



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/12, C07K 14/705, C12Q 1/68, C07K 16/28, A61K 31/70, 35/00, 38/00, 38/17, 48/00, G01N 33/53, 33/68	A1	(11) International Publication Number: WO 98/36062 (43) International Publication Date: 20 August 1998 (20.08.98)
(21) International Application Number: PCT/GB98/00434 (22) International Filing Date: 12 February 1998 (12.02.98) (30) Priority Data: 9703011.8 13 February 1997 (13.02.97) GB 97305485.1 22 July 1997 (22.07.97) EP <i>(34) Countries for which the regional or international application was filed:</i> AT et al. (71) Applicant (for all designated States except US): SMITHKLINE BEECHAM PLC [GB/GB]; New Horizons Court, Brentford, Middlesex TW8 9EP (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): TERRETT, Jonathan, Alexander [GB/GB]; SmithKline Beecham Pharmaceuticals, New Frontiers Science Park South, Third Avenue, Harlow, Essex CM19 5AW (GB). KENWRICK, Susan, Jane [GB/GB]; The University of Cambridge, School of Clinical Medicine, Dept. of Medicine, Addenbrookes Hospital, Level 5, P.O. Box 157, Hill Road, Cambridge, Cambridgeshire CB2 2QQ (GB). WANG, Bo [CN/CN]; The University of Cambridge, School of Clinical Medicine, Dept. of	Medicine, Addenbrookes Hospital, Level 5, P.O. Box 157, Hill Road, Cambridge, Cambridgeshire CB2 2QQ (GB). (74) Agent: CONNELL, Anthony, Christopher; SmithKline Beecham, Corporate Intellectual Property, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB). (81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: NEURAL CELL ADHESION MOLECULE SPLICING VARIANTS		
(57) Abstract <p>NrCAMvar polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing NrCAMvar polypeptides and polynucleotides in the design of protocols for the treatment of diabetes, obesity and cancer, among others, and diagnostic assays for such conditions.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

NEURAL CELL ADHESION MOLECULE SPLICING VARIANTS

FIELD OF INVENTION

5 This invention relates to newly identified splice-variant polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to the cell adhesion molecule family, hereinafter referred to as NrCAMvar. The invention also relates to inhibiting or activating the action of such polynucleotides and
10 polypeptides.

BACKGROUND OF THE INVENTION

 The NgCAM-related cell adhesion molecule NrCAM, also called bravo, was first identified and characterised in chick by Grumet *et al.*, (1991). Although the sequence of
15 rat NrCAM is not published, it has been cloned and sequenced (Davis and Bennett, 1994). This cell surface glycoprotein is a member of the immunoglobulin (Ig) superfamily, and is very similar in structure to chick NgCAM, human and mouse L1 and chick neurofascin. Each consists of six Ig domains, five fibronectin type III-like repeats, a transmembrane domain and an intracellular region. These neural cell surface proteins play a critical role
20 in nervous system development. Studies from Bennett *et al.*, (Bennett and Gilligan, 1993; Davis and Bennett, 1994) suggested that these molecules, including chick and rat NrCAM, have ankyrin binding activity suggesting that they may be important in membrane-cytoskeletal connections in brain. A role for NrCAM in the *in vivo* guidance of chick commissural neurones has been identified and distinguished from that of NgCAM (Stoeckli
25 and Landmesser, 1995). Chick NrCAM in floor plate cells together with axonin-1 on commissural growth cones is essential for accurate pathfinding at the midline whereas NgCAM is required for fasciculation of the commissural neurites. As well as interacting with axonin-1, NrCAM can also bind at the cell surface with F11, another member of the Ig superfamily (Morales *et al.*, 1993).

30 Recently a highly conserved human homologue to chick NrCAM was described (Lane *et al.*, Genomics 35 (3), 456-465 (1996)) with 82% amino acid identity to the chick protein. The transmembrane and intracellular domains of human NrCAM are 100% identical to the chick homologue while percent identities for individual extracellular domains vary from 66% for IgVI to 93% for IgIV. Lane *et al.* identified two alternatively
35 spliced exons, AE12 encoding a 12-amino-acid section 5' to FNIII-5, and AE93 encoding the 93-amino-acids corresponding to the whole of FNIII-5 (Figure 2). Four different isoforms were found: with both AE12 and AE93, with only AE12 or AE93, and without

either AE12 or AE93. In addition to AE12 and AE93, two more splice variants have been identified in chick, AE19 and AE10. AE19 encodes a 19-amino-acid section between IgII and IgIII while AE10 is a 10 amino-acid section between IgVI and FNIII-1 (Grumet *et al.*, 1991). Using human NrCAM probes, Lane *et al.* observed one major RNA band of ~7.0kb in multiple brain tissues including amygdala, caudate nucleus, corpus callosum, hippocampus, hypothalamus, substantia nigra, subthalamic nucleus, and thalamus. In chick, the same size of RNA was found in brain tissue but not in embryo heart, gizzard or liver on Northern blots.

This indicates that these cell adhesion molecules have an established importance in vertebrate development and are consequently candidates for therapeutic targets. Clearly there is a need for identification and characterization of further members and variants, including splice variants, of the cell adhesion molecule family which can play a role in preventing, ameliorating or correcting dysfunctions or diseases.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to NrCAMvar polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such NrCAMvar polypeptides and polynucleotides. Such uses include the treatment of diabetes, obesity and cancer, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with NrCAMvar imbalance with the identified compounds, including diabetes, obesity and cancer. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate NrCAMvar activity or levels, including diabetes, obesity and cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide and deduced amino acid sequence of a human NrCAMvar; SEQ ID NOS: 1 and 2, respectively.

Figure 2 shows a comparison of the sequences of human NrCAMvar of the present invention and human and chick NrCAM cDNAs.

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"NrCAMvar" refers, among others, generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 or an allelic variant thereof.

5 "NrCAMvar activity or NrCAMvar polypeptide activity" or "biological activity of the NrCAMvar or NrCAMvar polypeptide" refers to the metabolic or physiological function of said NrCAMvar including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said NrCAMvar.

"NrCAMvar gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

10 "Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from
15 its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or
20 polydeoxiribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-
25 stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons.

"Modified" bases include, for example, tritylated bases and unusual bases such as inosine.
30 A variety of modifications have been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

35 "Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres.

“Polypeptide” refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. “Polypeptides” include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, 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. See, for instance, *PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., *Posttranslational Protein Modifications: Perspectives and Prospects*, pgs. 1-12 in *POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS*, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, “Analysis for protein modifications and nonprotein cofactors”, *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, “Protein Synthesis: Posttranslational Modifications and Aging”, *Ann NY Acad Sci* (1992) 663:48-62.

“Variant” as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions,

deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al.*, *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al.*, *J Molec Biol* (1990) 215:403).

The invention discloses a new splice variant of NrCAM (termed NrCAMvar) which comprises the AE10K2 sequence, which is absent in the published human NrCAM sequence (Lane *et al.*, 1996) but which is present in the chick sequence. In addition the NrCAMvar does

not have the AE10K1 sequence which is present in the Lane *et al.* human sequence (Figure 2). NrCAMvar is expressed at high levels in the brain, pancreas and adrenal cortex and at lower levels in placenta, adrenal medulla, thyroid and testis. The published human NrCAM, however, appears not to be expressed in the pancreas.

5

Polypeptides of the Invention

In one aspect, the present invention relates to novel NrCAMvar polypeptides. The NrCAMvar polypeptides include the polypeptide of SEQ ID NO:2; as well as polypeptides comprising the amino acid sequence of SEQ ID NO: 2. Preferably NrCAMvar polypeptide exhibit at least one biological activity of NrCAMvar.

10

The NrCAMvar polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

15

Biologically active fragments of the NrCAMvar polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned NrCAMvar polypeptides. As with NrCAMvar polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region.

20

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of NrCAMvar polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Biologically active fragments are those that mediate NrCAMvar activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

25

30

Preferably, all of these polypeptide fragments retain the biological activity of the NrCAMvar, including antigenic activity. Variants of the defined sequence and fragments also

35

form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr.

