of 100-350 bp were designed using CloneManager software.

Total RNAs and poly(A)RNA derived from fetal and adult human tissues were purchased from Clontech, Dnase treated to remove residual chromosomal DNA and used as templates for cDNA synthesis using an RnaseH deficient reverse transcriptase. cDNA equivalent to 21 ng total RNA was used for each PCR reaction which were carried out using a DNA engine 2 Opticon light cycler from MJ research.

Real-time PCR was performed in an Opticon-2 thermocycler (MJ Research), using LightCycler-FastStart DNA Master SYBR Green I kit (Roche). Studies were carried out in duplicates using primers 5' oligo: 5'-ACTGGCTCTTTCTAGCCTAC-3', annealing at bp 159 in GenBank sequence: NM_182522 and 3' oligo: 5'-GGCAGCACTTTACAATCCTC-3', annealing at bp 468 in GenBank sequence: NM_182522 amplifying a 310 bp fragment of the NsG28 cDNA spanning two introns in the NsG28 ORF region. For Real-Time PCR, a standard curve was prepared by serial dilution of a gel-purified PCR product, prepared using the above primers. The standard curve was used to verify that crossing-point values (C(T)) of all samples were within the exponential range of the PCR reaction and to calculate final expression levels. All RT-PCR amplifications were performed in a total volume of 10 µl containing 3 mM MgCl2, 12 % sucrose and 1x reaction buffer included in the LightCycler kit. PCR cycling profile consisted of 95°C, 10' » 35 cycles: 95°C, 10" » 62°C, 20" » 72°C, 20" » plate read 72°C, 2". The specificity of the amplification reaction was determined by performing a melting curve analysis of the PCR fragments by slowly raising the temperature from 55 °C to 95°C with continuous data acquisition.

For normalization purposes, all cDNAs were subjected to real-time PCR using primers for β_2-microglobulin (B2M, 5'-TGTGCTCGCGCTACTCTCTC-3' and 5'-CTGAATGCTCCACTTTTTCAATTCT-3'). Standard curves for β_2-microglobulin were prepared similar to NsG28. β_2-microglobulin gene real-time PCR was done using the same kit as for the target gene, except a different annealing temperature was used.

β_2-microglobulin expression levels were determined from the standard curve and the relative expression levels were used to normalize expression levels of the target genes in the tissues that were analyzed. Following normalization, relative expression levels of the target gene were calculated using the tissue with the lowest expression as a reference. Normalized data in Fig. 14 should be interpreted with caution as β_2-microglobulin levels vary between some tissues.

Data are shown in Fig. 14 and Fig 15.

Analysis of total RNA samples

High to moderate expression (C(T) values< 26): Substantia Nigra, Retina, Spinal Cord, Brain

Low expression (29 ≥ C(T) values ≥26): Putamen, testis, cerebellum, Dorsal Root Ganglion, foetal liver

Very low or no expression (C(T) values >29): Pancreas, heart, muscle

Analysis of poly(A)RNA

High expression (C(T) values< 23): Thalamus, Corpus Callosum

Intermediate to low expression (26≥ C(T) values ≥23): Hippocampus, Amygdala, Caudate Nucleus

Very low or no expression (C(T) values >26): Pituitary

This spatial distribution of NsG28 expression indicates that NsG28 is a potential therapeutic candidate for the treatment of neurodegenerative and retinal disorders in general. Suitable assays for the verification of this function include the PC12 assay, the assay testing for protection of cerebellar granule cells from apoptosis induced by potassium deprivation, and assays for retinopathies.

Differential regional expression indicates possible presence of surface receptors and functional relevance in the regions with high expression levels, e.g., in substantia nigra. Thus NsG28 is a candidate for the treatment of Parkinson's Disease. This is based on the fact that Parkinson's Disease is caused by neural degeneration or malfunction in the substantia nigra and

the fact that NsG28 is a growth factor. Thus NsG28 could be important for maintaining normal function of neural cells of the adult substantia nigra. NsG28 expression is also seen in the developing human mesencephalon, further supporting a role for NsG28 in the normal development of the central midbrain. Verification of the function can be tested in the in vitro assay with mesencephalic cultures (example 10) and the in vivo (example 11) using striatal partial lesion model.

The high expression levels in spinal cord indicate that NsG28 is a potential candidate for the treatment of spinal cord injury. One possible verification of this function is the motorneuron assay (Example 9).

NsG28 may also be a candidate for peripheral neuropathies based on the finding of expression in dorsal root ganglion. Verification of this function can be done with the dorsal root ganglion assay described herein (Example 8).

NsG30

A: Quantitative Expression Data

Method

Primers amplifying cDNA fragments of 100-350 bp were designed using CloneManager software.

Total RNAs derived from foetal and adult human tissues were purchased from Clontech, Dnase treated to remove residual chromosomal DNA and used as templates for cDNA synthesis using an RnaseH deficient reverse transcriptase. cDNA equivalent to 21 ng total RNA was used for each PCR reaction which were carried out using a DNA engine 2 Opticon light cycler from MJ research.

Real-time PCR was performed in an Opticon-2 thermocycler (MJ Research), using LightCycler-FastStart DNA Master SYBR Green I kit (Roche). Studies were carried out in duplicates using primers 5' oligo: 5'-GGAAGGTGGTATGGAAGAAG-3', annealing at bp 2780 in GenBank sequence: NM178539 and 3' oligo: 5'-CCAGGAAAGTGTCTGAGTAG-3', annealing at bp 2953 in GenBank sequence: NM178539 amplifying a 194 bp fragment in the fourth exon of NsG30 in the GenBank sequence: NM178539. For Real-Time PCR, a standard curve was prepared by serial dilution of a gel-purified PCR product, prepared using the above

primers. The standard curve was used to verify that crossing-point values (C(T)) of all samples were within the exponential range of the PCR reaction and to calculate final expression levels. All RT-PCR amplifications were performed in a total volume of 10 µl containing 3 mM $MgCl_2$, 12 % sucrose and 1x reaction buffer included in the LightCycler kit. PCR cycling profile consisted of 95°C, 10' » 35 cycles: 95°C, 10" » 62°C, 20" » 72°C, 20" » plate read 72°C, 2". The specificity of the amplification reaction was determined by performing a melting curve analysis of the PCR fragments by slowly raising the temperature from 55 °C to 95°C with continuous data acquisition.

Expression levels were calculated from C(T) values and standard curves generated from serial dilutions of template DNA (plasmid or PCR product).

For normalization purposes, all cDNAs were subjected to real-time PCR using primers for β_2-microglobulin (B2M, 5'-TGTGCTCGCGCTACTCTCTC-3' and 5'-CTGAATGCTCCACTTTTTCAATTCT-3'). Standard curves for β_2-microglobulin were prepared similar to NsG30. β_2-microglobulin gene real-time PCR was done using the same kit as for the target gene, except a different annealing temperature was used.

β_2-microglobulin expression levels were determined from the standard curve and the relative expression levels were used to normalize expression levels of the target genes in the tissues that were analyzed. Following normalization, relative expression levels of the target gene were calculated using the tissue with the lowest expression as a reference. Normalized data in Fig. 16 should be interpreted with caution as β_2-microglobulin levels vary between some tissues.

Results are shown in Fig. 16 and Fig. 17.

Analysis of total RNA samples (Fig. 16)

<u>High to moderate expression (C(T) values< 24)</u>: Brain, Substantia Nigra

<u>Intermediate and low expression (28≥C(T) values ≥24)</u>: Putamen, Retina, Spinal Cord, DRG, testis, cerebellum

<u>Very low or no expression (C(T) values >28)</u>: Foetal liver, pancreas, muscle, Heart

Analysis of poly(A)RNA (Fig. 17)

<u>High expression (C(T) values< 21)</u>: Hippocampus, Amygdala, Thalamus

<u>Intermediate expression (C(T) values ≥21):</u> Corpus Callosum, Caudate Nucleus, Pituitary

B: Gene-Chip Experiments

The human material comes from discarded tissue pieces obtained from electively terminated pregnancies using the regular vacuum aspiration technique. The collection of residual tissue for the study is approved by the Human Ethics Committee of the Huddinge University Hospital, Karolinska Institute (Diary Nr. 259/00) and Lund University (970401), and is in accordance with the guidelines of the Swedish National Board of Health and Welfare (Socialstyrelsen), including an informed consent from the pregnant women seeking abortions. Recovered nervous tissue is micro-dissected within 2 hours of surgery and appropriate tissue fragments are further dissociated for cell isolation.

RNA isolation: Human foetal tissue (8 weeks) was obtained in two rounds, both 8-weeks gestation age. Dissected VM and DM regions were used for total RNA isolation with good results and yields.

Total RNA was isolated with the Trizol extraction following the manufacturer's instruction (Invitrogen) from ventral and dorsal mesencephalic regions subdissected from human foetal tissue, 8 weeks gestational age. To concentrate RNA and to remove traces of chromosomal DNA, Rneasy columns combined with the RNase-Free DNase Set are used following the manufacturer's instructions.

From 5 µg of total RNA, biotinylated cRNA is prepared and fragmented as described in Affymetrix protocols (GeneChip Expression Analysis, Technical Manual 2000) and hybridized (15 µg) to Affymetrix Human U133B GeneChips (containing approximately 22,000 genes) according to manufacturer's instructions. Scanned images are analyzed and converted to expression index values using the GenePublisher analysis software package (Knudsen S, Workman C, Sicheritz-Ponten T, Friis C. (2003) "GenePublisher: Automated analysis of DNA microarray data.", Nucleic Acids Res. 31(13):3471-6.).

In the Table below, probe signals obtained by hybridizing 15 µg biotinylated cRNA to Affymetrix Human U133B Gene Chips are shown.

NsG	Probe-id	Mes1	Mes2	Mes3	Mes4	Mes5	Mes6
30	241399_at	352	368	313	359	301	345

Probe regions for NsG30 with the id given in the Table were located in the 3'UTRs. The probe signals indicated significant expression of NsG30 in the developing human midbrain.

NsG32

A: Quantitative Expression Data

Method

Primers amplifying cDNA fragments of 100-350 bp were designed using CloneManager software.

Total RNAs derived from foetal and adult human tissues were purchased from Clontech, Dnase treated to remove residual chromosomal DNA and used as templates for cDNA synthesis using an RnaseH deficient reverse transcriptase. cDNA equivalent to 21 ng total RNA was used for each PCR reaction which were carried out using a DNA engine 2 Opticon light cycler from MJ research.

Real-time PCR was performed in an Opticon-2 thermocycler (MJ Research), using LightCycler-FastStart DNA Master SYBR Green I kit (Roche). Studies were carried out in duplicates using primers 5' oligo: 5'-GCGGGAGGATAAAGACCACC-3', annealing at bp 480 in GenBank sequence: NM_015381 and 3' oligo: 5'-CTGGACAGACCGAAGCTCAG-3', annealing at bp 692 in GenBank sequence: NM_015381 amplifying a 213 bp fragment of both forms of NsG32 spanning two introns between exons 2 and 4 of NsG32 in GenBank sequence: NM_015381. For Real-Time PCR, a standard curve was prepared by serial dilution of a gel-purified PCR product, prepared using the above primers. The standard curve was used to verify that crossing-point values (C(T)) of all samples were within the exponential range of the PCR reaction and to calculate final expression levels. All RT-PCR amplifications were performed in a total volume of 10 µl containing 3 mM MgCl2, 12 % sucrose and 1x reaction buffer included in the LightCycler kit. PCR cycling profile consisted of 98°C, 10' » 35 cycles: 98°C, 10" » 68°C, 20" » 72°C, 20" » plate read 82°C, 2". The specificity of the amplification reaction was determined by performing a melting curve analysis of the PCR fragments by slowly raising the temperature from 55°C to 95°C with continuous data acquisition.

Expression levels were calculated from C(T) values and standard curves generated from serial dilutions of template DNA (plasmid or PCR product).

For normalization purposes, all cDNAs were subjected to real-time PCR using primers for β_2-microglobulin (B2M, 5'-TGTGCTCGCGCTACTCTCTC-3' and 5'-CTGAATGCTCCACTTTTTCAATTCT-3'). Standard curves for β_2-microglobulin were

prepared similar to NsG32. β_2-microglobulin gene real-time PCR was done using the same kit as for the target gene, except a different annealing temperature was used.

β_2-microglobulin expression levels were determined from the standard curve and the relative expression levels were used to normalize expression levels of the target genes in the tissues that were analyzed. Following normalization, relative expression levels of the target gene were calculated using the tissue with the lowest expression as a reference. Normalized data in Fig. 18 should be interpreted with caution as β_2-microglobulin levels vary between some tissues.

Analysis of total RNA samples (Fig. 18)

High and Intermediate expression (C(T) values< 26): Cerebellum, Substantia Nigra, Brain, Dorsal Root Ganglion, Spinal Cord, Putamen, Foetal Brain, Retina, Testis, Kidney, Salivary Gland

Low expression (C(T) values >26): Prostate, Placenta, Small Intestines, Foetal liver, Stomach, Lung, Heart, Uterus, Skeletal muscle, Colon, Pancreas, Trachea, Thymus, Spleen

Analysis of poly(A)RNA (Fig. 19)

High expression (C(T) values< 23): Hippocampus, Amygdala, Thalamus, Corpus Callosum, Caudate Nucleus

Low expression (C(T) values ≥23): Pituitary

B: Gene-Chip Experiments

The human material comes from discarded tissue pieces obtained from electively terminated pregnancies using the regular vacuum aspiration technique. The collection of residual tissue for the study is approved by the Human Ethics Committee of the Huddinge University Hospital, Karolinska Institute (Diary Nr. 259/00) and Lund University (970401), and is in accordance with the guidelines of the Swedish National Board of Health and Welfare (Socialstyrelsen), including an informed consent from the pregnant women seeking abortions. Recovered nervous tissue is micro-dissected within 2 hours of surgery and appropriate tissue fragments are further dissociated for cell isolation.

RNA isolation: Human foetal tissue (8 weeks) was obtained in two rounds, both 8-weeks gestation age. Dissected VM and DM regions were used for total RNA isolation with good results and yields.

Total RNA was isolated with the Trizol extraction following the manufacturer's instruction (Invitrogen) from ventral and dorsal mesencephalic regions subdissected from human foetal tissue, 8 weeks gestational age. To concentrate RNA and to remove traces of chromosomal DNA, Rneasy columns combined with the RNase-Free DNase Set are used following the manufacturer's instructions.

From 5 μg of total RNA, biotinylated cRNA is prepared and fragmented as described in Affymetrix protocols (GeneChip Expression Analysis, Technical Manual 2000) and hybridized (15 μg) to Affymetrix Human U133B GeneChips (containing approximately 22,000 genes) according to manufacturer's instructions. Scanned images are analyzed and converted to expression index values using the GenePublisher analysis software package (Knudsen S, Workman C, Sicheritz-Ponten T, Friis C. (2003) "GenePublisher: Automated analysis of DNA microarray data.", Nucleic Acids Res. 31(13):3471-6.).

In the Table below, probe signals obtained by hybridizing 15 μg biotinylated cRNA to Affymetrix Human U133B Gene Chips are shown.

NsG	Probe-id	Mes1	Mes2	Mes3	Mes4	Mes5	Mes6
32	229655_at	505	587	544	645	583	579

Probe regions for NsG32 with the id given in the Table were located in the 3'UTRs. The probe signals indicated significant expression of NsG32 in the developing human midbrain.

Example 5: Testing for General Neuroprotective Effect (PC12 Assay)

Generation of virus stock: NsG28, NsG30, or NsG32 coding sequence is subcloned into pHsCXW using appropriate restriction sites as described in Example 3. To generate virus stocks, the resulting lentiviral transfer vector is cotransfected into 293T cells with two helper plasmids (pMD.G and pBR8.91) providing the necessary viral genes, gag-pol and env, respectively, in trans. Briefly, $2x10^6$ 293T cells are seeded in each of 20 T75 culture flasks. The next day, each T75 flask is transfected with 15 μg ppBR8.91, 5 μg pMD.G and 20 μg of transfer vector using Lipofectamine+ following the manufacturer's instructions (Invitrogen). Virus-containing medium is harvested 2-3 days after the transfection and filter-sterilized through a 0.45 μm cellulose acetate or polysulphonic filter. The virus is pelleted by double ultracentrifugation at 50,000xg for 90 minutes at 4°C and then resuspended in DMEM medium. Virus is titrated using

a reverse transcriptase (RT) assay (Current Protocols in Molecular Biology, Editors: Ausubel et al., Willey). The number of transducing units (TU)/ml is calculated from the resulting RT activity and frequency of fluorescent cells obtained by transduction of 293T cells with an equivalent GFP lentivirus. The virus stock is stored in aliquots at -80°C until use.

Transduction of PC12 cells: PC12 cells are cultured in Dulbecco´s modified Eagle´s medium (DMEM) with 4.5 g/l glucose and glutamax (Life Technologies #32430-027) with 7.5% donor horse serum (Life Technologies #16050-098) and 7.5% FBS (Life Technologies # 10099-141) in the presence of 5% CO_2 at 37°C. Medium is changed every 2-3 days and cells are subcultured 1:3 - 1:6 twice a week by tapping the flask and dispensing into new flasks. The day before transduction, cells are seeded in 48-well or 6-well plates coated with collagen. Virus is added from the stock solution to 1ml cell culture medium together with or without 5 µg/ml (final conc.) polybrene. The virus is incubated with the cells for 24 hours in a CO_2 incubator. A lentiviral GFP vector is added to a parallel culture to estimate transduction efficiency and to serve as control.

Effect on PC12 differentiation: Cultures in 6-well plates are followed and scored for the number of neurite bearing cells after 2-5 days.

Effect on PC12 survival: After transduction of cells in 48-well plates, medium is changed to serum-free DMEM and cell viability is measured after 3-4 days using the MTS assay following the manufacturer's instructions (Promega).

A positive effect in either the neurite outgrowth and/or the survival assay is indicative of a potential therapeutic effect of the encoded secreted protein in treating neurodegenerative disorders.

NsG30

PC12 cells transduced with cDNA for NsG30 show significantly higher MTS reduction compared with cells transduced with cDNA for EGFP after 4 days incubation in serum-free DMEM.

Results are shown in Fig. 25. Data shown are means ± SEM (n=6) from a representative experiment, and * indicates a significant difference from cells transduced with cDNA for EGFP ($P<0.05$, one way ANOVA, Dunnett´s Method). Transduction of PC12 cells with NsG30 lentivirus leads to a 3.5 fold improvement in cell survival compared to the control. MTS is a measure of the metabolic activity in the total cell population. Thus, the increase in MTS activity in the rLV-NsG30 relative to the control may reflect the presence of an increased number of viable cells in the culture and/or increased viability of the surviving cells.

NsG32

Fig. 34 shows the result of one experiment. Briefly, cells were seeded in collagen-coated 48-well plates, $2x10^4$ cells/well in growth medium. The following day, cells were transduced by incubation overnight with 10^5 transducing units virus/well (MOI=5) in the presence of 5 µg/ml polybrene. After transduction, medium was changed to serum-free DMEM (Invitrogen) and cell survival was then assayed after four days using the MTS assay. Data shown are means ± SEM (n=6) from a representative experiment, and * indicates a significant difference from cells transduced with cDNA for EGFP ($P<0.05$, one way ANOVA, Dunnett´s Method). EGFP: lentivirus EGFP transduced PC12 cells. NsG32a: PC12 cells transduced with human full length NsG32a. NsG32b: PC12 cells transduced with human full length NsG32b.

The MTS activity in PC12 cells transduced with a lentivirus containing full length NsG32a or NsG32b cDNA is significantly increased as compared to control cells transduced with a lentivirus carrying a marker-gene (EGFP). MTS is a measure of the metabolic activity in the total cell population. Thus, the increase in MTS activity in the rLV-NsG32a and -b relative to the control culture may reflect the presence of an increased number of viable cells in the culture and/or increased viability of the surviving cells.

Example 6: Protection of Cerebellar Granule Cells from Glutamate Toxicity

Testing for survival effects is carried out by transducing cultures of cerebellar granule cells that subsequently is exposed to toxic concentrations of glutamate essentially as described (Daniels and Brown, 2001; J. Biol. Chem. 276: 22446-22452).

Cerebellar granule neurons (CGN) are dissected from 7-8 days old mouse pups. Cells are dissociated from freshly dissected cerebella by enzymatic disruption in the presence of trypsin

and DNase and then plated in poly-D-lysine-precoated 24-well plates (Nunc) at a density of 1-2 x 10^6 cells/cm^2 in DMEM medium supplemented with 10% heat-inactivated fetal calf serum. Cells are cultured at 37°C in a humidified atmosphere and Cytosine arabinoside (10 μM) is added to the culture medium after 24 hr to arrest the growth of non-neuronal cells.

Cultures are transduced with an NsG28, NsG30, or NsG32-containing lentivirus prepared as described in Example 5 on DIV1 by the addition of virus stock solution to DMEM medium containing 10 % Fetal bovine serum and 4 μg/ml Polybrene. Parallel control cultures are transduced with a Green Fluorescent Protein (GFP) lentivirus. Five hours after the transduction, medium is replaced with medium preconditioned on CGNs.

At DIV5, glutamate (0.1-1 mM) is added the culture and after two additional days cell survival is assayed using the MTT assay. The extent of MTT reduction to formazane is measured spectrophotometrically at 570 nm. Briefly, culture medium is removed, and cells are washed in sodium saline solution (140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2 . 6H_2O$, 1 mM NaH_2PO_4, 1.5 mM $CaCl_2$, 5.6 mM glucose, 20 mM HEPES, pH 7.4). MTT (final concentration 0.5 mg/ml), prepared just before using and maintained in the dark in sodium saline solution, is then added to the cells. After a 3 h incubation at 37°C, an equal volume of acid-isopropanol (0.04 M HCl in isopropanol) is added and mixed thoroughly at room temperature until all formazan crystals were dissolved. Cell viability is expressed as a percentage of the optical density of control cells. Parallel cultures are left untreated.

This assay can be considered as a general assay for testing of protection against excitotoxic damage as well as an assay predictive for factors with therapeutic potential in the treatment of cerebellar disorders.

Example 7: Protection of Cerebellar Granule Cells from Apoptosis Induced by Potassium Deprivation

A: Rat Cerebellar Granule Neurons

Testing for survival effects is carried out by transducing cerebellar granule cells deprived of potassium essentially as described (Nomura et al., 2001; Dev. Neurosci. 23: 145-152).

Cerebellar granule neurons (CGN) are dissected from 8-d-old Sprague-Dawley rat pups. Cells are dissociated from freshly dissected cerebella by enzymatic disruption in the presence of

trypsin and DNase and then plated in poly-L-lysine-precoated 96-well plates (Nunc) at a density of 3.5×10^5 cells/cm^2 in Eagle's basal medium containing 25 mM KCl and supplemented with 10% heat-inactivated foetal calf serum, 2 mM glutamine. Cells are cultured at 37°C in a humidified atmosphere and Cytosine arabinoside (10 μM) is added to the culture medium after 24 hr to arrest the growth of non-neuronal cells.

Cultures are transduced with an NsG28, NsG30, or NsG32 containing lentivirus prepared as described in Example 5 on DIV1 by the addition of virus stock solution to DMEM medium containing 10 % Foetal bovine serum and 4 μg/ml Polybrene. Parallel control cultures are transduced with a GFP lentivirus. Five hours after the transduction, medium is replaced with medium preconditioned on CGNs.

