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(54) Titre : POLYMORPHISMES GENETIQUES DANS LE GENE DE LA PREPROTACHYKININE
(54) Title: GENETIC POLYMORPHISMS IN THE PREPROTACHYKININ GENE

(57) **Abrégé/Abstract:**

The present invention relates to a method for correlating single nucleotide polymorphisms in the preprotachykinin (NKNA) gene with the efficacy and compatibility of a pharmaceutically active compound administered to a human being. The invention further relates to a method for determining the efficacy and compatibility of a pharmaceutically active compound administered to a human being which method comprises determining at least one single nucleotide polymorphism in the NKNA gene. Said methods are based on determining specific single nucleotide polymorphisms in the NKNA gene and determining the efficacy and compatibility of a pharmaceutically active compound in the human by reference to polymorphism in NKNA. The invention further relates to isolated nucleic acids comprising within their sequence the polymorphisms as defined herein, to nucleic acid primers and oligonucleotide probes capable of hybridizing to such nucleic acids and to a diagnostic kit comprising one or more of such primers and probes for detecting a polymorphism in the NKNA gene, to a pharmaceutical pack comprising NK-1 receptor antagonists and instructions for administration of the drug to human beings tested for the polymorphisms as well as to a computer readable medium with the stored sequence information for the polymorphisms in the NKNA gene.



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(54) Title: GENETIC POLYMORPHISMS IN THE PREPROTACHYKININ GENE

(57) **Abstract:** The present invention relates to a method for correlating single nucleotide polymorphisms in the preprotachykinin (NKNA) gene with the efficacy and compatibility of a pharmaceutically active compound administered to a human being. The invention further relates to a method for determining the efficacy and compatibility of a pharmaceutically active compound administered to a human being which method comprises determining at least one single nucleotide polymorphism in the NKNA gene. Said methods are based on determining specific single nucleotide polymorphisms in the NKNA gene and determining the efficacy and compatibility of a pharmaceutically active compound in the human by reference to polymorphism in NKNA. The invention further relates to isolated nucleic acids comprising within their sequence the polymorphisms as defined herein, to nucleic acid primers and oligonucleotide probes capable of hybridizing to such nucleic acids and to a diagnostic kit comprising one or more of such primers and probes for detecting a polymorphism in the NKNA gene, to a pharmaceutical pack comprising NK-1 receptor antagonists and instructions for administration of the drug to human beings tested for the polymorphisms as well as to a computer readable medium with the stored sequence information for the polymorphisms in the NKNA gene.



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Genetic polymorphisms in the preprotachykinin gene

The present invention relates to a method for correlating single nucleotide polymorphisms in the preprotachykinin (NKNA) gene with the efficacy and compatibility of a pharmaceutically active compound administered to a human being. The invention further relates to a method for determining the efficacy and compatibility of a pharmaceutically active compound administered to a human being which method comprises determining at least one single nucleotide polymorphism in the NKNA gene. Said methods are based on determining specific single nucleotide polymorphisms in the NKNA gene and determining the efficacy and compatibility of a pharmaceutically active compound in the human by reference to polymorphism in NKNA. The invention further relates to isolated nucleic acids comprising within their sequence the polymorphisms as defined herein, to nucleic acid primers and oligonucleotide probes capable of hybridizing to such nucleic acids and to diagnostic kits comprising one or more of such primers and probes for detecting a polymorphism in the NKNA gene, to a pharmaceutical pack comprising NK-1 receptor antagonists and instructions for administration of the drug to human beings tested for the polymorphisms as well as to a computer readable medium with the stored sequence information for the polymorphisms in the NKNA gene.

Pharmacogenetics is an approach to use the knowledge of polymorphisms to study the role of genetic variation among individuals in variation to drug response, a variation that often results from individual differences in drug metabolism. Pharmacogenetics helps to identify patients most suited to therapy with particular pharmaceutical agents. This approach can be used in pharmaceutical research to assist the drug selection process. Details on pharmacogenetics and other uses of polymorphism detection can be found in Linder et al. (1997), *Clinical Chemistry*, 43, 254; Marshall (1997), *Nature Biotechnology*, 15, 1249; International Patent Application WO 97/40462, Spectra Biomedical; and Schafer et al. (1998), *Nature Biotechnology*, 16, 33.

Moreover, polymorphisms are implicated in over 2000 human pathological syndromes resulting from DNA insertions, deletions, duplications and nucleotide substitutions. Finding genetic polymorphisms in individuals and following these variations in families provides a means to confirm clinical diagnoses and to diagnose

both predispositions and disease states in carriers, as well as preclinical and subclinical affected individuals. Counseling based upon accurate diagnoses allows patients to make informed decisions about potential parenting, ongoing pregnancy, and early intervention in affected individuals.

5 Polymorphisms associated with pathological syndromes are highly variable and, consequently, can be difficult to identify. Because multiple alleles within genes are common, one must distinguish disease-related alleles from neutral (non-disease-related) polymorphisms. Most alleles are neutral polymorphisms that produce indistinguishable, normally active gene products or express normally variable characteristics like eye color.
10 In contrast, some polymorphic alleles are associated with clinical diseases such as sickle cell anemia. Moreover, the structure of disease-related polymorphisms are highly variable and may result from a single point mutation such as occurs in sickle cell anemia, or from the expansion of nucleotide repeats as occurs in fragile X syndrome and Huntington's chorea.

15 Neurokinin-1 (NK-1) or substance P is a naturally occurring undecapeptide belonging to the tachykinin family of peptides, the latter being so-named because of their prompt contractile action on extravascular smooth muscle tissue. The 3 known tachykinins in man are encoded by 2 genes, NKNA and NKNB. The NKNA gene encodes a precursor containing both substance P and neurokinin A, while the NKNB gene
20 encodes a precursor containing only neurokinin B. (Neurokinin A was formerly known as substance K). Using probes derived from the cloned human genes and a panel of rodent-human somatic cell hybrids, Bonner et al. (Cytogenet. Cell Genet., 1987, 46, 584) assigned the NKNA gene to 7q21-q22 and the NKNB gene to 12q13-q21. Molecular characterization of the tachykinins show that they arise from common precursor
25 molecules known as preprotachykinins by proteolytic processing. Three forms of message (alpha, beta, and gamma) arise by alternative splicing events (Proc. Nat. Acad. Sci., 1987, 84, 881-885). The beta and gamma forms of preprotachykinins encode both substance P and neurokinin A, while the alpha form contains only the substance P sequence.

The neuropeptide receptor for substance P (NK-1 receptor) is a member of the
30 superfamily of G protein-coupled receptors. It is widely distributed throughout the mammalian nervous system (especially brain and spinal ganglia), the circulatory system and peripheral tissues (especially the duodenum and jejunum) and is involved in regulating a number of diverse biological processes.

The central and peripheral actions of the mammalian tachykinin substance P have
35 been associated with numerous inflammatory conditions including migraine, rheumatoid arthritis, asthma, and inflammatory bowel disease as well as mediation of the

emetic reflex and the modulation of central nervous system (CNS) disorders such as Parkinson's disease (Neurosci. Res., 1996, 7, 187-214), anxiety (Can. J. Phys., 1997, 75, 612-621) and depression (Science, 1998, 281, 1640-1645).

Evidence for the usefulness of NK-1 receptor antagonists in pain, headache,
5 especially migraine, Alzheimer's disease, multiple sclerosis, attenuation of morphine withdrawal, cardiovascular changes, oedema, such as oedema caused by thermal injury, chronic inflammatory diseases such as rheumatoid arthritis, asthma/bronchial hyperreactivity and other respiratory diseases including allergic rhinitis, inflammatory diseases of the gut including ulcerative colitis and Crohn's disease, ocular injury and
10 ocular inflammatory diseases has been reviewed in "Tachykinin Receptor and Tachykinin Receptor Antagonists", J. Auton. Pharmacol., 1993, 13, 23-93.

Furthermore, NK-1 receptor antagonists are being developed for the treatment of a number of physiological disorders associated with an excess or imbalance of tachykinin, in particular substance P. Examples of conditions in which substance P has been
15 implicated include disorders of the central nervous system such as anxiety, depression and psychosis (WO 95/16679, WO 95/18124 and WO 95/23798).

The NK-1 receptor antagonists are further useful for the treatment of motion sickness and for treatment induced vomiting.

In addition, in The New England Journal of Medicine, 1999, 340(3), 190-195, the
20 reduction of cisplatin-induced emesis by a selective NK-1 receptor antagonist has been described.

The usefulness of NK-1 receptor antagonists for the treatment of certain forms of urinary incontinence is further described in Neuropeptides, 1998, 32(1), 1-49, and Eur. J. Pharmacol., 1999, 383(3), 297-303.

25 Furthermore, US 5,972,938 describes a method for treating a psychoimmunologic or a psychosomatic disorder by administration of a tachykinin receptor antagonist, such as NK-1 receptor antagonist.

Life Sci., 2000, 67(9), 985-1001, describes, that astrocytes express functional receptors to numerous neurotransmitters including substance P, which is an important
30 stimulus for reactive astrocytes in CNS development, infection and injury. In brain tumors malignant glial cells originating from astrocytes are triggered by tachykinins via NK-1 receptors to release soluble mediators and to increase their proliferative rate. Therefore, selective NK-1 receptor antagonists may be useful as a therapeutic approach to treat malignant gliomas in the treatment of cancer.

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In Nature (London), 2000, 405(6783), 180-183, it is described that mice with a genetic disruption of NK-1 receptor show a loss of the rewarding properties of morphine. Consequently, NK-1 receptor antagonists may be useful in the treatment of withdrawal symptoms of addictive drugs such as opiates and nicotine and reduction of their
5 abuse/craving.

NK-1 receptor antagonists have been reported to have also a beneficial effect in the therapy of traumatic brain injury (oral disclosure by Prof. Nimmo at the International Tachykinin Conference 2000 in La Grande Motte, France, October 17-20, 2000 with the title "Neurokinin 1 (NK-1) Receptor Antagonists Improve the Neurological Outcome
10 Following Traumatic Brain Injury" (Authors: A.J. Nimmo, C.J. Bennett, X.Hu, I. Cernak, R. Vink).

Another indication for treatment with NK-1 antagonists is benign prostatic hyperplasia (BPH), which can be progressive and lead to urinary retention, infections, bladder calculi and renal failure and has been reported in EP 01109853.0.

15 Clinical trials have shown that patient response to treatment with pharmaceuticals, e.g. NK-1 receptor antagonists, is often heterogeneous. Thus there is a need for improved approaches to pharmaceutical agent design and therapy.

Surprisingly it has now been found that single nucleotide polymorphisms in the NKNA gene can be used to determine the efficacy and compatibility of a
20 pharmaceutically active compound, e.g. a NK-1 receptor antagonists, administered to a human being.

The present invention relates to a method for correlating single nucleotide polymorphisms in the NKNA gene with the efficacy and compatibility of a
25 pharmaceutically active compound administered to a human being. The invention further relates to a method for determining the efficacy and compatibility of a pharmaceutically active compound administered to a human being which method comprises determining at least one single nucleotide polymorphism in the NKNA gene. Said methods are based on the determination of at least one single nucleotide
30 polymorphism in the NKNA gene in the sample of said human being, which method comprises determining the nucleotide at position 41172 in intron 1 of the NKNA gene as defined by the position in Figure 2 and determining the status of the human being by reference to polymorphism in the NKNA gene. Alternatively or, in addition thereto, the method comprises determining the sequence of the nucleic acid of the human being at

positions 41112 in intron 1 of the NKNA gene, 37434 in intron 5 of the NKNA gene, 37114, 37025, 33949 in intron 6 of the NKNA gene and 33612 in the 3'UTR of the NKNA gene as defined by Figure 2. The invention further relates to isolated nucleic acids comprising within their sequence the polymorphisms at the positions as defined before, to nucleic acid primers and oligonucleotide probes capable of hybridizing to such nucleic acids and to diagnostic kits comprising one or more of such primers and probes for detecting a polymorphism in the NKNA gene, to a pharmaceutical pack comprising NK-1 receptor antagonists and instructions for administration of the drug to human beings tested for the polymorphisms as well as to a computer readable medium with the stored sequence information for the polymorphisms in the NKNA gene.