The NrCAMvar polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to NrCAMvar polynucleotides. NrCAMvar polynucleotides include isolated polynucleotides which encode the NrCAMvar polypeptides and fragments, and polynucleotides closely related thereto. More specifically, NrCAMvar polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO:1 encoding a NrCAMvar polypeptide of SEQ ID NO: 2, and polynucleotides having the particular sequence of SEQ ID NO:1. Also included under NrCAMvar polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such NrCAMvar polynucleotides.

NrCAMvar of the invention is structurally related to other proteins of the cell adhesion molecules, as shown by the results of sequencing the cDNA encoding human NrCAMvar. The cDNA sequence contains an open reading frame encoding a polypeptide of 1304 amino acids. Amino acid of sequence of Figure 1 (SEQ ID NO:2) has about >99% identity (using BlastP) in 1299 amino acid residues with Human NrCAM (Lane, RP et al, Genomics 35 (3), 456-465 (1996)). Nucleotide sequence of Figure 1 (SEQ ID NO:1) has about >99% identity (using BlastN) in 3897 nucleotide residues with Human NrCAM (Genomics 35 (3), 456-465 (1996)). Figure 2 shows the splice variant AE10K.

One polynucleotide of the present invention encoding NrCAMvar may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human adrenal using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature*, (1992) 355:632-634; Adams, M.D., *et al., Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding NrCAMvar polypeptide of SEQ ID NO:2 may be identical over its entire length to the coding sequence set forth in Figure 1 (SEQ ID NO:1), or may be a degenerate form of this nucleotide sequence encoding the polypeptide of SEQ ID NO:2, or may be highly identical to a nucleotide sequence that encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of NrCAMvar polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding NrCAMvar variants comprise the amino acid sequence NrCAMvar polypeptide of Figure 1 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination.

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding NrCAMvar polypeptide and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the NrCAMvar gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 70% identical, preferably 80% identical, more preferably 90% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

In one embodiment, to obtain a polynucleotide encoding NrCAMvar comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof, and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Stringent hybridization conditions are as defined above or alternatively conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements,

from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the NrCAMvar polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If NrCAMvar polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

NrCAMvar polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of NrCAMvar polynucleotides for use as diagnostic reagents. Detection of a mutated form of NrCAMvar gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of NrCAMvar. Individuals carrying mutations in the NrCAMvar gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used

directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled
5 NrCAMvar nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be
10 revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising NrCAMvar nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general
15 applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee *et al.*, *Science*, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to Diabetes, obesity and cancer through detection of mutation in the NrCAMvar gene by the
20 methods described.

In addition, Diabetes, obesity and cancer, can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of NrCAMvar polypeptide or NrCAMvar mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the
25 quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an NrCAMvar polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include
radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

30

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to
35 chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise

chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the NrCAMvar polypeptides. The term "immunospecific" means that the antibodies have substantial greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the NrCAMvar polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against NrCAMvar polypeptides may also be employed to treat Diabetes, obesity and cancer, among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with NrCAMvar polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from Diabetes, obesity and cancer, among others. Yet
5 another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering NrCAMvar polypeptide via a vector directing expression of NrCAMvar polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation
10 (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a NrCAMvar polypeptide wherein the composition comprises a NrCAMvar polypeptide or NrCAMvar gene. The vaccine formulation may further comprise a suitable carrier. Since NrCAMvar polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular,
15 intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be
20 presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the
25 vaccine and can be readily determined by routine experimentation.

Screening Assays

The NrCAMvar polypeptide of the present invention may be employed in a screening process for compounds which activate (agonists) or inhibit activation of (antagonists, or
30 otherwise called inhibitors) the NrCAMvar polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess identify agonist or antagonists from, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These agonists or antagonists may be natural substrates, ligands, receptors, etc., as the case may be, of the polypeptide of the present invention; or may be structural or functional mimetics of the
35 polypeptide of the present invention. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

NrCAMvar polypeptides are ubiquitous in the mammalian host and are responsible for many biological functions, including many pathologies. Accordingly, it is desirable to find compounds and drugs which stimulate NrCAMvar polypeptide on the one hand and which can inhibit the function of NrCAMvar polypeptide on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as Diabetes, obesity and cancer. Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as Diabetes, obesity and cancer.

In general, such screening procedures may involve using appropriate cells which express the NrCAMvar polypeptide or respond to NrCAMvar polypeptide of the present invention. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells which express the NrCAMvar polypeptide (or cell membrane containing the expressed polypeptide) or respond to NrCAMvar polypeptide are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. The ability of the cells which were contacted with the candidate compounds is compared with the same cells which were not contacted for NrCAMvar activity.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the NrCAMvar polypeptide is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the NrCAMvar polypeptide, using detection systems appropriate to the cells bearing the NrCAMvar polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Standard methods for conducting such screening assays are well understood in the art.

Examples of potential NrCAMvar polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, receptors, etc., as the case may be, of the NrCAMvar polypeptide, e.g., a fragment of the ligands, substrates, receptors, or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Prophylactic and Therapeutic Methods

This invention provides methods of treating an abnormal conditions related to both an excess of and insufficient amounts of NrCAMvar polypeptide activity, including diabetes, obesity and cancer.

If the activity of NrCAMvar polypeptide is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as

hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the NrCAMvar polypeptide, or by inhibiting a second signal, and thereby alleviating the abnormal condition.

5 In another approach, soluble forms of NrCAMvar polypeptides still capable of binding the ligand in competition with endogenous NrCAMvar polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the NrCAMvar polypeptide.

10 In still another approach, expression of the gene encoding endogenous NrCAMvar polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360. These oligomers
15 can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of NrCAMvar and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates NrCAMvar
20 polypeptide, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of NrCAMvar by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct
25 may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular
30 Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

Formulation and Administration

35 Peptides, such as the soluble form of NrCAMvar polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the

polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 $\mu\text{g/kg}$ of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Examples

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

Example 1

Cloning of human NrCAMvar cDNA

The HGS EST database was screened using the chick NrCAM sequence and three HGS EST clones (EST99669, EST237133, and EST373834) were obtained. EST99669 and EST237133 clones were from human adrenal cDNA library while EST373834 was from human striatum cDNA library. cDNA clones EST237133 and EST373834 contained several *Eco*RI or *Eco*RI/*Xho*I fragments, suggesting that inserts from several different genes may be present. Only those fragments containing the EST sequence homologous to NrCAM were subcloned and used for further characterisation. These clones were end sequenced and used as probes labelled with [α -³²P]dCTP (Amersham) to screen a human fetal brain (20-wk) λ gt11 cDNA library (Clontech). Four positive cDNA clones were isolated and the inserts were cloned into pBluescript plasmids (Maniatis *et al.*, 1982). Additional sections of the gene were isolated using gene-specific primers to amplify cDNA from the human fetal brain MarathonTM cDNA (Clontech) and the λ gt11 fetal brain cDNA library. All sequencing was performed on an ABI373 sequencer using the ABI PRISMTM dye terminator cycle sequencing ready reaction kit. Sequences were assembled using the Wisconsin GCG package.