At DIV2, apoptosis is induced in *immature* cultures by switching the cells to serum-free medium containing 5 mM KCl, while the untreated cells received conditioned medium containing 25 mM KCl. Survival is measured on DIV3, using the MTS assay.

At DIV8, apoptosis is induced in *differentiated (neuronal)* cultures by switching the cells to serum-free medium containing 5 mM KCl, while the untreated cells received conditioned medium containing 25 mM KCl. Survival is measured after 24-72 hr, using the MTS assay.

The MTS assay is carried out using the The CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Promega) following the manufacturer's instructions.

This assay can be considered as a general assay for neuroprotective effects as well as an assay predictive for factors with therapeutic potential in the treatment of cerebellar disorders.

B: Mouse Cerebellar Granule Neurons

Testing for survival effects was carried out by transducing cerebellar granule cells deprived of potassium essentially as described (Nomura et al., 2001; Dev. Neurosci. 23: 145-152).

Cerebellar granule neurons (CGN) were dissected from 9-day-old NMRI mouse pups. Cells were dissociated from freshly dissected cerebella by enzymatic disruption in the presence of trypsin and DNase and then plated in poly-L-lysine-precoated 48-well plates (Nunc) at a density of 3.5×10^5 cells/cm^2 in Eagle's basal medium containing 25 mM KCl and supplemented with 10% heat-inactivated fetal calf serum, and 2 mM glutamine. Cells were cultured at 37°C in

a humidified atmosphere and Cytosine arabinoside (10 μM) was added to the culture medium after 24 hr to arrest the growth of non-neuronal cells.

At DIV1 cultures were transduced with lentivirus carrying cDNA for NsG28, NsG32a, or EGFP (prepared as described in Example 5) by the addition of virus stock solution to the cultures. Separate plates were used for each gene and non-transduced cells serve as controls. 24 hours after addition of virus, medium in all wells was replaced with medium preconditioned on parallel cerebellar granule cell cultures.

At DIV4, apoptosis was induced in the cultures by switching the cells to serum-free medium and a depolarising level of potassium, 5 mM KCl ("5-S"). High potassium levels as well as the growth factor Insulin-like growth factor-I (IGF1) have been found to protect cells from apoptosis (D´Mello et al. 1993, Proc. Natl. Acad. Sci. USA, 90, 10989-10993) and some of the cells were therefore incubated in serum-free medium with 100 ng/ml IGF ("5-S+I") or 25 mM KCl ("25-S"). To see the effect of the lack of serum medium and the medium change, some wells were left unchanged ("U"). All media types contains Cytosine arabinoside (10 μM).

Survival was measured on DIV6, using the MTS assay (CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay from Promega) according to the manufacturer's instructions.

A significant difference in MTS reduction was detected between ± virus for rLV-NsG28 in the presence or absence of IGF1 and for rLV-NsG32a in the absence of IGF1 (Fig. 36) (one way ANOVA on Ranks, all multiple comparison procedures, Student-Newman-Keuls Method). Though not significant, there was also a tendency for a higher MTS reduction in cells transduced with rLV-NsG32a in the presence of IGF1. In contrast, there was no significant difference in MTS values between non-transduced cells and cells transduced with rLV-EGFP in low potassium, and maybe even a tendency for a negative effect of rLV-EGFP. The findings demonstrate that members of the Cys10 family (NsG28 and NsG32a) show a small, but significant neuroprotective effect in cerebellar granule cells deprived of serum and potassium.

This assay can be considered as a general assay for neuroprotective effects as well as an assay predictive for factors with therapeutic potential in the treatment of cerebellar disorders.

Example 8: Effect on DRG Cultures

Preparation of conditioned media from transduced ARPE-19 cells. To transduce ARPE-19 cells with a lentivirus containing cDNA encoding the NsG28, NsG30, or NsG32 gene (prepared as described in Example 5), cells are plated at a density of 1 x10^5 cells/well in a 6-well plate in DMEM/F12 medium supplemented with 10 % Fetal Bovine Serum. Next day virus is added from the stock solution to the cell culture medium together with 5 μg/ml (final conc.) polybrene. The virus is incubated with the cells overnight in a CO_2 incubator. GFP lentivirus is added to a parallel culture. The next day, cultures are changed to serum-free UltraCULTURE medium (1 ml/well) and conditioned media are harvested after two additional days of incubation.

Isolation and culture of P1 DRG cells. DRGs from all spinal levels are removed from P1 (post-natal day 1) Sprague-Dawley. Tissues are enzymatically dissociated in 125-250 U/ml type 1 collagenase (Worthington, Freehold, N.J.) at 37°C for 30 minutes. Samples are triturated with fire-polished Pasteur pipettes and filtered though 70 μm sterile mesh to produce single cell suspensions. Cells are pre-plated on non-coated tissue-culture-ware dishes for 2 hours to remove non-neuronal cells. Non adherent cells are plated at 15,000 cells/well in 24-well tissue culture dishes that had been coated with poly-d-ornithine (Life Technologies) and laminin (Collaborative Biomedical). Negative controls are cultured in UltraCULTURE™ serum-free media, (BioWhittaker, Walkersville, MD) containing 2.5μg/ml sheep-neutralizing anti-NGF pAb (Chemicon, Temecula, CA). NGF-treated positive controls lacked the neutralizing anti-NGF pAb. Different dilutions of conditioned medium collected from NsG28, NsG30, or NsG32-transduced or GFP-transduced ARPE-19 cells are added to the cultures after centrifugation and filtering through a 0.4 μm sterilfilter. Cultures are fed every second day by replacing the media.

Immunocytochemistry. After seven days in culture, cells are fixed in 4% formaldehyde in PBS for 10 minutes at room temperature. Cells are pre-blocked in 4% goat serum, 0.1% NP40 for 30 minutes at room temperature and then incubated with mouse anti-ßIII tubulin (1:100) overnight at 4°C. After rinsing in pre-block solution, the cultures are incubated with a secondary Cy-3 coupled anti-murine antibody for 1 hour at room temperature. Following a final rinse in pre-block solution, cells from a strip through the middle of each well are counted using fluorescence optics. All ßIII-tubulin positive cells are scored as neurons and survival is determined by the number of neurons counted per well. All antibodies are diluted in pre-block solution.

Interpretation of results: Protective effects in this assay indicates therapeutic potential in peripheral neuropathies and neuropathic pain.

Example 9: Effect on Motoneuron Cultures

Testing for survival effects on motoneuron cultures is carried out using NsG28, NsG30, or NsG32-containing lentivirus essentially as described in Cisterni et al. 200 (J. Neurochem. 74, 1820-1828). Briefly, ventral spinal cords of embryonic day 14.5 (E14.5) Sprague Dawley rat embryos are dissected and dissociated. Motoneurons are purified using a protocol based on the immunoaffinity purification of motoneurons with antibodies against the extracellular domain of the neurotrophin receptor, p75, followed by cell sorting using magnetic microbeads (Arce et al. 1999). Purified motoneurons are seeded on 4-well tissue culture dishes precoated with polyornithine/laminin at density of 500 cells per well. Culture medium is Neurobasal culture medium (Life Technologies) supplemented with the B27 supplement (Life Technologies), horse serum (2% v/v), L-glutamine (0.5 mM), and 2-mercaptoethanol (25 μM). L-Glutamate (25 μM) is added to the medium during the first 4 d of culture and subsequently omitted.

Motoneurons cultured for 16 h are transduced with an NsG28, NsG30, or NsG32-containing lentivirus prepared as described in Example 5 by the addition of virus stock solution to the culture medium (corresponding to MOI=4). Parallel control cultures are transduced with a GFP lentivirus. Eight hours after the transduction, medium is replaced with fresh medium (DIV1).

Motoneuron survival is quantified at DIV3 by counting the number of large phase-bright neurons with long axonal processes in a predetermined area of 1.5 cm^2 in the center of duplicate dishes.

Interpretation of results: Protective effects in this assay indicates therapeutic potential in motoneuron diseases including ALS, Spinal Cord injury, SMA (spinal muscular atrophy), DMD (Duchenne muscular dystrophy).

Example 10: Bioassay for Dopaminergic Neurotrophic Activities

Culture conditions:

Dissociated mesencephalic cell cultures are prepared as previously described (Friedman and Mytilineou 1987 Neurosci. Lett. 79:65-72), with minor modifications. Briefly, rostral mesencephalic tegmentum from brains of Sprague-Dawley rat embryos, at the 13^{th}-16th day of gestation, are dissected under the microscope in sterile conditions, collected in Ca^{2+} - and Mg^{2+} -

free Dulbecco's phosphate buffered saline (Gibco, Gaithersburg, Md.) and dissociated mechanically by mild trituration. The cells are plated in 100 µl per well onto 16-mm diameter tissue culture wells (Falcon, Lincoln Park, N.J., 24-well plate) containing 400 µl medium to give a density of 2.5-3.5x10^5 cells per well. The culture wells have been previously exposed to 0.1 mg/ml solution of poly L-ornithine in 10 mM sodium borate, pH 8.4, for 3 hours at 37°C., washed 3 times in milli-Q H_2O and once in Earle's balanced salt solution (Gibco). The feeding medium (10/10) consists of minimal essential medium (MEM, Gibco) supplemented with glucose (33 mM), sodium bicarbonate (24.5 mM), glutamine (2 mM), HEPES (15 mM), penicillin G (5 U/ml), streptomycin (5 µg/ml), 10% heat-inactivated fetal calf serum (Gibco) and 10% heat inactivated horse serum (Gibco). The cultures are kept at 37°C in a water-saturated atmosphere containing 6.5% CO_2. After 3 hours, when most of the cells have adhered to the bottom of the well, the medium is replaced with 500 µl of fresh medium. At this time, a serial dilution of the sample to be assayed for dopaminergic neurotrophic activity (conditioned medium) is added to each well in duplicate and the plates are incubated in the 37°C incubator. After a week, the cultures are treated for 24 hours with fluorodeoxyuridine (13 µg/ml) and uridine (33 µg/ml) to prevent excessive glial proliferation and subsequently fed with the above medium without fetal calf serum. The feeding medium is changed weekly.

Alternatively, chemically defined serum-free medium is used in which serum is replaced by a mixture of proteins, hormones and salts. The defined medium (DM) consists of a mixture of MEM and F12 nutrient mixture (both Gibco, 1:1; vol/vol) with glucose (33 mM), glutamine (2 mM) $NaHCO_3$ (24.5 mM), HEPES (15 mM), supplemented with transferrin (100 µg/ml), insulin (25 µg/ml), putrescine (60 µM), progesterone (20 nM), sodium selenite (30 nM), penicillin G (5 U/ml) and streptomycin (5 µg/ml). The osmolarity of the DM is adjusted to 325 by the addition of milli-Q H_2O. (110-125 ml H_2O/l).

The functional status of the dopaminergic neurons may be assayed in these cultures by measuring dopamine uptake through specific "scavenger" transporters in the dopaminergic neurons and by counting the number of neurons positive for the dopamine synthetic enzyme tyrosine hydroxylase using immunohistochemistry as described in Karlsson et al, 2002, Brain Res. 2002 Nov 15;955(1-2):268-80.

Sample preparation: Prior to being assayed for dopaminergic neurotrophic activity in the mesencephalic cell cultures, all the samples of conditioned medium are desalted as follows. One hundred µl of the medium 10/10 (as a carrier) is added to a Centricon-10 (Amicon) and allowed

to sit for 10 minutes. Aliquots of the sample to be assayed are added to the Centricon, followed by 1 ml of Dulbecco's high glucose Modified Eagle medium, without bicarbonate, but containing 10 mM HEPES, pH 7.2 (solution A), and centrifuged at 5,000Xg for 70 minutes. The retentate (about 0.1 ml) is brought back to 1.1 ml with fresh solution A and reconcentrated twice. The sample is filtered through a 0.11 μm Ultrafree-MC sterile Durapore unit (Millipore, Bedford Mass.) prior to being added to the culture well.

^{3}H-dopamine uptake: Uptake of tritiated dopamine (^{3}H-DA) is performed in cultures at day 6 or day 7 as described previously (Friedman and Mytilineou (1987) Neurosci. Lett. 79:65-72) with minor modifications, and all the solutions are maintained at 37°C. Briefly, the culture medium is removed, rinsed twice with 0.25 ml of the uptake buffer which consists of Krebs-Ringer's phosphate buffer, pH 7.4, containing 5.6 mM glucose, 1.3 mM EDTA, 0.1 mM ascorbic acid and 0.5 mM pargyline, an inhibitor of monoamine oxidase. The cultures are incubated with 0.25 ml of 50 nM ^{3}H-DA (New England Nuclear, Boston, Mass. sp. act 36-37 Ci/mmol) for 15 minutes at 37°C. ^{3}H-DA uptake is stopped by removing the incubation mixture and cells are then washed twice with 0.5 ml of the uptake buffer. In order to release ^{3}H-DA from the cells, the cultures are incubated with 0.5 ml of 95% ethanol for 30 min at 37°C, and then added to 10 ml of EcoLite (ICN, Irvine, Calif.) and counted on a scintillation counter. Blank values are obtained by adding to the uptake buffer 0.5 mM GBR-12909 (RBI), a specific inhibitor of the high-affinity uptake pump of the dopamine neurons (Heikkila et al. 1984 Euro J. Pharmacol. 103:241-48).

The number of TH positive neurons can be quantified by staining for TH as described previously by Grasbon-Frodl and Brundin, Experimental brain research, 1997 113:138-143 and quantified according to the method described by Karlsson et al, Brain Research, 1998, 805:155-168.

An increase in the number of TH positive neurons and/or an increase in 3H-dopamine uptake compared to a control treatment is an indication of a possible function of NsG28, NsG30, or NsG32 in the treatment of Parkinson's disease.

The mesencephalic cultures described here also comprise motoneurons. By measuring the increase in ChAT (Choline acetyltransferase), an indication of the effect of NsG28, NsG30, or NsG32 on the cholinergic system can be obtained. An activation of ChAT over background is an indication of potential therapeutic use in the treatment of human motoneruron diseases such as ALS (Zurn et al, Neuroreport, 1994, 30:113-118).

Example 11: Assessment of Neuroprotection of Nigral Dopamine Neurons *in vivo* in the Instrastriatal 6-OHDA Lesion Model

VSV-G pseudotyped (rLV) vectors are produced as described in Example 5.

All work involving experimental animals are conducted according to the guidelines set by the Ethical Committee for Use of Laboratory Animals at Lund University. Animals are housed in 12:12 hour light/dark cycle with access to rat chow and water. Female Sprague Dawley rats (~220g by the time of surgery) are used. For stereotaxic surgery animals are anesthetized using halothane and a total of two microliters rLV-GFP (n=8) or rLV- NsG28, NsG30, or NsG32 of a 1:2 viral stock (1.0-1.2 x 10^5 TU) are injected into two tracts in the right striatum at the following coordinates: (1) AP = +1.0 mm, ML = -2.6 mm, DV = -5.0 and –4.5 mm, Tb = 0.0 and (2) AP = 0.0 mm, ML = -3.7 mm, DV = -5.0 and –4.5 mm, Tb = 0.0. After two weeks the animals are again anesthetized and placed in the stereotaxic frame. An injection of 6-hydroxydopamine (20 µg [calculated as free base] per 3 µl vehicle [saline with 0.2% ascorbic acid]) is made into the right striatum at the following coordinates: AP = +0.5 mm, ML = -3.4 mm, DV = -5.0 and –4.5 mm, Tb = 0.0.

At four weeks post-lesion the animals are deeply anesthetized with pentobarbital (70 mg/kg, Apoteksbolaget, Sweden), and transcardially perfused with 50ml saline at room temperature, followed by 200 ml ice-cold phosphate-buffered 4% paraformaldehyde (pH 7.2-7.4). The brains are postfixed for 3-6 hours in the same fixative, transferred to 30% sucrose for 24 hours and cut into 6 series of 40 µm thick sections on a freezing microtome.

Immunohistochemistry for detection of tyrosine hydroxylase-immunoreactive, in the substantia nigra is performed as described previously (Rosenblad et al., Molecular and Cellular Neuroscience, 2000, 15:199-214). The number of TH-IR and VMAT-IR nigral neurons is assessed by counting under microscope all immunoreactive neurons lateral to the medial terminal nucleus of the accessory optic tract in three consecutive sections through the SN, as described previously (Sauer & Oertel, Neuroscience 1994, 59:401-415).

An increase in the number of TH-IR compared to the GFP control is a strong indication of a function in the treatment of Parkinson's disease. An increase in the number of VMAT-IR further strengthens the conclusion.

Example 12: Effect on Neuronal Differentiation of Human Neural Progenitor Cells

To test for effect on neuronal differentiation, human neural progenitor cells are plated on different substrates after lentiviral transduction.

Establishment of cultures from first trimester human forebrain tissue has been described in the literature (Carpenter et al., 1999 Exp. Neurol. 158, 265-278). Cells are growing as floating spheres in N2 medium supplemented with 20 ng/ ml human EGF(R&D Systems), 20 ng/ml human bFGF (R&D Systems), 10 ng/ml human CNTF (R&D Systems) and 2 µg/ml heparin. In some cases EGF are omitted from the growth medium. N2 medium consists of DMEM: F12 (1:1) (Life Technologies) supplemented with 0.6% glucose, 2 mM glutamine, 5 mM HEPES and N2 supplement (containing insulin, transferrin, progesterone, putrescine and selenium chloride available from Life Technologies).

Cultures are transduced with a NsG28, NsG30, or NsG32 containing lentivirus prepared as described in Example 5. Furthermore, parallel transduction with a control virus containing EGFP are carried out. Briefly, cultures are triturated to a single cell suspension three days before transduction. On the day of transduction, the small spheres of cells are collected by centrifugation and resuspended in growth medium supplemented with 10 % human serum albumin (HSA) at a density of $1x10^6$ cells per ml. Virus is added (MOI=1-5) and cultures are incubated for 4 h. After transduction, cells are collected by centrifugation, resuspended in N2 medium without growth factors and plated on 12-mm glass coverslips coated with poly-L-lysine (PLL, from Sigma, 100 µg/ml) and laminin (LN, from Sigma, 50 µg/ml). Approximately 100,000 cells are plated per coverslip.

After 1-4 days after plating, cells are fixed in 4% paraformaldehyde in PBS for 20 min at room temperature. The cells are then washed three times with PBS, followed by overnight incubation with primary antibodies diluted in PBS incubation buffer which contained 10% normal goat serum, 0.3% Triton X-100 and 1% BSA at 4°C in a humidified chamber. The cells are washed with PBS, and incubated for 1 h at room temperature in the dark with secondary antibodies diluted in incubation buffer. After washing with PBS, nuclei are counterstained with Hoechst 33342. Negative controls (omission of the primary antibody) are included in each experiment.

The primary antibodies used are; mouse anti-ß-tubulin (Sigma 1:400), rabbit anti-GFAP (DAKO 1:100), mouse-antiGalC (Chemicon), and rabbit anti-tyrosine hydroxylase (TH, Chemicon). The secondary antibodies used in these experiments are: anti-mouse FITC (Sigma 1:128) and anti-rabbit Cy3 (Chemicon 1:500).

Interpretation of results

After 1-4 days of differentiation, on PLL/laminin substrate control cells transduced with lenti-EGFP have migrated out from the spheres and differentiated to a mixture of neurons (ß-tubulin positive cells) and astrocytes (GFAP positive cells). Only few or no TH- or GalC-positive cells are seen.

An increase in the neuron/astrocyte ratio indicates that NsG28, NsG30, or NsG32 has an effect on neuronal differentiation and/or survival.

The presence of GalC positive cells after differentiation indicates that NsG28, NsG30, or NsG32 has an effect on oligodendrocyte specification/differentiation.

The presence of TH positive cells after differentiation indicates that NsG28, NsG30, or NsG32 has an effect on dopaminergic specification/differentiation.

Example 13: Real-Time PCR on Cys10 family Mouse Orthologues

Materials & Methods:

Primers: The following primers were used for real-time PCR:

mNsG28:
mNsG28 bp224 intronspan 5': 5'-TCTGGACTCCCGGATCTGAC-3'
mNsG28 bp495 intronspan 3': 5'-CGAGGCGGACATCAGCTTAC-3'
mNSG30:
mNsG30 bp259 intronspan 5': 5'-TGGACACTGGGACTGAACTG-3'
mNsG30 bp572 intronspan 3': 5'-CAGGAGCACTTGACCGTTTG-3'
mNsG32:
mNsG32a bp65 intronspan 5': 5'-CTGCCCAGCATGTCCTCAAC-3'
mNsG32a bp404 intronspan 3': 5'-TGGTCTTTATCCGCCCTCCG-3'
GAPDH:
mGAPDH-s904: 5'-AACAGCAACTCCCACTCTTC-3'
mGAPDH-as1067: 5'-TGGTCCAGGGTTTCTTACTC-3'
mALDH1A1:
mALDH1A1_590fwd, 5'-AAACTCCTCTCACGGCTCTTC-3'
mALDH1A1_859rev, 5'-CAATGTCCAAGTCGGCATCTG-3'
mOTX2:

mOTX2_269fwd, 5'-CCGCCTTACGCAGTCAATGG-3'

mOTX2_611rev, 5'-TCACTTCCCGAGCTGGAGAG-3'

mGDNF:

mGDNF_95s, TATGGGATGTCGTGGCTGTC

mGDNF_341as, GCTGCCGCTTGTTTATCTGG

Preparation of expression panel:

Tissue from different brain regions of E10.5, E11.5, E13.5, P1 and adult mice was isolated and RNA prepared by Trizol extraction. Subsequent on-column DNAse treatment using RNeasy spin columns was done to remove traces of gDNA and to further clean the RNA. Aliqouts of 2.5µg RNA was used as template for cDNA synthesis with an RNAseH deficient reverse transcriptase derived from MoMLV (SuperScript) and poly-dT pimer. cDNA from all samples were synthesised at the same time using the same mastermix to avoid variations. The final volume of the cDNA reaction was 120µl, which was stored in aliquots at -80°C to avoid repeated thawing and freezing. The expression panel consists of cDNA prepared from the following tissues; dorsal forebrain (DFB), ventral forebrain (VFB), ventral mesencephalon (VM), dorsal mesencephalon (DM) and spinal cord (SC) from 10.5 and 11.5 days old embryos. In addition, cortex (CTX), medial and lateral ganglionic eminences (MGE/LGE), DM, VM and SC from 13.5 days old embryos were included. Furthermore, from newborn mouse (P1), cerebellum (Cb), CTX, VM, DM, and MGE/LGE were used and finally Cb, CTX, VM, DM, and SC were used from adult mouse.

Real time PCR expression analysis:

For real-time PCR expression analysis, approximately 20ng of each cDNA was used as template. Real-time PCR was performed in an Opticon-2 thermocycler (MJ Research), using LightCycler-FastStart DNA Master SYBR Green I kit (Roche). Studies were carried out in duplicates using the primers described above. For real-time PCR, a standard curve was prepared by serial dilution of a gel-purified PCR product, prepared using the above primers. The standard curve was used to verify that crossing-point values (C_T) of all samples were within the exponential range of the PCR reaction and to calculate final expression levels. All real-time PCR amplifications were performed in a total volume of 10 µl containing 3 mM $MgCl_2$, 12 % sucrose and 1x reaction buffer included in the LightCycler kit. PCR cycling profile consisted of a 10

minutes pre-denaturation step at 98°C and 35 three-step cycles at 98°C for 10 seconds, at 62°C (mGAPDH), 65°C (mALDH1A1), 65°C (mOTX2), 60°C (mGDNF), 62°C (mNsG28-32) for 20 seconds and at 72°C for 20 seconds. Following the extension step of each cycle, a plate reading step was added (80°C, 2 seconds) to quantify the newly formed PCR products. The specificity of the amplification reaction was determined by performing a melting curve analysis of the PCR fragments by slowly raising the temperature from 52°C to 95°C with continuous data acquisition.