The present invention is based on the discovery of single nucleotide polymorphisms in the NKNA gene.

The term "polymorphisms" is broadly defined to include all variations that are known to occur in nucleic acid sequences including insertions, deletions, substitutions and repetitive sequences including duplications.

"Polynucleotide" and "nucleic acid" refer to single or double-stranded molecules which may be DNA, comprised of the nucleotide bases A, T, C and G, or RNA, comprised of the bases A, U (substitutes for T), C, and G. The polynucleotide may represent a coding strand or its complement. Polynucleotide molecules may be identical in sequence to the sequence which is naturally occurring or may include alternative codons which encode the same amino acid as that which is found in the naturally occurring sequence (See, Lewin "Genes V" Oxford University Press Chapter 7, 1994, 171-174. Furthermore, polynucleotide molecules may include codons which represent conservative substitutions of amino acids as described. The polynucleotide may represent genomic DNA or cDNA.

As defined herein, the "NKNA gene" is the sequence present within the nucleic acid sequences shown in Figure 2 and in SEQ ID NO.1 located on human chromosome 7q21.1-q31.1. The NKNA gene includes 7 exon regions, 6 intron sequences intervening the exon sequences and 3' and 5' untranslated regions (3'UTR and 5'UTR) including the promotor element of the NKNA gene illustrated in Figure 1. The first in frame ATG occurs in exon 2 (or at position 41031 in Figure 2) while the TAG stop codon occurs in exon 7 (or at position 33724 in Figure 2) for the putative 129 amino acid protein.

The present invention relates to a method for correlating single nucleotide polymorphisms in the NKNA gene with the efficacy and compatibility of a

pharmaceutically active compound administered to a human being which method comprises determining single nucleotide polymorphisms in the NKNA gene of a human being and determining the status of said human being to which a pharmaceutically active compound was administered by reference to polymorphism in the NKNA gene.

5 According to a further aspect of the present invention there is provided a method for correlating single nucleotide polymorphisms in the NKNA gene with the efficacy and compatibility of a pharmaceutically active compound administered to a human being which method comprises determining single nucleotide polymorphisms in the NKNA gene of a human being and determining the status of said human being to which a
10 pharmaceutically active compound was administered by reference to polymorphism at at least one or more positions in Figure 2 comprising the NKNA gene including positions 33612, 33949, 37025, 37114, 37434, 41112 and/or 41172.

 The status of the human being may be determined by reference to allelic variation at one, two, three, four, five, six or all seven positions. The status of the human being may
15 also be determined by one or more of the specific polymorphisms identified herein in combination with one or more other single nucleotide polymorphisms.

 The status of the human being comprises any response of the human being to the drug therapy, comprising physiological and psychological responses.

 The term human being includes a human having or suspected of having a NK-1
20 receptor ligand mediated disease. At each position the human being may be homozygous for an allele or the human being may be heterozygous for an allele.

 The allelic variation may have a direct effect on the response of an individual to drug therapy. The methods of the invention may therefore be useful both to predict the clinical response to such agents and to determine therapeutic dose.

25 Pharmaceutically active compounds may belong to the group of NK-1 receptor antagonists. Neurokinin receptor antagonists have been reviewed in Exp. Opin. Ther. Patents, 1996, 6, 367-378, and in Exp. Opin. Ther. Patents, 1997, 7, 43-54. The term "NK-1 receptor antagonist" as used herein refers to any natural or synthetic chemical compound that inhibits binding of substance P to the NK-1 receptor. A large number of
30 such receptor antagonists are known and have been described. NK-1 receptor antagonists may be selected from the group consisting of 4-phenyl-pyridine derivatives, 3-phenyl-pyridine derivatives, 2-phenyl-substituted benzene derivatives, biphenyl derivatives, 4-phenyl-pyrimidine derivatives, 5-phenyl-pyrimidine derivatives, 1,4-diazepan-2,5-dione derivatives, 1,3,8-triaza-spiro[4.5]decan-4-one derivatives and piperidine derivatives as
35 described in EP1035115, WO0050401, WO0050398, WO0053572, WO0073279,

WO0073278, EP1103546, EP1103545, and WO0206236. These document as well as all documents referred to below are herewith incorporated by reference in their entirety.

Further preferred NK-1 receptor antagonists useful in connection with the present invention are the following NK-1 receptor antagonists currently under drug
5 development:

GR205171: 3-Piperidinamine, N-[[2-methoxy-5-[5-(trifluoromethyl)-1H-tetrazol-1-yl]phenyl]methyl]-2-phenyl-, (2S-cis)- (Gardner et al. Regul. Pep. 65:45, 1996)

HSP-117: 3-Piperidinamine, N-[[2,3-dihydro-5-(1-methylethyl)-7-benzofuranyl]methyl]-2-phenyl-, dihydrochloride, (2S-cis)-

10 L 703,606: 1-Azabicyclo[2.2.2]octan-3-amine, 2-(diphenylmethyl)-N-[(2-iodophenyl)methyl]-, (2S-cis)-, oxalate (Cascieri et al., Mol. Pharmacol. 42, 458, 1992)

L 668,169: L-Phenylalanine, N-[2-[3-[[N-[2-(3-amino-2-oxo-1-pyrrolidinyl)-4-methyl-1-oxopentyl]-L-methionyl-L-glutaminy]-D-tryptophyl-N-methyl-L-phenylalanyl]amino]-2-oxo-1-pyrrolidinyl]-4-methyl-1-oxopentyl]-L-methionyl-L-glutaminy]-D-tryptophyl-N-methyl-, cyclic (8->1)-peptide, [3R-[1[S*[R*(S*)]],3R*]]-
15

LY 303241: 1-Piperazineacetamide, N-[2-[acetyl[(2-methoxyphenyl)methyl]amino]-1-(1H-indol-3-yl-methyl)ethyl]-4-phenyl-, (R)-

LY 306740: 1-Piperazineacetamide, N-[2-[acetyl[(2-methoxyphenyl)methyl]amino]-1-(1H-indol-3-yl-methyl)ethyl]-4-cyclohexyl-, (R)-

20 MK-869: 3H-1,2,4-Triazol-3-one, 5-[[2-[1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2-dihydro-, [2R-[2 α (R*),3 α]]-

R-544: Ac-Thr-D-Trp(FOR)-Phe-N-MeBzl

Spantide III: L-Norleucinamide, N6-(3-pyridinylcarbonyl)-D-lysyl-L-prolyl-3-(3-pyridinyl)-L-alanyl-L-prolyl-3,4-dichloro-D-phenylalanyl-L-asparaginy]-D-tryptophyl-L-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-leucyl-
25

WIN-62,577: 1H-Benzimidazo[2,1-b]cyclopenta[5,6]naphtho[1,2-g]quinazolin-1-ol, 1-ethynyl-2,3,3a,3b,4,5,15,15a,15b,16,17,17a-dodecahydro-15a,17a-dimethyl-, (1R,3aS,3bR,15aR,15bS,17aS)-

GR 103,537

L 758,298: Phosphonic acid, [3-[[2-[1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-2,5-dihydro-5-oxo-1H-1,2,4-triazol-1-yl]-, [2R-[2 α (R*),3 α]]-

NKP608: (2R,4S)-N-[1-{3,5-bis(trifluoromethyl)-benzoyl}-2-(4-chloro-benzyl)-4-piperidinyl]-quinoline-4-carboxamide

CGP49823: (2R, 4S)-2-benzyl-1-(3, 5-dimethylbenzoyl)-N-[(4-quinolinyl)methyl]-4-piperineamine) dihydrochloride

CP-96,345: 2S, 3S)-cis-(2(diphenylmethyl)-N-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine (Snyder et al., Science 251:435, 1991)

CP-99,994: ((2S, 3S)-cis-3-(2-methoxybenzylamino)-2-phenyl-piperidine)dihydrochloride (Desai et al., J. Med. Chem. 35:4911, 1992)

CP-122,721: (+)-2S, 3S)-3-(2-methoxy-5-trifluoromethoxybenzyl)amino-2-phenylpiperidine

FK 888: (N2-[(4R)-4-hydroxy-1(1-methyl-1H-indol-3-yl)carbonyl-L-propyl]-N-methyl-N-phenylmethyl-L-3-(2-naphthyl)-alaninamide (Fujii et al., Br. J. Pharm. 107:785, 1992)

GR203040: (2S, 3S and 2R, 3R)-2methoxy-5-tetrazol-1-yl-benzyl-(2-phenyl-piperidin-3-yl)-amine

GR 82334: [D-Pro9,]spiro-gamma-lactam]Leu10, Trp11]physalaemin-(1-11)

GR 94800: PhCO-Ala-Ala-DTrp-Phe-DPe-DPro-Pro-NIe-NH₂

L 732,138: N-acetyl-L-tryptophan

L 733,060: ((2S,S)-3-((3, 5-bis(trifluoromethyl)phenyl)methoxy)-2-phenyl piperidine

L 742,694: (2-(S)-(3, 5-bis(trifluoromethyl)benzyloxy)-3-(S)-phenyl-4-(5-(3-oxo-1, 2, 4-triazolo)methylmorpholine

L 754,030: 2-(R)-(1-(R)-3, 5-bis(trifluoromethyl)phenylethoxy)-3-(S)-(4-fluoro)phenyl-4-(3-oxo-1,2,4-triazol-5-yl)methylmorpholine

LY 303870: (R)-1[N-(2-methoxybenzyl)acetylamino]-3-(1H-indol-3-yl)-2-[N-(2-(4-(piperidinyl)piperidin-1-yl)acetyl)amino]propane

MEN 11149: 2-(2-naphthyl)-1-N-[(1R, 2S)-2-N-[2(H)indol-3-ylcarbonyl]aminocyclohexanecarbonyl]-1-[N'-ethyl-N'-(4methylphenylacetyl)]diaminoethane (Cirillo et al., Eur. J. Pharm. 341:201, 1998)

PD 154075: (2-benzofuran)-CH₂OCO]-(R)-alpha-MeTrp-(S)-NHCH(CH₃) Ph

- 5 RP-67580: (3aR, 7aR)-7, 7-diphenyl-2[1-imino-2(2-methoxyphenyl)-ethyl]+++perhydroisoidol-4-one hydrochloride (Garret et al., PNAS 88:10208, 1991)

RPR 100893: (3aS, 4S, 7aS)-7, 7-diphenyl-4-(2-methoxyphenyl)-2-[(S)-2-(2-methoxyphenyl)propionyl]perhydroisoindol-4-ol

Spendide: Tyr-D-Phe-Phe-D-His-Leu-Met-NH₂

- 10 Spantide II: D-NicLys1, 3-PaI3, D-CI2Phe5, Asn6, D-Trp7.0, Nle11-substance P

SR140333: (S)-1-[2-[3-(3, 4-dichlorophenyl)-1(3-isopropoxyphenylacetyl) piperidin-3-yl]ethyl]-4-phenyl-1 azaniabicyclo [2.2.2]octane (Edmonds et al., Eur. J. Pharm. 250:403, 1993)

