

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
8 June 2006 (08.06.2006)

PCT

(10) International Publication Number  
**WO 2006/058889 A1**

- (51) International Patent Classification:  
*G01N 33/74* (2006.01)    *G01N 33/68* (2006.01)
- (21) International Application Number:  
PCT/EP2005/056350
- (22) International Filing Date:  
30 November 2005 (30.11.2005)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
04106220.9    1 December 2004 (01.12.2004)    EP
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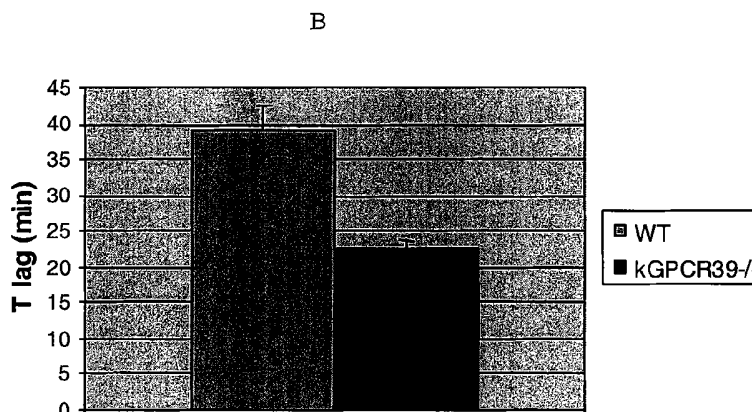
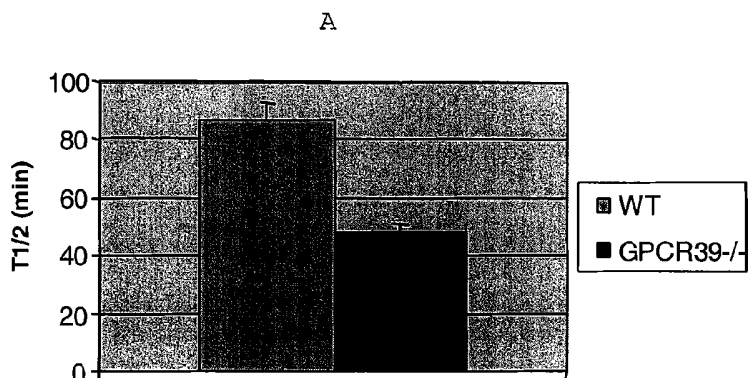
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG,

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(54) Title: G PROTEIN COUPLED RECEPTOR



(57) Abstract: The present invention relates to the functional characterization of the G protein coupled receptor GPR39 and to compounds, which modify or regulate GPR39 protein activity. In particular the present invention relates to methods of screening for agonists or antagonists of GPR39 in order to identify compounds capable of modulating Gastrointestinal Kinetics and/or cholesterol metabolism and to the therapeutic uses of these compounds. The invention also relates to transgenic animals bearing mutations in the GPR39 gene.



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SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US,  
UZ, VC, VN, YU, ZA, ZM, ZW.

**(84) Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17:**

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*  
— *of inventorship (Rule 4.17(iv))*

**Published:**

— *with international search report*  
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*  
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

G PROTEIN COUPLED RECEPTORField of the Invention

5 The present invention relates to the functional  
characterization of the G protein coupled receptor GPR39  
and to compounds, which modify or regulate GPR39 protein  
activity. In particular the present invention relates to  
methods of screening for agonists or antagonists of GPR39  
10 in order to identify Gastrointestinal Kinetics regulating  
agonists or antagonists and to the therapeutic uses of  
these compounds. In another embodiment the present  
invention relates to the involvement of GPR39 in  
metabolism homeostasis, in particular on the cholesterol  
15 levels observed in the GPR39 knockout mice. The invention  
also relates to transgenic animals bearing mutations in  
the GPR39 gene.

Background to the Invention

20 GTP-binding proteins (G proteins) act as intermediaries  
between binding of ligands such as hormones and other  
chemical mediators to G protein coupled receptors (GPCRs)  
and activation of intracellular effectors. Upon binding of  
25 a ligand to a GPCR, the cytoplasmic domains of the  
receptor undergo conformational changes, which enable  
interaction of the receptor with a G protein, which in  
turn enables activation of intracellular intermediaries  
such as adenylate cyclase, phospholipase C or ion  
30 channels. Such a system allows amplification of the  
original signal as many second messengers can be produced  
in response to the binding of a single ligand at the GPCR.  
Through this mechanism, cells are able to sense and  
respond to alterations in their external environment.

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G protein coupled receptors form a superfamily of integral plasma membrane proteins, each receptor sharing the common feature of seven hydrophobic transmembrane domains, each of which is 20 -30 amino acids long and which are linked  
5 by hydrophilic amino acid sequences of varied length. The amino terminus of the receptor is extracellular with the carboxy terminus found in the cytoplasm of the cell.

GPCRs are found in a wide range of tissues and cell types  
10 and are involved in many different physiological processes. They are activated by a broad range of ligands, for example, hormones such as luteinizing hormone, follicle stimulating hormone, chorionic gonadotrophin, thyrotropin, adrenocorticotrophin, glucagon and  
15 vasopressin; neurotransmitters such as 5-HT, acetylcholine (muscarinic AchR), histamine, prostaglandins, calcitonin, leukotrienes and  $Ca^{2+}$ . The broad distribution and wide variety of roles of GPCRs indicate that GPCRs may play important roles in a variety of pathological conditions.  
20 Indeed, GPCRs have been found to be involved in diseases related to bronchoconstriction, hypertension, inflammation, hormonal disturbance, diabetes, apoptosis, nociception, facilitation of neurotransmission and tremor disorders.

25

Within the G-protein-coupled receptor superfamily, the putative GPCRs for which the natural ligands are unknown are called "orphan receptors". G protein-coupled receptor have been proven to be valuable drug targets, since they  
30 are the target of about 50% of the marketed drugs. Therefore many orphan GPCR are being evaluated to identify new potential targets.

GPR39 was identified based upon its sequence similarity  
35 with the growth hormone secretagogue receptor (GHS-R) and

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the neurotensin receptors 1 and 2 (NT-R1 and NT-R2) (McKee et al., 1997). The predicted 453-amino acid GPR39 protein contains the 7 transmembrane domains characteristic of GPCRs. By sequence comparison with other GPCRs, McKee et al. (1997) found that the protein sequence of GPR39 is 27%, 29% and 32% identical to that of GSHR, MTLR1 (motilin receptor) and neurotensin receptor-1, respectively. Northern blot analysis revealed that GPR39 has a wide tissue distribution. A single hybridizing mRNA transcript of 1.8-2 kb, was detected in most brain regions tested. However, in addition to this species, an alternate transcript, 3-kb in length, was observed in several peripheral tissues such as stomach and small intestine, and in tissues such as pancreas, thyroid and colon, this 3-kb species was the only transcript detected (McKee et al., 1997). By fluorescence in situ hybridization, McKee et al. (1997) mapped the GPR39 gene to 2q21-q22. An acidic residue in TM3, essential for the binding and activation of the GHS-R by structurally dissimilar GHSs, is conserved in GPR39.