For normalization purposes, all cDNAs were subjected to real-time PCR using primers for the housekeeping gene GAPDH. Real-time PCR analysis of GAPDH was done as for the target genes. Housekeeping expression pattern was determined from the respective standard curves and the relative expression levels were used to normalize expression levels of the target genes in the tissues that were analysed. Following normalization with GAPDH, relative expression levels of the target genes were calculated using the tissue with the lowest expression as a reference.

Results:

To verify tissue dissections and subsequent RNA isolation and cDNA preparation, the expression profile of the marker genes OTX2 and ALDH1A were investigated. OTX2 is expressed in the forebrain and primarily in the dorsal part of the midbrain with a posterior boundary at the isthmic organiser. The retinoid synthesizing enzyme ALDH1A1, is a specific marker of developing dopaminergic neurons in the ventral midbrain. Hence, the expression profile of these two genes can be used to validate the cDNA panel. It is apparent from Fig. 22A, that the expression level of the housekeeping gene GAPDH differs less that 50% between tissues. In contrast, very differentiated expression profiles are observed for ALDH1A1 (Fig. 22B) and OTX2 (Fig. 22C). As expected, in the foetal tissues and at P1, ALDH1A1 is expressed almost exclusively in the ventral midbrain. Also as expected, during development, OTX2 is expressed in the forebrain and (dorsal) midbrain but not in the spinal cord. Together, this is evidence of a high quality expression panel of the developing mouse central nervous system (CNS).

Several proteins have been described that have been shown to have therapeutic properties relevant to the central nervous system. Common to these molecules are conservation across species, features of growth factors, and expression during development in specific regions of the nervous system. The regional and temporal expressions are also predictive for the therapeutic

indications in a majority of cases. For example, GDNF is one growth factor that is known to be therapeutically relevant for the nervous system. This molecule is conserved across species and, during development, GDNF is expressed in the ventral mesencephalon and the striatum at the time of terminal differentiation of the nigro-striatal dopaminergic system (Fig. 23). GDNF is in fact a therapeutic molecule for Parkinson's Disease and its therapeutic properties have been demonstrated in several animal models of PD.

NsG28 Results:

The real-time PCR results for mouse NsG28 are shown in Fig. 24. C_T values ranged from 23 to 31. From this figure it is apparent that the expression of the gene peaks in P1 VM (from which the substantia nigra develops) and P1 LGE/MGE (from which the striatum develops). Expression is also seen in the spinal cord (E11.5, E13.5, and adult) and cerebellum (P1).

NsG28 is a secreted molecule conserved across species with features of a growth factor or hormone with a regional and temporal expression pattern that in combination with its other features strongly predicts a therapeutic use for the treatment of neurological disorders. Looking at the expression pattern, this secreted growth factor is expressed during development, primarily in the ventral mesencephalon and the striatum during the time of terminal differentiation, strongly suggesting a survival and/or differentiation effect on dopaminergic neurons and striatal medium spiny neurons. Analogous to GDNF, the growth factor features, conservation across species, and the temporal and spatial expression pattern indicate that NsG28 has therapeutic relevance for the treatment of Parkinson's Disease and Huntington's Disease.

NsG30 Results:

The real-time PCR results for mouse NsG30 are shown in Fig. 28. C_T values for mNsG30 ranged from 19-30. From this figure it is apparent that the expression of all 5 genes all peak in P1 VM (from which the substantia nigra develops) and P1 LGE/MGE (from which the striatum develops).

The expression pattern of NsG30 in the developing mouse CNS resembles that of NsG28. There is high expression in the ventral mesencephalon and the striatum during the latter part of the development during the early postnatal period. This relatively restricted regional and temporal expression pattern indicates a role in the differentiation and termination of the projections between the VM and the striatum (LGE/MGE). Similar to GDNF (and NsG28) a

therapeutic effect on neurons involved in Huntingdon's and Parkinson's diseases is strongly indicated.

NsG32 Results:

The real-time PCR results for mouse NsG32a are shown in Fig. 35. C_T values for the expression varied from 22 to 28, i.e. evidence of an intermediate to high expression level consistent with the quantitative expression analysis in human tissues (Example 4a). From this figure it is apparent that the expression peaks in P1 VM (from which the substantia nigra develops) and P1 LGE/MGE (from which the striatum develops), i.e. expression peaks during the early postnatal period. This coincides with the differentiation and termination of projections between the striatum and the ventral mesencephalon. Similar to GDNF a therapeutic effect on neurons involved in Huntington's and Parkinson's disease is indicated.

Claims

1. An isolated polypeptide for medical use, said polypeptide comprising an amino acid sequence selected from the group consisting of:
   a) the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41;
   b) a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41, wherein the variant has at least 70% sequence identity to said SEQ ID NO; and
   c) a biologically active fragment of at least 50 contiguous amino acids of any of a) through b).

2. The polypeptide of claim 1 that is a naturally occurring allelic variant of the sequence selected from the group consisting of SEQ ID NO 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41.

3. The polypeptide of claim 2, wherein the allelic variant comprises an amino acid sequence that is the translation of a nucleic acid sequence differing by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID NO 2, 8, 17, 22, 26, 30, 35, 37, and 39.

4. The polypeptide of claim 1 that is a variant polypeptide described therein, wherein any amino acid specified in the chosen sequence is changed to provide a conservative substitution.

5. The polypeptide of claim 1, wherein the signal peptide has been replaced by a heterologous signal peptide.

6. The polypeptide of claim 1, having at least 70% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID NO 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41.

7. The polypeptide of claim 1, having at least 80% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID NO 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41.

8. The polypeptide of claim 1, having at least 95% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID NO 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41.

9. The polypeptide of claim 1, having at least 98% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID NO 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41.

10. The polypeptide of claim 1, wherein the fragment is selected from the group consisting of:

i) SEQ ID No 6, and polypeptides having from one to five additional amino acids from the mature polypeptide sequence in the C- and/or N-terminal, up to AA_{14}-AA_{98} of SEQ ID No 5;

ii) SEQ ID No 11 and polypeptides having from one to five additional amino acids from the mature polypeptide sequence in the C- and/or N-terminal, up to AA_{14}-AA_{98} of SEQ ID No 10;

iii) SEQ ID No 15 and polypeptides having from one to five additional amino acids from the mature polypeptide sequence in the C- and/or N-terminal, up to AA_{14}-AA_{98} of SEQ ID No 14

iv) SEQ ID No 20, and polypeptides having from one to five additional amino acids from the mature polypeptide sequence in the C- and/or N-terminal, up to AA_{9}-AA_{93} of SEQ ID No 19 or 24;

v) SEQ ID No 34, and polypeptides having from one to five additional amino acids from the mature polypeptide sequence in the C- and/or N-terminal, up to AA_{8}-AA_{92} of SEQ ID No 32; and

vi) variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed.

11. The polypeptide of claim 1, selected from the group consisting of:

i) SEQ ID No 7, and polypeptides having from one to five additional amino acids from the mature polypeptide sequence in the C- and/or N-terminal, up to AA_{12}-AA_{106} of SEQ ID No 5;

ii) SEQ ID No 12 and polypeptides having from one to five additional amino acids from the mature polypeptide sequence in the C- and/or N-terminal, up to AA_{12}-AA_{106} of SEQ ID No 10;

iii) SEQ ID No 16 and polypeptides having from one to five additional amino acids from the mature polypeptide sequence in the C- and/or N-terminal, i.e. up to AA_{12}-AA_{105} of SEQ ID No 14;

iv) SEQ ID No 21, and polypeptides having from one to five additional amino acids from the mature polypeptide sequence in the C- and/or N-terminal, up to AA_7-AA_{101} of SEQ ID No 19 or 24;

v) SEQ ID No 33, and polypeptides having from one to five additional amino acids from the mature polypeptide sequence in the C- and/or N-terminal, up to AA_6-AA_{100} of SEQ ID No 32; and

vi) variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed.

12. The polypeptide of any of the preceding claims, wherein the changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in any of Figs. 11, 12, 13A, and 13B.

13. The polypeptide of any of the preceding claims, wherein the changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in Fig. 1B, more preferably in Fig. 1A.

14. The polypeptide of any of the preceding claims, wherein any changed amino acid residue is changed to a residue found at the same or corresponding position in another Cys10 protein (Fig. 1A or 1B).

15. The polypeptide of any of the preceding claims, wherein any changed amino acid residue is changed to a residue found at the same or corresponding position in another NsG28, NsG30, or NsG32.

16. The polypeptide of any of the preceding claims, comprising the conserved cysteine residues of the Cys10 family at positions corresponding to the position of mature NsG28, mature NsG30, or mature NsG32.

17. The polypeptide of any of the preceding claims, comprising the following sequence: G-T-**C**-E-[V/I]-[V/I]-x(3)-R-x(5)-[R/K]-x(5)-Q-T-[V/A]-[K/R]-**C**-x-**C**-x(2)-G-x-[V/I]-A-G-T-T-R-x(2)-P-x-**C**-V-[D/E]-A-x-I-[V/I]-x(2)-[K/R]-x-W-**C**-x-M-x-P-**C**-L-x-G-E-x-**C**-x(2)-L-x(4)-G-W-x-**C**-x(2-3)-G-x-[K/R]-[V/I]-K-T-T.

18. The polypeptide of claim 17, comprising the following sequence: G-T-**C**-E-V-[V/I]-A-x-H-R-**C**-**C**-N-[K/R]-N-[R/K]-I-E-E-R-S-Q-T-V-K-**C**-S-**C**-x(2)-G-x-V-A-G-T-T-R-x(2)-P-S-**C**-V-[D/E]-A-x-I-V-x(2)-[K/R]-W-W-**C**-x-M-x-P-**C**-L-x-G-E-[E/D]-**C**-K-x-L-P-D-x(2)-G-W-x-**C**-x-[S/T]-G-x-K-[V/I]-K-T-T-[R/K].

19. The polypeptide of any of the preceding claims, being capable of forming at least one intramolecular cysteine bridge.

20. The polypeptide of any of the preceding claims, comprising a homodimer of NsG28, NsG30, NsG32a, or NsG32b linked through an intermolecular cysteine bridge.

21. The polypeptide of any of the preceding claims, comprising a heterodimer of (i) NsG28 and one of the other members of the Cys10 family, (ii) NsG30 and one of the other members of the Cys10 family, (iii) NsG32a and NsG32b, (iv) NsG32a and one of the other members of the Cys10 family, or (v) NsG32b and one of the other members of the Cys10 family linked through an intramolecular cysteine bridge.

22. The polypeptide according to any of the preceding claims, further comprising an affinity tag, such as a polyhis tag, a GST tag, a HA tag, a Flag tag, a C-myc tag, a HSV tag, a V5 tag, a

maltose binding protein tag, a cellulose binding domain tag.

23. The polypeptide according to any of the preceding claims, wherein the polypeptide inhibitis apoptosis.

24. An isolated nucleic acid molecule for medical use comprising a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence coding for a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41;

b) a nucleotide sequence coding for a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41, wherein the variant has at least 70% sequence identity to said SEQ ID No.;

c) a nucleotide sequence coding for a biologically active fragment of at least 50 contiguous amino acids of any of a) through b);

d) a nucleotide sequence selected from the group consisting of SEQ ID No. 2, 8, 17, 22, 26, 30, 35, 37, and 39;

e) a nucleotide sequence having at least 70% sequence identity to a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 2, 8, 17, 22, 26, 30, 35, 37, and 39;

f) a nucleic acid sequence of at least 150 contiguous nucleotides of a coding sequence selected from the group consisting of the coding sequence of SEQ ID No. 2, 8, 17, 22, 26, 30, 35, 37, and 39;

g) the complement of a nucleic acid capable of hybridising with a nucleic acid molecule having the sequence of the coding sequence of SEQ ID No. 2, 8, 17, 22, 26, 30, 35, 37, and 39 under conditions of high stringency;

h) the nucleic acid sequence of the complement of any of the above.

25. The nucleic acid molecule of claim 24, wherein the nucleic acid molecule comprises the nucleotide sequence of a naturally occurring allelic nucleic acid variant.

26. The nucleic acid molecule of claim 24 that encodes a variant polypeptide, wherein the variant polypeptide has the polypeptide sequence of a naturally occurring polypeptide variant.

27. The nucleic acid molecule of claim 24, wherein the nucleic acid molecule differs by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID No. 2, 8, 17, 22, 26, 30, 35, 37, and 39.

28. The nucleic acid molecule of claim 24, wherein the encoded polypeptide has at least 70% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41.

29. The nucleic acid molecule of claim 24, wherein the encoded polypeptide has at least 75% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41.

30. The nucleic acid molecule of claim 24, wherein the encoded polypeptide has at least 80% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41.

31. The nucleic acid molecule of claim 24, wherein the encoded polypeptide has at least 95% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41.

32. The nucleic acid molecule of claim 24, wherein the encoded polypeptide has at least 98% sequence identity to the protein having the sequence of SEQ ID No. 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 36, 38, 40, and 41.

33. The nucleic acid molecule of claim 24, wherein the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of

a) the nucleotide sequence selected from the group consisting of SEQ ID No. 2, 8, 17, 22, 26, 30, 35, 37, and 39;

b) a nucleotide sequence having at least 70% sequence identity to a nucleotide sequence selected from the group consisting of the coding sequence of SEQ ID No. 2, 8, 17, 22, 26, 30, 35, 37, and 39;

c) a nucleic acid sequence of at least 150 contiguous nucleotides of a sequence selected from the group consisting of the coding sequence of SEQ ID No. 2, 8, 17, 22, 26, 30, 35, 37, and 39;

d) the complement of a nucleic acid capable of hybridising with nucleic acid having the sequence selected from the group consisting of the coding sequence of SEQ ID No.: SEQ ID No. 2, 8, 17, 22, 26, 30, 35, 37, and 39 under conditions of high stringency; and

e) the nucleic acid sequence of the complement of any of the above.

34. The nucleic acid molecule of claim 24, comprising a nucleotide sequence having at least 70% sequence identity to a nucleotide sequence selected from the group consisting of the coding sequence of SEQ ID No. 2, 8, 17, 22, 26, 30, 35, 37, and 39.

35. The nucleic acid molecule of claim 24, comprising a nucleotide sequence having at least 75% sequence identity to the nucleic acid molecule having the sequence of the coding sequence of SEQ ID No. 2, 8, 17, 22, 26, 30, 35, 37, and 39.

36. The nucleic acid molecule of claim 24, comprising a nucleotide sequence having at least 80% sequence identity to the nucleic acid molecule having the sequence of the coding sequence of SEQ ID No. 2, 8, 17, 22, 26, 30, 35, 37, and 39.

37. The nucleic acid molecule of claim 24, comprising a nucleotide sequence having at least 95% sequence identity to the nucleic acid molecule having the sequence of the coding sequence of SEQ ID No. 2, 8, 17, 22, 26, 30, 35, 37, and 39.

38. The nucleic acid molecule of claim 24, comprising a nucleotide sequence having at least 98% sequence identity to the nucleic acid molecule having the sequence of the coding sequence of SEQ ID No. 2, 8, 17, 22, 26, 30, 35, 37, and 39.

39. The nucleic acid molecule of claim 24, comprising the nucleotide sequence of nucleotides 466-888 of SEQ ID No. 2 (long human NsG28 CDS), nucleotides 481-888 of SEQ ID No. 2 (human NsG28 CDS), nucleotides 568-885 of SEQ ID No. 2 (human mature NsG28 CDS), nucleotides 622-846 of SEQ ID No. 2 (human NsG28 cys1-cys10 fragment CDS), nucleotides 616-876 of SEQ ID No. 2 (human NsG28 core fragment CDS), nucleotides 206-613 of SEQ ID No. 8 (mouse NsG28 CDS), nucleotides 293-610 of SEQ ID No. 8 (mouse mature NsG28 CDS), nucleotides 347-571 of SEQ ID No. 8 (mouse NsG28 cys1-cys10 fragment CDS), or nucleotides 341-601 of SEQ ID No. 8 (mouse NsG28 core fragment CDS).

40. The nucleic acid molecule of claim 24, comprising sequence of nucleotides 156-551 of SEQ ID No. 17 (human NsG30 CDS), nucleotides 246-548 of SEQ ID No. 17 (human mature NsG30 CDS), nucleotides 285-509 of SEQ ID No. 17 (human Cys1-Cys10 fragment CDS), nucleotides 279-539 of SEQ ID No. 17 (human core fragment CDS), nucleotides 367-762 of SEQ ID No. 22 (mouse NsG30 CDS), nucleotides 457-759 of SEQ ID No. 22 (mouse mature NsG30 CDS), nucleotides 496-720 of SEQ ID No. 22 (mouse cys1-cys10 fragment CDS), or nucleotides 490-750 of SEQ ID No. 22 (mouse core fragment CDS).

41. The nucleic acid molecule of claim 24, comprising the nucleotide sequence of nucleotides 167-511 of SEQ ID No. 26, nucleotides 113-511 of SEQ ID No. 26, nucleotides 242-508 of SEQ ID No. 26, nucleotides 74-418 of SEQ ID No. 35, nucleotides 20-418 of SEQ ID No. 35, nucleotides 149-415 of SEQ ID No. 35, nucleotides 95-472 of SEQ ID No. 30, nucleotides 206-430 of SEQ ID No. 30, nucleotides 200-463 of SEQ ID No. 30, nucleotides 170-469 of SEQ ID No. 30, nucleotides 168-545 of SEQ ID No. 37, nucleotides 243-542 of SEQ ID No. 37, nucleotides 279-503 of SEQ ID No. 37, or nucleotides 273-536 of SEQ ID No. 37.

42. The nucleic acid molecule of any of the preceding claims 24-41, being codon optimised for expression in E. coli, Chinese Hamster, Baby Hamster, Yeast, insect and/or fungus.

43. The nucleic acid molecule of claim 24, wherein the nucleic acid molecule is a shuffled variant between (i) SEQ ID No 2 or 8 and a nucleic acid coding for NsG29, NsG30, NsG31,

or NsG32, (ii) SEQ ID No 17 or 22 and a nucleic acid coding for NsG28, NsG29, NsG31, or NsG32, (iii) SEQ ID No 26 or 35 and a nucleic acid coding for NsG28, NsG29, NsG30, or NsG31, or (iv) SEQ ID No 30 or 37 and a nucleic acid coding for NsG28, NsG29, NsG30, or NsG31.

44. The nucleic acid molecule of claim 24, wherein the nucleic acid molecule is a shuffled variant between SEQ ID No 2 and 8, SEQ ID No 17 and 22, SEQ ID No 26 and 35, or SEQ ID No 30 and 37.

45. A vector comprising the nucleic acid molecule of any of the preceding claims 24 to 44.

46. The vector of claim 45, further comprising a promoter operably linked to the nucleic acid molecule.

47. The vector of claim 46, wherein the promoter is selected from the group consisting of: CMV, human UbiC, JeT, RSV, Tet-regulatable promoter, Mo-MLV-LTR, Mx1, EF-1alpha.

48. The vector of claim 45 or 46, being selected from the group consisting of vectors derived from the Retroviridae family including lentivirus, HIV, SIV, FIV, EAIV, CIV.

49. The vector of claim 45 or 46, being selected from the group consisting of alphavirus, adenovirus, adeno associated virus, baculovirus, HSV, coronavirus, Bovine papilloma virus, Mo-MLV, preferably adeno associated virus.

50. An isolated host cell transformed or transduced with the vector of any of the claims 45 to 49.

51. The host cell of claim 50, being selected from the group consisting of E. coli, Yeast, Saccharomyces cerevisiae, Aspergillus, Sf9 insect cells.

52. The host cell of claim 50, being selected from the group consisting of mammalian cells, such as human, feline, porcine, simian, canine, murine, rat, mouse and rabbit.

53. The host cell of claim 52, being selected from the group consisting of immortalised retinal pigmented epithelial cells, such as ARPE-19 cells, immortalised human fibroblasts, and immortalised human astrocytes.

54. The host cell of claim 53, being attached to a matrix.

55. The host cell of claim 52, being selected from the group consisting of stem cells, including human neural stem or precursor cells, human glial stem or precursor cells, and foetal stem cells.

56. The host cell of claim 52, being selected from the group consisting of CHO, CHO-K1, HEI193T, HEK293, COS, PC12, HiB5, RN33b, BHK cells.

57. A packaging cell line capable of producing an infective virus particle, said virus particle comprising a Retroviridae derived genome comprising a 5' retroviral LTR, a tRNA binding site, a packaging signal, a promoter operably linked to a polynucleotide sequence encoding the polypeptide of any of the claims 1 to 23, an origin of second strand DNA synthesis, and a 3' retroviral LTR.

58. The packaging cell line of claim 57, wherein the genome is lentivirally derived and the LTRs are lentiviral.

59. An implantable biocompatible cell device, the device comprising:

i) a semipermeable membrane permitting the diffusion of a polypeptide as defined by any of the preceding claims 1 to 23 and/or a virus vector; and

ii) a composition of cells according to any of the claims 50 to 56 or a packaging cell line according to any of the claims 57 to 58.

60. The device of claim 59, wherein the semipermeable membrane is immunoisolatory.

61. The device of claim 59, wherein the semipermeable membrane is microporous.

62. The device of claim 59, wherein the device further comprises a matrix disposed within the semipermeable membrane.

63. The device of claim 59, wherein the device further comprises a tether anchor.

64. The device of claim 59, wherein said device comprises a core comprising living packaging cells that secrete a viral vector for infection of a target cell, wherein the viral vector is a retrovirus, the vector comprising a heterologous gene encoding a polypeptide according to any of claims 1 to 23, operably linked to a promoter that regulates the expression of said polypeptide in the target cell; and an external jacket surrounding said core, said jacket comprising a permeable biocompatible material, said material having a porosity selected to permit passage of retroviral vectors of approximately 100 nm diameter thereacross, permitting release of said viral vector from said capsule.

65. The device of claim 64, wherein the core additionally comprises a matrix, the packaging cells being immobilized by the matrix.

66. The device of claim 64, wherein the jacket comprises a hydrogel or thermoplastic material.

67. A pharmaceutical composition comprising

i) the polypeptide of any of the claims 1 to 23; or
ii) the isolated nucleic acid sequence of any of the claims 24 to 44; or
iii) the expression vector of any of the claims 45 to 49; or
iv) a composition of host cells according to any of the claims 50 to 56; or
v) a packaging cell line according to any of the claims 57 to 58; or
vi) an implantable biocompatible cell device according to any of the claims 59 to 66; and
vii) a pharmaceutically acceptable carrier.

68. The use of

i) the polypeptide of any of the claims 1 to 23; or
ii) the isolated nucleic acid sequence of any of the claims 24 to 44; or
iii) the expression vector of any of the claims 45 to 49; or

iv) a composition of host cells according to any of the claims 50 to 56; or

v) a packaging cell line according to any of the claims 57 to 58; or

vi) an implantable biocompatible cell device according to any of the claims 59 to 66;

for the manufacture of a medicament.