- 15 WIN-41,708: (17beta-hydroxy-17alpha-ethynyl-5alpha-androstano[3.2-b]pyrimido[1,2-a]benzimidazole

WIN-62,577: 1H-Benzimidazo[2,1-b]cyclopenta[5,6]naphtho[1,2-g]quinazolin-1-ol, 1-ethynyl-2,3,3a,3b,4,5,15,15a,15b,16,17,17a-dodecahydro-15a,17a-dimethyl-,(1R, 3aS, 3bR,15aR, 15bS, 17aS)-

- 20 SR-48,968: (S)-N-methyl-N[4-(4-acetylamino-4-[phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl]benzamide

L-659,877: cyclo[Gln, Trp, Phe, Gly, Leu, Met]

MEN 10627: cyclo(Met-Asp-Trp-Phe-Dap-Leu)cyclo(2beta-5beta)

SR 144190: (R)-3(1-[2-(4-benzoyl-2-(3,4-difluorophenyl)-morpholin-2-yl)ethyl]-4-phenylpiperidin-4-yl)-1-dimethylurea

- 25 GR 94800: PhCO-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH₂

SR-142,801: (S)-(N)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-N-methyl acetamide

R820: 3-indolylcarbonyl-Hyp-Phg-N(Me)-Bzl

R486: H-Asp-Ser-Phe-Trp-beta-Ala-Leu-Met-NH₂

SB 222200: (S)-(-)-N-(a-ethylbenzyl)-3-methyl-2-phenylquinoline-4-carboximide

L 758,298: Phosphonic acid, [3-[[2-[1-[3, 5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]-2, 5-dihydro-5-oxo-1H-1,2,4-triazol-1-yl]-, [2R-[2a(R*),
5 3a]]-

NK-608: (2R,4S)-N-[1-{3, 5-bis(trifluoromethyl)-benzoyl}-2-(4-chloro-benzyl)-4-piperidinyl]-quinoline-4-carboxamide

CGP 47899: Shilling et al., Pers. Med. Chem. 207, 1993

MEN 11467: Evangelista et al., XXIX Nat. Congr. of the Ital. Pharmacological Soc.,
10 Florence 20-23.06.1999.

Any reference herein to the compound specifically named above includes also the pharmaceutically acceptable acid addition salts thereof.

Further information on these NK-1 receptor antagonists under drug development can be found in the published literature.

15 Additional suitable NK-1 receptor antagonists are described in the following published patents and patent applications.

U.S. Patent No. 5,990,125 in particular the compounds Ia to Ie, X and XVI to XXI, as well as other antagonists comprising quinuclidine, piperidine ethylene diamine, pyrrolidine and azabornane derivatives and related compounds that exhibit activity as
20 substance P receptor antagonists as described in column 33 of USP 5,990,125. These antagonists are preferably used in dosages as specified in column 34 of USP 5,990,125.

Further suitable NK-1 receptor antagonists are described in the following publications:

U.S. Patent Nos. (USP)

5,977,104	5,162,339	4,481,139	5,232,929
5,998,444	5,242,930	5,373,003	5,981,744
5,387,595	5,459,270	5,494,926	5,496,833
5,637,699			

Europ. Patent Application, Publ. Nos. (EP-A-)

0 360 390	0 394 989	0 428 434	0 429 366
0 430 771	0 436 334	0 433 132	0 482 539
0 498 069	0 499 313	0 512 901	0 512 902
0 514 273	0 514 274	0 514 275	0 514 276
0 515 681	0 517 589	0 520 555	0 522 808
0 528 495	0 532 456	0 533 280	0 536 817
0 545 478	0 558 156	0 577 394	0 585 913
0 590 152	0 599 538	0 610 793	0 634 402
0 686 629	0 639 489	0 694 535	0 699 655
0 699 674	0 707 006	0 708 101	0 709 375
0 709 376	0 714 891	0 723 959	0 733 632
0 776 893			

PCT Int. Patent Publ. Nos.(WO)

90/05525	90/05729	91/09844	91/18899
92/01688	92/06079	92/12151	92/15585
92/17449	92/20661	92/20676	92/21677
92/22569	93/00330	93/00331	93/01159
93/01165	93/01169	93/01170	93/06099
93/09116	93/10073	93/14084	93/14113
93/18023	93/19064	93/21155	93/21181
93/23380	93/24465	94/00440	94/01402
94/02461	94/02595	94/03429	94/03445
94/04494	94/04496	94/05625	94/07843
94/08997	94/10165	94/10167	94/10168
94/10170	94/11368	94/13639	94/13663
94/14767	94/15903	94/19320	94/19323
94/20500	94/26735	94/26740	94/29309
95/02595	95/04040	95/04042	95/06645
95/07886	95/08908	95/08549	95/11880
95/14017	95/15311	95/16679	95/17382
95/18124	95/18129	95/19344	95/20575
95/21819	95/22525	95/23798	95/26338
95/28418	95/30674	95/30687	95/33744
96/05181	96/05193	96/05203	96/06094
96/07649	96/10562	96/16939	96/18643

96/20197	96/21661	69/29304	96/29317
96/29326	96/29328	96/31214	96/32385
96/37489	97/01553	97/01554	97/03066
97/08144	97/14671	97/17362	97/18206
97/19084	97/19942	97/21702	97/49710

British Patent Publ. Nos. (GB)

2 266 529	2 268 931	2 269 170	2 269 590
2 271 774	2 292 144	2 293 168	2 293 169
2 302 689			

Indications for the NK-1 receptor antagonists described above are treatment of
 5 pain, headache, especially migraine, Alzheimer's disease, disorders of the central nervous
 system such as certain depressive disorders, anxiety, and emesis, psychosis, multiple
 sclerosis, attenuation of morphine withdrawal, cardiovascular changes, oedema, such as
 oedema caused by thermal injury, chronic inflammatory diseases such as rheumatoid
 arthritis, asthma/bronchial hyperreactivity and other respiratory diseases including
 10 allergic rhinitis, inflammatory diseases of the gut including ulcerative colitis and Crohn's
 disease, ocular injury and ocular inflammatory diseases, benign prostatic hyperplasia,
 motion sickness, treatment induced vomiting, cancer such as malignant gliomas,
 traumatic brain injury.

Preferably, the NK-1 receptor antagonist is 2-(3,5-bis-trifluoromethyl-phenyl)-N-
 15 [6-(1,1-dioxo-1 λ^6 -thiomorpholin-4-yl)-4-o-tolyl-pyridin-3-yl]-N-methyl-isobutyramide
 or 2-(3,5-bis-trifluoromethyl-phenyl)-N-[6-(1,1-dioxo-1 λ^6 -thiomorpholin-4-yl)-4-(4-
 fluoro-2-methyl-phenyl)-pyridin-3-yl]-N-methyl-isobutyramide as disclosed in
 EP1035115.

Most preferably, the NK-1 receptor antagonist is 2-(3,5-bis-trifluoromethyl-
 20 phenyl)-N-methyl-N-(6-morpholin-4-yl-4-o-tolyl-pyridin-3-yl)-isobutyramide as
 disclosed in EP1035115.

Preferred indications for these NK-1 receptor antagonists are those, which include
 disorders of the central nervous system, for example the treatment or prevention of
 certain depressive disorders, anxiety or emesis. A major depressive episode has been
 25 defined as being a period of at least two weeks during which, for most of the day and

nearly every day, there is either depressed mood or the loss of interest or pleasure in all, or nearly all activities.

In another aspect the present invention provides a method for determining the efficacy and compatibility of a pharmaceutically active compound for a human being, which method comprises determining the presence of single nucleotide polymorphisms in the NKNA genomic sequence obtained from the human being which single nucleotide polymorphisms are correlated with the efficacy and compatibility of the pharmaceutically active compound, and thereby determining the efficacy and compatibility of the pharmaceutically active compound for the human being.

Preferably, the present invention relates to a method for determining the efficacy and compatibility of a pharmaceutically active compound for a human being, which method comprises determining the nucleotide at at least one or more of the positions 33612, 33949, 37025, 37114, 37434, 41112 and/or 41172 of the nucleotide sequence in Figure 2 comprising the NKNA gene in the NKNA genomic sequence obtained from the human being which single nucleotide polymorphisms are correlated with the efficacy and compatibility of the pharmaceutically active compound, and thereby determining the efficacy and compatibility of the pharmaceutically active compound for the human being.

Preferably, the present invention relates to a method for determining the efficacy and compatibility of a pharmaceutically active compound for a human being, which method comprises determining in the NKNA genomic sequence obtained from the human being the presence of a single nucleotide polymorphism selected from the group of a C or a T at position 33612 of the nucleotide sequence in Figure 2 comprising the NKNA gene, a T or a C at position 33949 in Figure 2, a C or a T at position 37025 in Figure 2, a G or an A at position 37114 in Figure 2, an A or a C at position 37434 in Figure 2, a T or a G at position 41112 in Figure 2, a G or an A at position 41172 in Figure 2, and combinations thereof as well as their reverse complement, which single nucleotide polymorphisms are correlated with the efficacy and compatibility of the pharmaceutically active compound, and thereby determining the efficacy and compatibility of the pharmaceutically active compound for the human being.

In this method, the pharmaceutically active compound may be a NK-1 receptor antagonist. The NK-1 receptor antagonist may be any NK-1 receptor antagonist as described beforehand.

The method in accordance with the present invention can be performed using any suitable method for detecting single nucleotide variations, such as e.g. direct mass-analysis of PCR products using mass spectrometry, direct analysis of invasive cleavage products, direct sequence analysis, allele specific amplification (i.e. ARMSTM-allele specific amplification; ARMS referring to amplification refractory mutation system), ALEXTM (amplification refractory mutation system linear extension) and COPS (competitive oligonucleotide priming system), allele specific hybridization (ASH), oligonucleotide ligation assay (OLA), Invader Assay, DNA chip analysis and restriction fragment length polymorphism (RFLP) (for review see Genome Research, 1998, 8, 769-776; Pharmacogenomics, 2000, 1, 95-100; Human Mutation, 2001, 17, 475-492).

The test sample of the nucleic acid carrying the said polymorphism is conveniently a sample of blood, bronchoalveolar lavage fluid, sputum, urine or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the sequence in the test sample, that is to say that all or a part of the region in the sample nucleic acid may firstly be amplified using any convenient technique, e.g. polymerase chain reaction (PCR) or ligase chain reaction (LCR), before analysis of allelic variation.

Polymorphisms in the NKNA gene can be identified by sequencing a nucleic acid sample of patients and comparing the sequence to controls or by PCR-amplification of 400-600 base pair fragments (covering coding and regulatory regions of the NKNA gene) in the DNA of unrelated individuals of different ethnic origin. Fragments can be sequenced in these samples with a forward and reverse primer, polymorphisms can be detected by using the PolyPhred software (licensed from University of Washington) and allele frequencies for the variants can be established (Human Mutation, 2001, 17, 243-254).

It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more polymorphic positions of the invention. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and optionally a signal generation system. International patent application WO 00/06768 lists a number of amplification techniques and mutation detection techniques, some based on PCR. These may be used in combination with a number of signal generation systems, a selection of which is also listed in WO 00/06768. Many current methods for the detection of allelic variation are reviewed by Nollau et al., Clin. Chem., 1997, 43, 1114-1120; and in standard textbooks, for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren, Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

The invention further provides an isolated nucleic acid molecule selected from the following polymorphism containing sequences:

- the nucleic acid sequence of Figure 2 with T at position 33612 as defined by the position in Figure 2;
- 5 the nucleic acid sequence of Figure 2 with C at position 33949 as defined by the position in Figure 2;
- the nucleic acid sequence of Figure 2 with T at position 37025 as defined by the position in Figure 2;
- the nucleic acid sequence of Figure 2 with A at position 37114 as defined by the position
- 10 in Figure 2;
- the nucleic acid sequence of Figure 2 with C at position 37434 as defined by the position in Figure 2;
- the nucleic acid sequence of Figure 2 with G at position 41112 as defined by the position in Figure 2;
- 15 the nucleic acid sequence of Figure 2 with G at position 41172 as defined by the position in Figure 2; or
- a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the polymorphisms.