Based on these tissue distribution studies GPR39 has been hypothesized to be involved in cardiovascular disease states (WO2001/081634 & WO2004/004279), cancers and in particular brain cancers such as glioblastoma (WO2001/036685 & WO01042288), inflammation and neurological disease states (US 2003/ 232769 & WO2004/004279) and in gastrointestinal and liver diseases (WO2004/004279). Nonetheless, in none of the cited references a functional characterization based on ligand identification for GPR39 has been provided. For said reasons, the present functional annotation of GPR39 is speculative and requires further studies to identify the ligand-binding and functional properties of GPR39.

Given the wide tissue distribution of GPR39 and the fact that most G protein coupled receptors play an important role in a wide variety of cellular and physiological processes, it would be interesting to gain an understanding of the normal physiological role of this receptor.

#### Summary of the Invention

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As noted above, the present invention concerns identification of novel functions of the GPR39 receptor. As shown in the examples hereinafter, GPR39 mutations in mammals affect gastric emptying and designate GPR39 as a key element in the regulation of gastrointestinal kinetics. Given the fact that down regulation of the GPR39 gene leads to an increase in gastric emptying it is to be expected that an increase in GPR39 expression will attenuate gastric emptying. This discovery provides an avenue for new therapeutic approaches in the regulation of gastrointestinal kinetics through modulation of GPR39 activity. This discovery also provides new model systems for studying gastrointestinal kinetics and diseases involving gastrointestinal kinetics disorders, it also provides for new screening methods for identifying compounds useful for the prevention or the treatment of these diseases.

In addition to the above, phenotyping of the GPR39 knockout mice further revealed an involvement of this receptor on cholesterol metabolism. A down regulation of the GPR39 gene leads to increased cholesterol levels. It is accordingly to be expected that GPR39 will be involved in disease conditions with excess cholesterol levels such as the metabolic syndrome, including obesity, diabetes,

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obesity related cardiovascular diseases, and glaucoma.

Therefore, a first aspect of the invention provides for the use of all or part of GPR39 protein in a method for  
5 identifying compounds that modulate gastrointestinal kinetics or which are effective for preventing and/or treating pathologies related with gastrointestinal kinetics disorders. In a further aspect the present invention provides the use of all or part of the GPR39  
10 protein in a method to identify compounds that modulate cholesterol formation or which are effective for preventing and/or treating pathologies related with excess cholesterol formation. Alternatively, the invention provides for the use of cells expressing all or part of  
15 the GPR39 protein in such method. In a specific embodiment GPR39 is an isolated protein having an amino acid sequence selected from the group consisting of SEQ ID No:2, SEQ ID NO:4, a splice variant of the proteins having the aforementioned SEQ ID's, and an amino acid sequence  
20 having at least 50% and preferably at least 60%, 70%, 80%, 90%, 95% or 98% sequence identity to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

Parts of the GPR39 protein, as used hereinbefore, are  
25 meant to include fragments of the polypeptide of SEQ ID NO:2 or SEQ ID NO:4, said fragments being of at least 10, for example at least 20, 30 40, 50, 75, 100 or 150 or more amino acids in size. Such fragments may be derived from the N-terminal region of SEQ ID NO:2 or SEQ ID NO:4  
30 respectively. Fragments including the N-terminal region may be used to reconstitute the extracellular portion of the receptor to provide receptor binding sites. Preferably, fragments will retain the ability to bind adenine.

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The present invention also provides the use of an isolated nucleic acid sequence encoding all or part of the polypeptide of SEQ ID NO:2 or SEQ ID NO:4 in a method for identifying compounds that modulate gastrointestinal kinetics or which are effective for preventing and/or  
5 treating pathologies related with gastrointestinal kinetics disorders and disease states related to high levels of cholesterol such as the metabolic syndrome, including diabetes and cardiovascular diseases. The  
10 nucleic acid sequences as used in the methods of the present invention are meant to include the isolated nucleic acid sequences consisting of SEQ ID NO:1 or SEQ ID NO:3, and nucleic acid sequences having at least 50% and preferably at least 60%, 70%, 80%, 90%, 95% or 98%  
15 sequence identity to the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3.

Nucleic acids of the invention further include nucleic acids which comprise a sequence having at least 50% and  
20 preferably at least 60%, 70%, 80%, 90%, 95% or 98% sequence identity to the nucleic acid sequences of SEQ ID NO: 1, or SEQ ID NO: 3 or their complements. Preferably, these sequences will hybridise to the corresponding nucleic acid under conditions controlled to minimise non-  
25 specific binding. Preferably stringent to moderately stringent hybridisation conditions are preferred. Suitable conditions include, e.g. for detection of sequences that are about 80-90% identical, hybridization overnight at 42 °C in 0.25M Na<sub>2</sub>HPO<sub>4</sub>, pH 7.2, 6.5% SDS, 10% dextran sulfate  
30 and a final wash at 55 °C in 0.1X SSC, 0.1% SDS. For detection of sequences that are greater than about 90% identical, suitable conditions include hybridization overnight at 65 °C in 0.25M Na<sub>2</sub>HPO<sub>4</sub>, pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 60 °C in 0.1X SSC, 0.1%  
35 SDS.



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It will be appreciated that such nucleic acids do not necessarily encode "full length" polypeptides, and will thus include nucleic acids which represent, for example, mutant forms of the GPR39 gene in which the coding  
5 sequence has been prematurely terminated by either a substitution resulting in a stop codon or a frameshift mutation. These are also nucleic acids of the invention.

The invention also provides the use of nucleic acids that are fragments of the nucleic acids encoding a polypeptide  
10 of the invention. In one aspect, the invention provides nucleic acids primers which consist essentially of from 15 to 50, for example from 15 to 35, 18 to 35, 15 to 24, 18 to 30, 18 to 21 or 21 to 24 nucleotides of a sequence  
15 encoding a polypeptide of the invention or its complement.

Nucleic acids and polypeptides of the invention may be used therapeutically to treat disease. In particular, they may be used to treat diseases, the pathology of which is  
20 associated with action at GPR39 receptors, particularly those associated with for preventing and/or treating pathologies related with gastrointestinal kinetics disorders and disease states related to increased cholesterol levels, such as the metabolic syndrome.

25 In a further aspect, there are provided vectors comprising the sequences of said nucleic acids, particularly expression vectors comprising a promoter operably linked to the nucleic acid sequences of the invention. The  
30 vectors may be carried by a host cell, and expressed within said cell. Following said expression, said cells can be used in the methods according to the invention.

As mentioned hereinbefore, it is an object of the  
35 invention to provide assay methods for the identification

of compounds (hereinafter also referred to as agents), which bind to or modulate the activity of polypeptides of the invention. In particular it is envisaged that such compounds are an organic or inorganic assembly of atoms of any size, and includes small molecules (less than about 2500 Daltons) or larger molecules such as peptides, polypeptides, whole proteins and polynucleotides, wherein said compounds may be used in methods of treatment as described above.