69. The use of claim 68, wherein said medicament is for the treatment of a disease, disorder, or damage associated with the nervous system.

70. The use of claim 69, wherein said medicament is for the treatment of a disease, disorder, or damage involving injury to the brain, brain stem, the spinal cord, and/or peripheral nerves, including but not limited to conditions such as stroke, traumatic brain injury, spinal cord injury, diffuse axonal injury, epilepsy, neuropathy, peripheral neuropathy and associated pain and other symptoms.

71. The use of claim 70, wherein the disorder is thalamic pain.

72. The use of claim 69, wherein the nervous system disorder involves degeneration of neurons and their processes in the brain, brain stem, the spinal cord, and/or the peripheral nerves, including but not limited to Parkinson's Disease, Alzheimer's Disease, senile dementia, Huntington's Disease, amyotrophic lateral sclerosis, neuronal injury associated with multiple sclerosis, and associated symptoms.

73. The use of claim 69, wherein the nervous system disorder is a disease, disorder, or damage involving dysfunction and/or loss of neurons in the brain, brain stem, the spinal cord, and/or peripheral nerves, including but not limited to conditions caused by metabolic diseases, nutritional deficiency, toxic injury, malignancy, and/or genetic or idiopathic conditions including but not limited to diabetes, renal dysfunction, alcoholism, chemotherapy, chemical agents, drug abuse, vitamin deficiency, and infection.

74. The use of claim 73, wherein the disease is essential tremor.

75. The use of claim 73 or 70, wherein the disease is peripheral neuropathy and associated pain.

76. The use of claim 69, wherein the nervous system disorder is a disorders, diseases, or damages associated with the Cerebellum, including but not limited to sensory ataxia, multiple sclerosis, neurodegenerative spinocerebellar disorders, hereditary ataxia, cerebellar atrophies (such as Olivopontocerebellar Atrophy (OPCA), Shy-Drager Syndrome (multiple systems atrophy)), and alcoholism

77. The use of claim 69, wherein the nervous system disorder is a disease, disorder, or damage involving degeneration or sclerosis of glia such as oligodendrocytes, astrocytes and Schwann cells in the brain, brain stem, the spinal cord, and the peripheral nerves, including but not limited to multiple sclerosis, optic neuritis, cerebral sclerosis, post-infectious encephalomyelitis, and epilepsy and associated symptoms.

78. The use of claim 77, wherein the disorder is multiple sclerosis.

79. The use of claim 69, wherein the nervous system disorder, disease, or damage involves the retina, photoreceptors, and associated nerves including but not limited to retinitis pigmentosa, macular degeneration, glaucoma, diabetic retinopathy, and associated symptoms.

80. The use of claim 69, wherein the nervous system disorder, disease, or damage involves the sensory epithelium and associated ganglia of the vestibuloacoustic complex including but not limited to noise-induced hearing loss, deafness, tinnitus, otitis, labyrintitis, hereditary and cochleovestibular atrophies, Menieres Disease, and associated symptoms.

81. The use of claim 68, wherein the medicament is for the treatment of a disease related to testis, including male sterility, impotence, erectile dysfunction, cancer, and germ cell tumours.

82. A method of treatment of a pathological condition in a subject comprising administering to an individual in need thereof a therapeutically effective amount of:

i) the polypeptide of any of the claims 1 to 23; or

ii) the isolated nucleic acid sequence of any of the claims 24 to 44; or

iii) the expression vector of any of the claims 45 to 49; or

iv) a composition of host cells according to any of the claims 50 to 56; or

v) a packaging cell line according to any of the claims 57 to 58; or

vi) an implantable biocompatible cell device according to any of the claims 59 to 66.

83. The method of claim 82, wherein the pathological condition is a disease, disorder, or damage associated with the nervous system.

84. The method of claim 83, wherein said method is for the treatment of a disease, disorder, or damage involving injury to the brain, brain stem, the spinal cord, and/or peripheral nerves, including but not limited to conditions such as stroke, traumatic brain injury, spinal cord injury, diffuse axonal injury, epilepsy, neuropathy, peripheral neuropathy and associated pain and other symptoms.

85. The method of claim 84, wherein said disorder is thalamic pain.

86. The method of claim 83, wherein the nervous system disorder involves degeneration of neurons and their processes in the brain, brain stem, the spinal cord, and/or the peripheral nerves, including but not limited to Parkinson's Disease, Alzheimer's Disease, senile dementia, Huntington's Disease, amyotrophic lateral sclerosis, neuronal injury associated with multiple sclerosis, and associated symptoms.

87. The method of claim 83, wherein the nervous system disorder is a disease, disorder, or damage involving dysfunction and/or loss of neurons in the brain, brain stem, the spinal cord, and/or peripheral nerves, including but not limited to conditions caused by metabolic diseases, nutritional deficiency, toxic injury, malignancy, and/or genetic or idiopathic conditions including but not limited to diabetes, renal dysfunction, alcoholism, chemotherapy, chemical agents, drug abuse, vitamin deficiency, and infection.

88. The method of claim 87, wherein the disease is essential tremor.

89. The method of claim 87, wherein the disease is peripheral neuropathy and associated pain.

90. The method of claim 83, wherein the nervous system disorder is a disorders, diseases, or damages associated with the Cerebellum, including but not limited to sensory ataxia, multiple sclerosis, neurodegenerative spinocerebellar disorders, hereditary ataxia, cerebellar atrophies (such as Olivopontocerebellar Atrophy (OPCA), Shy-Drager Syndrome (multiple systems atrophy)), and alcoholism

91. The method of claim 83, wherein the nervous system disorder is a disease, disorder, or damage involving degeneration or sclerosis of glia such as oligodendrocytes, astrocytes and Schwann cells in the brain, brain stem, the spinal cord, and the peripheral nerves, including but not limited to multiple sclerosis, optic neuritis, cerebral sclerosis, post-infectious encephalomyelitis, and epilepsy and associated symptoms.

92. The method of claim 91, wherein said disorder is multiple sclerosis.

93. The method of claim 83, wherein the nervous system disorder, disease, or damage involves the retina, photoreceptors, and associated nerves including but not limited to retinitis pigmentosa, macular degeneration, glaucoma, diabetic retinopathy, and associated symptoms.

94. The method of claim 83, wherein the nervous system disorder, disease, or damage involves the sensory epithelium and associated ganglia of the vestibuloacoustic complex including but not limited to noise-induced hearing loss, deafness, tinnitus, otitis, labyrintitis, hereditary and cochleovestibular atrophies, Menieres Disease, and associated symptoms.

95. The method of claim 82, wherein the pathological condition is a disease related to testis, including male sterility, impotence, erectile dysfunction, cancer, and germ cell tumours.

96. The method of claim 82, wherein the subject is a human being.

97. The use of

i) the polypeptide of any of the claims 1 to 23; or

ii) the isolated nucleic acid sequence of any of the claims 24 to 44; or

iii) the expression vector of any of the claims 45 to 49; or

iv) a composition of host cells according to any of the claims 50 to 56; or

v) an implantable biocompatible cell device according to any of the claims 59 to 66;

as a male contraceptive.

98. A method of expanding a composition of mammalian cells, comprising administering to said composition the polypeptide of any of the claims 1 to 23; or transducing/transfecting the cells with the expression vector of any of the claims 45 to 49.

99. A method of differentiating a composition of mammalian cells, comprising administering to said composition the polypeptide of any of the claims 1 to 23; or transducing/transfecting the cells with the expression vector of any of the claims 45 to 49.

100. An antibody capable of binding to a polypeptide of any of the claims 1 to 23.

101. The antibody of claim 100, being selected from the group consisting of: polyclonal antibodies, monoclonal antibodies, humanised antibodies, single chain antibodies, recombinant antibodies.

102. An immunoconjugate comprising the antibody of claim 100 and a conjugate selected from the group consisting of: a cytotoxic agent such as a chemotherapeutic agent, a toxin, or a radioactive isotope; a member of a specific binding pair, such as avidin or streptavidin or an antigen; an enzyme capable of producing a detectable product.

103. An isolated polypeptide having an amino acid sequence selected from the group consisting of SEQ ID No. 6, 7, 11, 12, 15, 16, 20, 21, 33, and 34, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15 of the amino acid residues in the sequence are so changed.

104. The polypeptide of claim 103, wherein no more than 10 of the amino acid residues in the chosen sequence are changed.

105. The isolated polypeptide of claim 103 or 104, wherein the changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in any of Figs. 11, 12, 13A, and 13B.

106. The isolated polypeptide of claim 103 or 104, wherein the changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in Fig. 1B, preferably in Fig. 1A.

107. The isolated polypeptide of claim 103 or 104, further comprising up to 5 additional amino acids in the C- or N-terminal, the additional amino acids preferably being selected from the amino acids at corresponding positions in mature NsG28, mature NsG30, mature NsG32b.

108. An isolated polynucleotide coding for a polypeptide according to any of claims 1 to 23 or 103 to 107.

109. A method of preventing apoptosis in a neuronal cell, the method comprising contacting a neuronal cell with an affective amount of a polypeptide according to any of claims 1 to 23, a nucleic acid according to any of claims 24 to 44, or a vector according to any of claims 45 to 49.

110. A method of treating a disorder characterized by neuronal apoptosis, the method comprising administering to a subject having a disorder characterized by neuronal apoptosis a therapeutically affective amount of a polypeptide according to any of claims 1 to 23, a nucleic acid according to any of claims 24 to 44, or a vector according to any of claims 45 to 49.

111. Use of a polypeptide according to any of claims 1 to 23, a nucleic acid according to any of claims 24 to 44, or a vector according to any of claims 45 to 49 for the preparation of a medicament for the prevention of neuronal apoptosis.

112. Use of a polypeptide according to any of claims 1 to 23, a nucleic acid according to any of claims 24 to 44, or a vector according to any of claims 45 to 49 for the preparation of a medicament for treating a disorder characterized by neuronal apoptosis.

```
NsG28       SSQHLRGHAGHHQIKQGTCEVVAVHRCCNKNRIEERSQTVKCSCFPGQVAGTTRAQPSCV
NsG28_m     SSQHLRGHAGHHLIKPGTCEVVAVHRCCNKNRIEERSQTVKCSCFPGQVAGTTRAQPSCV
NsG30       -----ANHHKAHHVKTGTCEVVALHRCCNKNKIEERSQTVKCSCFPGQVAGTTRAAPSCV
NsG30_m     -----ANHHKAHHVRTGTCEVVALHRCCNKNKIEERSQTVKCSCFPGQVAGTTRAAPSCV
NsG31       ---ALQPPTATVLVQQGTCEVIAAHRCCNRNRIEERSQTVKCSCFSGQVAGTTRAKPSCV
NsG31_m     ---SLQPPTSAVLVKQGTCEVIAAHRCCNRNRIEERSQTVKCSCLSGQVAGTTRAKPSCV
NsG29       SLQHTFQQHHLHRPEGGTCEVIAAHRCCNKNRIEERSQTVKCSCLPGKVAGTTRNRPSCV
NsG29_m     SLQHTFQQHHLHRPEGGTCEVIAAHRCCNKNRIEERSQTVKCSCLPGKVAGTTRNRPSCV
                        . *****:* *****:*:************:.*:******  ****

NsG28       EASIVIQKWWCHMNPCLEGEDCKVLPDYSGWSCSSGNKVKTTKVTR--
NsG28_m     EAAIVIEKWWCHMNPCLEGEDCKVLPDSSGWSCSSGNKVKTTKAS---
NsG30       DASIVEQKWWCHMQPCLEGEECKVLPDRKGWSCSSGNKVKTTRVTH--
NsG30_m     DASIVEQKWWCHMQPCLEGEECKVLPDRKGWSCSSGNKVKTTRVTH--
NsG31       DASIVLQRWWCQMEPCLPGEECKVLPDLSGWSCSSGHKVKTTKVTR--
NsG31_m     DASIVLQKWWCQMEPCLLGEECKVLPDLSGWSCSSGHKVKTTKVTR--
NsG29       DASIVIGKWWCEMEPCLEGEECKTLPDNSGWMCATGNKIKTTRIHPRT
NsG29_m     DASIVIGKWWCEMEPCLEGEECKTLPDNSGWMCATGNKIKTTRIHPRT
            :*:**  :***.*:*** **:**.*** .** *::*:*:***:
```

Fig. 1A

```
NsG29       SLQHTFQQHHLHRPEGGTCEVIAAHRCCNKNRIEERSQTVKCSCLPGKVAGTTRNRPSCV
NsG29_m     SLQHTFQQHHLHRPEGGTCEVIAAHRCCNKNRIEERSQTVKCSCLPGKVAGTTRNRPSCV
NsG28       SSQHLRGHAGHHQIKQGTCEVVAVHRCCNKNRIEERSQTVKCSCFPGQVAGTTRAQPSCV
NsG28_m     SSQHLRGHAGHHLIKPGTCEVVAVHRCCNKNRIEERSQTVKCSCFPGQVAGTTRAQPSCV
NsG30       -----ANHHKAHHVKTGTCEVVALHRCCNKNKIEERSQTVKCSCFPGQVAGTTRAAPSCV
NsG30_m     -----ANHHKAHHVRTGTCEVVALHRCCNKNKIEERSQTVKCSCFPGQVAGTTRAAPSCV
NsG31       ---ALQPPTATVLVQQGTCEVIAAHRCCNRNRIEERSQTVKCSCFSGQVAGTTRAKPSCV
NsG31_m     ---SLQPPTSAVLVKQGTCEVIAAHRCCNRNRIEERSQTVKCSCLSGQVAGTTRAKPSCV
NsG32b      ------QFLKEGQLAAGTCEIVTLDRDSSQPRRTIARQTARCACRKGQIAGTTRARPACV
NsG32b_m    ------QFLKEGQLAAGTCEIVTLDRDSSQPRRTIARQTARCACRKGQIAGTTRARPACV
NsG32a      ----------------TCEIVTLDRDSSQPRRTIARQTARCACRKGQIAGTTRARPACV
NsG32a_m    ----------------TCEIVTLDRDSSQPRRTIARQTARCACRKGQIAGTTRARPACV
                          ***::: .* ..: :     **.:*:*  *::*****  *:**

NsG29       DASIVIGKWWCEMEPCLEGEECKTLPDNSGWMCA-TGNKIKTTRIHPRT
NsG29_m     DASIVIGKWWCEMEPCLEGEECKTLPDNSGWMCA-TGNKIKTTRIHPRT
NsG28       EASIVIQKWWCHMNPCLEGEDCKVLPDYSGWSCS-SGNKVKTTKVTR--
NsG28_m     EAAIVIEKWWCHMNPCLEGEDCKVLPDSSGWSCS-SGNKVKTTKAS---
NsG30       DASIVEQKWWCHMQPCLEGEECKVLPDRKGWSCS-SGNKVKTTRVTH--
NsG30_m     DASIVEQKWWCHMQPCLEGEECKVLPDRKGWSCS-SGNKVKTTRVTH--
NsG31       DASIVLQRWWCQMEPCLPGEECKVLPDLSGWSCS-SGHKVKTTKVTR--
NsG31_m     DASIVLQKWWCQMEPCLLGEECKVLPDLSGWSCS-SGHKVKTTKVTR--
NsG32b      DARIIKTKQWCDMLPCLEGEGCDLLINRSGWTCTQPGGRIKTTTVS---
NsG32b_m    DARIIKTKQWCDMLPCLEGEGCDLLINRSGWTCTQPGGRIKTTTVS---
NsG32a      DARIIKTKQWCDMLPCLEGEGCDLLINRSGWTCTQPGGRIKTTTVS---
NsG32a_m    DARIIKTKQWCDMLPCLEGEGCDLLINRSGWTCTQPGGRIKTTTVS---
            :* *:  : **.* *** ** *. * : .** *: .* ::***
```

Fig. 1B

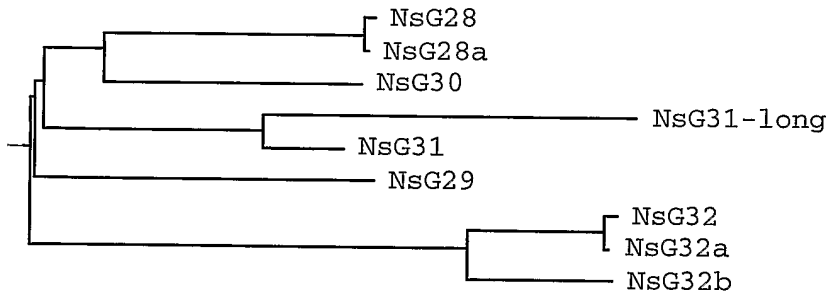
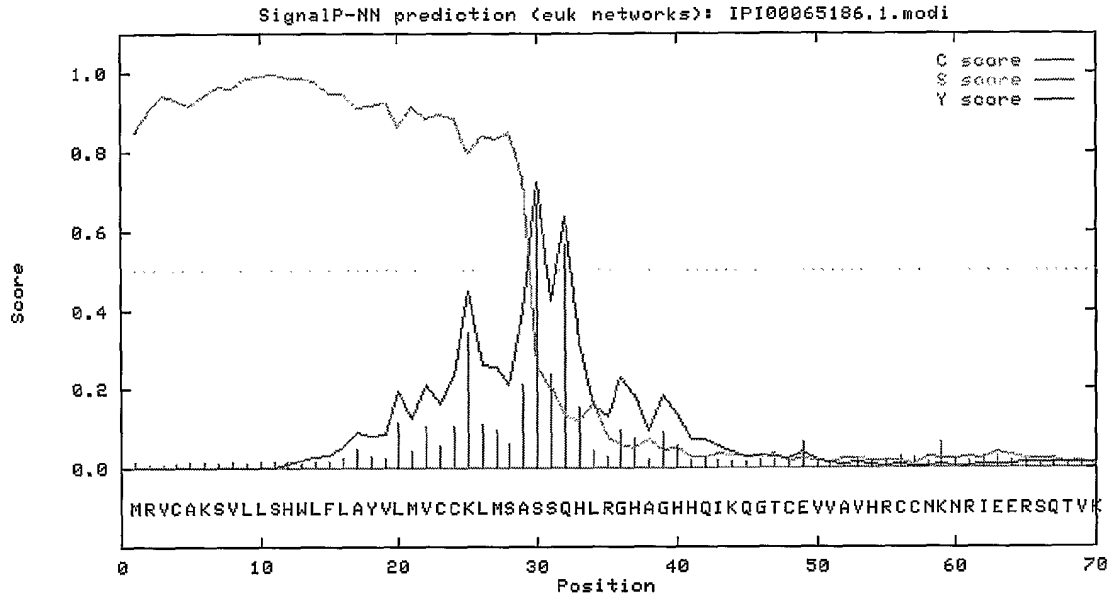

NsG28
NsG28a
NsG30
NsG31-long
NsG31
NsG29
NsG32
NsG32a
NsG32b

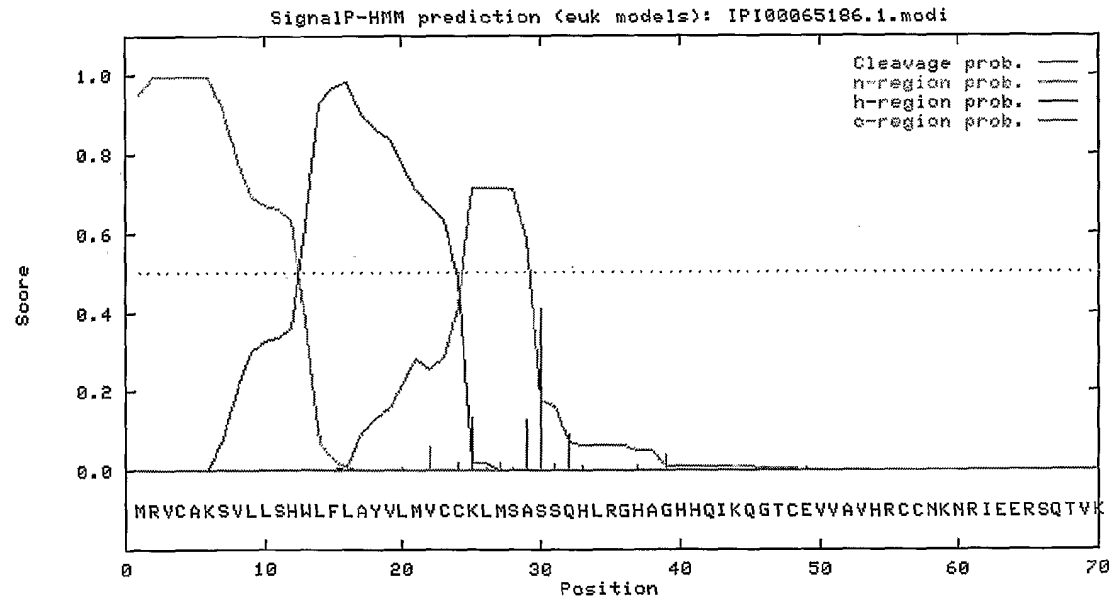
Fig.1C



# Measure	Position	Value	Cutoff	signal peptide?
max. C	30	0.666	0.33	YES
max. Y	30	0.724	0.32	YES
max. S	11	0.996	0.82	YES
mean S	1-29	0.914	0.47	YES

Most likely cleavage site between pos. 29 and 30: MSA-SS

Fig. 2A



Signal peptide probability: 0.954
Signal anchor probability: 0.044
Max cleavage site probability: 0.408 between pos. 29 and 30

Fig. 2B

Human NsG28a (SEQ ID No 4)

# Gene Ontology category	Odds
Signal_transducer	0.420
Receptor	0.026
Hormone	**2.750**
Structural_protein	0.181
Transporter	0.217
Ion_channel	0.147
Voltage-gated_ion_channel	0.161
Cation_channel	0.215
Transcription	0.356
Transcription_regulation	0.281
Stress_response	0.556
Immune_response	0.244
Growth_factor	**4.497**
Metal_ion_transport	0.020

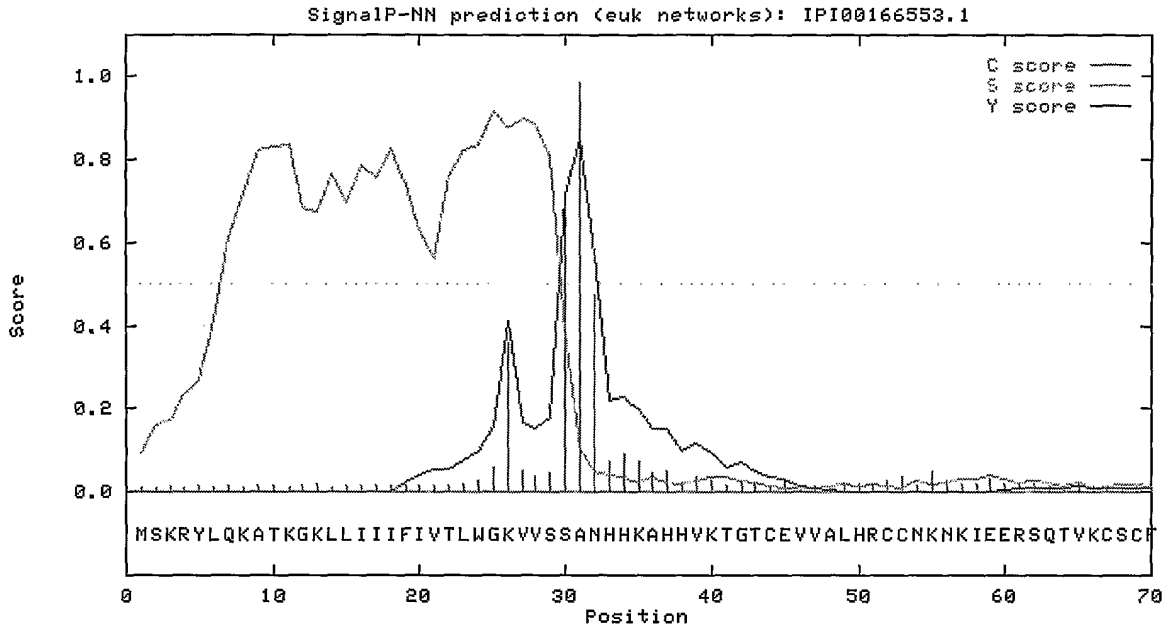
Mouse NsG28 (SEQ ID No 9)