An "isolated" NKNA nucleic acid molecule is a nucleic acid molecule that is

20 identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the NKNA nucleic acid. An isolated NKNA nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated NKNA nucleic acid molecules therefore are distinguished from the NKNA nucleic acid molecule as it exists in natural cells. However, an isolated NKNA

25 nucleic acid molecule includes NKNA nucleic acid molecules contained in cells that ordinarily express NKNA where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

Furthermore the invention relates to allele-specific nucleic acid primers which can be used as diagnostic primers for detecting a polymorphism in the NKNA gene capable of

30 hybridizing to nucleic acids comprising within their sequence the polymorphisms at positions 33612 in Figure 2, 33949 in Figure 2, 37025 in Figure 2, 37114 in Figure 2, 37434 in Figure 2, 41112 in Figure 2, and 41172 in Figure 2.

Another aspect of the present invention is a nucleic acid primer comprising the following sequences selected from the group of:

- 35 the nucleic acid sequence as defined by SEQ ID NO.8;
- the nucleic acid sequence as defined by SEQ ID NO.9;
- the nucleic acid sequence as defined by SEQ ID NO.10;

- 16 -

- the nucleic acid sequence as defined by SEQ ID NO.11;
the nucleic acid sequence as defined by SEQ ID NO.12;
the nucleic acid sequence as defined by SEQ ID NO.13;
the nucleic acid sequence as defined by SEQ ID NO.14;
5 the nucleic acid sequence as defined by SEQ ID NO.15; or
the nucleic acid sequence as defined by SEQ ID NO.16 and their reverse complement.

An allele specific primer is used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence
10 position e.g. as used for ARMSTM assays. The length of the allele specific primer is preferably 17-50 nucleotides, more preferably about 17-35 nucleotides, most preferably about 17-30 nucleotides.

Preferably, the allele specific primer corresponds exactly with the allele to be detected but derivatives thereof are also contemplated wherein about 6-8 of the
15 nucleotides at the 3' terminus correspond with the allele to be detected and wherein up to 10, such as up to 8, 6, 4, 2, or 1 of the remaining nucleotides may be varied without significantly affecting the properties of the primer. Often the nucleotide at the -2 and/or -3 position (relative to the 3' terminus) is mismatched in order to optimize differential primer binding and preferential extension from the correct allele discriminatory primer
20 only.

The invention also relates to oligonucleotide probes for detecting a polymorphism in the NKNA gene capable of hybridizing specifically to a nucleic acid comprising within its sequence the polymorphism at positions 33612 in Figure 2, 33949 in Figure 2, 37025 in Figure 2, 37114 in Figure 2, 37434 in Figure 2, 41112 in Figure 2, and 41172 in Figure
25 2.

The term "oligonucleotide probe" refers to a nucleotide sequence of at least 17 nucleotides in length which corresponds to part or all of the human NKNA gene, preferably a part of the human NKNA gene which expresses the polymorphism. A length of 17 to 50 nucleotides is preferred. In general such probes will comprise base sequences
30 entirely complementary to the corresponding wild type or variant locus in the gene. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection, such as in Molecular Beacons.

The present invention also encompasses a diagnostic kit comprising one or more nucleic acid primer(s) and/or one or more oligonucleotide probe(s) with a single nucleotide polymorphism of the NKNA gene selected from the group consisting of a C or a T at position 33612 in Figure 2, a T or a C at position 33949 in Figure 2, a C or a T at position 37025 in Figure 2, a G or an A at position 37114 in Figure 2, an A or a C at position 37434 in Figure 2, a T or a G at position 41112 in Figure 2, and a G or an A at position 41172 in Figure 2, and combinations thereof as well as their reverse complement.

The present invention further provides a pharmaceutical pack comprising NK-1 receptor antagonists and instructions for administration of the drug to human beings tested for a single nucleotide polymorphism at one or more positions of the NKNA gene.

The present invention further provides a pharmaceutical pack comprising NK-1 receptor antagonists, preferably 2-(3,5-bis-trifluoromethyl-phenyl)-N-methyl-N-(6-morpholin-4-yl-4-o-tolyl-pyridin-3-yl)-isobutyramide, and instructions for administration of the drug to human beings tested for a single nucleotide polymorphism at one or more positions of the NKNA gene according to a method of the present invention.

The present invention further provides the use of a NK-1 receptor antagonist for the preparation of a medicament for treating a NK-1 receptor ligand-mediated disease in a human being diagnosed as having a single polynucleotide polymorphism at one or more of position 33612, 33949, 37025, 37114, 37434, 41112 and 41172 in Figure 2 comprising the NKNA gene.

The present invention also includes a computer readable medium having stored thereon sequence information for the polymorphisms in the NKNA gene including polymorphisms at positions 33612 in Figure 2, 33949 in Figure 2, 37025 in Figure 2, 37114 in Figure 2, 37434 in Figure 2, 41112 in Figure 2, and 41172 in Figure 2.

A method for performing sequence identification is also provided in the present invention, said method comprising the steps of providing a nucleic acid sequence selected from the group consisting of the nucleic acid sequence of Figure 2 with T at position 33612; the nucleic acid sequence of Figure 2 with C at position 33949; the nucleic acid sequence of Figure 2 with T at position 37025; the nucleic acid sequence of Figure 2 with A at position 37114; the nucleic acid sequence of Figure 2 with C at position 37434; the nucleic acid sequence of Figure 2 with G at position 41112; the nucleic acid sequence of Figure 2 with G at position 41172; or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the polymorphisms, and comparing

said nucleic acid sequence to at least one other nucleic acid or polypeptide sequence to determine identity.

Having now generally described this invention, the same will become better understood by reference to the specific examples, which are included herein for purpose
 5 of illustration only and are not intended to be limiting unless otherwise specified, in connection with the following figures:

Figure 1: Genomic structure of the NKNA gene and polymorphisms found in the gene. Exon-intron boundaries are indicated with respect to the sequence depicted in
 10 Figure 2 (corresponding to parts of the DNA sequence of the accession no. EM_HUM1:AC004140.1). Positions of polymorphisms are indicated with arrows.

Figure 2: Part of the genomic sequence of the PAC clone DJ0841B21 defined by the accession no. EM_HUM1:AC004140.1 containing the preprotachykinin gene. Exon-intron boundaries are indicated in Figure 1. The sequence corresponds to SEQ ID NO.1
 15 whereas position 33301 corresponds to position 1 in SEQ ID NO.1, and position 41800 corresponds to position 8500 in SEQ ID NO.1.

Figure 3: Identified single nucleotide polymorphisms in the NKNA gene with positions as defined in Figure 2.

Figure 4: 2 x 2 Contingency table for 29 position 41172 genotype test results with
 20 concentration before emesis test > 20 ng/ml 2-(3,5-bis-trifluoromethyl-phenyl)-N-methyl-N-(6-morpholin-4-yl-4-o-tolyl-pyridin-3-yl)-isobutyramide. Effect = yes: number of vomits + retches $\leq$ 3. Fisher's Exact Test (two-sided): $p = 0.033$. The genotypes of the subjects were classified according to the following categories: 0-2 are allele 2 (nucleotide G) homozygotes (two copies of allele 2, no copies of allele 1); not 0-2
 25 include allele1 (nucleotide A) homozygotes 2-0 (two copies of allele 1, no copies of allele 2) and heterozygotes 1-1 (one copy of allele 1 and one copy of allele 2).

Examples

Commercially available reagents referred to in the Examples were used according to manufacturer's instructions unless otherwise indicated.

5 Example 1: Detection of polymorphisms

For all single nucleotide polymorphisms discovery was performed by double-stranded DNA sequencing using an ABI capillary sequencer and Big Dye chemistry (ABI). First the genomic organization of the NKNA gene was derived from a PAC clone found in the EMBL database with the accession no. EM_HUM1:AC004140.1 by a BLAST
10 search with the NKNA mRNA (accession no. U37529.1 in the EMBL database). Exon-intron boundaries were derived as indicated in Figure 1 and primers were designed to amplify all coding and regulatory regions of the gene. The primers used to amplify all exons are shown below and were also used as sequencing primers. All polymorphisms were targeted with these pair-of-primer sets:

15 Table 1: List of oligonucleotide primers for polymorphism detection

<u>Primer type</u>	<u>Nucleotide sequence</u>	<u>SEQ ID NO</u>
Primer 1	CATGTTTACAATACATATTGGCAC	SEQ ID NO. 2
Primer 2	GTATATGATGAATGATG	SEQ ID NO. 3
Primer 3	CACCCTCATTCTTCCCTGC	SEQ ID NO. 4
Primer 4	CTTCAGTCTCACCAAACTTG	SEQ ID NO. 5
Primer 5	GTGCCCCTTTCCATCCTCTC	SEQ ID NO. 6
Primer 6	GGTGTGGGTTGGTGGGTTAG	SEQ ID NO. 7

To detect polymorphisms the NKNA gene was PCR-amplified from 47 unrelated individuals of 5 different ethnic origins. Using fragment-specific primer pairs (length 18-27 bp), 200-700 bp fragments were amplified e.g. a 519 bp-PCR product was generated
20 with the primer pair 5 and 6. Fragments were designed covering coding and regulatory regions of the NKNA gene. After a column purification of the PCR products, the DNA was sequenced on an ABI capillary sequencer using ABI Dye terminator chemistry (fluorescence based sequencing). Polymorphisms in the DNA sequences were detected

using Polyphred software (Nickerson, D. et al. 1997: NAR 25(14): 2745-2751), which operates on the basis of Phred, Phrap and Consed (programs all licensed from the University of Washington, USA). This program is able to automatically detect the presence of heterozygous single nucleotide substitutions by fluorescence-based
 5 sequencing. In the example above the following 2 polymorphisms were detected in the 519bp fragment:

41112*	T or G
41172*	G or A

*defined by the position in accession no. EM_HUM1:AC004140.1

In total, seven single polynucleotide polymorphisms were detected in the NKNA gene as shown in Figure 3.

10

Example 2: Genotyping

a) Selection of subjects

The study protocol and the informed consent form were submitted for approval to the local ethical committee. All subjects provided written informed consent for their
 15 blood sample to be used for genotyping. The consent could be withdrawn up to a month later, if the subjects changed their mind.

All the samples were assigned new independent codes and within six months after clinical database closure the link between the new and original codes was deleted. This was an added measure to ensure patient confidentiality; however, as a consequence it is
 20 not possible to retrieve genotype information based on the patient's name or number used in the original clinical trial. In approximately 15 years time, all blood and DNA samples will be destroyed.

b) Genotyping Assay

Single blood samples (9ml) were collected in EDTA tubes. These were frozen and
 25 stored between -20 and -70 °C, before being sent to the Roche Central Sample Office (CSO) in Basel, Switzerland, where they were aliquoted into three tubes and assigned new, independent codes on bar code labels to assure patient anonymity. Two samples of blood (1ml and 4mls) were sent to the Roche Sample Repository (RSR) at Roche Molecular Systems (RMS) in Alameda, California, and stored at -80 °C. The remaining

4ml aliquot was stored at -80°C in the CSO in Basel, Switzerland. All procedures performed on the samples at the RSR were done according to established standard operating procedures in compliance with GCP guidelines.