10

As the present inventors are the first to identify GPR39 as a receptor as a key element in the regulation of gastrointestinal kinetics, the present invention opens up the possibility of using GPR39 itself and/or compounds agonising or antagonising this receptor in therapeutic applications. Therefore the invention further extends to a method of treatment of a human or animal body, said method comprising the use of a GPR39 agonist or antagonist. In particular, a method of treating disease conditions related with delayed gastric emptying such as for example, gastroparesis post-operative ileus, gastroparesis in diabetic, functional dyspepsia, post-vagotomy gastroparesis, slow transition constipation, constipation-IBS, mixed-IBS and idiopathic intestinal pseudo-obstruction. Said method comprising administering to the human or animal a therapeutically active dosage of a GPR39 receptor antagonist, in particular comprising the use of a GPR39 antagonist identifiable using a method of the present invention.

It is also an object to provide a method of treating disease conditions related with increased gastric emptying such as for example, dumping syndrome or increased intestinal motility such as diarrhoea, diarrhoea-IBS and mixed IBS, said method comprising administering to the human or animal a therapeutically active dosage of a GPR39

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receptor agonist, in particular comprising the use of a GPR39 agonist identifiable using a method of the present invention.

5 In addition, based on the observed effect of the down regulation of the GPR39 receptor on cholesterol homeostasis, in a further aspect the present invention opens up the possibility of using GPR39 itself and/or compounds agonising or antagonising this receptor in  
10 therapeutic applications with a defective cholesterol homeostasis. Therefore the invention also extends to a method of treatment of a human or animal body, said method comprising the use of a GPR39 agonists or antagonist in treating disease conditions related with a defective  
15 cholesterol homeostasis. In particular the use of a GPR39 agonist in the treatment of disease conditions related with an excess cortisol formation such as the metabolic syndrome, including obesity, diabetes, and cardiovascular diseases such as atherosclerosis related to high levels of  
20 cholesterol.

These and other aspects of the invention are described herein in more detail.

25 Description of sequences.

SEQ ID NO:1 is the nucleotide sequence for mice GPR39.  
SEQ ID NO:2 is the amino acid sequence for mice GPR39.  
SEQ ID NO:3 is the nucleotide sequence for human GPR39.  
30 SEQ ID NO:4 is the amino acid sequence for human GPR39.  
SEQ ID NO:5 is the GPR39 forward primer  
SEQ ID NO:6 is the GPR39 reverse primer  
SEQ ID NO:7 is a GPR39 probe sequence  
SEQ ID No:8 is human obestatin  
35 SEQ ID No:9 is monkey obestatin

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- SEQ ID No:10 is mouse obestatin  
SEQ ID No:11 is rat obestatin  
SEQ ID No:12 is gerbil obestatin  
SEQ ID No:13 is pig obestatin  
5 SEQ ID No:14 is cat obestatin  
SEQ ID No:15 is dog obestatin  
SEQ ID No:16 is goat obestatin  
SEQ ID No:17 is sheep obestatin  
SEQ ID No:18 is Cattle obestatin  
10 SEQ ID No:19 is the consensus sequence for obestatin

Brief Description of the Drawings

15 **Figure 1A** Real-Time Quantitative Reverse Transcription PCR of GPR39 in tissues derived from wild type mice.

**Figure 1B** Real-Time Quantitative Reverse Transcription PCR of GPR39 in four different tissues derived from wild type  
20 mice and heterozygote and homozygote GPR39 knock-out mice.

**Figure 2** Comparison of the half emptying time ( $t_{1/2}$ ) (A) and  $T_{lag}$  (B) for gastric emptying in GPCR39 knock-out mice vis-à-vis wild type mice.

25

**Figure 3** Comparison of the faecal pellets propulsion in wild type mice (A) vis-à-vis GPCR39 knock-out mice (B). The distribution of pellets is counted in bins of 15% of total colon length and the number of pellets expelled from  
30 each bin is counted during a 20 min time interval.

**Figure 4** Comparison of the cumulative food intake of not fasted mice (A) vis-à-vis fasted (19h) mice (B) for both young (17 weeks n=6) and older mice (56 weeks, n=6).

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**Figure 5** Amino acid sequence of preproghrelin from 11 mammalian species are shown with the signal peptide (italicized), mature ghrelin (shaded), and the flanking obestatin (underlined). Consensus basic residues representing putative convertase cleavage sites are shown as white letters on a black background. In the consensus sequence, individual residues with complete conservation are shown in upper case. GenBank (gi) numbers for individual ghrelin genes are 37183224 (human), 34541890 (monkey), 19224664 (mouse), 11067387 (rat), 27357900 (gerbil), 47523230 (pig), 52782813 (cat), 50978704 (dog), 52782814 (goat), 57526202 (sheep), and 27806613 (cattle).

15

#### Detailed Description of the Invention

##### Nucleic acid

20 Nucleic acid as used in the methods of the present invention includes DNA (including both genomic and cDNA) and RNA. Where nucleic acid according to the invention includes RNA, reference to the sequences shown in the accompanying listings should be construed as reference to 25 the RNA equivalent, with U substituted for T.

Nucleic acid of the invention may be single or double stranded. Single stranded nucleic acids of the invention include anti-sense nucleic acids. Thus it will be 30 understood that reference to SEQ ID NO:1 or sequences comprising SEQ ID NO:1 or fragments thereof include complementary sequences unless the context is clearly to the contrary. The same applies to SEQ ID NO:3.

35 Generally, nucleic acid according to the present invention

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is provided as an isolate, in isolated and/or purified form, or free or substantially free of material with which it is naturally associated, such as free or substantially free of nucleic acid flanking the gene in the human  
5 genome, except possibly one or more regulatory sequence(s) for expression. Nucleic acid may be wholly or partially synthetic and may include genomic DNA, cDNA or RNA.

The invention also provides nucleic acids that are  
10 fragments of the nucleic acids encoding a polypeptide of the invention. In one aspect, the invention provides nucleic acids primers which consist essentially of from 15 to 50, for example from 15 to 35, 18 to 35, 15 to 24, 18 to 30, 18 to 21 or 21 to 24 nucleotides of a sequence  
15 encoding a polypeptide of the invention or its complement.

The term Aconsist essentially of $\cong$  refers to nucleic acids which do not include any additional 5' or 3' nucleic acid sequences. In a further aspect of the invention, nucleic  
20 acids of the invention which consist essentially of from 15 to 30 nucleotides as defined above may however be linked at the 3' but preferably 5' end to short (e.g from 4 to 15, such as from 4 to 10 nucleotides) additional sequences to which they are not naturally linked. Such  
25 additional sequences are preferably linkers which comprise a restriction enzyme recognition site to facilitate cloning when the nucleic acid of the invention is used for example as a PCR primer.