# Gene Ontology category	Odds
Signal_transducer	0.342
Receptor	0.024
Hormone	**5.414**
Structural_protein	0.214
Transporter	0.217
Ion_channel	0.147
Voltage-gated_ion_channel	0.162
Cation_channel	0.215
Transcription	0.351
Transcription_regulation	0.276
Stress_response	0.339
Immune_response	0.945
Growth_factor	**2.930**
Metal_ion_transport	0.020

Rat NsG28 (SEQ ID No 13)

# Gene Ontology category	Odds
Signal_transducer	0.435
Receptor	0.028
Hormone	**2.375**
Structural_protein	0.194
Transporter	0.217
Ion_channel	0.147
Voltage-gated_ion_channel	0.161
Cation_channel	0.215
Transcription	0.380
Transcription_regulation	0.225
Stress_response	0.757
Immune_response	1.483
Growth_factor	**3.077**
Metal_ion_transport	0.020

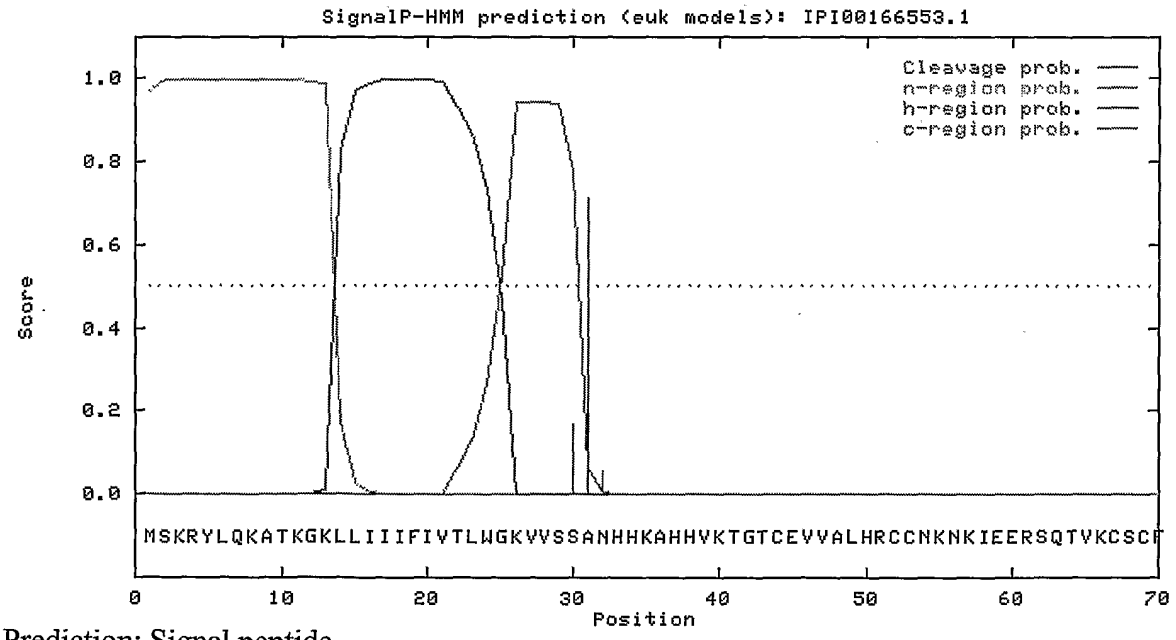
Fig. 3



# Measure	Position	Value	Cutoff	signal peptide?
max. C	31	0.986	0.33	YES
max. Y	31	0.849	0.32	YES
max. S	25	0.918	0.82	YES
mean S	1-30	0.649	0.47	YES

Most likely cleavage site between pos. 30 and 31: VSS-AN

Fig. 4A



Prediction: Signal peptide
Signal peptide probability: 0.969
Signal anchor probability: 0.028
Max cleavage site probability: 0.713 between pos. 30 and 31

Fig. 4B

Human NsG30 (SEQ ID No 18)

# Gene Ontology category	Odds
Signal_transducer	0.530
Receptor	0.052
Hormone	**2.396**
Structural_protein	0.529
Transporter	0.249
Ion_channel	0.149
Voltage-gated_ion_channel	0.198
Cation_channel	0.215
Transcription	0.316
Transcription_regulation	0.502
Stress_response	1.012
Immune_response	1.090
Growth_factor	**3.731**
Metal_ion_transport	0.020

Mouse NsG30 (SEQ ID No. 23)

# Gene Ontology category	Odds
Signal_transducer	0.654
Receptor	1.081
Hormone	**2.401**
Structural_protein	0.448
Transporter	0.333
Ion_channel	0.147
Voltage-gated_ion_channel	0.109
Cation_channel	0.215
Transcription	0.341
Transcription_regulation	0.481
Stress_response	0.846
Immune_response	1.171
Growth_factor	**3.897**
Metal_ion_transport	0.020

Fig. 5

NetStart 1.0 Prediction Results

```
Name: gi_24658289
GGCGGCGGAGGATGGCGCGCGCGGGGCCCGCACGTGGAGGCCGGCGCGGGGGCGCGGGCAGGGCCGGCTGCTGAGACGCG
CTGCTGCCCCCCGCGCGGGCGCCGCGGCTTCAATGGCGCCATCGCCCAGGACCGGCAGCCGGCAAGATGCGACCGCCCTG
CCCAGCATGTCCTCAACTTTCTGGGCGTTCATGATCCTGGCCAGCCTGCTCATCGCCTACTGCAGTCAGCTGGCCGCCGG
CACCTGTGAGATTGTGACCTTGGACCGGGACAGCAGCCAGCCTCGGAGGACGATCGCCCGGCAGACCGCCCGCTGTGCGT
GTAGAAAGGGGCAGATCGCCGGCACCACGAGAGCCCGGCCCGCCTGTGTGGACGCAAGAATCATCAAGACCAAGCAGTGG
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CGGGAGGATAAAGACCACCACGGTCTCCTGACAAACACAGCCCCTGAGGGGGCCCCGGGAGTGGCCTTGGCTCCCTGGAG
AGCCCACGTCTCAGCCACAGTTCTCCACTCGCCTCGGACTTCACCCGTTCTCTGCCGCCCGCCCACTCCGTTTCCCTGTG
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CGGGAGGCAGCGACGGCCCCCACGCAGACGCCGGGAACGCAGGCCGCTTTATTCCTCTGTACTTAGATCAACTTGACCGT
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TAACACTTGTTTGAAAGAGATCATTAAATGTATTTTGCAAAGCCCAAAAAAAAAAAAAAAA
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.....................................................................
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................................i....................................................
.........N...........................................................................
.........................N.....................................
```

Pos	Score	Pred
12	0.549	Yes
113	0.469	-
147	0.446	-
167	0.745	Yes
191	0.621	Yes
407	0.420	-
1070	0.672	Yes
1084	0.416	-
1314	0.600	Yes
1371	0.400	-
1468	0.064	-

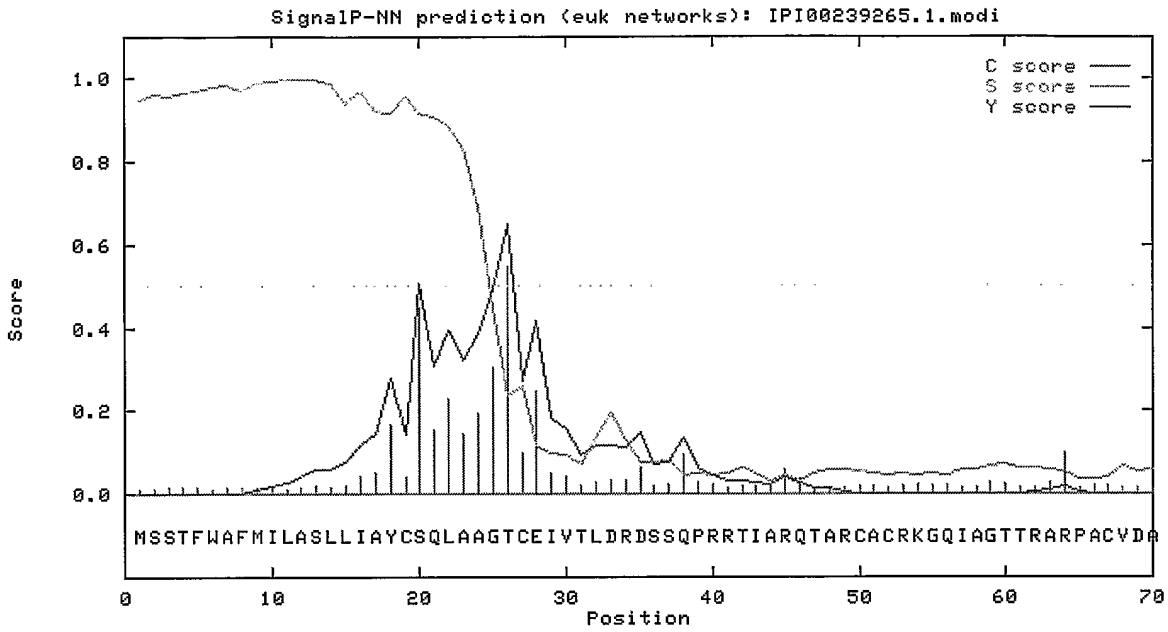
Fig. 6

NetStart 1.0 Prediction Results

```
Name: gi_26355279
GGCGGGCACCGCGGCTTCAATGGCGCCATCGCCCAGGACCAGCAGCCGGCAAGATGCGACCGCCCTGCCCAGCATGTCCT
CAACTTTTTGGGCATTCATGATCCTGGCCAGCCTGCTCATCGCCTACTGCAGTCAGCTGGCCGCTGGAACCTGTGAGATT
GTGACCCTAGACCGGGACAGCAGCCAGCCACGGAGGACGATCGCCCGGCAGACAGCACGCTGTGCATGCAGAAAGGGGCA
GATAGCAGGCACCACTCGAGCCCGGCCTGCTTGTGTGGACGCTCGAATTATCAAGACAAAGCAGTGGTGTGACATGCTTC
CTTGCCTGGAGGGGGAAGGCTGTGACTTGTTAATCAACCGGTCAGGCTGGACTTGCACACAGCCCGGAGGGCGGATAAAG
ACCACCACGGTCTCCTGACAATCGCAGCCCCTGCGGTGCCCCCTGGGAGTGGCTGTGGCTCCCTGGAAATTCTACACCTC
AGCCACAGTCCTGCATCCTATGTGGACACCGCTCATTCTCCCCCATCCACCCACTGTTTCCCAGAGGCCTAACAAAGACA
GCCCAATTCCTCGGAGCTCTGAGCTCAAGTCTGTCCAGCTGAACCCAGGAGGCTGGGACTCAGACACACATGTCAAGGGT
GGTACCTGGAATCAGCAATACCTGGTGTGTGGAGCCTGGCTGCATCCATCCTCACTAGCCGCTTGCTCCCTGGAGTTGGA
GGACCGGAACAAGGAAATTGTGGCCCGGCCAGCTGGGCCACAACGCAAGGTCCGGGGACTGTCAGGTGCAGAAGCCACCA
CCCCCTCTGTCCCAGACTGTCTGAATCGCTTTTATTTTCTTATACTTTCAGTATACTCAATAGACCAAAGAGCAAATCTA
TTTTAACGTGGACACATCCCTGCTGCCAAGAGTTCCCTGGGTTTGATTGTGGGGCAGGGGAGGGCGCAGTGGCAGACATG
CTGGTGGCCCAGGCAGCATGGAGAGGGCATCTGAGGCCAAGACCCCCGCCCCACGAACACTGCAGCGTGCCCTAAGAGGA
TGGCTTTGCCCAGAAGGACACCACCGTGGTGCACCTGCAGGACCCAGCAGGGGCATGGCCGGCGACGGCCCCCAGCGGAC
AGCAAGGGCCCCCGGACACAGACCCTTTGCCCCTGTGCTTAGACCAACTCTATCGTACTAAAATCACTTTCTGTTTTAAC
CAGTTAGACATGCCTCTTCCACAGCTCCATTTTTGATAGTTGGATAATCCACCATTTGCCAAGAGCATGCTGGGTCTCCT
GTGACTGCTATCTCATCTGATACACCATTTAGCTCCAGAAAGCAAATTAAAGAAAACTGAAGC
.....N.....N.....i.....
.....i.....
.....N.....
.....N.....
.....N.....
.....i.....
.....i..
.....i.....i
.....N.....
.....N.....N.....
```

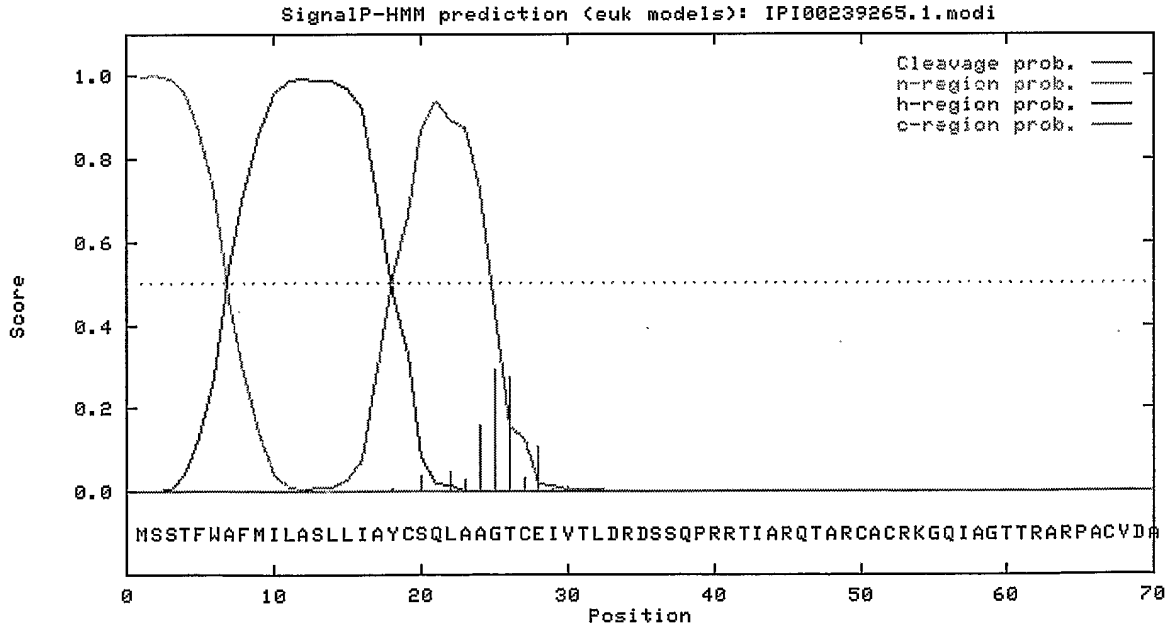
Pos	Score	Pred
20	0.322	-
54	0.411	-
74	0.777	Yes
98	0.626	Yes
226	0.291	-
314	0.415	-
500	0.496	-
630	0.543	Yes
958	0.725	Yes
978	0.660	Yes
1040	0.526	Yes
1095	0.425	-
1210	0.344	-
1267	0.179	-

Fig. 7




```
>Human NsG32                  length = 70
# Measure  Position  Value  Cutoff  signal peptide?
  max. C     26       0.544   0.33   YES
  max. Y     26       0.650   0.32   YES
  max. S     13       0.997   0.82   YES
  mean S      1-25    0.923   0.47   YES
# Most likely cleavage site between pos. 25 and 26: AAG-TC
```

Fig. 8A




```
>Human NsG32
Prediction: Signal peptide
Signal peptide probability: 0.999
Signal anchor probability: 0.001
Max cleavage site probability: 0.292 between pos. 24 and 25
```

Fig. 8B

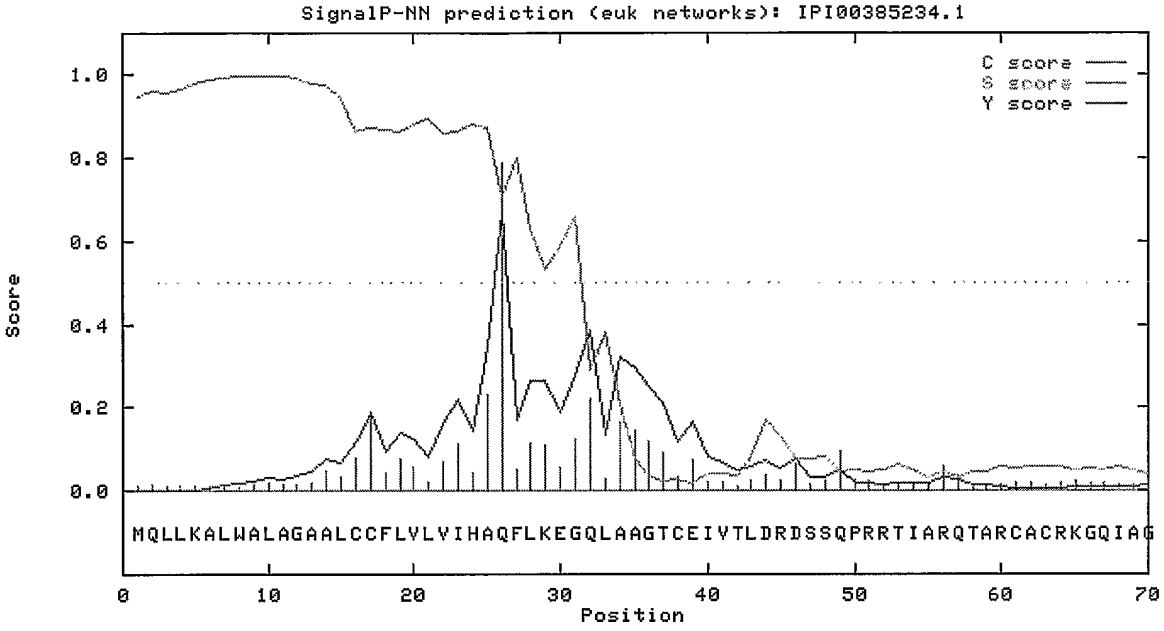

SignalP-NN prediction (euk networks): IPI00385234.1
C score
S score
Y score
Score
1.0
0.8
0.6
0.4
0.2
0.0
MQLLKALWALAGAALCCFLVLVIHAQFLKEGQLAAGTCEIVTLDRDSSQPRRTIARQTARCACRKGQIAG
0
10
20
30
40
50
60
70
Position

Fig. 9A

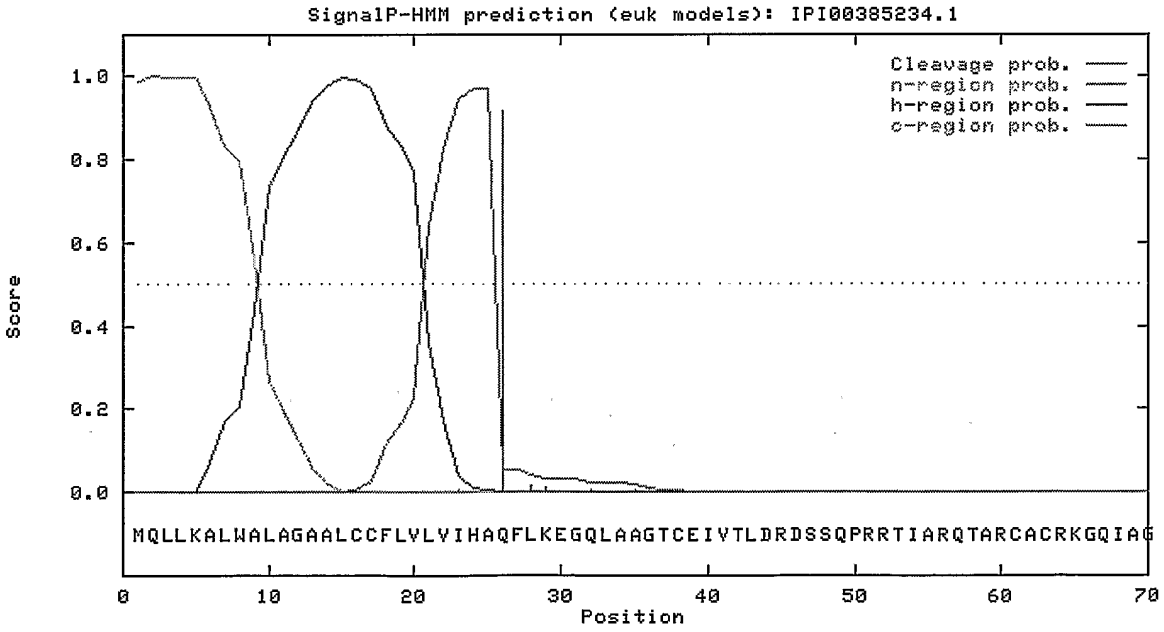

SignalP-HMM prediction (euk models): IPI00385234.1
Cleavage prob.
n-region prob.
h-region prob.
c-region prob.
Score
1.0
0.8
0.6
0.4
0.2
0.0
MQLLKALWALAGAALCCFLVLVIHAQFLKEGQLAAGTCEIVTLDRDSSQPRRTIARQTARCACRKGQIAG
0
10
20
30
40
50
60
70
Position

Fig. 9B

Human NsG32a (SEQ ID No 27)

# Gene Ontology category	Odds
Signal_transducer	0.770
Receptor	0.233
Hormone	**25.274**
Structural_protein	0.216
Transporter	0.220
Ion_channel	0.147
Voltage-gated_ion_channel	0.172
Cation_channel	0.215
Transcription	0.522
Transcription_regulation	0.786
Stress_response	0.804
Immune_response	0.419
Growth_factor	**4.122**
Metal_ion_transport	0.020

Human NsG32a (SEQ ID No. 28)

# Gene Ontology category	Odds
Signal_transducer	0.544
Receptor	0.073
Hormone	0.618
Structural_protein	0.427
Transporter	1.418
Ion_channel	0.149
Voltage-gated_ion_channel	0.173
Cation_channel	0.417
Transcription	0.702
Transcription_regulation	0.649
Stress_response	1.029
Immune_response	0.373
Growth_factor	**4.296**
Metal_ion_transport	0.033

Human NsG32b (SEQ ID No 31)

# Gene Ontology category	Odds
Signal_transducer	0.540
Receptor	0.018
Hormone	**14.523**
Structural_protein	0.177
Transporter	0.216
Ion_channel	0.147
Voltage-gated_ion_channel	0.140
Cation_channel	0.215
Transcription	0.266
Transcription_regulation	0.186
Stress_response	0.734
Immune_response	0.358
Growth_factor	0.372
Metal_ion_transport	0.020