Comparison of both DNA and amino acid sequences for human NrCAMvar (SEQ ID NO:1) and the published NrCAM sequence (Lane *et al.*) revealed that they were >99% identical in overlapping regions. The DNA sequence of human NrCAMvar of SEQ ID NO:1 is 77.1% identical to that of chick gene while the amino acid sequence (SEQ ID NO:2) is 80% identical. Evidence for alternative splicing of AE19, AE12 and AE93, was obtained either through sequencing of cDNA clones or PCR products from human cDNA and in addition two novel regions, AE10K and AE10L were found to be differentially absent in the present (AE10K) versus Lanes (AE10L) sequence (see Figure 2).

a) Exon structure:

EST99669 was found to contain a contiguous genomic sequence not homologous to any sequence of the chick gene. Examination of this sequence revealed a splice donor consensus sequence. This probably corresponds to an intron:exon boundary represented in cDNA due to incomplete mRNA processing. A donor and an acceptor were present in EST373834. In addition to alternatively spliced regions AE12 and AE93, identified by Lane *et al.* (1996), AE19 was also found to be absent from some cDNA fragments obtained from human fetal brain cDNA. In addition, two novel alternatively spliced regions, encoding 10-amino-acid sections, were identified (see Figure 2). AE93 was

absent in EST237133 which was from human adrenal cDNA library while both AE12 and AE93 were observed in PCR products from human adult brain cDNA.

b) Results from Northern blots:

- 5 A mRNA band of ~7.0kb was observed for human brain, placenta, pancreas, adrenal medulla and cortex, thyroid, and testis tissues. The results also showed that this gene is highly expressed in brain, pancreas, and adrenal cortex tissues (the levels of mRNA on the blots used are controlled at Clontech and samples are tested for their integrity by hybridisation with an actin gene probe).

10

c) Chromosomal localisation:

- To obtain precise localisation of the human NrCAM locus , primers from an intron (sbp12) and from an exon (sbp13) were designed according to the sequence of EST373834. These primers produced a PCR product of 214bp, and were used to screen
15 for the presence of this gene in the Genebridge 4 radiation hybrid panel. The results were analysed by the WIGCR experimental mapping server and are shown in Table 1. These data place the human NrCAM gene on the long arm of chromosome 7 at 7q21-22 between D7S666 and D7S658.

- 20 *Table 1:* Data vectors obtained from testing Genebridge 4 Radiation Hybrids panel using primers sbp12 and 13.

D7S666	00000 00100 10000 00010 00000 10110 00001 10000 00100 00021
10101	00000 01100 10010 11000 01000 11120 01000 210
25 NrCAM	00000 00100 10001 00010 00000 10110 00000 00000 01100 00000
10101	00000 01100 10010 11000 11000 11110 01000 010
D7S658	00000 00100 10000 00011 00000 10110 00001 10000 01100 00001
10101	00000 01100 10010 11000 01002 11120 01000 210
30	

Each digit corresponds to one of 93 cell lines in the radiation hybrid panel. 0 and 1 represent negative and positive PCR assays respectively. 2 shows that the assay was contradictory between duplicate experiments or was untested.

35

Since pancreatic function is intimately involved in the development of diabetes, NrCAMvar becomes a target molecule in the management of this disease. This suggestion is supported by the genomic mapping data. A locus for non-insulin-dependent diabetes mellitus (NIDDM), also called Type II diabetes, has been mapped to the same region of

5 chromosome 7 as NrCAMvar (Prochazka, 1995).

SEQUENCE LISTING

INFORMATION FOR SEQ ID NO:1:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3997 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

10

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

15

CCATCGTAAT	TCGCCTAATG	CAGCTTAAAA	TAATGCCGAA	AAAGAAGCGC	TTATCTGCGG	60
GCAGAGTGCC	CCTGATTCTC	TTCTGTGCC	AGATGATTAG	TGCACTGGAA	GTACCTCTTG	120
ATCCAAAAC	TCTTGAAGAC	TTGGTACAGC	CTCCAACCAT	CACCCAACAG	TCTCCAAAAG	180
ATTACATTAT	TGACCCTCGG	GAGAATATTG	TAATCCAGTG	TGAAGCCAAA	GGGAAACCGC	240
CCCCAAGCTT	TTCTGGACC	CGTAATGGGA	CTCATTTTGA	CATCGATAAA	GACCCTCTGG	300
TCACCATGAA	GCCTGGCACA	GGAACGCTCA	TAATTAACAT	CATGAGCGAA	GGGAAAGCTG	360
AGACCTATGA	AGGAGTCTAT	CAGTGTACAG	CAAGGAACGA	ACGCGGAGCT	GCAGTTTCTA	420
ATAACATTGT	TGTCCGCCCA	TCCAGATCAC	CATTGTGGAC	CAAAGAAAAA	CTTGAACCAA	480
TCACACTTCA	AAGTGGTCAG	TCTTTAGTAC	TTCCCTGCAG	ACCCCCAATT	GGATTACCAC	540
CACCTATAAT	ATTTTGGATG	GATAATTCCT	TTCAAAGACT	TCCACAAAGT	GAGAGAGTTT	600
CTCAAGGTTT	GAATGGGGAC	CTTTATTTTT	CCAATGTCCT	CCCAGAGGAC	ACCCGCGAAG	660
ACTATATCTG	TTATGCTAGA	TTTAATCATA	CTCAAACCAT	ACAGCAGAAG	CAACCTATTT	720
CTGTGAAGGT	GATTTCAGTG	GATGAATTGA	ATGACACTAT	AGCTGCTAAT	TTGAGTGACA	780
CTGAGTTTTA	TGGTGCTAAA	TCAAGTAGAG	AGAGGCCACC	AACATTTTTA	ACTCCAGAAG	840
GCAATGCAAG	TAACAAAGAG	GAATTAAGAG	GAAATGTGCT	TTCACTGGAG	TGCATTGCAG	900
AAGGACTGCC	TACCCCAATT	ATTTACTGGG	CAAAGGAAGA	TGGAATGCTA	CCCAAAAACA	960
GGACAGTTTA	TAAGAACTTT	GAGAAAACCT	TGCAGATCAT	TCATGTTTCA	GAAGCAGACT	1020
CTGGAAATTA	CCAATGTATA	GCAAAAAATG	CATTAGGAGC	CATCCACCAT	ACCATTTCTG	1080
TTAGAGTTAA	AGCGGCTCCA	TACTGGATCA	CAGCCCCTCA	AAATCTTGTG	CTGTCCCCAG	1140
GAGAGGATGG	GACCTTGATC	TGCAGAGCTA	ATGGCAACCC	CAAACCCAGA	ATTAGCTGGT	1200
TAACAAATGG	AGTCCCAATA	GAAATTGCCC	CTGATGACCC	CAGCAGAAAA	ATAGATGGCG	1260