30 Primers of the invention are desirably capable of selectively hybridising to nucleic acids encoding the polypeptides of the invention. By Aselective $\cong$ , it is meant selective with respect to sequences encoding other purine receptors and in particular with respect to

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receptors other than adenine receptors. The ability of the sequence to hybridise selectively may be determined by experiment or calculated.

5 For example, one way to calculate  $T_m$  of a primer is by reference to the formula for calculating the  $T_m$  of primers to a homologous target sequence. This formula is  $T_m(^{\circ}\text{C}) = 2(A+T) + 4(G+C) - 5$ . This will provide the  $T_m$  under conditions of 3xSSC and 0.1% SDS (where SSC is 0.15M NaCl,  
10 0.015M sodium citrate, pH 7). This formula is generally suitable for primers of up to about 50 nucleotides in length. In the present invention, this formula may be used as an algorithm to calculate a nominal  $T_m$  of a primer for a specified sequence derived from a sequence encoding  
15 a polypeptide of the invention. The  $T_m$  may be compared to a calculated  $T_m$  for GPCR sequences of humans and rats, based upon the maximum number of matches to any part of these other sequences.

20 Suitable conditions for a primer to hybridise to a target sequence may also be measured experimentally. Suitable experimental conditions comprise hybridising a candidate primer to both nucleic acid encoding a polypeptide of the invention and nucleic acid encoding other adenine  
25 receptors on a solid support under low stringency hybridising conditions (e.g. 6xSSC at 55 $^{\circ}$ C), washing at reduced SSC and/or higher temperature, for example at 0.2xSSC at 45 $^{\circ}$ C, and increasing the hybridisation temperature incrementally to determine hybridisation  
30 conditions which allow the primer to hybridise to nucleic acid encoding a polypeptide of the invention but not other purine receptor encoding nucleic acids.

Nucleic acids of the invention, particularly primers, may  
35 carry a revealing label. Suitable labels include

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radioisotopes such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , fluorescent labels, enzyme labels, or other protein labels such as biotin. Such labels may be added to polynucleotides or primers of the invention and may be detected using by techniques  
5 known per se.

Primers of the present invention may be comprised of synthetic nucleic acids, such as those with modified backbone structures intended to improve stability of the  
10 nucleic acid in a cell. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes  
15 of the present invention, it is to be understood that the polynucleotides described herein may be modified by any method available in the art. Such modifications may be carried out in order to enhance the in vivo activity or lifespan of polynucleotides of the invention.

20

Also included within the scope of the invention are antisense sequences based on the nucleic acid sequences described herein, preferably in the form of oligonucleotides, particularly stabilized  
25 oligonucleotides, or ribozymes.

Antisense oligonucleotides may be designed to hybridise to the complementary sequence of nucleic acid, pre-mRNA or mature mRNA, interfering with the production of  
30 polypeptide encoded by a given target DNA sequence, so that its expression is reduced or prevented altogether. Ribozymes will be designed to cleave mRNA encoded by an GPR39 GPCR encoding nucleic acid sequence of the invention, desirably at a target sequence specific to the  
35 GPR39 GPCR, i.e one which is not common to other GPCR



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sequences. The construction of antisense sequences and their use is described in Peyman and Ulman, *Chemical Reviews*, 90:543-584, (1990), Crooke, *Ann. Rev. Pharmacol. Toxicol.*, 32:329-376, (1992), and Zamecnik and Stephenson, *P.N.A.S.*, 75:280-284, (1974). The construction of ribozymes and their use is described in for instance Gibson and Shillito, *Molecular Biotechnology* 7(2): 125-137, (1997).

10 In one embodiment, RNA of the invention can be used for induction of RNA interference (RNAi), using double stranded (dsRNA) (Fire et al., *Nature* 391: 806-811. 1998) or short-interfering RNA (siRNA) sequences (Yu et al., *Proc Natl Acad Sci USA.* 99:6047-52. 2002). "RNAi" is the process by which dsRNA induces homology-dependent degradation of complimentary mRNA. In one embodiment, a nucleic acid molecule of the invention is hybridized by complementary base pairing with a "sense" ribonucleic acid of the invention to form the double stranded RNA. The dsRNA antisense and sense nucleic acid molecules are provided that correspond to at least about 20, 25, 50, 100, 250 or 500 nucleotides or an GPR39 coding strand, or to only a portion thereof. In an alternative embodiment, the siRNAs are 30 nucleotides or less in length, and more preferably 21- to 23-nucleotides, with characteristic 2- to 3-nucleotide 3'-overhanging ends, which are generated by ribonuclease III cleavage from longer dsRNAs. See e.g. Tuschl T. (*Nat Biotechnol.* 20:446-48. 2002).

30 Intracellular transcription of small RNA molecules can be achieved by cloning the siRNA templates into RNA polymerase III (Pol III) transcription units, which normally encode the small nuclear RNA (snRNA) U6 or the human RNase P RNA H1. Two approaches can be used to express siRNAs: in one embodiment, sense and antisense

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strands constituting the siRNA duplex are transcribed by individual promoters (Lee, et al. Nat. Biotechnol. 20, 500-505. 2002); in an alternative embodiment, siRNAs are expressed as stem-loop hairpin RNA structures that give rise to siRNAs after intracellular processing (Brummelkamp et al. Science 296:550-553. 2002) (herein incorporated by reference).

The dsRNA/siRNA is most commonly administered by annealing sense and antisense RNA strands in vitro before delivery to the organism. In an alternate embodiment, RNAi may be carried out by administering sense and antisense nucleic acids of the invention in the same solution without annealing prior to administration, and may even be performed by administering the nucleic acids in separate vehicles within a very close timeframe. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of a GPR39 or antisense nucleic acids complementary to a GPR39 nucleic acid sequence are additionally provided.

Antisense, siRNAs and ribozyme sequences of the invention may be introduced into mammalian cells lines in culture to study the function of GPR39 GPCR, for example by causing down-regulation of this gene and observing phenotypic effects, or the expression or location of proteins described herein which associate with GPR39 GPCR. In cells where aberrant expression of GPR39 GPCR occurs, such antisense, siRNA and ribozyme sequences may be used to down-regulate the expression of the gene.

The cDNA sequence of the GPCR of the invention may be cloned using standard PCR (polymerase chain reaction) cloning techniques. This involves making a pair of primers to 5' and 3' ends on opposite strands of SEQ ID

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NO:1, bringing the primers into contact with mRNA or cDNA obtained from a mammalian cortical cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the reaction mixture on an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable cloning vector. The same applies to SEQ ID NO:3.

Polynucleotides which are not 100% homologous to the sequence of SEQ ID NO:1 or SEQ ID NO:3 but which encode either SEQ ID NO:2 or SEQ ID NO:4 or other polypeptides of the invention can be obtained in a number of ways.

For example, site directed mutagenesis of the sequence of SEQ ID NO: 1 or SEQ ID NO:3 may be performed. This is useful where for example silent codon changes are required to sequences to optimise codon preferences for a particular host cell in which the polynucleotide sequences are being expressed. Other sequence changes may be desired in order to introduce restriction enzyme recognition sites, or to alter the property or function of the polypeptides encoded by the polynucleotides. Further changes may be desirable to represent particular coding changes which are required to provide, for example, conservative substitutions.