Fig. 10

```
CLUSTAL W (1.82) multiple sequence alignment

Human           MRVCAKSVLLSHWLFLAYVLMVCCKLMSASSQHLRGHAGHHQIKQGTCEVVAVHRCCNKN
Mouse           MRVCAKWVLLSRWLVLTYVLMVCCKLMSASSQHLRGHAGHHLIKPGTCEVVAVHRCCNKN
Rat             MRVCTKWVLLSHWLVLAYMLMVCCKLMSASSQHLRGHAGHHVIKQGTCEVVAVHRCCNKN
                ****:* ****:**.*:*:********************** ** **************

Human           RIEERSQTVKCSCFPGQVAGTTRAQPSCVEASIVIQKWWCHMNPCLEGEDCKVLPDYSGW
Mouse           RIEERSQTVKCSCFPGQVAGTTRAQPSCVEAAIVIEKWWCHMNPCLEGEDCKVLPDSSGW
Rat             RIEERSQTVKCSCFPGQVAGTTRAQPSCVEASIVIEKWWCHMDPCLEGEDCKVLPDSSGW
                ********************************:***:******:************* ***

Human           SCSSGNKVKTTKVTR
Mouse           SCSSGNKVKTTKVTR
Rat             SCSSGNKVKTTKAS-
                ************.:
```

Fig. 11

```
CLUSTAL W (1.82) multiple sequence alignment

Mouse           MNKRYLQKATQGKLLIIIFIVTLWGKAVSSANHHKAHHVRTGTCEVVALHRCCNKNKIEE
Rat             ----------------------------------AHHVRTGTCEVVALHRCCNKNKIEE
Human           MSKRYLQKATKGKLLIIIFIVTLWGKVVSSANHHKAHHVKTGTCEVVALHRCCNKNKIEE
                                                  ****:********************

Mouse           RSQTVKCSCFPGQVAGTTRAAPSCVDASIVEQKWWCHMQPCLEGEECKVLPDRKGWSCSS
Rat             RSQTVKCSCFPGQVAGTTRAAPSCVDASIVEQKWWCHMQPCLEGEECKVLPDRKGWSCSS
Human           RSQTVKCSCFPGQVAGTTRAAPSCVDASIVEQKWWCHMQPCLEGEECKVLPDRKGWSCSS
                ************************************************************

Mouse           GNKVKTTRVTH
Rat             GNKVKTTRVS-
Human           GNKVKTTRVTH
                *********:
```

Fig. 12

```
CLUSTAL W (1.82) multiple sequence alignment

Human           MSSTFWAFMILASLLIAYCSQLAAGTCEIVTLDRDSSQPRRTIARQTARCACRKGQIAGT
Mouse           MSSTFWAFMILASLLIAYCSQLAAGTCEIVTLDRDSSQPRRTIARQTARCACRKGQIAGT
Rat             MSSTFWAFMILASLLIAYCSQLAAGTCEIVTLDRDSSQPRRTIARQTARCACRKGQIAGT
                ************************************************************

Human           TRARPACVDARIIKTKQWCDMLPCLEGEGCDLLINRSGWTCTQPGGRIKTTTVS
Mouse           TRARPACVDARIIKTKQWCDMLPCLEGEGCDLLINRSGWTCTQPGGRIKTTTVS
Rat             TRARPACVDARIIKTKQWCDMLPCLEGEGCDLLINRSGWTCTQPGGRIKTTTVS
                ***************************************************
```

Fig. 13A

```
CLUSTAL W (1.82) multiple sequence alignment

Human           MQLLKALWALAGAALCCFLVLVIHAQFLKEGQLAAGTCEIVTLDRDSSQPRRTIARQTAR
Mouse           MQLLKALWALAGAALCCFLVLVIHAQFLKEGQLAAGTCEIVTLDRDSSQPRRTIARQTAR
                ************************************************************

Human           CACRKGQIAGTTRARPACVDARIIKTKQWCDMLPCLEGEGCDLLINRSGWTCTQPGGRIK
Mouse           CACRKGQIAGTTRARPACVDARIIKTKQWCDMLPCLEGEGCDLLINRSGWTCTQPGGRIK
                ************************************************************

Human           TTTVS
Mouse           TTTVS
                *****
```

Fig. 13B

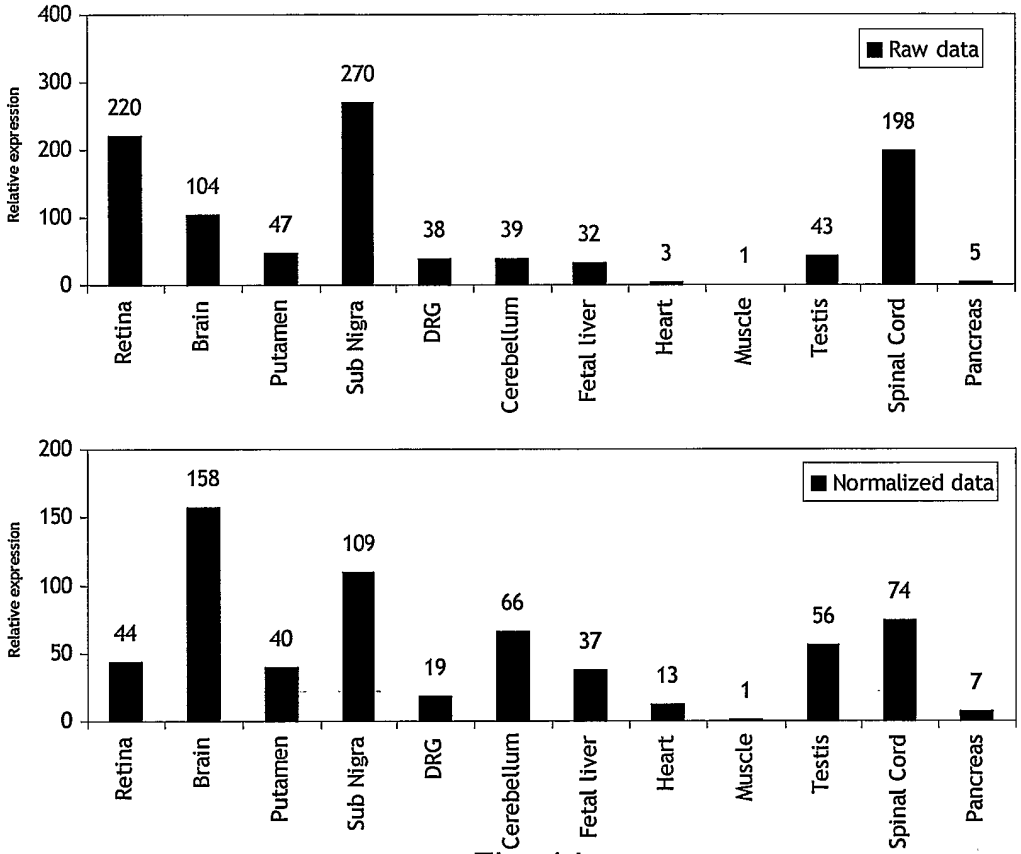

Relative expression
Raw data
400
300
200
100
0
220
104
47
270
38
39
32
3
1
43
198
5
Retina
Brain
Putamen
Sub Nigra
DRG
Cerebellum
Fetal liver
Heart
Muscle
Testis
Spinal Cord
Pancreas
Normalized data
200
150
100
50
0
44
158
40
109
19
66
37
13
1
56
74
7

Fig. 14

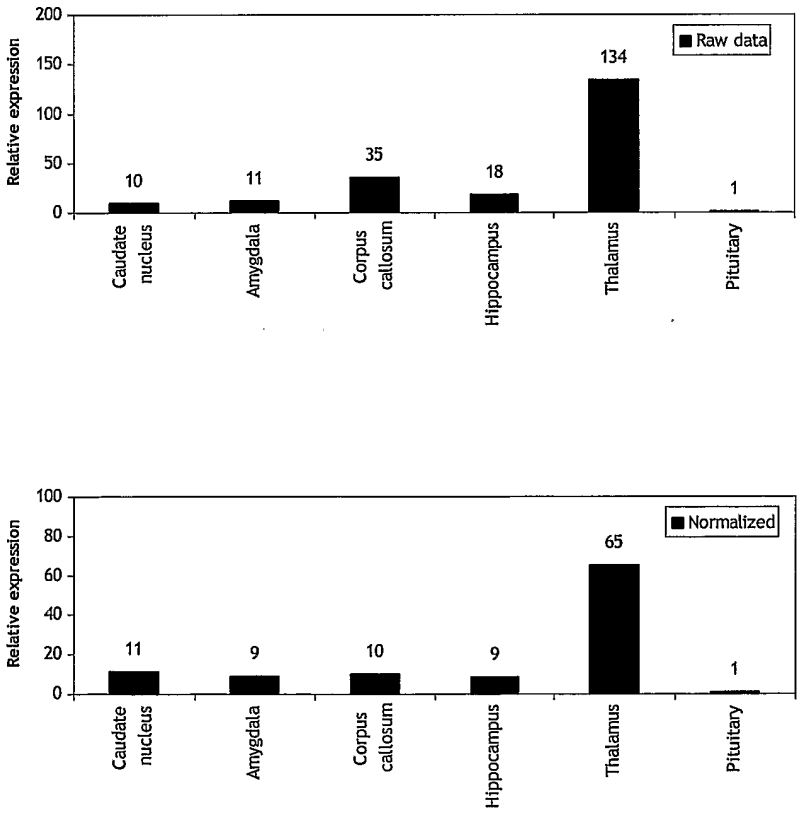

Relative expression
Raw data
200
150
100
50
0
10
11
35
18
134
1
Caudate nucleus
Amygdala
Corpus callosum
Hippocampus
Thalamus
Pituitary
Normalized
100
80
60
40
20
0
11
9
10
9
65
1

Fig. 15

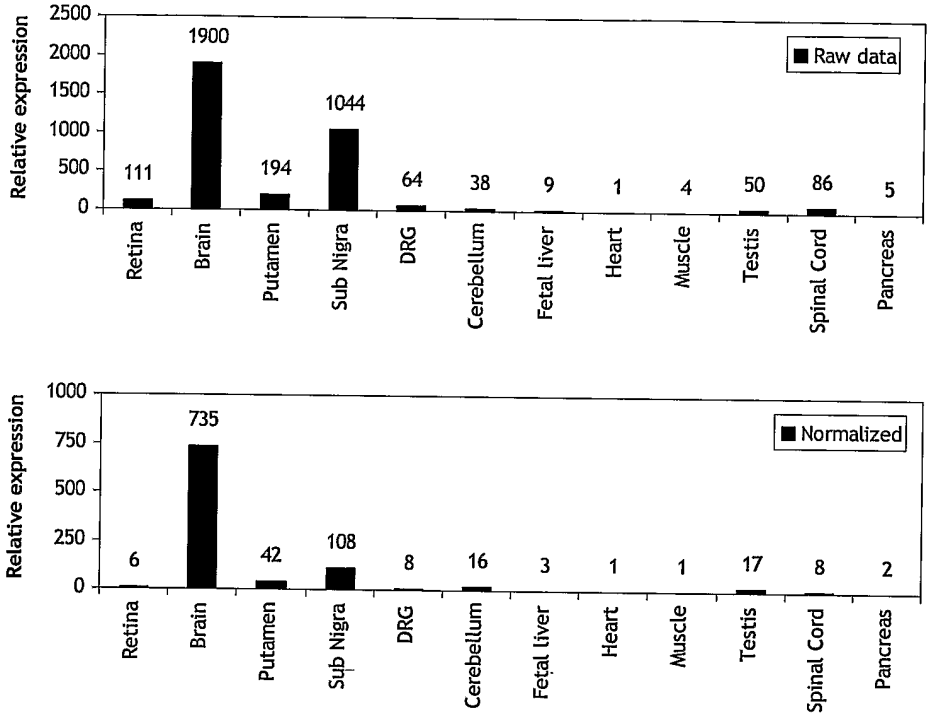

Relative expression
2500
2000
1500
1000
500
0
Raw data
111
1900
194
1044
64
38
9
1
4
50
86
5
Retina
Brain
Putamen
Sub Nigra
DRG
Cerebellum
Fetal liver
Heart
Muscle
Testis
Spinal Cord
Pancreas
Relative expression
1000
750
500
250
0
Normalized
6
735
42
108
8
16
3
1
1
17
8
2
Retina
Brain
Putamen
Sub Nigra
DRG
Cerebellum
Fetal liver
Heart
Muscle
Testis
Spinal Cord
Pancreas

Fig. 16

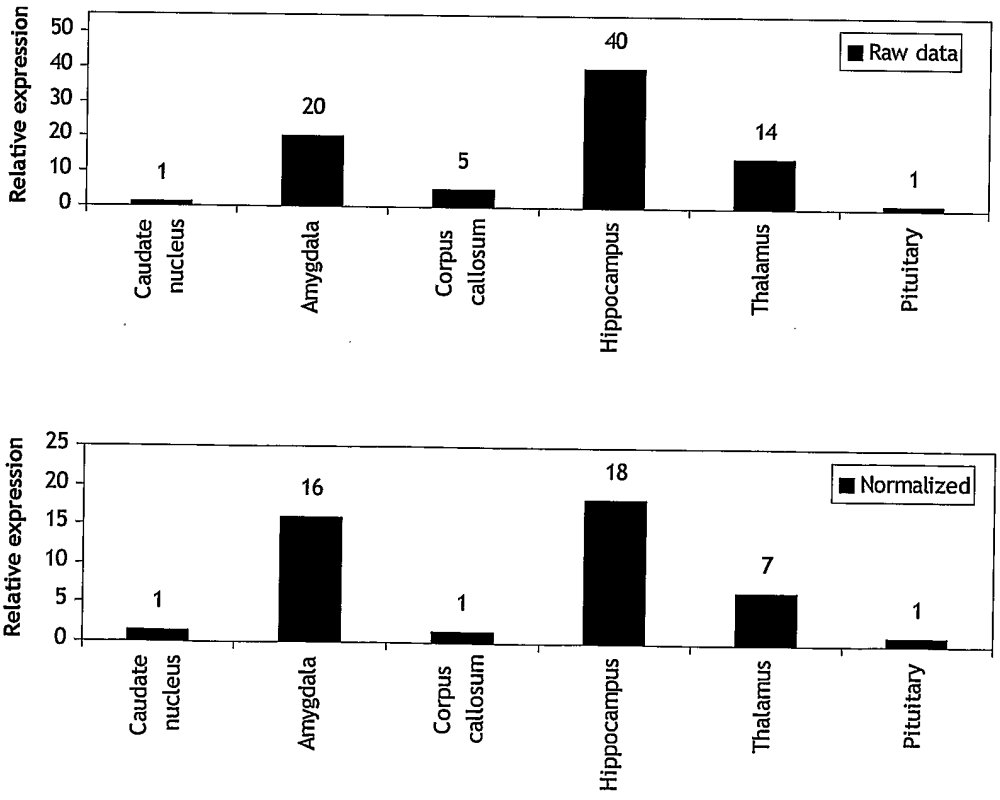

Relative expression
50
40
30
20
10
0
Raw data
1
20
5
40
14
1
Caudate nucleus
Amygdala
Corpus callosum
Hippocampus
Thalamus
Pituitary
Relative expression
25
20
15
10
5
0
Normalized
1
16
1
18
7
1
Caudate nucleus
Amygdala
Corpus callosum
Hippocampus
Thalamus
Pituitary

Fig. 17

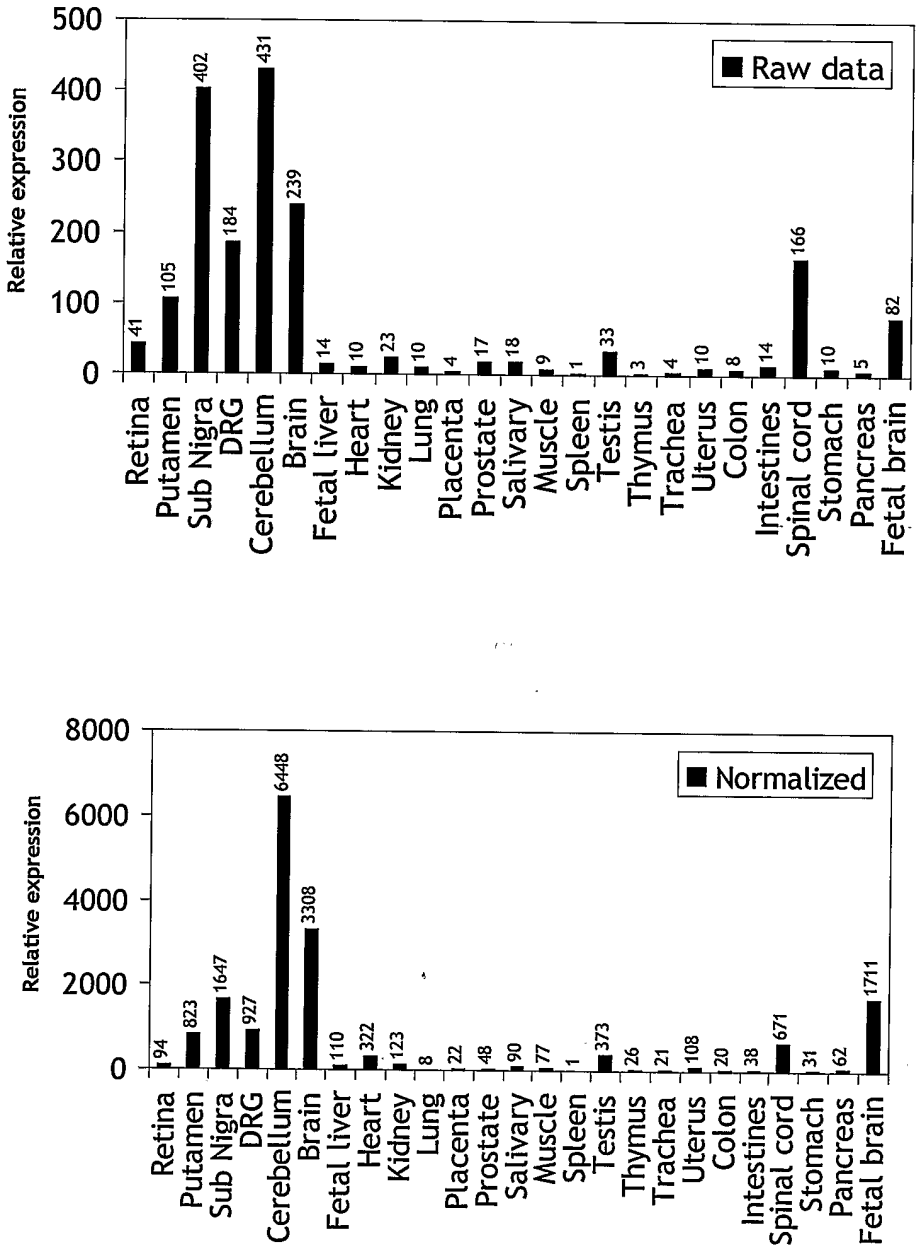

Relative expression
500
400
300
200
100
0
Raw data
41
105
402
184
431
239
14
10
23
10
4
17
18
9
1
33
3
4
10
8
14
166
10
5
82
Retina
Putamen
Sub Nigra
DRG
Cerebellum
Brain
Fetal liver
Heart
Kidney
Lung
Placenta
Prostate
Salivary
Muscle
Spleen
Testis
Thymus
Trachea
Uterus
Colon
Intestines
Spinal cord
Stomach
Pancreas
Fetal brain
Relative expression
8000
6000
4000
2000
0
Normalized
94
823
1647
927
6448
3308
110
322
123
8
22
48
90
77
1
373
26
21
108
20
38
671
31
62
1711
Retina
Putamen
Sub Nigra
DRG
Cerebellum
Brain
Fetal liver
Heart
Kidney
Lung
Placenta
Prostate
Salivary
Muscle
Spleen
Testis
Thymus
Trachea
Uterus
Colon
Intestines
Spinal cord
Stomach
Pancreas
Fetal brain

Fig. 18

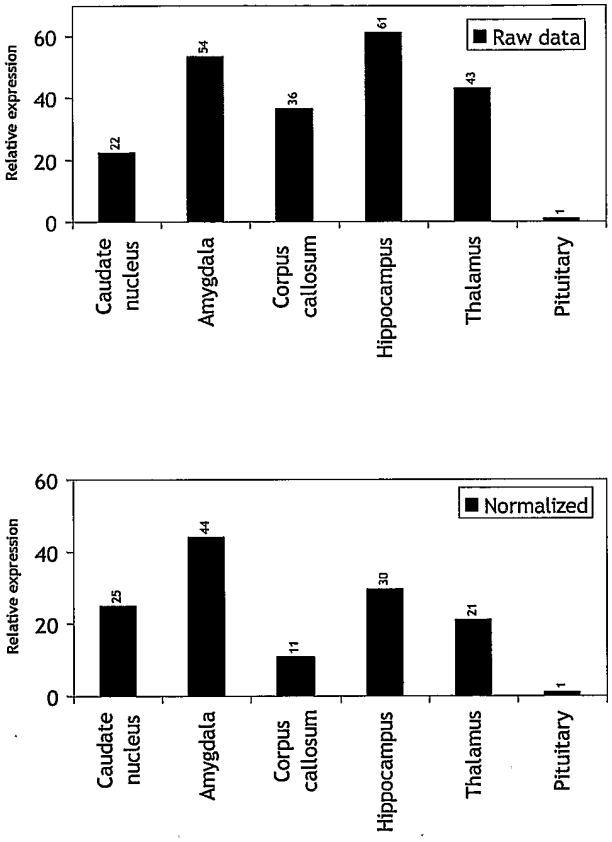

Raw data
Relative expression
60
40
20
0
22
54
36
61
43
1
Caudate nucleus
Amygdala
Corpus callosum
Hippocampus
Thalamus
Pituitary
Normalized
Relative expression
60
40
20
0
25
44
11
30
21
1
Caudate nucleus
Amygdala
Corpus callosum
Hippocampus
Thalamus
Pituitary