35

	ATACCATTAT	TTTTTCAAAT	GTTCAAGAAA	GATCAAGTGC	AGTATATCAG	TGCAATGCCT	1320
	CTAATGAATA	TGGATATTTA	CTGGCAAACG	CATTTGTAAA	TGTGCTGGCT	GAGCCACCAC	1380
	GAATCCTCAC	ACCTGCAAAC	ACACTCTACC	AGGTCATTGC	AAACAGGCCT	GCTTTACTAG	1440
	ACTGTGCCTT	CTTTGGGTCA	CCTCTCCCAA	CCATCCAGTG	GTTTAAAGGA	GCTAAAGGAA	1500
5	GTGCTCTTCA	TGAAGATATT	TATGTTTTAC	ATGAAAATGG	AACTTTGGAA	ATTCTGTGG	1560
	CCCAAAGGA	CAGTACAGGA	ACTTATACGT	GTGTTGCAAG	GAATAAATTA	GGGATGGCGA	1620
	AGAATGAAGT	TCACCTAGAA	ATCAAAGATC	CTACATGGAT	CGTTAAACAG	CCCGAATATG	1680
	CAGTTGTGCA	AAGAGGGAGC	ATGGTGTCTT	TTGAATGCAA	AGTGAAACAT	GATCACACCT	1740
	TATCCCTCAC	TGTCCTGTGG	CTGAAGGACA	ACAGGGAAC	GCCCAGTGAT	GAAAGGTTCA	1800
10	CTGTTGACAA	GGATCATCTA	GTGGTAGCTG	ATGTCAGTGA	CGATGACAGC	GGGACCTACA	1860
	CGTGTGTGGC	CAACACCACT	CTGGACAGCG	TCTCCGCCAG	CGCTGTGCTT	AGCGTTGTTG	1920
	CTCCTACTCC	AACTCCAGCT	CCCGTTTACG	ATGTCCCAA	TCCTCCGCTT	GACTTAGAAC	1980
	TGACAGATCA	ACTTGACAAA	AGTGTTGAGC	TGTCATGGAC	CCCAGGCGAT	GACAACAATA	2040
	GCCCCATTAC	AACAATTCAT	GACGAATATG	AAGATGCAAT	GCACAAGCCA	GGGCTGTGGC	2100
15	ACCACCAAAC	TGAAGTTTCT	GGAACACAGA	CCACAGCCCA	GCTGAAGCTG	TCTCCTTACG	2160
	TGAACTACTC	CTTCCGCGTG	ATGGCAGTGA	ACAGCATTTG	GAAGAGCTTG	CCCAGCGAGG	2220
	CCTCTGAGCA	GTATTTGACG	AAAGCCTCAG	AACCAGATAA	AAACCCCA	GCTGTGGAAG	2280
	GACTGGGATC	AGAGCCTGAT	AATTTGGTGA	TTACGTGGAA	GCCCTTGAAT	GGTTTCGAAT	2340
	TTAATGGGCC	AGGCCTTCAG	TACAAAGTTA	GCTGGCGCCA	GAAAGTTGGT	GATGATGAAT	2400
20	GGACATCTGT	GGTTGTGGCA	AATGTATCCA	AATATATTGT	TTCAGGCACG	CCAACCTTTG	2460
	TTCCATACCT	GATCAAAGTT	CAGGCCCTGA	ATGACATGGG	GTTTGCCCCC	GAGCCAGCTG	2520
	TAGTCATGGG	ACATTCTGGA	GAAGACCTCC	CAATGGTGGC	TCCTGGGAAC	GTGCGTGTGA	2580
	ATGTGGTGAA	CAGTACCTTA	GCCGAGGTGC	ACTGGGACCC	AGTACCTCTG	AAAAGCATCC	2640
	GAGGACACCT	ACAAGGCTAT	CGGATTTACT	ATTGGAAGAC	CCAGAGTTCA	TCTAAAAGAA	2700
25	ACAGACGTCA	CATTGAGAAA	AAGATCCTCA	CCTTCCAAGG	CAGCAAGACT	CATGGCATGT	2760
	TGCCGGGGCT	AGAGCCCTTT	AGCCACTACA	CACTGAATGT	CCGAGTGGTC	AATGGGAAAG	2820
	GGGAGGGCCC	AGCCAGCCCT	GACAGAGTCT	TTAATACTCC	AGAAGGAGTC	CCCAGCGTTC	2880
	CCTCGTCTTT	GAAGATTGTG	AATCCAACAC	TGGACTCTCT	CACTTTGGAA	TGGGATCCAC	2940
	CGAGCCACCC	GAATGGCATT	TTGACAGAGT	ACACCTTAAA	GTATCAGCCA	ATTAACAACA	3000
30	CACATGAATT	AGGCCCTCTG	GTAGATTTGA	AAATTCCTGC	CAACAAGACA	CGGTGGACTT	3060
	TAAAAAATTT	AAATTTCAAC	ACTCGATATA	AGTTTTATTT	CTATGCACAA	ACATCAGCAG	3120
	GATCAGGAAG	TCAAATTACA	GAGGAAGCAG	TAACAACGTG	GGATGAAGCT	GGTATTCTTC	3180
	CACCTGATGT	AGGTGCAGGC	AAAGTTCAAG	CAGTAAATCC	CAGGATCAGC	AATCTTACTG	3240
	CTGCAGCTGC	TGAAACCTAT	GCCAATATCA	GTTGGGAATA	TGAGGGACCA	GAGTATGCCA	3300
35	ACTTTTATGT	TGAATATGGT	GTAGCAGGCA	GCAAAGAAGA	ATGGAGAAAA	GAAATTGTAA	3360
	ATGGTTCTCG	GAGCTTCTTT	GGGTAAAGG	GTCTAATGCC	AGGAACAGCA	TACAAGTTTC	3420

5 GAGTTGGTGC TGTGGGGGGA CCCC GTTTG TGAGTTCAGA GGGTGTGTTT GAGACAGGCC 3480
 CAGCGATGGC AAGCCGGCAG GTGGATATTG CAACTCAGGG CTGGTTCATT GGTCTGATGT 3540
 GTGCTGTTGC TCTCCTTATC TTAATTTTGC TGATTGTTTG CTTTCATCAGA AGAAACAAGG 3600
 GTGGTAAATA TCCAGTTAAA GAAAAGGAAG ATGCCCATGC TGACCCTGAA ATCCAGCCTA 3660
 10 TGAAGGAAGA TGATGGGACA TTTGGAGAAT ACAGTGATGC AGAAGACCAC AAGCCTTTGA 3720
 AAAAAGGAAG TCGAACTCCT TCAGACAGGA CTGTGAAAAA AGAAGATAGT GACGACAGCC 3780
 TACTTGACTA TGGAGAAGGG GTTAATGGCC AGTTCAATGA GGATGGCTCC TTTATTGGAC 3840
 AATACAGTGG TAAAAAAGAG AAAGAGCCGG CTGAAGGAAA CGAAAGCTCA GAGGCACCTT 3900
 CTCCTGTCAA CGCCATGAAT TCCTTTGTTT AATCATAGAA CTTGATTCCG ATGATGTCTT 3960
 10 TACAGTTTGT TTGCTATTGT CCATCCAGGT TGTA CTG 3997

INFORMATION FOR SEQ ID NO:2:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1304 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

25 Met Gln Leu Lys Ile Met Pro Lys Lys Lys Arg Leu Ser Ala Gly Arg
 1 5 10 15
 Val Pro Leu Ile Leu Phe Leu Cys Gln Met Ile Ser Ala Leu Glu Val
 20 25 30
 Pro Leu Asp Pro Lys Leu Leu Glu Asp Leu Val Gln Pro Pro Thr Ile
 35 40 45
 30 Thr Gln Gln Ser Pro Lys Asp Tyr Ile Ile Asp Pro Arg Glu Asn Ile
 50 55 60
 Val Ile Gln Cys Glu Ala Lys Gly Lys Pro Pro Pro Ser Phe Ser Trp
 65 70 75 80
 Thr Arg Asn Gly Thr His Phe Asp Ile Asp Lys Asp Pro Leu Val Thr
 35 85 90 95
 Met Lys Pro Gly Thr Gly Thr Leu Ile Ile Asn Ile Met Ser Glu Gly

	100	105	110
	Lys Ala Glu Thr Tyr Glu Gly Val Tyr Gln Cys Thr Ala Arg Asn Glu		
	115	120	125
	Arg Gly Ala Ala Val Ser Asn Asn Ile Val Val Arg Pro Ser Arg Ser		
5	130	135	140
	Pro Leu Trp Thr Lys Glu Lys Leu Glu Pro Ile Thr Leu Gln Ser Gly		
	145	150	155
	Gln Ser Leu Val Leu Pro Cys Arg Pro Pro Ile Gly Leu Pro Pro Pro		160
	165	170	175
10	Ile Ile Phe Trp Met Asp Asn Ser Phe Gln Arg Leu Pro Gln Ser Glu		
	180	185	190
	Arg Val Ser Gln Gly Leu Asn Gly Asp Leu Tyr Phe Ser Asn Val Leu		
	195	200	205
	Pro Glu Asp Thr Arg Glu Asp Tyr Ile Cys Tyr Ala Arg Phe Asn His		
15	210	215	220
	Thr Gln Thr Ile Gln Gln Lys Gln Pro Ile Ser Val Lys Val Ile Ser		
	225	230	235
	Val Asp Glu Leu Asn Asp Thr Ile Ala Ala Asn Leu Ser Asp Thr Glu		240
	245	250	255
20	Phe Tyr Gly Ala Lys Ser Ser Arg Glu Arg Pro Pro Thr Phe Leu Thr		
	260	265	270
	Pro Glu Gly Asn Ala Ser Asn Lys Glu Glu Leu Arg Gly Asn Val Leu		
	275	280	285
	Ser Leu Glu Cys Ile Ala Glu Gly Leu Pro Thr Pro Ile Ile Tyr Trp		
25	290	295	300
	Ala Lys Glu Asp Gly Met Leu Pro Lys Asn Arg Thr Val Tyr Lys Asn		
	305	310	315
	Phe Glu Lys Thr Leu Gln Ile Ile His Val Ser Glu Ala Asp Ser Gly		320
	325	330	335
30	Asn Tyr Gln Cys Ile Ala Lys Asn Ala Leu Gly Ala Ile His His Thr		
	340	345	350
	Ile Ser Val Arg Val Lys Ala Ala Pro Tyr Trp Ile Thr Ala Pro Gln		
	355	360	365
	Asn Leu Val Leu Ser Pro Gly Glu Asp Gly Thr Leu Ile Cys Arg Ala		
35	370	375	380
	Asn Gly Asn Pro Lys Pro Arg Ile Ser Trp Leu Thr Asn Gly Val Pro		