Nucleic acids of the invention may comprise additional sequences at the 5' or 3' end. For example, synthetic or natural 5' leader sequences may be attached to the nucleic acid encoding polypeptides of the invention. The additional sequences may also include 5' or 3' untranslated regions required for the transcription of

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nucleic acid of the invention in particular host cells.

In addition, other animal, particularly mammalian (e.g. humans or rabbits), more particularly primate including  
5 human, homologues of GPR39 may be obtained and used in the methods of the present invention. Such sequences may be obtained by making or obtaining cDNA libraries made from dividing cells or tissues or genomic DNA libraries from other animal species, and probing such libraries with  
10 probes comprising all or part of SEQ ID NO:1 or of SEQ ID NO:3 under conditions of medium to high stringency (for example 0.03M sodium chloride and 0.03M sodium citrate at from about 50<sup>N</sup>C to about 60<sup>N</sup>C).

15 The present invention further extends to an isolated DNA sequence comprising sequences encoding a polypeptide of the invention but in which the encoding sequences are divided up into two or more (preferably no more than five, e.g. four or three) exons. Such exon sequences may be  
20 natural and obtained from genomic clones, or synthetic. Exon sequences may be used in the construction of mini-gene sequences which comprise nucleic acid encoding polypeptides of the invention which sequences are interrupted by one or more exon sequences.

25

Mini-genes may also be constructed using heterologous exons, derived from any eukaryotic source.

#### Polypeptides.

30

Isolated polypeptides used in the methods of the present invention will be those as defined above in isolated form, free or substantially free of material with which it is naturally associated such as other polypeptides with which

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it is found in the cell: The polypeptides may of course be formulated with diluents or adjuvants and still for practical purposes be isolated - for example the polypeptides will normally be mixed with gelatin or other carriers if used to coat microtitre plates for use in immunoassays. The polypeptides may be glycosylated, either naturally or by systems of heterologous eukaryotic cells, or they may be (for example if produced by expression in a prokaryotic cell) unglycosylated.

10 Polypeptides may be phosphorylated and/or acetylated.

A polypeptide of the invention may also be in a substantially purified form, in which case it will generally comprise the polypeptide in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the polypeptide in the preparation is a polypeptide of the invention.

Polypeptides of the invention may be modified for example by the addition of histidine residues to assist their purification or by the addition of a signal sequence to promote their secretion from a cell.

Polypeptides having at least 50%, for example 60%, 70%, 80%, 90%, 95% or 98% sequence identity to SEQ ID NO:2 or SEQ ID NO:4 may be polypeptides which are amino acid sequence variants, alleles, derivatives or mutants of SEQ ID NO:2 or SEQ ID NO:4 respectively, and are also provided by the present invention. For example such a polypeptide may have an amino acid sequence which differs from that given in SEQ ID NO:2 or SEQ ID NO:4 by one or more of addition, substitution, deletion and insertion of one or more (such as from 1 to 20, for example 2, 3, 4, or 5 to 10) amino acids.

35

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The percentage identity of polypeptide sequences can be calculated using commercially available algorithms which compare a reference sequence( e.g. SEQ ID NO:2 or SEQ ID NO:4 of the present invention) with a query sequence.

5 Further details of assessing identity are described below.

Where a query sequence is determined to have an identity to that of SEQ ID NO:2 or SEQ ID NO:4 of at least 50% and preferably at least 60%, 70%, 80%, 90%, 95% or 98% said  
10 sequence being that of a polypeptide retaining GPR39 receptor activity, such a sequence forms part of the present invention.

A polypeptide according to the present invention may be  
15 isolated and/or purified (e.g. using an antibody) for instance after production by expression from encoding nucleic acid. The isolated and/or purified polypeptide may be used in formulation of a composition, which may include at least one additional component, for example a  
20 pharmaceutical composition including a pharmaceutically acceptable excipient, vehicle or carrier.

A polypeptide according to the present invention may be used as an immunogen or otherwise in obtaining specific  
25 antibodies. Antibodies are useful in purification and other manipulation of polypeptides, diagnostic screening and therapeutic contexts.

A polypeptide according to the present invention may be  
30 used in screening for molecules which bind to it or modulate its activity or function. Such molecules may be useful in a therapeutic (possibly including prophylactic) context.

35 A polypeptide or labelled polypeptide of the invention or

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fragment thereof may also be fixed to a solid phase, for example the surface of an immunoassay well or dipstick.

Such labelled and/or immobilized polypeptides may be  
5 packaged into kits in a suitable container along with  
suitable reagents, controls, instructions and the like.

Such polypeptides and kits may be used in methods of  
detection of antibodies to such polypeptides present in a  
10 sample or active portions or fragments thereof by  
immunoassay.

Immunoassay methods are well known in the art and will  
generally comprise:

- 15 (a) providing a polypeptide comprising an epitope  
bindable by an antibody against said protein;
- (b) incubating a biological sample with said  
polypeptide under conditions which allow for the  
formation of an antibody-antigen complex; and
- 20 (c) determining whether antibody-antigen complex  
comprising said polypeptide is formed.

#### Sequence Identity

25 The percentage identity of nucleic acid and polypeptide  
sequences can be calculated using commercially available  
algorithms which compare a reference sequence with a query  
sequence. The following programs (provided by the  
National Center for Biotechnology Information) may be used  
30 to determine homologies/identities: BLAST, gapped BLAST,  
BLASTN and PSI-BLAST, which may be used with default  
parameters.

The algorithm GAP (Genetics Computer Group, Madison, WI)  
35 uses the Needleman and Wunsch algorithm to align two

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complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, the default parameters are used, with a gap creation penalty = 12 and gap extension penalty = 4.

5

Another method for determining the best overall match between a nucleic acid sequence or a portion thereof, and a query sequence is the use of the FASTDB computer program based on the algorithm of Brutlag et al (Comp. App. Biosci., 6; 237-245 (1990)). The program provides a global sequence alignment. The result of said global sequence alignment is in percent identity. Suitable parameters used in a FASTDB search of a DNA sequence to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty=0.05, and Window Size=500 or query sequence length in nucleotide bases, whichever is shorter. Suitable parameters to calculate percent identity and similarity of an amino acid alignment are: Matrix=PAM 150, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty=0.05, and Window Size=500 or query sequence length in nucleotide bases, whichever is shorter.

25

### Vectors

Nucleic acid sequences of the present invention may be incorporated into vectors, particularly expression vectors. The vector may be used to replicate the nucleic acid in a compatible host cell. Thus in a further embodiment, the invention provides a method of making polynucleotides of the invention by introducing a polynucleotide of the invention into a replicable vector, introducing the vector into a compatible host cell, and

35



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growing the host cell under conditions which bring about replication of the vector. The vector may be recovered from the host cell. Suitable host cells are described below in connection with expression vectors.