Fig. 19

```
   1 aattttcggg tcgacgattt cgtgggctcg gctccgcact gctagctgcg cgccgccctg gacgggggcgc accgactgcg cgcgcggctg cgggcaaaca
 101 tcgggagtcc tgcctcagct gccgcttctc cagcagcagc ttcaggcttc tcccgcagga gcttcgggct tctcctggta gagacgtggg aacttttctt
 201 ctcctggcga ggctgcagag gtgatgggcc gctcccgggg ctcccgcggg gaggcggcac ggtgagcgtc ctcgggctcc ggtgcggcga tcagtaccta
 301 gttccggacg cgccggtccg acttggatgc cggctcttct gagctacgat aatttttggg aacggcagaa atgattggtt ctagcaacag
 401 atgggaattt ggagtcactc tgaaatatat cctggaataa gtgtgtttga ctagaaccac atcttatgag gtcccccaagg atgagagtct gtgctaagtc
                                                                                  >>.....CDS.....>
                                                                                    m r v   c a k
 501 agtgttgctg tcgcactggc tctttctagc ctacgtgtta atggtgtgct gtaagctgat gtccgcctca agccagcacc tccggggaca tgcaggtcac
     >.....CDS.....>
     s v l l   s h w   l f l   a y v l   m v c   c k l   m s a a s   s q h   l r g   h a g h
 601 caccaaatca agcaagggac ctgtgaggtg gtcgccgtgc acaggtgctg caataagaac cgcatagaag agcggtcaca aacggtcaag tgctcttgct
     >.....CDS.....>
       h q i   k q g   t c e v   v a v   h r c   c n k n   r i e   e r s   q t v k   c s c
 701 tcccgggaca ggtggcgggc acaactcggg ctcaaccttc ttgtgttgaa gcttccattg tgattcagaa atggtggtgt cacatgaatc cgtgtttgga
     >.....CDS.....>
       f p g   q v a g   t t r   a q p   s c v e   a s i   v i q   k w w c   h m n   p c l
 801 aggagaggat tgtaaagtgc tgccagatta ctcaggttgg tcctgtagca gtggcaataa agtcaaaact acgaaggtaa cgcggtagcg aagagaggag
     >.....CDS.....>>
       e g e d   c k v   l p d   y s g w   s c s   s g n   k v k t   t k v   t r -
 901 tgtgcttcaa tcctggaggg gcagcaggag gcggagctct tttgcttgga ttcccataca tggccctct gcagaaattt gtctaggatt tcagcaactt
1001 catatttgta tattgtgagc tgtgagaagg tggcattcac ttaactggcc cagccctctc tgcttcgtga tttattttca ttgaattata accacaagcc
1101 acaacccatt tgacatcctc tctcggattc ccaaggagca tacctccaaa atccgagaag agcaaatcag agtcttcaaa atggatcacc actaagggca
1201 tgttcattct tcacttttct tctgctttta caaaagaact tggatgtatg ttccaaaggg tcctcattct gttcctcttt tgaacttttc ctttgtcct
1301 tgtattaaag tggttttaaa ggggtctaaa aagattttgg caaaacatat ttgcagatgt agattagctg gtgaagaaaa ttactgctag agatcaactg
1401 attaactggt aaagaacgtt tattttataa ccctgaaaga atagaaggac atagttggat tattgtgtgt gcattgtatt tttacttcta ttttttttt
1501 gctttccatt ttccagttag cagagataaa atgagagcgt tttaacttca atgtaccatt ttactgagtg ctaaggaagc atatcaattc caatatttta
1601 taaccaaagc tctatcagaa catatttata aaacttgttg gaatttttac ggctttggtg tagtcatgta ggtaaatcat ttaaaatata aaacaatctc
1701 aatttagatc aagggttatt tcttagatca aatttatgcc aattatatga aaagatttta actccgagac aggagtcttt cagtgctgaa ttttttagact
1801 gtaaatgagt tcttctttaac ttagctgttt ccctacttct gtgacttctg tgttagccat cttatttctt taaaatctga gtcctgattg gcttaatgat
1901 tttgcagcag acatgtctcc acatattctc aaatgctgtc atgcggaaac gtatgaaaca gatgaagaat gactgaccca gattttagat gtataatgtt
2001 gttaaagtac atactactgt aaaaatatgg gatgaatttt atatattaag aaatgccaaa aacatagttt ctgcaccaag ttaattatcc ctgtcctttc
2101 acatttatag ggggaaaata aatactttat agcctaacag ttatttgatg ttatttcttgc agagggaatg gaaaggaatg gaaagatttg ttggcgtaat
2201 ttttgaatat ttgttatgat catatgaata agtaaaaaaa ttcatcctgc tgatggcaaa aaaaaaaaaa aaaa
```

Fig. 20

```
   1  cagtgctgtg atcggcgctt cactctggac tcccggatct gactcggctg ctctttctag tctaacaggt cctccaagcc caagaagttt tgggggctg
 101  cagagatgat tggtcccagt tacaaaggga cattcacggt cactcttccag gaccttcttg agtaacagtg tttgactgga accatatact gtgaactctc
 201  caaggatgag agtctgtgct aagtgggtgc tgctgtcacg ctggctggtt ctgacctatg tgttaatggt ctgctgtaag ctgatgtccg cctcgagcca
                                                                                                          >>.....>
                                                                                                             m   r  v  v  c  a   k  w  v   l  l  s   r  w  l  v   l  t  y   v  l  m   v  c  c  k   l  m  s   a  s  s
 301  gcacctccgg ggacatgcag gtcaccacct gatcaagcca ggtacctgcg aggtggtcgc cgtgcaccgc tgctgcaata agaaccgcat agaggagcgc
      >.....>
      q  h  l  r   g  h  a   g  h  h   l  i  k  p   g  t  c   e  v  v   a  v  h  r   c  c  n   k  n  r   i  e  e  r
 401  tctcagacgg tcaaatgttc ctgcttccca ggccaggtgg ctggcacaac tcgggcacag ccttcttgtg ttgaagctgc cattgtgatt gagaagtggt
      >.....>
        s  q  t   v  k  c   s  c  f  p   g  q  v   a  g  t   t  r  a  q   p  s  c   v  e  a   a  i  v  i   e  k  w
 501  ggtgccacat gaatccttgc ttggaaggag aggactgtaa agtgcttcca gactcttcgg gttggtcctg tagcagtggc aataaagtca aaaccaccaa
      >.....>
        w  c  h   m  n  p  c   l  e  g   e  d  c   k  v  l  p   d  s  s   g  w  s   c  s  s  g   n  k  v   k  t  t
 601  ggtgacccgg tagcagacac aaaagtgttt tgaccccgag gggcatcggg acgcagagct gtgctgtgtg gattaccaga ctagtgagaa ctcactcacc
      >....CDS....>>
      k  v  t  r  -
 701  ttggaacttg ctcagaagtt catagccaaa atatgtgcag tgtggaaggt ggcatttacg tggctgttcc cgtttgtctg ctttgtgatc tcatcacact
 801  gaactacagc ttaggtggcc actcaactga ggtgttacct ggatctcaaa aggagcctgc ctagcacaag aaatgctgga gaggacatcg gaggtgctaa
 901  t
```

Fig. 21

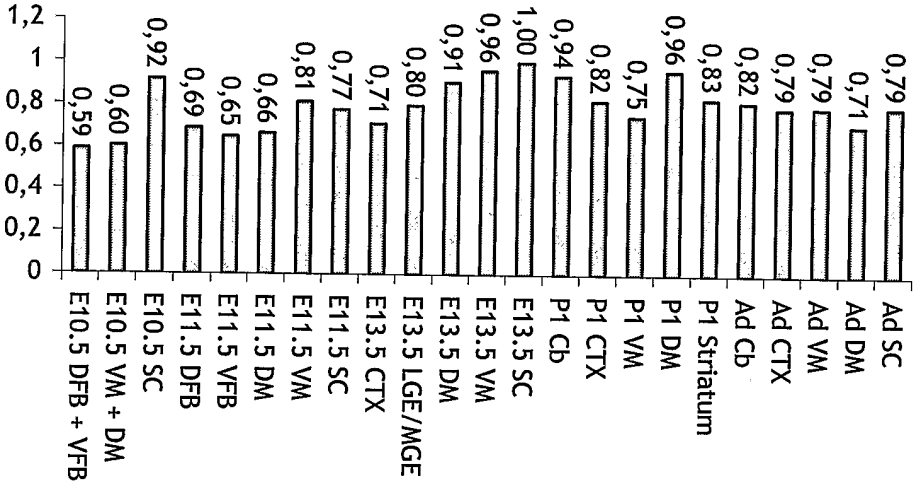


Fig. 22A

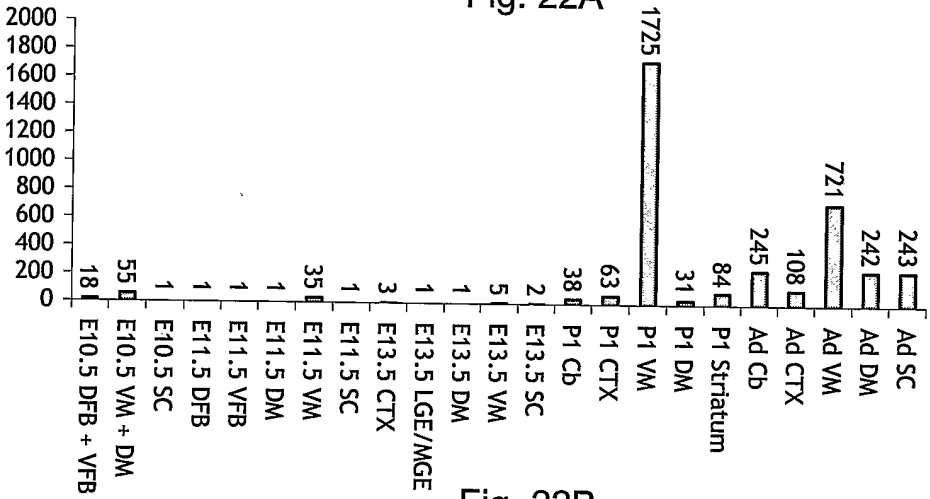


Fig. 22B

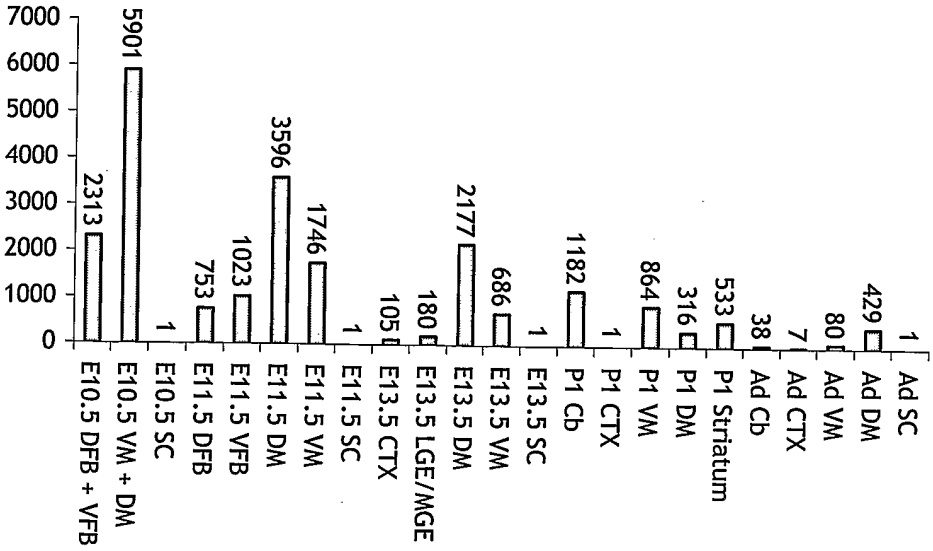


Fig. 22C

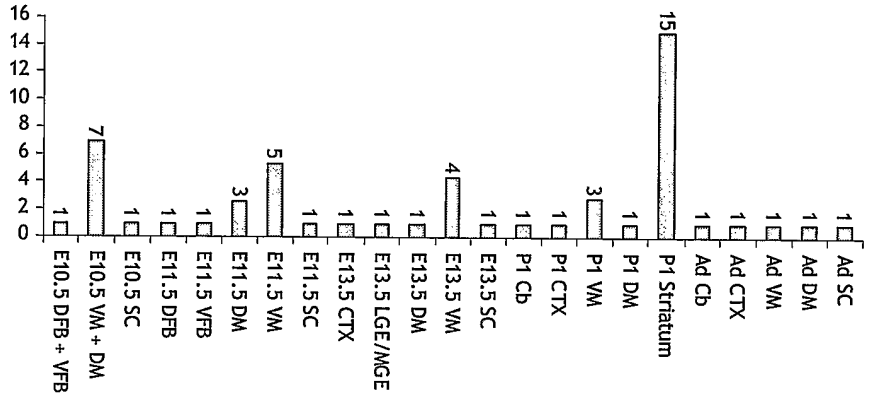

16
14
12
10
8
6
4
2
0
1
7
1
1
1
3
5
1
1
1
4
1
1
3
1
15
1
1
1
1
1
E10.5 DFB + VFB
E10.5 VM + DM
E10.5 SC
E11.5 DFB
E11.5 VFB
E11.5 DM
E11.5 VM
E11.5 SC
E13.5 CTX
E13.5 LGE/MGE
E13.5 DM
E13.5 VM
E13.5 SC
P1 Cb
P1 CTX
P1 VM
P1 DM
P1 Striatum
Ad Cb
Ad CTX
Ad VM
Ad DM
Ad SC

Fig. 23

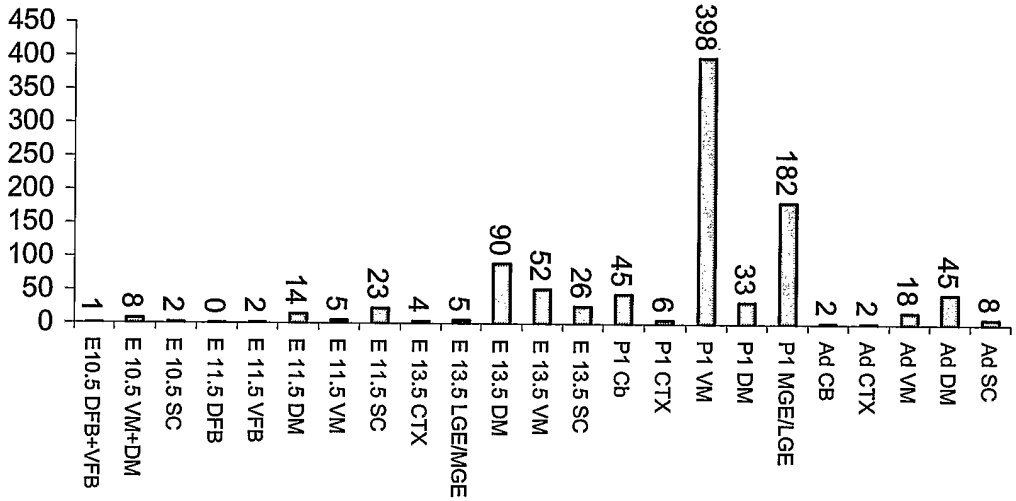

450
400
350
300
250
200
150
100
50
0
1
8
2
0
2
14
5
23
4
5
90
52
26
45
6
398
33
182
2
2
18
45
8
E10.5 DFB+VFB
E 10.5 VM+DM
E 10.5 SC
E 11.5 DFB
E 11.5 VFB
E 11.5 DM
E 11.5 VM
E 11.5 SC
E 13.5 CTX
E 13.5 LGE/MGE
E 13.5 DM
E 13.5 VM
E 13.5 SC
P1 Cb
P1 CTX
P1 VM
P1 DM
P1 MGE/LGE
Ad CB
Ad CTX
Ad VM
Ad DM
Ad SC

Fig. 24

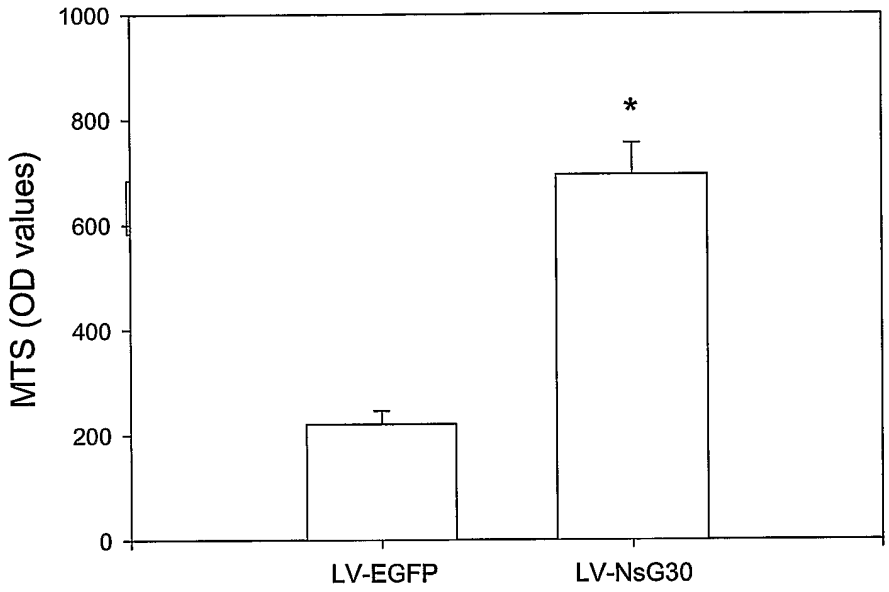


Fig. 25

```
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 101 aaaagctgca ggagcatcgc taggtgttgc cgccaccggg aagcgggggct gcaggatgag taagagatac ttacagaaag caacaaaagg aaaactgcta
                                                                                  >>.....CDS.....>
                                                                                    m   s  k  r  y   l  q  k   a  t  k   g  k  l  l
 201 ataataatat ttattgtaac cttgtgggggg aaagttgtat ccagtgcaaa ccatcataaa gctcaccatg ttaaaacggg aacttgtgag gtggtggcac
     >.....CDS.....>
       i  i  i   f  i  v   t  l  w  g   k  v  v   s  s  a   n  h  h  k   a  h  h   v  k  t   g  t  c  e   v  v  a
 301 tccacagatg ctgtaataag aacaagatag aagaacggtc acaaacagtc aagtgctcct gcttccctgg gcaggtggca ggcaccacgc gagctgctcc
     >.....CDS.....>
       l  h  r   c  c  n  k   n  k  i   e  e  r   s  q  t  v   k  c  s   c  f  p   g  q  v  a   g  t  t   r  a  a
 401 atcatgtgtg gatgcttcaa tagtggaaca gaaatggtgg tgccatatgc agccatgtct agagggagaa gaatgtaaag ttcttccgga tcggaaagga
     >.....CDS.....>
       p  s  c  v   d  a  s   i  v  e   q  k  w  w   c  h  m   q  p  c   l  e  g  e   e  c  k   v  l  p   d  r  k  g
 501 tggagctgtt cctctgggaa taaagtcaaa acaactaggg taacccatta acccaggaga aatcaagtga tcctcaaggc tgatgacatt gaacatgcgc
     >.....CDS.....>>
       w  s  c   s  s  g   n  k  v  k   t  t  r   v  t  h   -
 601 atagaaactt aactcaactc ctgaggtgat cttgaagatt tttataccac ttgaaagagg cgctcaatag t
```

Fig. 26

```
   1  cacacaccgg cggacttgag gtccctatgc ccctggatgg tgccagcgga ccggcatctc ggaagcgatg caggagcggt gaggctggcg tcggcccgcg
 101  gaagctaccg gaccatcgct aggtgctgcc gcccccggga cgcccggctg cagggtctct cactggacgt ggaaataagc tggtgaccag caagccctag
 201  cgtctctgtc cagtgactgt tacaggagca caggaccatg ctgcccatca gttttatgtg gacactgggg ctgaactggt acctgtgctt gtgcagcgag
 301  ggctcttacc cactgcgctg tctcttcagc ttgcaatgag tatcttattca gtgaatgatc accaagatga ataagagata cttgcagaaa gcaacacaag
                                                          >>>.....................CDS.....................>
                                                                m   n  k  r   y  l  q  k   a  t  q
 401  gaaagcttct gataattatt tttatagtga ccttgtgggg gaaagccgtt tccagcgcca accatcacaa agctcaccat gttagaactg ggacttgcga
      >.....................................CDS.....................................>
        g  k  l   l  i  i  i   f  i  v   t  l  w   g  k  a  v   s  s  a   n  h  h   k  a  h  h   v  r  t   g  t  c
 501  ggttgtggcg ctgcacagat gctgtaataa gaacaagata gaagaacggt cccaaacggt caagtgctcc tgcttccctg ggcaggtggc aggcactacc
      >.....................................CDS.....................................>
      e  v  v  a   l  h  r   c  c  n   k  n  k  i   e  e  r   s  q  t   v  k  c  s   c  f  p   g  q  v   a  g  t  t
 601  cgagctgctc cgtcttgtgt ggatgcatcc atagtggaac aaaagtggtg gtgtcatatg cagccatgcc tggagggaga ggaatgtaaa gtccttccag
      >.....................................CDS.....................................>
        r  a  a   p  s  c   v  d  a  s   i  v  e   q  k  w   w  c  h  m   q  p  c   l  e  g   e  e  c  k   v  l  p
 701  atcgcaaagg atggagctgt tcctctggaa acaaagtaaa aacaactagg gtaacccatt aaccactcct aaatcaaata atactgatgg ctgggttcat
      >.....................................CDS.....................>>
        d  r  k   g  w  s  c   s  s  g   n  k  v   k  t  t  r   v  t  h   -
 801  ggagacacaa gaatagaaac tggactcctg ccgtgacctt gaagattttt atactgctta gaagagacat ttaaaatcca tttccaagga attctatggc
 901  ttttcatcta cttcttagtg aaactaagac tttacagtga acgttgggtc ctgaagactt catccgctgt gaactaacgc ttggctcaca
1001  acacttcttga gggaaggggg cgcagttctc cacggagctg ggatagagtt ggttttctgg ggtgacacca gagactgtca cctttaaggt tccttgcttc
1101  a
```

Fig. 27

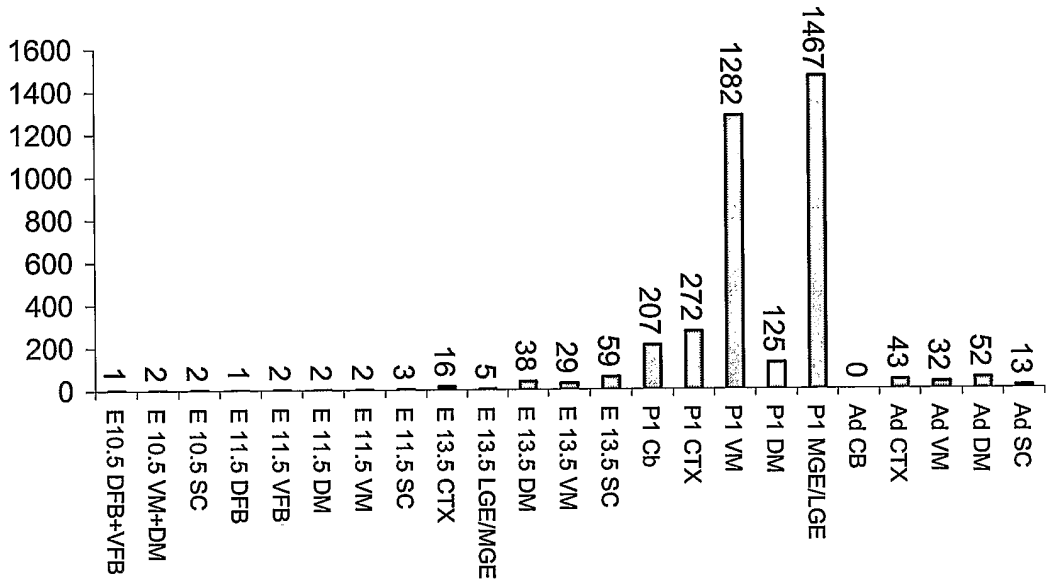

1600
1400
1200
1000
800
600
400
200
0
1
2
2
1
2
2
2
3
16
5
38
29
59
207
272
1282
125
1467
0
43
32
52
13
E10.5 DFB+VFB
E 10.5 VM+DM
E 10.5 SC
E 11.5 DFB
E 11.5 VFB
E 11.5 DM
E 11.5 VM
E 11.5 SC
E 13.5 CTX
E 13.5 LGE/MGE
E 13.5 DM
E 13.5 VM
E 13.5 SC
P1 Cb
P1 CTX
P1 VM
P1 DM
P1 MGE/LGE
Ad CB
Ad CTX
Ad VM
Ad DM
Ad SC