	385		390		395		400									
	Ile	Glu	Ile	Ala	Pro	Asp	Asp	Pro	Ser	Arg	Lys	Ile	Asp	Gly	Asp	Thr
				405						410					415	
	Ile	Ile	Phe	Ser	Asn	Val	Gln	Glu	Arg	Ser	Ser	Ala	Val	Tyr	Gln	Cys
5				420					425					430		
	Asn	Ala	Ser	Asn	Glu	Tyr	Gly	Tyr	Leu	Leu	Ala	Asn	Ala	Phe	Val	Asn
				435					440					445		
	Val	Leu	Ala	Glu	Pro	Pro	Arg	Ile	Leu	Thr	Pro	Ala	Asn	Thr	Leu	Tyr
		450					455						460			
10	Gln	Val	Ile	Ala	Asn	Arg	Pro	Ala	Leu	Leu	Asp	Cys	Ala	Phe	Phe	Gly
	465					470					475				480	
	Ser	Pro	Leu	Pro	Thr	Ile	Gln	Trp	Phe	Lys	Gly	Ala	Lys	Gly	Ser	Ala
				485					490					495		
	Leu	His	Glu	Asp	Ile	Tyr	Val	Leu	His	Glu	Asn	Gly	Thr	Leu	Glu	Ile
15			500						505					510		
	Pro	Val	Ala	Gln	Lys	Asp	Ser	Thr	Gly	Thr	Tyr	Thr	Cys	Val	Ala	Arg
		515						520					525			
	Asn	Lys	Leu	Gly	Met	Ala	Lys	Asn	Glu	Val	His	Leu	Glu	Ile	Lys	Asp
		530				535						540				
20	Pro	Thr	Trp	Ile	Val	Lys	Gln	Pro	Glu	Tyr	Ala	Val	Val	Gln	Arg	Gly
	545					550					555				560	
	Ser	Met	Val	Ser	Phe	Glu	Cys	Lys	Val	Lys	His	Asp	His	Thr	Leu	Ser
			565						570					575		
	Leu	Thr	Val	Leu	Trp	Leu	Lys	Asp	Asn	Arg	Glu	Leu	Pro	Ser	Asp	Glu
25			580						585					590		
	Arg	Phe	Thr	Val	Asp	Lys	Asp	His	Leu	Val	Val	Ala	Asp	Val	Ser	Asp
		595						600					605			
	Asp	Asp	Ser	Gly	Thr	Tyr	Thr	Cys	Val	Ala	Asn	Thr	Thr	Leu	Asp	Ser
		610					615						620			
30	Val	Ser	Ala	Ser	Ala	Val	Leu	Ser	Val	Val	Ala	Pro	Thr	Pro	Thr	Pro
	625					630					635				640	
	Ala	Pro	Val	Tyr	Asp	Val	Pro	Asn	Pro	Pro	Leu	Asp	Leu	Glu	Leu	Thr
			645						650					655		
	Asp	Gln	Leu	Asp	Lys	Ser	Val	Gln	Leu	Ser	Trp	Thr	Pro	Gly	Asp	Asp
35			660						665					670		
	Asn	Asn	Ser	Pro	Ile	Thr	Thr	Ile	His	Asp	Glu	Tyr	Glu	Asp	Ala	Met

	675		680		685
	His Lys Pro Gly Leu Trp	His His Gln Thr Glu Val Ser Gly Thr Gln			
	690	695	700		
5	Thr Thr Ala Gln Leu Lys Leu Ser Pro Tyr Val Asn Tyr Ser Phe Arg				
	705	710	715	720	
	Val Met Ala Val Asn Ser Ile Gly Lys Ser Leu Pro Ser Glu Ala Ser				
	725	730	735		
	Glu Gln Tyr Leu Thr Lys Ala Ser Glu Pro Asp Lys Asn Pro Thr Ala				
	740	745	750		
10	Val Glu Gly Leu Gly Ser Glu Pro Asp Asn Leu Val Ile Thr Trp Lys				
	755	760	765		
	Pro Leu Asn Gly Phe Glu Phe Asn Gly Pro Gly Leu Gln Tyr Lys Val				
	770	775	780		
	Ser Trp Arg Gln Lys Val Gly Asp Asp Glu Trp Thr Ser Val Val Val				
15	785	790	795	800	
	Ala Asn Val Ser Lys Tyr Ile Val Ser Gly Thr Pro Thr Phe Val Pro				
	805	810	815		
	Tyr Leu Ile Lys Val Gln Ala Leu Asn Asp Met Gly Phe Ala Pro Glu				
	820	825	830		
20	Pro Ala Val Val Met Gly His Ser Gly Glu Asp Leu Pro Met Val Ala				
	835	840	845		
	Pro Gly Asn Val Arg Val Asn Val Val Asn Ser Thr Leu Ala Glu Val				
	850	855	860		
	His Trp Asp Pro Val Pro Leu Lys Ser Ile Arg Gly His Leu Gln Gly				
25	865	870	875	880	
	Tyr Arg Ile Tyr Tyr Trp Lys Thr Gln Ser Ser Ser Lys Arg Asn Arg				
	885	890	895		
	Arg His Ile Glu Lys Lys Ile Leu Thr Phe Gln Gly Ser Lys Thr His				
	900	905	910		
30	Gly Met Leu Pro Gly Leu Glu Pro Phe Ser His Tyr Thr Leu Asn Val				
	915	920	925		
	Arg Val Val Asn Gly Lys Gly Glu Gly Pro Ala Ser Pro Asp Arg Val				
	930	935	940		
	Phe Asn Thr Pro Glu Gly Val Pro Ser Val Pro Ser Ser Leu Lys Ile				
35	945	950	955	960	
	Val Asn Pro Thr Leu Asp Ser Leu Thr Leu Glu Trp Asp Pro Pro Ser				

	965	970	975
	His Pro Asn Gly Ile Leu Thr Glu Tyr Thr Leu Lys Tyr Gln Pro Ile		
	980	985	990
5	Asn Asn Thr His Glu Leu Gly Pro Leu Val Asp Leu Lys Ile Pro Ala		
	995	1000	1005
	Asn Lys Thr Arg Trp Thr Leu Lys Asn Leu Asn Phe Thr Thr Arg Tyr		
	1010	1015	1020
	Lys Phe Tyr Phe Tyr Ala Gln Thr Ser Ala Gly Ser Gly Ser Gln Ile		
	1025	1030	1035
10	Thr Glu Glu Ala Val Thr Thr Val Asp Glu Ala Gly Ile Leu Pro Pro		
	1045	1050	1055
	Asp Val Gly Ala Gly Lys Val Gln Ala Val Asn Pro Arg Ile Ser Asn		
	1060	1065	1070
	Leu Thr Ala Ala Ala Ala Glu Thr Tyr Ala Asn Ile Ser Trp Glu Tyr		
15	1075	1080	1085
	Glu Gly Pro Glu Tyr Ala Asn Phe Tyr Val Glu Tyr Gly Val Ala Gly		
	1090	1095	1100
	Ser Lys Glu Glu Trp Arg Lys Glu Ile Val Asn Gly Ser Arg Ser Phe		
	1105	1110	1115
20	Phe Gly Leu Lys Gly Leu Met Pro Gly Thr Ala Tyr Lys Phe Arg Val		
	1125	1130	1135
	Gly Ala Val Gly Gly Pro Arg Phe Val Ser Ser Glu Gly Val Phe Glu		
	1140	1145	1150
	Thr Gly Pro Ala Met Ala Ser Arg Gln Val Asp Ile Ala Thr Gln Gly		
25	1155	1160	1165
	Trp Phe Ile Gly Leu Met Cys Ala Val Ala Leu Leu Ile Leu Ile Leu		
	1170	1175	1180
	Leu Ile Val Cys Phe Ile Arg Arg Asn Lys Gly Gly Lys Tyr Pro Val		
	1185	1190	1195
30	Lys Glu Lys Glu Asp Ala His Ala Asp Pro Glu Ile Gln Pro Met Lys		
	1205	1210	1215
	Glu Asp Asp Gly Thr Phe Gly Glu Tyr Ser Asp Ala Glu Asp His Lys		
	1220	1225	1230
	Pro Leu Lys Lys Gly Ser Arg Thr Pro Ser Asp Arg Thr Val Lys Lys		
35	1235	1240	1245
	Glu Asp Ser Asp Asp Ser Leu Leu Asp Tyr Gly Glu Gly Val Asn Gly		