5

Preferably, a polynucleotide of the invention in a vector is operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host cell, i.e. the vector is an expression vector.

10

The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is  
15 ligated in such a way that expression of the coding sequence is achieved under condition compatible with the control sequences.

Suitable vectors can be chosen or constructed, containing  
20 appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. phage, phagemid or baculoviral, cosmids, YACs, BACs,  
25 or PACs as appropriate. Vectors include gene therapy vectors, for example vectors based on adenovirus, adeno-associated virus, retrovirus (such as HIV or MLV) or alpha virus vectors.

30 The vectors may be provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin resistance gene in the  
35 case of a bacterial plasmid or a neomycin resistance gene

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for a mammalian vector. Vectors may be used in vitro, for example for the production of RNA or used to transfect or transform a host cell. The vector may also be adapted to be used in vivo, for example in methods of gene therapy.

5 Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, eukaryotic cells such as mammalian and yeast, and baculovirus systems. Mammalian cell lines available in the art for expression of a

10 heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, COS cells and many others.

Promoters and other expression regulation signals may be selected to be compatible with the host cell for which the expression vector is designed. For example, yeast promoters include *S. cerevisiae* GAL4 and ADH promoters, *S. pombe* nmt1 and adh promoter. Mammalian promoters include the metallothionein promoter which can be induced

20 in response to heavy metals such as cadmium. Viral promoters such as the SV40 large T antigen promoter or adenovirus promoters may also be used. All these promoters are readily available in the art.

25 The vectors may include other sequences such as promoters or enhancers to drive the expression of the inserted nucleic acid, nucleic acid sequences so that the polypeptide is produced as a fusion and/or nucleic acid encoding secretion signals so that the polypeptide

30 produced in the host cell is secreted from the cell.

Vectors for production of polypeptides of the invention of for use in gene therapy include vectors which carry a mini-gene sequence of the invention. It is accordingly an

35 object of the present invention to provide a method for

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treating abnormal conditions related to an under-expression of GPR39 GPCR and its activity, said method comprising the use of a polynucleotide encoding a GPR39 GPCR. In particular in a method to treat increased gastric emptying such as for example, dumping syndrome or increased intestinal motility such as diarrhoea, diarrhoea-IBS and mixed-IBS. In gene therapy a polynucleotide of the invention is used to effect the endogenous production of GPR39 GPCR by the relevant cells in the subject. For example, a polynucleotide encoding a GPR39 GPCR may be engineered for expression in a replication defective retroviral vector as provided above.

The retroviral expression construct 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 review of gene therapy, see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, in Human Molecular Genetics, T. Strachan and A.P. Read, BIOS Scientific Publishers Ltd. (1996).

For further details see, for example, Molecular Cloning: a Laboratory Manual: 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Current Protocols in Molecular Biology, Ausubel et al. eds., John Wiley & Sons, 1992.

35

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Vectors may be transformed into a suitable host cell as described above to provide for expression of a polypeptide of the invention. Thus, in a further aspect the invention provides a process for preparing polypeptides according to the invention which comprises cultivating a host cell transformed or transfected with an expression vector as described above under conditions to provide for expression by the vector of a coding sequence encoding the polypeptides, and recovering the expressed polypeptides. Polypeptides may also be expressed in vitro systems, such as reticulocyte lysate.

A further embodiment of the invention provides host cells transformed or transfected with the vectors for the replication and expression of polynucleotides of the invention. The cells will be chosen to be compatible with the said vector and may for example be bacterial, yeast, insect or mammalian. The host cells may be cultured under conditions for expression of the gene, so that the encoded polypeptide is produced. If the polypeptide is expressed coupled to an appropriate signal leader peptide it may be secreted from the cell into the culture medium. Following production by expression, a polypeptide may be isolated and/or purified from the host cell and/or culture medium, as the case may be, and subsequently used as desired, e.g. in the formulation of a composition which may include one or more additional components, such as a pharmaceutical composition which includes one or more pharmaceutically acceptable excipients, vehicles or carriers

Polynucleotides according to the invention may also be inserted into the vectors described above in an antisense orientation in order to provide for the production of antisense RNA or ribozymes.

35

Assays

It is an object of the present invention to provide an assay for identifying compounds that modulate  
5 gastrointestinal kinetics and cholesterol homeostasis, which assay comprises: providing all or part of a GPR39 receptor protein according to the invention, contacting said protein with a putative binding compound; and  
10 determining whether said compound is able to interact with said protein.

In one embodiment of the assay, the receptor or subunits of the receptor may be employed in a binding assay. Binding assays may be competitive or non-competitive.  
15 Such an assay can accommodate the rapid screening of a large number of compounds to determine which compounds, if any, are capable of binding to the polypeptides. Subsequently, more detailed assays can be carried out with those compounds found to bind, to further determine  
20 whether such compounds act as agonists or antagonists of the polypeptides of the invention.

It is accordingly an object of the present invention to provide for a method for identifying a compound that  
25 modulate gastrointestinal kinetics and cholesterol homeostasis, which method comprises:

- (i) contacting host cells expressing all or part of the GPR39 receptor protein according to the invention, with said compound under conditions  
30 suitable for binding, and
- (ii) detecting binding of the compound to said receptor protein.

In a further embodiment this invention provides a method  
35 for identifying a compound that modulate gastrointestinal

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kinetics, which method comprises:

- (i) contacting membrane preparations from host cells expressing all or part of the GPR39 receptor protein according to the invention, with said compound under conditions suitable for binding, and
- (ii) detecting binding of the compound to said receptor protein.

10 In a still further embodiment the present invention provides a method for identifying a compound that modulate gastrointestinal kinetics, which method involves a competitive binding assay wherein:

- (i) host cells expressing all or part of the GPR39 receptor protein according to the invention are contacted with a compound known to bind to the GPR39 receptor protein both in the presence and absence of the compound to be tested, and
- (ii) the effect of said compound on the binding of the compound known to bind to the GPR39 receptor protein is being assessed.

A decrease in the binding of the compound known to bind to the GPR39 receptor protein in the presence of the compound to be tested is an indication that said compound binds to the GPR39 receptor protein. Obestatin, the natural ligand for the GPR39 receptor protein has recently been identified (Zhang, J.V. et al., 2004 Science Vol.310;996-999) as another peptide derived from the same prohormone as ghrelin. In a particular embodiment the compound known to bind to the receptor consists of obestatin, more in particular selected from one of the obestatin sequences disclosed in Fig.5, i.e. selected from SEQ ID No's 8 to 19, more in particular SEQ ID No.8 or SEQ ID No.10. Alternatively the obestatin as used herein refers to a peptide having at least 60%, 70%, 80%, 90%, 95%, 98% or

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99% sequence identity to the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:10.

In an alternative embodiment, the aforementioned  
5 competitive binding assay is performed on membrane preparations of host cells expressing all or part of the GPR39 receptor protein according to the invention. Methods for preparing said host cells and for preparing membrane preparations from such cells are described  
10 hereinafter.