DNA was extracted from 400 μl of the whole blood using a silica membrane-based extraction method (QiaAmp 96 DNA Blood kit, Valencia, CA). Controls included 10 mM Tris pH 8.0, 0.1 mM EDTA (TE) buffer and whole blood from a blood unit with a known yield of DNA.

Three genetic markers were selected based on the results from polymorphism discovery in the NKNA gene. Samples were genotyped for these single nucleotide polymorphisms by a kinetic PCR method described by Germer et al., Genome Res. (2000), 10, 258–266 with the modification of using single sample for each reaction instead of pooling samples. This method allows discrimination of single nucleotide polymorphisms without the use of fluorescent probes.

In the kinetic thermal cycler (KTC) format, the generation of double-stranded amplification product is monitored using a DNA intercalating dye and a thermal cycler which has a fluorescence-detecting CCD camera attached (PE-Biosystems GeneAmp 5700 Sequence Detection System). Fluorescence in each well of the PCR amplification plate is measured at each cycle of annealing and denaturation. The cycle at which the relative fluorescence reached a threshold of 0.5 using the SDS software from PE-Biosystems was defined as the C_t .

The amplification reactions were designed to be allele-specific, so that the amplification reaction was positive if the allele was present and the amplification reaction was negative if the allele was absent. For each bi-allelic polymorphism, one well of the amplification plate was set up to be specific for allele 1 and a second well was set up to be specific for allele 2. For each polymorphism to be detected, three primers were designed – two allele-specific primers and one common primer (Table 2). Reactions for allele 1 contained allele 1-specific primer and the common primer and reactions for allele 2 contained allele 2-specific primer and the common primer. The C_t values for each pair of wells is used to calculate the delta C_t which is used to determine the allele call.

Table 2: List of oligonucleotide primers used for polymorphism screening

Marker	Primer ID	Nucleotide sequence	SEQ ID NO	Primer concentration (in μ M)	Annealing temperature
411172	PPT1/ 411172	CCGGTACAGGTGAGACTTT	SEQ ID NO.8	0.4	58°C
411172	PTT2/ 41172	CCGGTACAGGTGAGACTTC	SEQ ID NO.9	0.4	58°C
411172	PTT3/ 41172	CAACGGATGAACCAAGATC	SEQ ID NO.10	0.4	58°C
37114	PTT4/ 37114	AGAGAAATAGACAGATACTGTGGTAG	SEQ ID NO.11	0.2	58°C
37114	PPT 5 /37114	AGAGAAATAGACAGATACTGTGGTAA	SEQ ID NO.12	0.2	58°C
37114	PTT6 / 37114	ATGGATTTATAGCTGGTTAAGC	SEQ ID NO.13	0.2	58°C
37025	PTT7 /37025	CAGATCTATAGGAAAGAATATAGCAC	SEQ ID NO.14	0.2	58°C
37025	PTT8 /37025	CAGATCTATAGGAAAGAATATAGCAT	SEQ ID NO.15	0.2	58°C
37025	PTT9 /37025	CCATTTAATCATTACCAACCTGAATC	SEQ ID NO.16	0.2	58°C

The amplification conditions were as follows: 10 mM Tris pH 8.0, 40 mM KCl, 2 mM MgCl₂, 50 μ M each of dATP, dCTP, and dGTP, 25 μ M of dTTP and 75 μ M of dUTP, 4% DMSO, 0.2X SYBR Green I (Molecular Probes, Eugene, OR), 2% glycerol, 2 units of uracil N-glycosylase (UNG), 15 units of Stoffel Gold DNA polymerase (for reference see Nature (1996), 381, 445-6) and primers in an 85 μ l volume for each well. The concentration of the primers used for each assay are listed in Table 2. 30 ng of DNA in a 15 μ l volume was then added to each well.

To reduce the possibility of contamination by pre-existing amplification product, the assay procedure included the incorporation of dUTP into the amplification product and an incubation step for UNG degradation of pre-existing dU-containing products (Longo et al, Gene (1990), 93,125-128).

Amplification reactions were prepared using an aliquoting robot (Packard Multiprobe II, Meriden, CT) in 96-well amplification plates identified by barcode labels generated by the experiment management database. Parameters for procedures performed by the robot were set to minimize the possibility of cross-contamination. For
5 each plate of 81 samples, 5 samples were run in duplicate and the duplicate results were analyzed to determine that they matched.

The thermal cycling conditions were as follows: 2 minutes at 50 °C for UNG degradation of any previously contaminating PCR products, 12 minutes at 95 °C for Stoffel Gold polymerase activation, 55 cycles of denaturation at 95 °C for 20 seconds and
10 annealing at 58°C for 20 seconds, followed by a dissociation step of 0.57 minute at 1 degree increments from 60 °C to 95 °C. The amplification reactions were run in PE Biosystems GeneAmp 5700 Sequence Detection Systems (SDS) instruments (Foster City, CA). The first derivatives of the dissociation curves were produced by the SDS software and examined as needed to confirm that the fluorescence in a given reaction was due to
15 amplification of a specific product with a well-defined dissociation peak rather than non-specific primer-dimer. Product differentiation was done by Analysis of DNA Melting Curves during PCR following the method of K.M. Ririe et al., Anal. Biochem. (1997), 245, 154 – 160.

The C_t of each amplification reaction was determined and the difference between
20 the C_t for allele 1 and allele 2 (ΔC_t) was used as the assay result. Samples with ΔC_t s between -3.0 and 3.0 were considered heterozygous (A1/A2). Samples with ΔC_t s below -3.0 were considered homozygous for A1 (A1/A1); samples with ΔC_t s above 3.0 were considered homozygous for A2 (A2/A2). In most cases, the ΔC_t differences between the three groups of genotypes were well-defined and samples with C_t values close
25 to 3.0 were re-tested as discrepant.

Each assay was run on a panel of 20 cell line DNAs to identify cell lines with the appropriate genotypes for use as controls on each assay plate (A1/A1, A1/A2, and A2/A2). The cell line DNA was obtained from the Culture Collection in R & D Service, Roche Molecular Systems (RMS) Alameda, CA and was extracted using the Qiagen extraction
30 kits (QiaAmp DNA Blood kits, Valencia, CA). The genotypes of the cell line DNAs were confirmed by DNA sequencing. Three cell line DNAs (A1/A1, A1/A2, and A2/A2) were run as controls on each plate of clinical trial samples and used to determine the between-plate variability. The C_t values obtained for the control cell lines were analyzed to determine the cutoff for the ΔC_t values obtained for the clinical trial samples.

35 A data file containing the C_t values for each well was generated by the SDS software for each plate and entered into the experiment management database. For all the SNP

assays ran for the clinical trial, a data file with C_t values for all the samples identified by the independent code was extracted from the database and interpreted to the final genotypes by a in-house developed program. The genotype results were sent to the statistician and matched to the clinical data also identified by the independent code for
5 statistical analysis.

Example 3: Emesis test

The described emesis test was performed in two studies. A Single Ascending Dose study (SAD) and a Multiple Ascending Dose study (MAD). In the SAD the emesis test
10 was performed 6 and / or 24 hrs after intake of 2-(3,5-bis-trifluoromethyl-phenyl)-N-methyl-N-(6-morpholin-4-yl-4-o-tolyl-pyridin-3-yl)-isobutyramide. In the MAD the emesis test was performed after 14 once daily doses, 6 or 24 hrs after the last dose.

SAD

In the SAD on study day 1 doses of 5, 10, 20, 40, 80, 160, 230 and 400 mg 2-(3,5-bis-trifluoromethyl-phenyl)-N-methyl-N-(6-morpholin-4-yl-4-o-tolyl-pyridin-3-yl)-isobutyramide were administered to the subjects orally as a drinking emulsion. At either
15 6 or 24 hours after the administration of the drug the subjects received a subcutaneous injection of 50 µg/kg of apomorphine in the lower part of the abdomen. The time of apomorphine administration was recorded. The subjects were brought into an upright sitting position immediately after the injection. They remained in this position until
20 vomiting occurred or for at least 1 hour after the apomorphine injection. Vomiting is defined as regurgitation of approximately 25 ml or more of gastric contents. A retch is defined as a regurgitation producing less or no gastric content.

Nausea and/or vomiting was expected to occur on average within 10 minutes after
25 the injection. The duration of nausea and/or vomiting after a subcutaneous dose of 50 µg/kg apomorphine was approximately 5 to 30 minutes. The number of vomits and retches were recorded.

The test groups were ranked in following order of "plasma concentration at the time of emesis test". The plateau that was reached with the blockade had an average of 3
30 retches and vomits. If one assumes this as the point where efficacy is reached the test predicts that efficacious levels are reached at a concentration of 20 ng/ml plasma concentration.

- 25 -

The Spearman correlation test for the correlation between plasma concentration and the number of retches and vomits suggests a highly statistically significant relationship ($p < 0.01$).

MAD

- 5 Subjects were dosed for 14 days with 2-(3,5-bis-trifluoromethyl-phenyl)-N-methyl-N-(6-morpholin-4-yl-4-o-tolyl-pyridin-3-yl)-isobutyramide. On day 14 the test was carried out as described in the SAD.

The MAD showed results that were consistent with the SAD.

Genotyping

- 10 All participants of the SAD and MAD were tested for the single nucleotide polymorphism at position 41172 of the NKNA gene as defined by position in Figure 2.

- As the minimal concentration to achieve efficacy was found to be 20 ng/ml plasma concentration it was tested whether the single nucleotide polymorphism was found preferably in those individuals that had plasma concentration > 20 ng/ ml and who
15 responded to the treatment, which is having ≤ 3 retches and vomits. The results are shown in Figure 4.

- Subjects containing the single nucleotide polymorphism G at position 41172 of the NKNA gene as defined by position in Figure 2 in their genome were responding with a higher efficacy to the treatment as subjects not containing the single nucleotide
20 polymorphism or those who are heterozygous only.

Claims

1. A method for correlating single nucleotide polymorphisms in the NKNA gene with the efficacy and compatibility of a pharmaceutically active compound administered to a human being which method comprises determining single
5 nucleotide polymorphisms in the NKNA gene of a human being and determining the status of said human being to which a pharmaceutically active compound was administered by reference to polymorphism in the NKNA gene.
2. The method according to claim 1, wherein the nucleotide at at least one or more of the positions 33612, 33949, 37025, 37114, 37434, 41112 and 41172 of
10 the nucleotide sequence in Figure 2 comprising the NKNA gene is determined.
3. The method according to claim 1, wherein the nucleotide at position 41172 of the NKNA gene as defined by the position of the nucleotide in Figure 2 is determined.
4. The method according to claim 1 to 3, wherein the single nucleotide
15 polymorphism in the NKNA gene is selected from the group consisting of a C or a T at position 33612 in Figure 2, and/or a T or a C at position 33949 in Figure 2, and/or a C or a T at position 37025 in Figure 2, and/or a G or an A at position 37114 in Figure 2, and/or
20 an A or a C at position 37434 in Figure 2, and/or a T or a G at position 41112 in Figure 2, and/or a G or an A at position 41172 in Figure 2, and combinations thereof as well as their reverse complement.
5. The method according to claim 1 to 4, wherein the pharmaceutically active
25 compound is a NK-1 receptor antagonist.
6. The method according to claim 5, wherein the NK-1 receptor antagonist is a compound selected from the group consisting of 2-(3,5-bis-trifluoromethyl-phenyl)-N-methyl-N-(6-morpholin-4-yl-4-o-tolyl-pyridin-3-yl)-
isobutyramide, 2-(3,5-bis-trifluoromethyl-phenyl)-N-[6-(1,1-dioxo-1 λ^6 -
30 thiomorpholin-4-yl)-4-o-tolyl-pyridin-3-yl]-N-methyl-isobutyramide, 2-(3,5-bis-trifluoromethyl-phenyl)-N-[6-(1,1-dioxo-1 λ^6 -thiomorpholin-4-yl)-4-(4-fluoro-2-methyl-phenyl)-pyridin-3-yl]-N-methyl-isobutyramide; and a pharmaceutically acceptable acid addition salt thereof.