1250 1255 1260
Gln Phe Asn Glu Asp Gly Ser Phe Ile Gly Gln Tyr Ser Gly Lys Lys
1265 1270 1275 128
Glu Lys Glu Pro Ala Glu Gly Asn Glu Ser Ser Glu Ala Pro Ser Pro
5 1285 1290 1295
Val Asn Ala Met Asn Ser Phe Val
1300

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence encoding the NrCAMvar polypeptide of SEQ ID:NO2; or a nucleotide sequence
5 complementary to said nucleotide sequence.
2. The polynucleotide of claim 1 which is DNA or RNA.
3. The polynucleotide of claim 2 wherein said nucleotide sequence comprises
10 the NrCAMvar polypeptide encoding sequence contained in SEQ ID:NO2.
4. The polynucleotide of SEQ ID NO: 1.
5. A polynucleotide probe or primer comprising at least 15 contiguous
15 nucleotides of the polynucleotide of claim 3.
6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a NrCAMvar polypeptide comprising an amino acid sequence of SEQ ID:NO2 when said expression system is present in a compatible host
20 cell.
7. A host cell comprising the expression system of claim 7.
8. A process for producing a NrCAMvar polypeptide comprising culturing a
25 host of claim 7 and under conditions sufficient for the production of said polypeptide.
9. The process of claim 8 which further includes recovering the polypeptide from the culture.
- 30 10. A process for producing a cell which produces a NrCAMvar polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a NrCAMvar polypeptide.
- 35 11. Cells produced by the process of claim 10.

12. A polypeptide which comprises the amino acid sequence of SEQ ID NO:2.

13. The polypeptide encoded in SEQ ID:NO2.

14. A NrCAMvar polypeptide prepared by the method of claim 9.

15. An antibody immunospecific for the NrCAMvar polypeptide of claim 12.

16. A method for the treatment of a subject in need of enhanced NrCAMvar polypeptide activity comprising:

(a) administering to the subject a therapeutically effective amount of an agonist to said polypeptide; and/or

(b) providing to the subject NrCAMvar polynucleotide in a form so as to effect production of said polypeptide activity *in vivo*.

17. A method for the treatment of a subject having need to inhibit NrCAMvar polypeptide activity comprising:

(a) administering to the subject a therapeutically effective amount of an antagonist to said polypeptide; and/or

(b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said polypeptide; and/or

(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said polypeptide for its ligand, substrate, or receptor.

18. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of NrCAMvar polypeptide in a subject comprising:

(a) determining the presence or absence of a mutation in the nucleotide sequence encoding said NrCAMvar polypeptide in the genome of said subject; and/or

(b) analyzing for the presence or amount of the NrCAMvar polypeptide expression in a sample derived from said subject.

19. A method for identifying compounds which inhibit (antagonize) or agonize the NrCAMvar polypeptide which comprises:

(a) contacting a candidate compound with cells which express the NrCAMvar polypeptide (or cell membrane expressing NrCAMvar polypeptide) or respond to NrCAMvar polypeptide; and

5 (b) observing the binding, or stimulation or inhibition of a functional response; or
comparing the ability of the cells (or cell membrane) which were contacted with the candidate compounds with the same cells which were not contacted for NrCAMvar polypeptide activity.

20. An agonist identified by the method of claim 19.

10 21. An antagonist identified by the method of claim 19.

22. A polynucleotide consisting essentially of a DNA sequence obtainable by screening an appropriate library containing the NrCAMvar gene under stringent hybridization conditions with a probe having the sequence of SEQ ID:NO 1 or a fragment
15 thereof; and isolating said DNA sequence.

23. A polypeptide obtainable by expressing a nucleotide sequence comprising that of SEQ ID NO:1

20 24. A method for the treatment of diabetes, obesity or cancer which comprises administering to the subject a therapeutically effective amount of a modulator of NrCAMvar polypeptide activity.

25 25. A process according to claim 20 for diagnosing presence of or susceptibility to diabetes, obesity or cancer.

Figure 1 DNA sequence and amino acid sequence of NrCAMvar (SEQ ID NOS : 1 and 2 respectively)

5 SEQ ID NO:1

CCATCGTAATTCGCCTAATGCAGCTTAAAATAATGCCGAAAAAGAAGCGCTTATCTGCGG
GCAGAGTGCCCCCTGATTCTCTTCCTGTGCCAGATGATTAGTGCACTGGAAGTACCTCTTG
ATCCAAAACCTCTTGAAGACTTGGTACAGCCTCCAACCATCACCCAACAGTctccAAAAG
ATTACATTATTGACCCTCGGGAGAATATTGTAATCCAGTGTGAAGCCAAAGGGAAACCGC
10 CCCCAGCTTTTCCTGGACCCGTAATGGGACTCATTTTGACATCGATAAAGACCCCTCTGG
TCACCATGAAGCCTGGCACAGGAACGCTCATAATTAACATCATGAGCGAAGGGAAAGCTG
AGACCTATGAAGGAGTCTATCAGTGTACAGCAAGGAACGAACGCGGAGCTGCAGTTTCTA
ATAACATTGTTGTCCGCCCATCCAGATCACCATTGTGGACCAAAGAAAAAACTTGAACCAA
TCACACTTCAAAGTGGTCAGTCTTTAGTACTTCCCTGCAGACCCCAATTGGATTACCAC
15 CACCTATAATATTTTGGATGGATAATTCCTTTCAAAGACTTCCACAAAGTGAGAGAGTTT
CTCAAGGTTTGAATGGGGACCTTTATTTTCCAATGTCTCCAGAGGACACCCGCGAAG
ACTATATCTGTTATGCTAGATTTAATCATACTCAAACCATACAGCAGAAGCAACCTATTT
CTGTGAAGGTGATTTTCAGTGGATGAATTGAATGACACTATAGCTGCTAATTTGAGTGACA
CTGAGTTTATGGTGCTAAATCAAGTAGAGAGAGGCCACCAACATTTTAACTCCAGAAG
20 GCAATGCAAGTAACAAAGAGGAATTAAGAGGAAATGTGCTTTCACTGGAGTGCATTGCAG
AAGGACTGCCTACCCCAATTATTTACTGGGCAAAGGAAGATGGAATGCTACCCAAAAACA
GGACAGTTTATAAGAACTTTGAGAAAACCTTGCAGATCATTCATGTTTCAGAAGCAGACT
CTGGAAATTACCAATGTATAGCAAAAAATGCATTAGGAGCCATCCACCATACCATTCTG
TTAGAGTTAAAGCGGCTCCATACTGGATCACAGCCCCCTCAAAATCTTGTGCTGTCCCCAG
25 GAGAGGATGGGACCTTGATCTGCAGAGCTAATGGCAACCCCAACCCAGAATTAGCTGGT
TAACAAATGGAGTCCCAATAGAAATGCCCCCTGATGACCCAGCAGAAAAATAGATGGCG
ATACCATTATTTTTTCAAATGTTCAAGAAAGATCAAGTGCAGTATATCAGTGCAATGCCT
CTAATGAATATGGATATTTACTGGCAAACGCATTTGTAAATGTGCTGGCTGAGCCACCAC
GAATCCTCACACCTGCAAACACACTCTACCAGGTCATTGCAAACAGGCCTGCTTTACTAG
30 ACTGTGCCTTCTTTGGGTCACCTCTCCCAACCATCCAGTGGTTTAAAGGAGCTAAAGGAA
GTGCTCTTCATGAAGATATTTATGTTTTACATGAAAATGGAACCTTTGGAAATTCCTGTGG
CCCCAAAGGACAGTACAGGAACTTATACGTGTGTTGCAAGGAATAAATTAGGGATGGCGA
AGAATGAAGTTCACCTAGAAATCAAAGATCCTACATGGATCGTTAAACAGCCGAATATG
CAGTTGTGCAAAGAGGGAGCATGGTGTCTTTGAATGCAAAGTGAAACATGATCACACCT
35 TATCCCTCACTGTCTGTGGCTGAAGGACAACAGGGAAGTGGCCAGTGATGAAAGGTTCA
CTGTTGACAAGGATCATCTAGTGGTAGCTGATGTCAGTGACGATGACAGCGGGACCTACA