In a specific embodiment the GPR39 receptor protein in the aforementioned binding assays is an isolated protein having an amino acid sequence selected from the group  
15 consisting of SEQ ID No:2, SEQ ID NO:4, a splice variant of the proteins having the aforementioned SEQ ID's, and an amino acid sequence having at least 50% and preferably at least 60%, 70%, 80%, 90%, 95% or 98% sequence identity to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

20 Parts of the GPR39 protein, as used hereinbefore, are meant to include fragments of the polypeptide of SEQ ID NO:2 or SEQ ID NO:4, said fragments being of at least 10, for example at least 20, 30 40, 50, 75, 100 or 150 or more  
25 amino acids in size. Such fragments may be derived from the N-terminal region of SEQ ID NO:2 or SEQ ID NO:4 respectively. Fragments including the N-terminal region may be used to reconstitute the extracellular portion of the receptor to provide receptor binding sites.  
30 Preferably, fragments will retain the ability to bind the compound known to bind to the GPR39 receptor protein.

#### Membrane preparations

Cell membranes expressing the receptor protein of this  
35 invention are useful for certain types of assays including

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but not restricted to ligand binding assays, GTP- $\gamma$ -S binding assays and others. The specifics of preparing such cell membranes may in some cases be determined by the nature of the ensuing assay but typically involve

5 harvesting whole cells and disrupting the cell, for example by sonication in ice cold buffer (e.g. 20 mM Tris HCl, 1 mM EDTA, pH 7.4 at 4°C). The resulting crude cell lysate is subsequently cleared of cell debris by low speed centrifugation, for example at 200xg for 5 min at 4°C.

10 Further clearance and membrane enrichment is finally done using a high speed centrifugation step, such as for example 40,000xg for 20 min at 4°C, and the resulting membrane pellet is washed by suspending in ice cold buffer and repeating the high speed centrifugation step. The

15 final washed membrane pellet is resuspended in assay buffer. Protein concentrations are determined by the method of Bradford (1976) using bovine serum albumin as a standard. The membranes may be used immediately or frozen for later use.

20

#### Radiolabeled ligand binding assays

Cells expressing the receptor of this invention may be used to screen for ligands for said receptor. The same assays may be used to identify agonists or antagonists of

25 the receptor that may be employed for a variety of therapeutic purposes.

Radioligand binding assays are performed by diluting membranes prepared from cells expressing the receptor in an appropriate buffer, such as for example 50 mM Tris

30 buffer (pH = 7.4 at 0°C) containing 2.1% bovine serum albumin (Sigma), aprotinin (0.005 mg/ml, Boehringer Mannheim) and bestatin (0.1 mM, Sigma) as protease inhibitors. The final protein concentration in the assay is typically within 12-40  $\mu$ g/ml.



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Membranes are then incubated with radiolabeled ligand either in the presence or absence of competing ligands on ice for 60 min in a total volume of 250  $\mu$ l 96 well  
5 microtiter plates. The bound ligand is then separated from free ligands by filtration through GF/B filters presoaked in 0.5% polyethyleneimine (PEI), using a Tomtec (Wallac) vacuum filtration device. After addition of Ready Safe (Beckman) scintillation fluid, bound radioactivity is  
10 quantitated using a Trilux (Wallac) scintillation counter (approximately 40% counting efficiency of bound counts). Alternatively, it may be preferable to collect bound ligand and then separate ligand from receptor using procedures well known in the art. Data is fit to non-  
15 linear curves using GraphPad prism.

In this manner, agonist or antagonist compounds that bind to the receptor may be identified as they inhibit the binding of the radiolabeled ligand to the membrane protein  
20 of cells expressing the said receptor. Non-specific binding is defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of 100 nM of the unlabeled peptide corresponding to the radioligand used. In equilibrium saturation binding  
25 assays membrane preparations or intact cells transfected with the receptor are incubated in the presence of increasing concentrations of the labeled compound to determine the binding affinity of the labeled ligand. The binding affinities of unlabeled compounds may be  
30 determined in equilibrium competition binding assays, using a fixed concentration of labeled compound in the presence of varying concentrations of the displacing ligands.

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In a particular embodiment the aforementioned radioligand binding assay is performed using radiolabeled obestatin as defined hereinbefore. Labeling of obestatin and receptor binding has been described in Zhang, J.V. et al. (2004  
5 Science Vol.310;996-999). Briefly;

Iodination of obestatin was performed using the Iodogen (Pierce, Upland, IN) procedure. Mixtures of the peptide (20  $\mu$ g) and 1 mCi [ $^{125}$ I] NaI were transferred to precoated Iodogen vials and incubated for 4 min. The  $^{125}$ I-labeled peptide was applied to a Sep-Pak C18 cartridge (Waters) before elution with 60% acetonitrile/0.1%TFA.  
10 For radioligand binding assays, rat jejunum or other tissues were washed with buffer A (20 mM Hepes, 5 mM EDTA, 1 mM dithiothreitol (DTT), 10  $\mu$ M amidinophenylmethanesulfonyl fluoride, 5 mg/L leupeptin, 100 mM KCl, pH 7.5), cut into small pieces, and homogenized using a motorized homogenizer. The homogenates were centrifuged at 1,000 g for 5 min. and the supernatant was centrifuged at 300,000 g  
15 for 1 hour at 2°C. The pellets (crude membrane fractions) were resuspended with buffer A without KCl, quickly frozen under liquid nitrogen, and stored at -80°C until use. Tissue homogenates were incubated in 100  $\mu$ l of phosphate buffered saline containing 0.1% bovine serum albumin for 18 hours at room temperature with varying concentrations of  $^{125}$ I-obestatin in the presence or absence of unlabeled obestatin at  
20 1,000-fold excess. After incubation, the tubes were centrifuged for 10 min. at 10,000 g, and pellets were washed twice in ice-cold PBS before quantitation of radioactivity with a  $\gamma$ -spectrophotometer. Specific binding was calculated by subtracting nonspecific binding from total binding. For displacement curves, a fixed concentration of  $^{125}$ I-obestatin was incubated with or without increasing concentrations of obestatin or other  
25 peptides.

In addition to the aforementioned binding assays, it is also an object of the present invention to provide functional assays for identifying compounds that modulate  
30 gastrointestinal kinetics, characterized in that said compounds modulate the activity of the GPR39 receptor proteins according to the invention. Such an assay comprises the steps of;

- (i) contacting all or parts of the GPR39 receptor  
35 protein with the compound to be tested, and
- (ii) measuring the activity of the GPR39 receptor

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protein, wherein the change of GPR39 activity in the presence of the test compound is an indication of the compounds capability to modulate gastrointestinal kinetics.

5

Given the observed involvement of GPR39 in cholesterol homeostasis, in all of the disclosed embodiments to identify compounds that bind to and/or modulate GPR39 activity, said assays can be used to identify compounds that may affect cholesterol homeostasis in a subject, including humans and warm-blooded animals.