7. The method according to claim 5, wherein the NK-1 receptor antagonist is a compound selected from the group of NK-1 receptor antagonists under drug development designated GR205171, HSP-117, L 703,606, L 668,169, LY 303241, LY 306740, MK-869, R-544, Spantide III, WIN-62,577, GR 103,537, L 758,298, NKP608, CGP49823, CP-96,345, CP-99,994, CP-122,721, FK 888, GR203040, GR 82334, GR 94800, L 732,138, L 733,060, L 742,694, L 754,030, LY 303870, MEN 11149, PD 154075, RP-67580, RPR 100893, Spendide, Spantide II, SR140333, WIN-41,708, WIN-62,577, SR-48,968, L-659,877, MEN 10627, SR 144190, GR 94800, SR-142,801, R820, R486, SB 222200, L 758,298, NK-608, CGP 47899 and MEN 11467; or is a pharmaceutically acceptable acid addition salt thereof.
8. The method according to claim 5 to 7, wherein the NK-1 receptor antagonist is used for the treatment of a disease or a disorder selected from the group consisting of pain, headache, especially migraine, Alzheimer's disease, disorders of the central nervous system such as certain depressive disorders, anxiety, and emesis, psychosis, multiple sclerosis, attenuation of morphine withdrawal, cardiovascular changes, oedema, such as oedema caused by thermal injury, chronic inflammatory diseases such as rheumatoid arthritis, asthma/bronchial hyperreactivity and other respiratory diseases including allergic rhinitis, inflammatory diseases of the gut including ulcerative colitis and Crohn's disease, ocular injury and ocular inflammatory diseases, benign prostatic hyperplasia, motion sickness, treatment induced vomiting, cancer such as malignant gliomas, and traumatic brain injury.
9. The method according to claim 5 to 7, wherein the NK-1 receptor antagonist is used for the treatment or prevention of disorders of the central nervous system such as certain depressive disorders, anxiety, and emesis.
10. The method according to claim 1 to 9, wherein an assay comprising a differential nucleic acid analysis technique selected from the group consisting of direct mass-analysis of PCR products using mass spectrometry, direct analysis of invasive cleavage products, direct sequence analysis, allele specific amplification, allele specific hybridization, oligonucleotide ligation assay, Invader Assay, DNA chip analysis and restriction fragment length polymorphism is used.

11. A method for determining the efficacy and compatibility of a pharmaceutically active compound for a human being, which method comprises determining the presence of single nucleotide polymorphisms in the NKNA genomic sequence obtained from the human being which single nucleotide polymorphisms are correlated with the efficacy and compatibility of the pharmaceutically active compound, and thereby determining the efficacy and compatibility of the pharmaceutically active compound for the human being.
12. The method according to claim 11, wherein the nucleotide at at least one or more of the positions 33612, 33949, 37025, 37114, 37434, 41112 and 41172 of the nucleotide sequence in Figure 2 comprising the NKNA gene is determined.
13. The method according to claim 11, wherein the nucleotide at position 41172 of the NKNA gene as defined by the position of the nucleotide in Figure 2 is determined.
14. The method according to claim 11 to 13, wherein the single nucleotide polymorphism in the NKNA gene is selected from the group of a C or a T at position 33612 in Figure 2, and/or a T or a C at position 33949 in Figure 2, and/or a C or a T at position 37025 in Figure 2, and/or a G or an A at position 37114 in Figure 2, and/or an A or a C at position 37434 in Figure 2, and/or a T or a G at position 41112 in Figure 2, and/or a G or an A at position 41172 in Figure 2, and combinations thereof as well as their reverse complement.
15. The method according to claim 11 to 14, wherein the pharmaceutically active compound is a NK-1 receptor antagonist.
16. The method according to claim 15, wherein the NK-1 receptor antagonist is a compound selected from the group consisting of 2-(3,5-bis-trifluoromethyl-phenyl)-N-methyl-N-(6-morpholin-4-yl-4-o-tolyl-pyridin-3-yl)-isobutyramide, 2-(3,5-bis-trifluoromethyl-phenyl)-N-[6-(1,1-dioxo-1 λ^6 -thiomorpholin-4-yl)-4-o-tolyl-pyridin-3-yl]-N-methyl-isobutyramide, 2-(3,5-bis-trifluoromethyl-phenyl)-N-[6-(1,1-dioxo-1 λ^6 -thiomorpholin-4-yl)-4-(4-fluoro-2-methyl-phenyl)-pyridin-3-yl]-N-methyl-isobutyramide; and a pharmaceutically acceptable acid addition salt thereof.

17. The method according to claim 15, wherein the NK-1 receptor antagonist is a compound selected from the group of NK-1 receptor antagonists under drug development designated GR205171, HSP-117, L 703,606, L 668,169, LY 303241, LY 306740, MK-869, R-544, Spantide III, WIN-62,577, GR 103,537, L 758,298, NKP608, CGP49823, CP-96,345, CP-99,994, CP-122,721, FK 888, GR203040, GR 82334, GR 94800, L 732,138, L 733,060, L 742,694, L 754,030, LY 303870, MEN 11149, PD 154075, RP-67580, RPR 100893, Spendide, Spantide II, SR140333, WIN-41,708, WIN-62,577, SR-48,968, L-659,877, MEN 10627, SR 144190, GR 94800, SR-142,801, R820, R486, SB 222200, L 758,298, NK-608, CGP 47899 and MEN 11467; or is a pharmaceutically acceptable acid addition salt thereof.
18. The method according to claim 15 to 17, wherein the NK-1 receptor antagonist is used for the treatment of a disease or a disorder selected from the group consisting of pain, headache, especially migraine, Alzheimer's disease, disorders of the central nervous system such as certain depressive disorders, anxiety, and emesis, psychosis, multiple sclerosis, attenuation of morphine withdrawal, cardiovascular changes, oedema, such as oedema caused by thermal injury, chronic inflammatory diseases such as rheumatoid arthritis, asthma/bronchial hyperreactivity and other respiratory diseases including allergic rhinitis, inflammatory diseases of the gut including ulcerative colitis and Crohn's disease, ocular injury and ocular inflammatory diseases, benign prostatic hyperplasia, motion sickness, treatment induced vomiting, cancer such as malignant gliomas, and traumatic brain injury.
19. The method according to claim 15 to 17, wherein the NK-1 receptor antagonist is used for the treatment or prevention of disorders of the central nervous system such as certain depressive disorders, anxiety, and emesis.
20. The method according to any of claims 11 to 19, wherein an assay comprising a differential nucleic acid analysis technique selected from the group consisting of direct mass-analysis of PCR products using mass spectrometry, direct analysis of invasive cleavage products, direct sequence analysis, allele specific amplification, allele specific hybridization, oligonucleotide ligation assay, Invader Assay, DNA chip analysis and restriction fragment length polymorphism is used.
21. An isolated nucleic acid molecule selected from the group of:
the nucleic acid sequence of Figure 2 with T at position 33612 as defined by the position in Figure 2;

- the nucleic acid sequence of Figure 2 with C at position 33949 as defined by the position in Figure 2;
- the nucleic acid sequence of Figure 2 with T at position 37025 as defined by the position in Figure 2;
- 5 the nucleic acid sequence of Figure 2 with A at position 37114 as defined by the position in Figure 2;
- the nucleic acid sequence of Figure 2 with C at position 37434 as defined by the position in Figure 2;
- the nucleic acid sequence of Figure 2 with G at position 41112 as defined by the position in Figure 2;
- 10 the nucleic acid sequence of Figure 2 with G at position 41172 as defined by the position in Figure 2; or
- a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the single nucleotide polymorphisms.
- 15 22. An allele-specific nucleic acid primer capable of detecting a polymorphism in the NKNA gene at one or more of the positions of the nucleotide sequence in Figure 2 as defined in claim 12.
23. The nucleic acid primer according to claim 22, wherein the primer is selected from the group consisting of SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 15 and SEQ ID NO. 16.
- 20 24. An oligonucleotide probe for detecting a polymorphism in the NKNA gene capable of hybridizing specifically to a nucleic acid having the polymorphisms as defined in claim 14.
- 25 25. The oligonucleotide probe according to claim 24, wherein the oligonucleotide probe has a length of 17 to 50 nucleotides.
26. A diagnostic kit comprising one or more nucleic acid primer(s) as defined in claim 22 and 23 and/or one or more oligonucleotide probe(s) as defined in claim 24 and 25.
- 30 27. A pharmaceutical pack comprising NK-1 receptor antagonists and instructions for administration of the drug to human beings tested for a single nucleotide polymorphism at one or more positions of the NKNA gene.

28. A pharmaceutical pack comprising NK-1 receptor antagonists and instructions for administration of the drug to human beings tested for a single nucleotide polymorphism at one or more positions of the NKNA gene as defined in claim 12.
- 5 29. A pharmaceutical pack comprising NK-1 receptor antagonists and instructions for administration of the drug to human beings tested for a single nucleotide polymorphism according to a method as claimed in claims 11 to 20.
- 10 30. Use of a NK-1 receptor antagonist for the preparation of a medicament for treating a NK-1 receptor ligand-mediated disease in a human being diagnosed as having a single nucleotide polymorphism at one or more of position 33612, 33949, 37025, 37114, 37434, 41112 and 41172 of the nucleotide sequence in Figure 2 comprising the NKNA gene.
- 15 31. A computer readable medium having stored thereon sequence information for the polymorphisms in the NKNA gene comprising polymorphisms at position 33612 as defined by the position in Figure 2, and/or at position 33949 as defined by the position in Figure 2, and/or at position 37025 as defined by the position in Figure 2, and/or at position 37114 as defined by the position in Figure 2, and/or at position 37434 as defined by the position in Figure 2, and/or at position 41112 as defined by the position in Figure 2, and/or at position 41172 as defined by the position in Figure 2.
- 20 32. A method for performing sequence identification, said method comprising the steps of providing a nucleic acid sequence as claimed in claim 21 and comparing said nucleic acid sequence to at least one other nucleic acid sequence to determine identity.
- 25 33. The methods, nucleic acid molecules, nucleic acid primers, oligonucleotide probes, diagnostic kits and pharmaceutical packs, uses and computer readable medium substantially as herein before described especially with reference to the foregoing Examples.