CTCCTACTCCAACCTCCAGCTCCCGTTTACGATGTCCCAAATCCTCCGCTTGACTTAGAAC
TGACAGATCAACTTGACAAAAGTGTTTACAGCTGTCATGGACCCCAGGCGATGACAACAATA
GCCCCATTACAACAATTCATGACGAATATGAAGATGCAATGCACAAGCCAGGGCTGTGGC
ACCACCAAACCTGAAGTTTCTGGAACACAGACCACAGCCCAGCTGAAGCTGTCTCCTTACG
5 TGAACACTCCTTCCGCGTGATGGCAGTGAACAGCATTGGGAAGAGCTTGCCCAGCGAGG
CCTCTGAGCAGTATTTGACGAAAGCCTCAGAACCAGATAAAAACCCCACAGCTGTGGAAG
GACTGGGATCAGAGCCTGATAATTTGGTGATTACGTGGAAGCCCTTGAATGGTTTTGAAT
TTAATGGGCCAGGCCTTCAGTACAAAGTTAGCTGGCGCCAGAAAGTTGGTGATGATGAAT
GGACATCTGTGGTTGTGGCAAATGTATCCAAATATATTGTTTCAGGCACGCCAACCTTTG
10 TTCCATACCTGATCAAAGTTCAGGCCCTGAATGACATGGGGTTTGCCCCGAGCCAGCTG
TAGTCATGGGACATTCTGGAGAAGACCTCCCAATGGTGGCTCCTGGGAACGTGCGTGTGA
ATGTGGTGAACAGTACCTTAGCCGAGGTGCACTGGGACCCAGTACCTCTGAAAAGCATCC
GAGGACACCTACAAGGCTATCGGATTTACTATTGGAAGACCCAGAGTTCATCTAAAAGAA
ACAGACGTCACATTGAGAAAAAGATCCTCACCTTCCAAGGCAGCAAGACTCATGGCATGT
15 TGCCGGGGCTAGAGCCCTTTAGCCACTACACACTGAATGTCCGAGTGGTCAATGGGAAAG
GGGAGGGCCCAGCCAGCCCTGACAGAGTCTTTAATACTCCAGAAGGAGTCCCCAGCGTTC
CCTCGTCTTTGAAGATTGTGAATCCAACACTGGACTCTCTCACTTTGGAATGGGATCCAC
CGAGCCACCCGAATGGCATTTTGACAGAGTACACCTTAAAGTATCAGCCAATTAACAACA
CACATGAATTAGGCCCTCTGGTAGATTTGAAAATTCCTGCCAACAAGACACGGTGGACTT
20 TAAAAAATTTAAATTTTACCACCTCGATATAAGTTTTATTTCTATGCACAAACATCAGCAG
GATCAGGAAGTCAAATTACAGAGGAAGCAGTAACAACTGTGGATGAAGCTGGTATTCTTC
CACCTGATGTAGGTGCAGGCAAAGTTCAAGCAGTAAATCCCAGGATCAGCAATCTTACTG
CTGCAGCTGCTGAAACCTATGCCAATATCAGTTGGGAATATGAGGGACCAGAGTATGCCA
ACTTTTATGTTGAATATGGTGTAGCAGGCAGCAAAGAAGAATGGAGAAAAGAAATTGTAA
25 ATGGTTCTCGGAGCTTCTTTGGGTAAAGGGTCTAATGCCAGGAACAGCATACAAGTTTC
GAGTTGGTGTCTGTGGGGGACCCCGTTTGTGAGTTCAGAGGGTGTGTTTGAGACAGGCC
CAGCGATGGCAAGCCGGCAGGTGGATATTGCAACTCAGGGCTGGTTCATTGGTCTGATGT
GTGCTGTTGCTCTCCTTATCTTAATTTTGCTGATTGTTTGCTTCATCAGAAGAAACAAGG
GTGGTAAATATCCAGTTAAAGAAAAGGAAGATGCCCATGCTGACCCTGAAATCCAGCCTA
30 TGAAGGAAGATGATGGGACATTTGGAGAATACAGTGATGCAGAAGACCACAAGCCTTTGA
AAAAAGGAAGTCGAACCTCCTTCAGACAGGACTGTGAAAAAAGAAGATAGTGACGACAGCC
TACTTGACTATGGAGAAGGGGTAAATGGCCAGTTCAATGAGGATGGCTCCTTTATTGGAC
AATACAGTGGTAAAAAAGAGAAAGAGCCGGCTGAAGGAAACGAAAGCTCAGAGGCACCTT
CTCCTGTCAACGCCATGAATTCCTTTGTTTAAATCATAGAACTTGATTCCGATGATGTCTT
35 TACAGTTTGTGCTATTGTCCATCCAGGTGTACTG

SEQ ID NO:2

5 MQLKIMPKKKRLSAGRVPLILFLCQMISALEVPLDPKLLLEDLVQPPTITQQSPKDYIIDP
 RENIVIQCEAKGKPPPSFSWTRNGTHFDIDKDPLVTMKPGTGTLIINIMSEGKAETYEYGV
 YQCTARNERGAAVSNNIVVRPSRSPLWTKEKLEPITLQSGQSLVLPKRPPIGLPPPIIFW
 MDNSFQRLPQSERVSQGLNGDLYFSNVLPEDTREDYICYARFNHTQTIQKQPISVKVIS
 VDELNDTIAANLSDTEFYGAKSSRERPPTFLTPEGNASNKEELRGNVLSLECIAEGLPTP
 10 I IYWAKEDGMLPKNRTVYKNFEKTLQIIHVSEADSGNYQCI AKNALGAIHHTISVRVAAA
 PYWITAPQNLVLS PGEDGT LICRANGNPKPRISWLTNGVPIE IAPDDPSRKIDGDTIIFS
 NVQERS SAVYQCNASNEYGYLLANAFVNVLAEP PRILTPANTLYQVIANRPALLDCAFFG
 SPLPTIQWFKGAKGSALHEDIYVLHENGTL EIPVAQKDSTGTYTCVARNKLGMAKNEVHL
 EIKDPTWIVKQPEYAVVQRGSMVSFECKVKHDHTLSLTVLWLKDNRELPSDERFTVDKDH
 LVVADVSDDDSGTYTCVANTTLD SVSASAVLSVVAPTPT PAPVYDVPNPPLDLELTDQLD
 15 KSVQLSWTPGDDNNSPITTIHDEYEDAMHKPGLWHHQTEVSGTQTTAQLKLSPVVNY SFR
 VMAVNSIGKSLPSEASEQYLT KASEPDKNPTAVEGLGSEPDNLVITWKPLNGFEFNGPGL
 QYKVS WRQKVGDEWTSVVVANVSKYIVSGTPTFPYLIKQALNDMGFAPEPAVVMGHS
 GEDLPMVAPGNVRVNVNSTLA EVHWDPVPLKSIRGHLQGYRIYYWKTQSSSKRNR RHIE
 KKILTFQSGSKTHGMLPGLEPPSHYTLNVRV VNGKGE GPASPD RVFNTPEGVPSVPSSLKI
 20 VNPTLDSLTL EWDPPSHPNGILTEYTLKYQPINNTHELGPLVDLKI PANKTRWTLKLNLF
 TTRYKFYFYAQT SAGSGSQITEEAVTTVDEAGILPPDVGAGKVQAVNPRISNLTA AAAAET
 YANISWEYEGPEYANFYVEYGVAGSKEEWRKEIVNGSR SFFGLKGLMPGTAYKFRVGAVG
 GPRFVSSEGVFETGPAMASRQVDIATQGWFI GLMCAVALLILILLIVCFIRRNKGGKYPV
 KEKEDAHADPEIQPMKEDDGT FG EYSDAEDHKPLKKSRTPSDRTVKKEDSDDSLLDYGE
 25 GVNGQFNEDGSFIGYSGKKEKEPAEGNESSEAPSPVNAMNSFV