Fig. 28

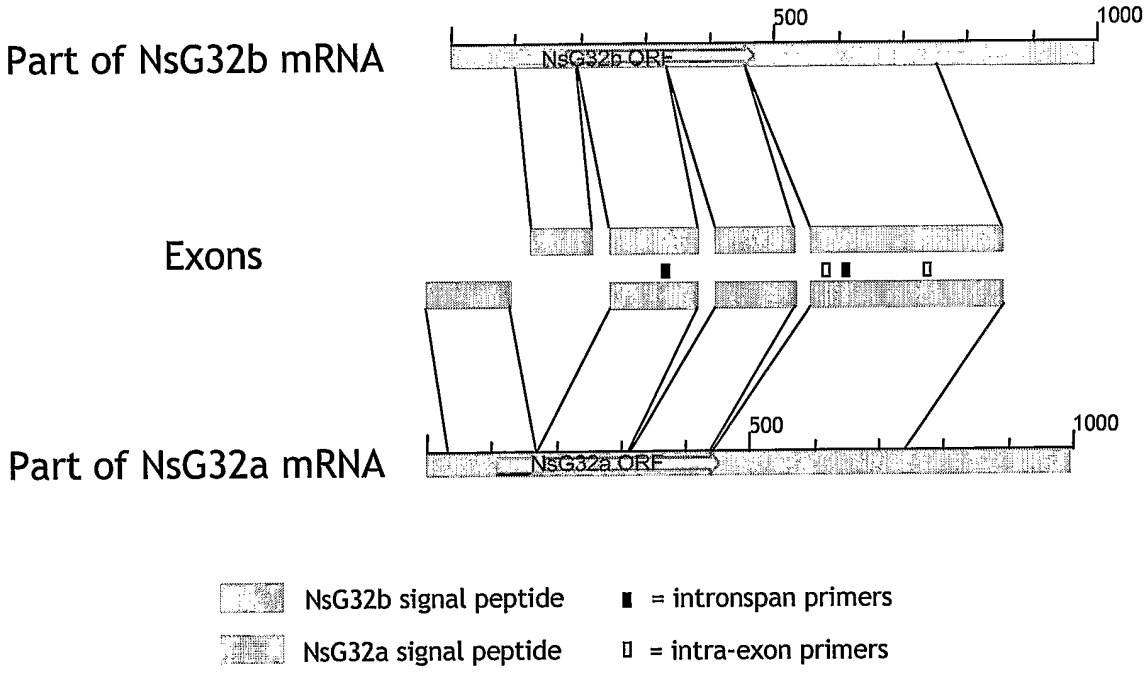

Part of NsG32b mRNA
500
1000
NsG32b ORF
Exons
Part of NsG32a mRNA
500
1000
NsG32a ORF
NsG32b signal peptide
NsG32a signal peptide
= intronspan primers
= intra-exon primers

Fig. 29

```
   1 ggcggcggag gatggcgcgc gcggggcccg cacgtggagg ccggcgcggg ggcgcgggca gggccggctg ctgagacgcg ctgctgcccc ccgcgcgggc
 101 gccgcggctt caatggcgcc atcgcccagg accggcagcc ggcaagatgc gaccgccctg cccagcatgt cctcaacttt ctgggcgttc atgatcctgg
                                                                                 >>.....................CDS.....................>
                                                                                    m   s   s   t   f   w   a   f   m   i   l
 201 ccagcctgct catcgcctac tgcagtcagc tggccgccgg cacctgtgag attgtgacct tggaccggga cagcagccag cctcggagga cgatcgcccg
 >.....................................................CDS.....................................................>
      a   s   l   l   i   a   y   c   s   q   l   a   a   g   t   c   e   i   v   t   l   d   r   d   s   s   q   p   r   r   t   i   a
 301 gcagaccgcc cgctgtgcgt gtagaaaggg gcagatcgcc ggcaccacga gagcccggcc cgcctgtgtg gacgcaagaa tcatcaagac caagcagtgg
 >.....................................................CDS.....................................................>
      r   q   t   a       r   c   a       c   r   k       g   q   i   a       g   t   t       r   a   r       p   a   c   v       d   a   r       i   i   k       t   k   q   w
 401 tgtgacatgc ttccgtgtct ggaggggaaa ggctgcgact tgttaatcaa ccggtcaggc tggacgtgca cgcagcccgg cgggaggata aagaccacca
 >.....................................................CDS.....................................................>
        c   d   m       l   p   c       l   e   g   e       g   c   d       l   l   i       n   r   s   g       w   t   c       t   q   p       g   g   r   i       k   t   t
 501 cggtctcctg acaaacacag cccctgaggg ggccccggga gtggccttgg ctccctggag agcccacgtc tcagccacag ttctccactc gcctcggact
 >...CDS...>>
      t   v   s   -
 601 tcacccgttc tctgccgccc gcccactccg ttccctgtg gtccgtgaag gacggcctca ggccttggca tcctgagctt cggtctgtcc agccgacccg
 701 aggaggccgg actcagacac ataggcgggg ggcggcacct ggcatcagca atacgcagtc tgtgggagcc cggccgcgcc cagcccccgc cgaccgtggc
 801 gttggccctg ctgtcctcag aggaggagga ggaggaggca gctccggcag ccacagaagg ctgcagccca gcccgcctga gacacgacgc ctgccccagg
 901 ggactgtcag gcacagaagc ggcctcctcc cgtgccccag actgtccgaa ttgctttttat tttcttatac ttcagtatac ctcccatagac caaagagcaa
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1101 ggagcggaga gggcggccgt ggtggaggcc tccaccccag gagcaccccg cacaccctcg gaggacgggc ttcggctgcg cggaggccgt ggcacacctg
1201 cgggaggcag cgacggcccc cacgcagacg ccgggaacgc aggccgcttt attcctctgt acttagatca acttgaccgt actaaaatcc ctttctgttt
1301 taaccagtta aacatgcctc ttctacagct tagttggata atccagtatc tgccaagagc atgttgggtc tcccgtgact gctgcctcat
1401 cgataccccca tttagctcca gaaagcaaag aaaactcgag taacacttgt ttgaaagaga tcattaaatg tattttgcaa agcccaaaaa aaaaaaaaaa
1501 a
```

Fig. 30A

```
   1  ggcggcggag gatggcgcgc gcggggcccg cacgtggagg ccggcgcggg ggcgcgggca gggccggctg ctgagacgcg ctgctgcccc ccgcgcgggc
 101  gccgcggctt caatggcgcc atcgcccagg accggcagcc ggcaagatgc gaccgccctg cccagcatgt cctcaacttt ctgggcgttc atgatcctgg
                >>.........................................CDS.............................................>
                  m  a   p  s  p  r   t  g  s   r  q  d   a  t  a  l   p  s  m   s  s  t   f  w  a  f   m  i  l
 201  ccagcctgct catcgcctac tgcagtcagc tggccgccgg cacctgtgag attgtgacct tggaccggga cagcagccag cctcggagga cgatcgcccg
>..................................................CDS...............................................>
        a  s  l   l  i  a  y   c  s  q   l  a  a   g  t  c  e   i  v  t   l  d  r   d  s  s  q   p  r  r   t  i  a
 301  gcagaccgcc cgctgtgcgt gtagaaaggg gcagatcgcc ggcaccacga gagcccggcc cgcctgtgtg gacgcaagaa tcatcaagac caagcagtgg
      >...............................................CDS..............................................>
      r  q  t  a   r  c  a   c  r  k   g  q  i  a   g  t  t   r  a  r   p  a  c  v   d  a  r   i  i  k   t  k  q  w
 401  tgtgacatgc ttccgtgtct ggagggggaa ggctgcgact tgttaatcaa ccggtcaggc tggacgtgca cgcagcccgg cgggaggata aagaccacca
      >...............................................CDS..............................................>
        c  d  m   l  p  c   l  e  g  e   g  c  d   l  l  i   n  r  s  g   w  t  c   t  q  p   g  g  r  i   k  t  t
 501  cggtctcctg acaaacacag cccctgaggg ggccccggga gtggccttgg ctccctggag agcccacgtc tcagccacag ttctccactc gcctcggact
      >.........>>
       t  v  s   -
 601  tcacccgttc tctgccgccc gcccactccg tttccctgtg gtccgtgaag gacggcctca ggccttggca tcctgagctt cggtctgtcc agccgacccg
 701  aggaggccgg actcagacac ataggcgggg ggcggcacct ggcatcagca atacgcagtc tgtgggagcc cggccgcgcc cagcccccgc cgaccgtggc
 801  gttggccctg ctgtcctcag aggaggagga ggaggaggca gctccggcag ccacagaagg ctgcagccca gcccgcctga gacacgacgc ctgccccagg
 901  ggactgtcag gcacagaagc ggcctcctcc cgtgccccag actgtccgaa ttgcttttat tttcttatac tttcagtata ctccatagac caaagagcaa
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1101  ggagcggaga gggcggccgt ggtggaggcc tccaccccag gagcaccccg cacaccctcg gaggacgggc ttcggctgcg cggaggccgt ggcacacctg
1201  cgggaggcag cgacggcccc cacgcagacg ccgggaacgc aggccgcttt attcctctgt acttagatca acttgaccgt actaaaatcc ctttctgttt
1301  taaccagtta aacatgcctc ttctacagct ccatttttga tagttggata atccagtatc tgccaagagc atgttgggtc tcccgtgact gctgcctcat
1401  cgatacccca tttagctcca gaaagcaaag aaaactcgag taacacttgt ttgaaagaga tcattaaatg tattttgcaa agcccaaaaa aaaaaaaaaa
1501  a
```

Fig. 30B

```
   1  ggcgggcacc gcggcttcaa tggcgccatc gcccaggacc agcagccggc aagatgcgac cgccctgccc agcatgtcct caacttttg ggcattcatg
                                                                                  >>.....CDS.....>
                                                                                    m  s   s  t  f   w  a  f  m
 101  atcctggcca gcctgctcat cgcctactgc agtcagctgg ccgctggaac ctgtgagatt gtgaccctag accgggacag cagccagcca cggaggacga
      >.....CDS.....>
        i  l  a   s  l  l   i  a  y  c   s  q  l   a  a  g   t  c  e  i   v  t  l   d  r  d   s  s  q  p   r  r  t
 201  tcgcccggca gacagcacgc tgtgcatgca gaaaggggca gatagcaggc accactcgag cccggcctgc ttgtgtggac gctcgaatta tcaagacaaa
      >.....CDS.....>
        i  a  r   q  t  a  r   c  a  c   r  k  g   q  i  a  g   t  t  r   a  r  p   a  c  v  d   a  r  i   i  k  t
 301  gcagtggtgt gacatgcttc cttgcctgga ggggaaggc tgtgacttgt taatcaaccg gtcaggctgg acttgcacac agcccggagg gcggataaag
      >.....CDS.....>
        k  q  w  c   d  m  l   p  c  l   e  g  e  g   c  d  l   l  i  n   r  s  g  w   t  c  t   q  p  g   g  r  i  k
 401  accaccacgg tctcctgaca atcgcagccc ctgcggtgcc ccctgggagt ggctgtggct ccctggaaat tctacacctc agccacagtc ctgcatccta
      >.....CDS.....>>
        t  t  t   v  s  -
 501  tgtggacacc gctcattctc ccccatccac ccactgtttc ccagaggcct aacaaagaca gcccaattcc tcggagctct gagctcaagt ctgtccagct
 601  gaacccagga ggctgggact cagacacaca tgtcaagggt ggtacctgga atcagcaata cctggtgtgt ggagcctggc tgcatccatc ctcactagcc
 701  gcttgctccc tggagttgga ggaccggaac aaggaaattg tggcccggcc agctgggcca caacgcaagg tccggggact gtcaggtgca gaagccacca
 801  ccccctctgt cccagactgt ctgaatcgct ttatatttct tatactttca gtatactcaa tagaccaaag agcaaatcta ttttaacgtg gacacatccc
 901  tgctgccaag agttccctgg gtttgattgt ggggcagggg agggcgcagt ggcagacatg ctggtggccc aggcagcatg gagagggcat ctgaggccaa
1001  gaccccccgcc ccacgaacac tgcagcgtgc cctaagagga tggctttgcc cagaaggaca ccaccgtggt gcacctgcag gacccagcag gggcatggcc
1101  ggcgacggcc cccagcggac agcaagggcc cccggacaca gacccttggc ccctgtgctt agaccaactc tatcgtacta aaatcacttt ctgttttaac
1201  cagttagaca tgcctcttcc acagctccat tttgatagt tggataatcc accatttgcc aagagcatgc tgggtctcct gtgactgcta tctcatctga
1301  tacaccattt agctccagaa agcaaattaa agaaaactga agc
```

Fig. 31

```
   1  gcggccgcgg aggctgcacc cggcggggcg ctgatgcggc gcctggacct tcgctgcgcg acttcggggg cgtcggccga gttgggactc cgcgatgcag
                                                                                                CDS >>...>
                                                                                                      m  q
 101  ctcctgaagg cgctctgggc actggcaggg gccgcgctct gctgcttcct cgtcctagtg atccacgcgc agttcctcaa agaaggtcag ctggccgccg
      >.....................................................CDS.....................................................>
        l  l  k   a  l  w   a  l  a  g    a  a  l    c  c  f   l  v  l  v    i  h  a    q  f  l    k  e  g  q    l  a  a
 201  gcacctgtga gattgtgacc ttggaccggg acagcagcca gcctcggagg acgatcgccc ggcagaccgc ccgctgtgcg tgtagaaagg ggcagatcgc
      >.....................................................CDS.....................................................>
         g  t  c   e  i  v  t   l  d  r   d  s  s    q  p  r  r   t  i  a    r  q  t    a  r  c  a    c  r  k   g  q  i
 301  cggcaccacg agagcccggc ccgcctgtgt ggacgcaaga atcatcaaga ccaagcagtg gtgtgacatg cttccgtgtc tggaggggga aggctgcgac
      >.....................................................CDS.....................................................>
      a  g  t  t   r  a  r   p  a  c   v  d  a  r   i  i  k   t  k  q   w  c  d  m   l  p  c   l  e  g   e  g  c  d
 401  ttgttaatca accggtcagg ctggacgtgc acgcagcccg gcgggaggat aaagaccacc acggtctcct gacaaacaca gcccctgagg ggccccggga
      >.....................................CDS.....................................>>
        l  l  i   n  r  s   g  w  t  c   t  q  p   g  g  r   i  k  t  t   t  v  s   -
 501  gtggccttgg ctccctggag agcccacgtc tcagccacag ttctccactc gcctcggact tcacccgttc tctgc
```

Fig. 32



Fig. 33

SUBSTITUTE SHEET (RULE 26)

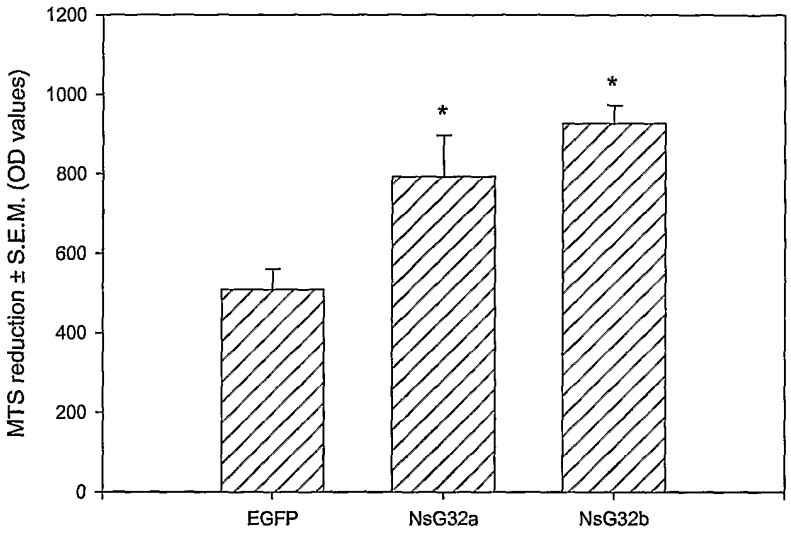

MTS reduction ± S.E.M. (OD values)
1200
1000
800
600
400
200
0
*
*
EGFP
NsG32a
NsG32b

Fig. 34

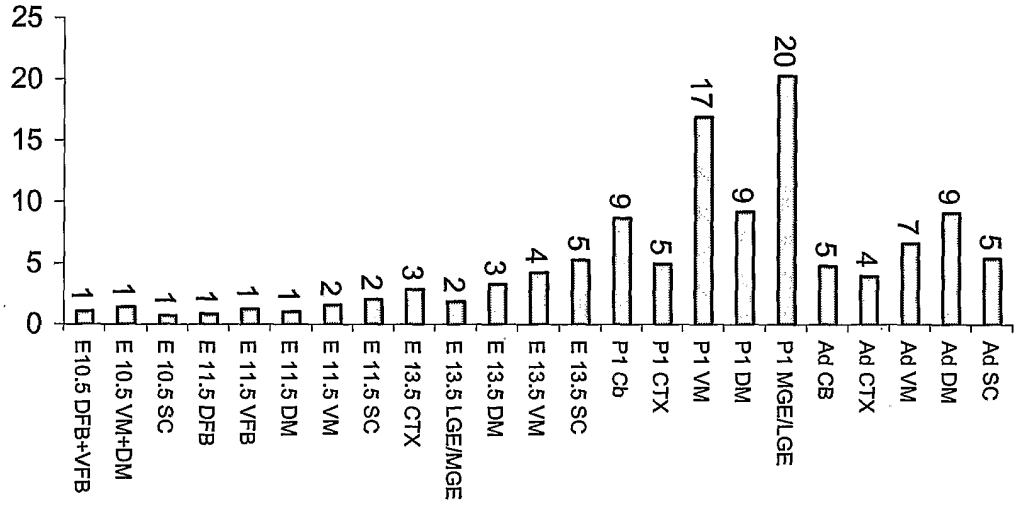
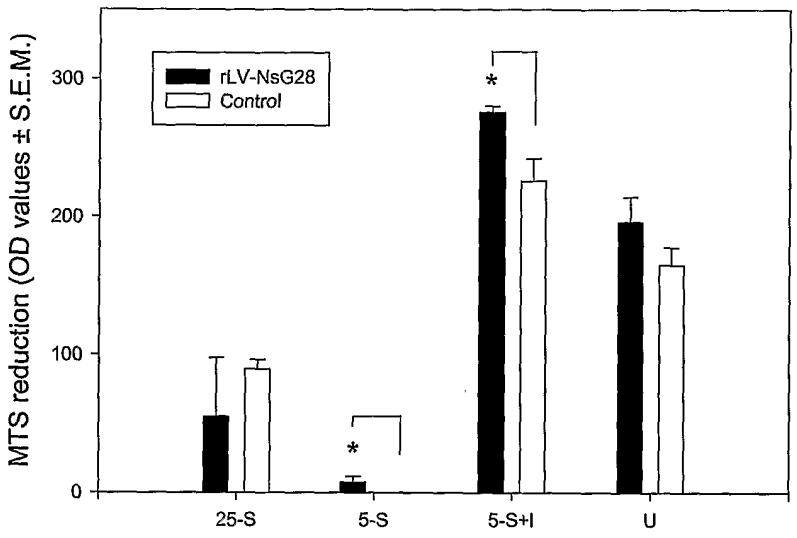
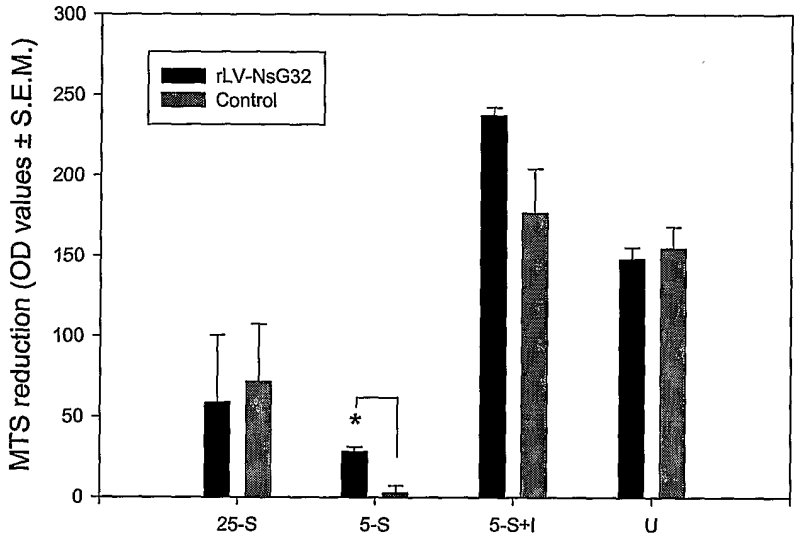

25
20
15
10
5
0
1
1
1
1
1
1
2
2
3
2
3
4
5
9
5
17
9
20
5
4
7
9
5
E10.5 DFB+VFB
E 10.5 VM+DM
E 10.5 SC
E 11.5 DFB
E 11.5 VFB
E 11.5 DM
E 11.5 VM
E 11.5 SC
E 13.5 CTX
E 13.5 LGE/MGE
E 13.5 DM
E 13.5 VM
E 13.5 SC
P1 Cb
P1 CTX
P1 VM
P1 DM
P1 MGE/LGE
Ad CB
Ad CTX
Ad VM
Ad DM
Ad SC

Fig. 35


MTS reduction (OD values ± S.E.M.)
rLV-NsG28
Control
300
200
100
0
*
*
25-S
5-S
5-S+I
U


MTS reduction (OD values ± S.E.M.)
rLV-NsG32
Control
300
250
200
150
100
50
0
*
25-S
5-S
5-S+I
U

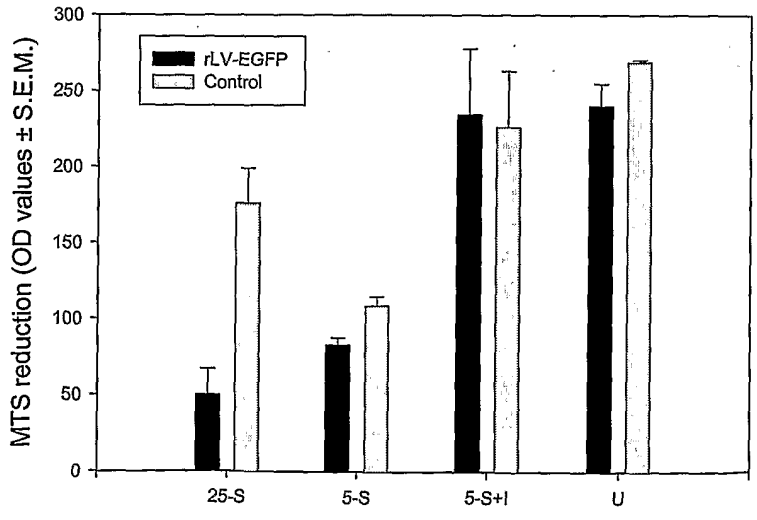

MTS reduction (OD values ± S.E.M.)
rLV-EGFP
Control
300
250
200
150
100
50
0
25-S
5-S
5-S+I
U

Fig. 36