Fig. 1

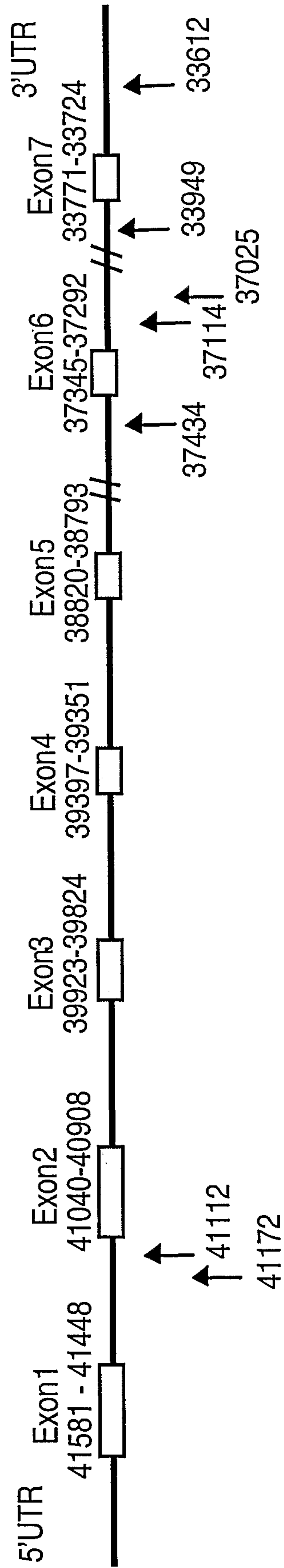


Fig.2

33301	TCAGAGATAC	AAATACAGTG	TAATACCAAA	ATCTCAGTAC	ATTTTCACCA
33351	TGAAAACAAA	TAGGAAAAGC	ACAAGACTGT	CAGGAGTTTC	CTTCCTTTTC
33401	CGCAGTAGCT	GACACAACCTG	CTTCAAAGCA	ATGATTTTTT	TTCTTTAGGG
33451	ACTGTATCAT	TGACAGGTTT	TACAATTTCA	TTTAACACTG	TGCTTTGTTG
33501	AGAAATAACC	ACTTATTTCT	ATTTTAAGGG	AGTCATCATA	TTGGAATTAA
33551	AACACATGTT	TACAATACAT	ATTGGCACAA	TATGAAAAAT	AAACACTTTT
33601	TGAATTTTCA	ACCCCAAACA	ATTGTTATTA	AATAAATAAT	TATTCCTCAT
33651	AGTGCATGTC	TTAATTTACC	TGTCATTGCC	CATTGACACA	AATGAAGCTG
33701	AATAAATAAT	GTTAGGTAGT	TTATTAACGT	CTTCTTTCAT	AATTCTGCAT
33751	TGCACTCCTT	TCATAAGCCA	CTGAAAACAA	AGAAGAGATA	AAGTGTTTTT
33801	TAAGTGAATT	CACTGAGGGA	GTTACACAAC	TAAAGTTCAG	GGCATATAGT
33851	AATTTATTTT	AAACTCATAA	TGACATTTTC	CCTCTACAGA	AGTATTTCTA
33901	AGAATGTTAT	CTGCACATCT	TCCAATCCTG	CCTCAGTCTC	TATTCTGATT
33951	GGAAATGATC	TAACCAACAGA	ACTCGGTTTC	ATTCATCATT	CATCATATAC
34001	TTGGTGCCCC	CAAAGCATAT	TTCCACAATA	TATTGCAGTG	TTGATTCTCA
34051	GCAGCTTTCT	CAAGCAGTGG	CAGATACCCA	TAGTGAATTC	TAGAAAGGAC
34101	AATGCCTAAA	ATAACCAGGA	ATACTAGTAG	GATAAAAACC	AAATGTGGGA
34151	CTTTTATTCT	AGCAGAGACC	ATGGGCCAAA	TCTAGAATAC	CATTTGTTCT
34201	TGTAAAGAGG	TTTATAGGAA	TACAGACACA	CTCATTCATT	TACATATTGT
34251	CTATGGCTGC	TTTGGCCTAA	AATAACAGAT	TTGGGTTAGT	GCAACAGAGA
34301	TTACATGGCT	CACAAAGCCT	AAAATATTTA	TAATCTGTTT	CTTTTCAGGA
34351	AAAGTTTGCT	GATCTTTGTT	CAAGGGGCAT	CTAATTGTGT	AGGGGTCTCT
34401	TCCTACCTTG	TGGATACCTC	CCAGTGATGG	ACTGCCCTCA	AGACTAAAAA
34451	CAGCCCATTT	ACCATAGGTA	AGTCAGTTGA	GCCATCTAAA	TACCATACTG
34501	TTTAACTTTT	CACTAGCAC	TTAATGGCCT	GTGAAGCATT	AATTATTGCT
34551	TCCAAAGGTA	TTTAACATAT	AGCTAGCAAG	TAATTTAAAT	ACATTGATTA
34601	TCTGGACAAA	AAAATGCCAA	AATTGTATAT	TATATTATAT	TATATTTATT
34651	ATATATAATT	TCAAAGGGAT	CTGTAACCAC	AAAGGGGTAA	AAATCAGACT
34701	GTTGCAATAA	AAGTATTTTG	GGATATTTTT	CATCCTTAAC	GAATACCTTA
34751	TAGACTTAGA	CGTATGGTAT	AGTGGAACATA	CTATGCTTTT	TGAAATTCAG
34801	TATGGCCTGT	ATAAAATACT	GAATGTAAAC	AGAAAGAGTA	GAAAGATCTG
34851	TGAAGGATCT	GTAGGAATAA	TAAGTGCTAC	TCTAAACAGT	TTCTTCAGTG
34901	AGAAGATTTG	CTGACAGAGA	AGACAGGTAA	AGGCACCAGT	ATTATCACTT
34951	CCCTCTTTTA	ATACTGGTCT	TAAACACCCA	GCTATCCTTT	AAGTGCGGTT
35001	AACAATAAGC	TGCATATTTT	ACATCAATTA	AGTATGCACT	TGTGAGGCAG
35051	AACAGTCTGA	GCCAACACTG	GACAGGCGTG	AGGGAAGGAA	ACAGTTTATT
35101	TCTCATTTCTG	GTTTGATTAT	ATTAGGAACA	GGATGAATGG	CCCAAGTTCA
35151	GTCTCTGACA	AGCTTTAGGT	AACATGACTC	TATTCCTCAG	AGAAATTAGA
35201	TGAAAGCTAA	TGTAAACCAA	CTAAAGACAG	ACAGTGATTT	AGCTGGAACA
35251	TAATGTCCTC	TTACATGGAA	GCAGAGTTTC	TTACCTGGTC	TTTGAACCCT
35301	CTGAAGCCTG	ATATAAATTT	TTGAGTGTAT	GAGCTGATGA	GGGTTTCTCT
35351	GGGAAAGGGC	ATCTATCTAG	ATTTATCAGA	TTGTCAAAGG	GGTCCCTGAC
35401	CATGAGTGGG	TAAAGATCAG	AGGGCTATAG	ATTAAGGCTA	TGATAACCTT
35451	GCCTCAATGA	GGTACATAAA	AAGATCCCTG	ATTGACTGTG	GATCATATCA
35501	CATTTTTTTTC	CACATTTTCAC	CTATTTATAT	TTCTTAACT	AAAATGGAGG
35551	AACAAAGATT	TAGTTATAAA	TTGATCCTGA	TTCACTACAG	TGATTTTTTAT
35601	CACTACTTAA	AATTATGTTA	TTATTTTAAG	TACATTTAAT	TGTATTTAAT
35651	CTGTTCTAAC	TTGTACAATA	TCTACATTTG	TAAAGTTAAG	CATGTTCTGA
35701	AACCTATATC	AATTCCTGGAA	ATGGAAAGTT	GATGACTAAT	ATGATTATCA
35751	AGAAAACCTAC	AATTAAGTGA	GGACTAGTAT	GTATTGGTAC	AAATAAAAGC
35801	CATTGTGGTT	TAAATTTTATA	TTTCATTTCC	AAAAGTGGGA	AGATAATACA
35851	TATTAATAAA	ACAATTACAA	GTTTTTCATT	AAGGCACATT	GATAATTTCT
35901	TACACTTTTA	CACTTCGGCA	GCCCAAAATG	ACTGGTGAGA	AGATGCAAAG
35951	GTAGTGTCTA	AATAGAAAGT	GGGGACCCAA	TAAGAACACT	AGGAAGTCCT
36001	GTGGACTTCA	CCAAGAACTT	CAAATTCAG	ATTTATTGGA	AATTTTGTAT
36051	CAAAAGGTCA	ATTATGAAAC	ACTTAGAACT	TTTCAATTAT	ATGAATATAT

36101	TCTCTGGCTT	CTGCCGTAGC	CCAGATTGTG	TTAACTTTTT	TTTTCTATTG
36151	CGCGTTTTGA	TTTTTGCCCC	ACAGAAAGTG	GTTTTATTTA	ATGTACCCAA
36201	GTCTTACATT	TCACAATAAA	GAAAAAATC	TCCATTAAAA	AACACTAGGA
36251	TAATTATTTT	TCCTAGGATC	CAGATAATTA	AGGATTAAGC	CAGCACATCT
36301	TATAGATTGT	CTAGAAACTG	GAAATATTCT	AAATGGGTAA	GACAGCTGCT
36351	GATTACTTTA	TAATTAATAA	CGTATTTAGT	ATTTTAATTC	TATTTTCACT
36401	GAAGGCAAAA	TAGAATTTGA	ATAGCTTTTC	CAAGAAGCCC	TAGAGGTAAG
36451	AGCTATAAGG	TCTTAACAAA	AAGTGAGCTT	CTATTAAAAT	GATCTAGGGG
36501	TTATTTACAT	TGAAATCAGT	GTGATGAATG	AGTTAACTAA	TAATGGTTTT
36551	CAGATATATG	TACGTGGTAT	TTACAAAGAA	GATGGAAATT	GAAGAGAAGG
36601	AAACCTGCAG	CCTTTATATC	ATACGAAAAA	GTTGTTCAGA	ATTAAAATGA
36651	GAATTGTGGC	ATCCTTTCTC	TCTAAGGAGT	TTATAGCCAG	AACCAATTTA
36701	TAACACTCAA	GGATATTTAA	TTGTGTTTCT	TTTTGAAAGT	ACTGGAATAA
36751	ACCGTATCAC	TTTTGTCTCT	TCCAACATCC	ATCAGGATGT	TTAGATTTTG
36801	AATTTCTACA	ACTTCATAAA	AAGTGATTAA	TTTGCATAAA	GAATTTGTTT
36851	TGTGAATTTA	TTTTTGTATT	GGAAGATCGC	CAAATGTAAT	GTCTTGTAAG
36901	ACTTTCAAAT	TACTTTGATA	ACAAGTGAAA	ATCACCTCTA	TTCTTCCCTG
36951	CACTCCCCAC	TCCCCCTTAG	TTCAGTAAGG	TTAAACACGG	CTTATTTAAC
37001	AGATCTATAG	GAAAGAATAT	AGCACTAACC	CAAAGATTCA	GGTTGGTAAT
37051	GATTAAATGG	GGCAGACACT	GGTAAGAATG	GTGATTGGAG	AGAAATAGAC
37101	AGATACTGTG	GTAGTATTAG	CTTAACCAGC	TATAAATCCA	TTTGTGGTTA
37151	CAAGTACAAC	AATTAAATTT	TTCCACCATA	ATACTCATTT	TTATATCTAC
37201	CACTCCTTTT	TAATATTTTA	AAACTATGTC	TTAGAAAAAA	TAGAAATAGA
37251	TTTGAGATAC	TGTCTATTTT	CAGTCATAAT	TTCATACATA	CCAGAATTTA
37301	AAGCTCTTTT	GCCCATTAGT	CCAACAAAGG	AATCTGTTTT	ATGTCCTGGA
37351	AAAATACATT	TAGGGGTATT	TTAATATGTA	TATCTTTGAG	GAAACAAACT
37401	ATTATAACCA	GAACAAAGCA	AGCTTCTGAA	TATATGTCTG	TATAATAAAT
37451	ATCTATCTAC	TTCAAGTTTT	GGTGAGACTG	AAGAAATAAA	GATTCCTTAA
37501	AAGTTTTTATA	AATTACACTA	AAACAATACA	ATGAAACCAC	TTTTCTTAAT
37551	TCTATATTTA	ATATTAGCAA	GCACTCTCTA	TTCATATATT	TTATCTGTTA
37601	TGTAAGGGTC	TAAAATATTT	TATATAAAAT	ATTGTTATTT	GAGCTAGGTT
37651	TGATTTTTTTA	GGAAGTCAAT	TTCACATTTG	TGTGAGAGTG	AAATATCCAA
37701	GCATAATTCC	TTGTGTTATT	AATAAGTTGT	ACTTCTCAGC	AAGTAGACCT
37751	TAATTAAACC	AGATTCCTTT	CCTTGATGAT	TATAATCTTC	TAAAAGGTCA
37801	CTGTTTTTTAT	TTTATTGGAT	ATTAAAAGAT	ACTCCTTTCA	GAAAAGTTTA
37851	TTAGTTTTCA	CATGAACAGA	GTACATTTTT	TATTACATAG	AATTTTTATA
37901	TATAATTCTC	TTCAAAGTCA	AATCCTTAGA	AAGTAAATAT	GAATACTATT
37951	TATTTTTTCAA	AGATGAAGTC	TTTGGAAGG	TTACTATTTT	AATGCTAGTG
38001	AGAGAATTTT	TCTTGTTT	TATTTTAATA	GTTGAGAATA	GAGCTGCTAC
38051	GTTAATGTGG	CCTGCTCTGC	CACCCAATGA	ACCAAATCCA	TGACAGGGTA
38101	CAATAGGTGA	GTGGACAATA	CGATGAAGAT	AGACCCTTTC	CACTTCTTGT
38151	CAACTCCGGT	TCCATTAACT	GAGAAACAAA	TTTAATGACC	TTCACATACC
38201	ACAATTTTGC	CATCTTTGCT	GCCCTAAGAA	ATGTTTTACT	GATCGAGTTT
38251	TCCCATTTCAA	TTAGGGGATT	AAAAAACTGC	ATCTCTACCG	TCTTCATTTT
38301	TAGTATGGAT	TCTCTACTCA	TGTTCACTCA	CTCATTTCCAT	TTAATTGGCT
38351	GCTGGATGAC	CCTAATAAAT	GACATCATTA	TTGGACCAAG	CATACTAAAA
38401	TGAGTTAGGA	GATGCCTATA	AATGCTTGTG	GGGGTTTCGT	GTTTGTTTGT
38451	TTGTTTGT	ACAGTGAAAG	TAAGCTTAGG	GGGTGGGTGG	CTGGGAAGAA
38501	ATATGTATGG	TAGATTTCTA	ATCCTAGGAT	TGAAATTCTT	CTCTAAAACT
38551	AACAATTGAA	GGGACTTAAA	AGACATTTTT	TTCTTTTTTTA	ATATAATGCA
38601	TCTCTTTGCT	AAGTACAATA	TGTCTAAACA	ACCATATTCT	GGATGTAATA
38651	TGAATCTCCC	CCATTCTTAT	ATTGTCTTCA	TTAAACATAT	TAAGCCCTAG
38701	AGTCATGACA	GAAAATAAGG	TAAGATAAAT	AAAGTGAAAT	TCAATATAAT
38751	TTTACATTTA	AATTTGATAA	ATGTCAAAAT	AATTTTGAAC	TTACTTTTGT
38801	GAGAGATCTG	GCCATGTCCT	AGAAGAAAGA	TAAAATTAGC	AAACACATAT
38851	AGATTTGTTT	TTAAAGATGC	TACATTATAT	CTTTTATAAA	ATAATTTGAT
38901	TCTCAAAATA	TGTAACATTT	TCTATCTATA	TATTATCCTG	AGGAATAAAG
38951	CAGAACCATA	AATGCTAAAC	AAATTATTAA	TCATAAAAAC	TAAATATTTA