30 Figure 2. Comparison of human NrCAM, NrCAMvar and chick NrCAM. The sequences compared are those of the current invention (Human1), of Lane *et al* (Human2) and chick. The nucleotide numbering refers to that of the Human1 sequence.

	1512		AE10K1
Human1	GAAGATATTT ATGTTT TACA TGAAAATGGA ACTTTG----		-----
35 Human2	GAAGATATTT ATGTTT TACA TGAAAATGGA ACTTTG GAAA		TCAAAGATGC
Chick	GGAAATGAAT ATGTTT TCCA TGATAATGGA ACCTTG----		-----
Human1	-----GAAA TTCCTGTGGC CCAAAAGGAC AGTACAGGAA		
Human2	TACATGGATC GTTAAAGAAA TTCCTGTGGC CCAAAAGGAC AGTACAGGAA		
40 Chick	-----GAAA TTCCAGTGGC TCAGAAGGAT AGTACTGGCA		
Human1	CTTATACGTG TGTGCAAGG AATAAATTAG GGATGGCGAA GAATGAAGTT		
Human2	CTTATACGTG TGTGCAAGG AATAAATTAG GGATGGCAAA GAATGAAGTT		
Chick	CATACACATG TGTGCAAGG AATAAATTAG GGAAGACGCA AAATGAAGTA		
45		AE10K2	
Human1	CACTTAGAAA TCAAAGATCC TACATGGATC GTTAAACAGC CCGAATATGC		
Human2	CACTTA-----CAGC CCGAATATGC		
Chick	CAACTG GAA G TTAAAGACCC AACGATGATA ATTAAACAGC CACAGTACAA		

Human1 AGTTGTGCAA AGAGGGAGCA TGGTGTCCCTT TGAATGCAAA GTGAAACATG
 Human2 AGTTGTGCAA AGAGGGAGCA TGGTGTCCCTT TGAATGCAAA GTGAAACATG
 Chick AGTGATTCAG AGATCTGCCC AGGCTTCATT TGAGTGTGTA ATAAAAACATG
 5
 Human1 ATCACACCTT ATCCCTCACT GTCCTGTGGC TGAAGGACAA CAGGGAAC TG
 HUman2 ATCACACCTT ATCCCTCACT GTCCTGTGGC TGAAGGACAA CAGGGAAC TG
 Chick ATCCTACCTT AATACCAACA GTTATATGGC TGAAAGACAA TAATGAACTA
 10
 Human1 CCCAGTGATG AAAGGTTTAC TGTGACAAG GATCATCTAG TGGTAGCTGA
 Human2 CCCAGTGATG AAAGGTTTAC TGTGACAAG GATCATCTAG TGGTAGCTGA
 Chick CCAGATGATG AAAGGTTTCT AGTTGGTAAA GACAACTTGA CCATTATGAA
 15
 Human1 TGTCA GTGAC GATGACAGCG GGACCTACAC GTGTGTGGCC AACACCACTC
 Humna2 TGTCA GTGAC GATGACAGCG GGACCTACAC GTGTGTGGCC AACACCACTC
 Chick TGTA ACTGAT AAAGATGATG GAACATATAC TTGCATAGTT AATACTACTC
 20
 Human1 TGGACAGCGT CTCCGCCAGC GCTGTGCTTA GCGTTGTTGC **TCCTACTCCA**
 Human2 TGGACAGCGT CTCCGCCAGC GCTGTGCTTA GCGTTGTTGC **TCCTACTCCA**
 Chick TGGACAGTGT TTCAGCAAGT GCTGTGCTTA CTGTTGTTGC **TGCTCCCCCA**
 AE10 1956
 Human1 **ACTCCAGCTC CCGTTTACGA TGTC**
 Human2 **ACTCCAGCTC CCGTTTACGA TGTC**
 Chick **ACTCCAGCTA TCATTTACGC TCGG**

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 98/00434

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/705 C12Q1/68 C07K16/28 A61K31/70
A61K35/00 A61K38/00 A61K38/17 A61K48/00 G01N33/53
G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K C12Q A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LANE R.P. ET AL.: "Characterization of a highly conserved human homolog to the chicken neural cell surface protein Bravo/Nr-CAM that maps to chromosome band 7q31" GENOMICS, vol. 35, no. 3, 1 August 1996, pages 456-465, XP002066828 cited in the application	5,15,18, 22,25
Y	see abstract see page 458 - page 459; figure 1 see page 461, left-hand column, paragraph 2 - page 464, left-hand column; figure 3 --- -/--	1-4, 6-14,16, 17, 19-21,23

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

4 June 1998

Date of mailing of the international search report

03.07.98

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2

NL - 2280 HV Rijswijk

Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,

Fax: (+31-70) 340-3016

Authorized officer

Macchia, G

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/00434

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DAVIS J.Q. ET AL.: "Molecular composition of the node of Ranvier: identification of Ankyrin-binding cell adhesion molecules Neurofascin (Mucin+/third FNIII domain-) and NrCAM at nodal axon segments" THE JOURNAL OF CELL BIOLOGY, vol. 135, no. 5, December 1996, pages 1355-1367, XP002066829	5,15,22
Y	see page 1357, right-hand column, paragraph 3 see page 1362; figure 7 ---	1-4, 6-14,23
Y	WO 96 32959 A (ACORDA THERAPEUTICS) 24 October 1996 see page 4, line 20 - page 6, line 25 see page 9, line 6 - page 10, line 14 see page 36, line 24 - page 40, line 31 see page 76 - page 84; tables 1-3 see page 103 - page 107; claims ---	16,17, 19-21
X	VOLKMER H. ET AL.: "Neurofascin induces neurites by heterophilic interactions with axonal NrCAM while NrCAM requires F11 in the axonal surface to extend neurites" THE JOURNAL OF CELL BIOLOGY, vol. 135, no. 4, November 1996, pages 1059-1069, XP002066882 see page 1062, left-hand column, paragraph 2 - page 1063; figure 2 ---	15,19-21
X	SUTER D.M. ET AL.: "Binding between the neural cell adhesion molecules Axonin-1 and Nr-CAM/Bravo is involved in neuron-glia interaction" THE JOURNAL OF CELL BIOLOGY, vol. 131, no. 4, November 1995, pages 1067-1081, XP002066830 see page 1074; figure 5 see page 1072, right-hand column see page 1075; figure 6 ---	15,19-21
P,X	Database EMBL, entry HSC7NRCAM, Accession number AJ001057, 30 November 1997, 100% identity with Seq.ID:1 nt.33-3932 XP002066831 see the whole document -----	1-5, 12-14, 22,23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 98/00434

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 16, 17, 24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/GB 98/00434

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9632959 A	24-10-1996	AU 5557296 A	07-11-1996
		EP 0821591 A	04-02-1998
		NO 974811 A	19-12-1997
		PL 322947 A	02-03-1998