39001	AACAAACAAA	TGGAATTTTT	ATTAA ^h GTGCC	TTCTATGGGA	TTCAAACCTA
39051	GGACTATGAG	ACTTCTGAGG	CCTCTTATTA	ACTATAAGAC	TGTAACACAT
39101	CCCACCTTCA	AGGCTGGCCA	CCCCTGAGA	GAGAGAGAGA	AAGAGAGAGA
39151	GAGAGACTGA	GAGAGAGTGA	CAGAGAGAGA	GAGACAGAGA	GAGTGACAGA
39201	GAGAGAGAGA	AACCGAGAGA	GAGTGCGCAA	ATCCATCTCT	TTATTAAACA
39251	GGCATGACCC	AGATCTTGTA	TTTCAGCCCA	TAAAAGATTT	TATTTCACTT
39301	TCCTACATGT	GCTTTCACAA	TAGTAAAATA	AAGATTTATA	GGAATGTTTA
39351	CCATAAAGAG	CCTTTAACAG	GGCCACTTGT	TTTTCAATTG	AGGAATCTGA
39401	CAAGAGACGA	ATTGTCATTA	ACTACATGTT	CCACTGACCG	TGCGATGAAT
39451	TCAAGGAAAC	GATATTTCTA	TCCCAAGACA	AATGTACTCT	CACCTCAGGG
39501	AATGCTATAA	AATCCTCTTC	CACATGCACT	TGGATCATCC	GCAGTACTAC
39551	CCCTGGAGGC	CCGTGCCTTC	CCTTTGTCAG	TTTGATCTTT	TAGTCAAGGG
39601	AGCGCTTCAC	TCGAATACAG	TGAGGCATGC	GAACCAGATC	TTTCCCTCCC
39651	CCCGCGGGCC	CGTGCGTGCC	TTAGTGCGGT	GTGGGCCAAT	GTGCGCGCCTG
39701	GGGAATTACA	CCCCTCTTGG	AGTGGAATC	TCTATTCAGA	GCGAGAGACC
39751	CTGGGGTACT	CCAGGAAGGA	GTGAGCAGAC	AGGGCGGGCT	GCCCAAGACA
39801	CCTAGGGACG	GTCGCCTATC	TCACCAGCAT	CCCGTTTGCC	CATTAATCCA
39851	AAGAACTGCT	GAGGCTTGGG	TCTCCGGGCG	ATTCTCTGCA	GAAGATGCTC
39901	AAAGGGCTCC	GGCAGTTCCT	CCTGGGAGAC	AAAAGCACGG	ACAAAGGGCG
39951	TGTAAGGCAA	CCAGAGCGAG	GCACCCAGCA	ACCCGAGCCC	TTCCCCCCGC
40001	TGTACTCCGG	CCCCGAGGCG	TGGGGAGGCG	GCCTTCCCCA	CGCTCGGGCT
40051	GAGCGGGGTC	CCTCCCGAGG	CGCTGCCTCT	CTGCGCCCCT	CCCCGCCGAA
40101	CTCGCGTCCC	GCCCCCGCA	AGCCTGCCAG	GGCGAGTTAG	CAGCTGGCTC
40151	GGCGTCTTCA	AGCTTCGAGC	GCTTTCCGCT	CTTTCCTCCC	AGCCCGGGGA
40201	CCTTCCCTCA	TCTGCTGCGC	CGCCACCTGA	CTTTGTCTTG	TCTCGGTGCC
40251	GGCTTTGTAG	AGTCCTCACC	GCCCTAAATG	CACACAGGTG	TGTGGGATCT
40301	GGGGCTGGGG	CTCCGGTGGG	GAAGGGTGAC	AACGCTTTAC	AGACGACTTT
40351	CACCGTTTTT	CCGAGGCGCG	CTCCCCAGCG	CCTGTAGCCT	ACGAGTTTCT
40401	AAAGGGAGAC	TTCAAGCTAC	ATCCTCAGGT	CCGCAAAACA	AACGTGAAGG
40451	CAGGAAATTA	ACTTCCCTCC	AGCCTCCGGC	CCTCCAATCC	CCCTGCAGCT
40501	CGCGCGTGTG	CCCGCTGCTG	CCCGCGACCC	AGGCGCCCTC	CTCTGCCCCG
40551	GCCCAGACGC	TAGGGCAGTG	CAGCTGGACC	GGAGCCCCCG	AAATCCCAGG
40601	TCCTGGGGGA	TGAAATCTTA	CGAGATTTCC	CGCCTTGTGA	GGATCTCGGG
40651	CTCATCCGGG	AGGGCATCCC	TACTGCAGGG	AGTTCCTGGG	CCGGACGGTG
40701	CAGCCCTCGG	GAAGAACTT	GAATCGTGGG	CACAAAATCC	CGTGGGGAAC
40751	CCGGGAAATA	GTGCCCCCTT	CCATCCTCTC	GCACGCAACC	GCTGCCTCTT
40801	TCCGTGGTGG	CGGTGCGTGC	CACGTTAACT	AGGGTGCTGT	TGCGTGGGAA
40851	GGTGACAGCT	CCCGAGCCCA	GGAAGAAGGG	TGCGGGCCGT	CCTGGGAAGG
40901	GGCCTCACCT	TGATCTGGTC	GCTGTCGTAC	CAGTCGGACC	AGTAATTCAG
40951	ATCATCATTG	GCTCCTATTT	CTTCTGCAAA	CAGCTGAGTG	GAGACAAGAA
41001	AAAAGACTGC	CAAGGCCACG	AGGATTTTCA	TGTTGGATTT	CTGGGAAGGG
41051	GTAGGAGGAG	AAGACACCAA	AGAGAGAAAT	AATGTATCTA	TTAGGGGTGG
41101	TTATCCAAGA	GTTAAAGCAA	CAGAATTTTC	TGCCCCACAA	CGGATGAACC
41151	AAGATCAAAA	ACATAAGGAC	TGAAGTCTCA	CCTGTACCGG	TTAGGCGAGA
41201	GAATCGCGTC	CCAAAGATTC	TCCAACCCTC	TTCTCCCTGA	ATCCCAACTC
41251	CCCCAGCCCC	CTAACCCACC	AACCCACACC	TTCAAACCAG	GAAACTCTGC
41301	AGGTGGAAGA	CAAAGAGGGG	CGTTGGCGTT	TGGGAAGCCA	GTCTCCCCAC
41351	TGTCCCTCGC	CGCAGCGGCG	CCCCCAGCGC	GGCCACCTCG	GACAGATGCG
41401	GGGCGGGGTG	ACCCGGGCAG	CCGCGGCCAG	CACCGCGCGG	GCACTTACTG
41451	CGACGGACAG	TCCCGCGGGG	TGCTGAGTTT	CTCTGGTTCC	TCCGAGCGCA
41501	CGCTGGTTCG	TCCGCACTCT	CGGTAGCTGC	CGCCGGGAAG	GAGGTCCGAG
41551	CGCGCTCTTT	CGCCTGCTCA	GTGCCTTGCG	GTATTTATCC	CAGCCTCCTT
41601	GAGCCTCCAG	ACCCACGTGA	CATTCCTCCC	ACGCGACTTT	CTGCTCAATA
41651	TTTAATTAGC	CGCCTCCTCT	CCTTTCGCTT	GCGTGATTGA	GAGTTTCCGA
41701	GACGGTAACT	CGTCGATGCC	CATAACATCT	GGACCCAATT	GGGTTCTAAA
41751	TGACGCAATT	TTAGGAAAAT	CGAGGTCTCG	CCATCCAATC	CGGAGCGGCC

Fig. 3

33612*	C or T
33949*	T or C
37025*	C or T
37114*	G or A
37434*	A or C
41112*	T or G
41172*	G or A

*defined by the position in accession no. EM_HUM1:AC004140.1

Fig. 4

G41172* genotypes			
Effect	0-2	Not 0-2	Total
yes	8 (100 %)	12 (57 %)	20
no	0 (0 %)	9 (43 %)	9
Total	8	21	29

*defined by the position in accession no. EM_HUM1:AC004140.1