A compound that modulates the activity of a polypeptide of the invention refers to a compound that alters the activity of the polypeptide so that it behaves differently in the presence of the compound than in the absence of the compound. Compounds affecting modulation include agonists and antagonists. An agonist encompasses a compound which activates GPR39 GPCR function. Alternatively, an antagonist includes a compound that interferes with GPR39 GPCR function. Typically, the effect of an antagonist is observed as a blocking of agonist-induced receptor activation, however in the case of GPR39, which has recently been described as a constitutive active receptor, the compounds that interferes with GPR39 GPCR function also include inverse agonists, i.e. agent which binds to the same receptor binding-site as an agonist for that receptor but exerts the opposite pharmacological effect. Antagonists include competitive as well as non-competitive antagonists. A competitive antagonist (or competitive blocker) interacts with or near the site specific for agonist binding. A non-competitive antagonist or blocker inactivates the function of the receptor by interacting with a site other than the agonist interaction site.

It is thus an object of the present invention to provide a method for identifying compounds capable to increase gastric emptying and/or colonic motility said method

5 comprising;

- (i) contacting host cells expressing all or part of the GPR39 receptor protein with the compound to be tested under conditions permitting the activation of said receptor protein, and
- 10 (ii) detecting any increase in GPR39 receptor activity, thereby identifying the test compound as a compound capable to increase gastric emptying and/or colonic motility.

15 In a further embodiment the present invention provides a method for identifying compounds capable to decrease gastric emptying and/or colonic motility said method comprising;

- 20 (i) contacting host cells expressing all or part of the GPR39 receptor protein with the compound to be tested under conditions permitting the activation of said receptor protein, and
- (ii) detecting any decrease in GPR39 receptor activity, thereby identifying the test compound
- 25 as a compound capable to decrease gastric emptying and/or colonic motility.

Again, as for the binding assays mentioned hereinbefore, the aforementioned activity assays can also be used to

30 identify compounds that would modulate cholesterol metabolism. Based on the observed phenotype it is to be expected that compounds that increase GPR39 activity would result in a lowering of cholesterol levels and compounds that decrease GPR39 activity would result in an increase

35 in cholesterol levels. In a particular embodiment the

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present invention provides a method for identifying compounds capable to decrease cholesterol levels said method comprising;

- 5 (i) contacting host cells expressing all or part of the GPR39 receptor protein with the compound to be tested under conditions permitting the activation of said receptor protein, and
  - (ii) detecting any increase in GPR39 receptor activity, thereby identifying the test compound as a compound capable to decrease cholesterol levels.
- 10

The effect of the compounds on GPR39 activity may be an increase or decrease of an intracellular second messenger formation that is mediated by GPR39 such as intracellular calcium, cAMP or a reporter gene product. GPR39 belongs to the class of proteins known as G-protein coupled receptors (GPCRs). GPCRs transmit signals across cell membranes upon the binding of the ligand. The ligand-bound GPCR activates intracellular signalling events mediated by heterotrimeric G proteins, such as activation of the adenylate cyclase pathway or activation of the phospholipase C- $\beta$  pathway. Assay to assess the activation of the aforementioned intracellular signalling events are generally known in the art and include amongst others cell based assays for signal transduction comprising chimeric ligand-inducible transcription factors, binding assays for G-protein-coupled receptors using fluorescence intensity distribution analysis, cell-signaling assays using cyclic nucleotides coupled to luminophores or measurement of responses from G protein coupled receptors using a multiple response element or cAMP response element-directed reporter assay. Description of several such

15

20

25

30

assays follows.

The present invention also provides a bioassay for  
5 identifying compounds which modulate the regulatory  
regions of the GPR39 GPCR gene. Such an assay is  
conducted utilising rat or human cells capable of  
expressing a polypeptide of the invention (preferably of  
SEQ ID NO:2 or SEQ ID NO:4). The cells are contacted with  
10 at least one compound wherein the ability of said compound  
to modulate the regulatory region is known. Thereafter,  
the cells are monitored for expression of the nucleic acid  
of the invention. Alternatively, the promoter may be  
linked to a reporter gene. Suitable reporter genes that  
15 may be employed include, for example, the chloramphenicol  
acetyltransferase gene, the luciferase gene, and the like.

As understood by those of skill in the art, bioassay  
methods for identifying compounds that modulate the  
20 activity of receptors such as polypeptides of the  
invention generally require comparison to a control. One  
type of Acontrol $\cong$  is a cell or culture that is treated  
substantially the same as the test cell or test culture  
exposed to the compound, with the distinction that the  
25 Acontrol $\cong$  cell or culture is not exposed to the compound.

Another type of Acontrol $\cong$  cell or culture that can be  
employed is a cell or culture that is identical to  
transfected cells, the exception that the Acontrol $\cong$  cell  
or culture does not express functional GPR39 GPCR.  
30 Accordingly, the response of the transfected cell can be  
compared with that of the Acontrol $\cong$  cell or culture to the  
same compound under the same reaction conditions.

Particularly preferred types of assays include binding

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assays and functional assays which may be performed as follows:

Binding assays

5

Over-expression of nucleic acid encoding polypeptides of the invention in cell lines (including mammalian HEK 293 cells, CHO cells and Sf9 insect cells) may be used to produce membrane preparations bearing said polypeptides (referred to in this section as GPR39 GPCR for 10 convenience) for ligand binding studies. These membrane preparations can be used in conventional filter-binding assays (eg. Using Brandel filter assay equipment) or in high throughput Scintillation Proximity type binding 15 assays (SPA and Cytostar-T flashplate technology; Amersham Pharmacia Biotech) to detect binding of radio-labelled ligand and displacement of such radio-ligands by competitors for the binding site. Radioactivity can be measured with Packard Topcount, or similar 20 instrumentation, capable of making rapid measurements from 96-, 384-, 1536- microtitre well formats. SPA/Cytostar-T technology is particularly amenable to high throughput screening and therefore this technology is suitable to use as a screen for compounds able to displace standard 25 ligands.

Another approach to study binding of ligands to GPR39 GPCR protein in an environment approximating the native situation makes use of a surface plasmon resonance effect 30 exploited by the Biacore instrument (Malmqvist M., Biochem Soc Trans. 1999 Feb;27(2):335-40). GPR39 GPCR in membrane preparations or whole cells could be attached to the biosensor chip of a Biacore and binding of ligands examined in the presence and absence of compounds to 35 identify competitors of the binding site.

Functional assays

Since GPR39 GPCR acts via  $G_i$  or  $G_o$  (inhibitory G protein),  
5 which usually interacts with GIRK (inward rectifying  
potassium channels), potassium ion flux should result on  
activation of these receptors. This flux of ions may be  
measured in real time using a variety of techniques to  
determine the agonistic or antagonistic effects of  
10 particular compounds. Therefore, recombinant GPR39 GPCR  
receptors expressed in cell lines or, for example, *Xenopus*  
oocytes can be characterised using whole cell and single  
channel electrophysiology to determine the mechanism of  
action of compounds of interest. Electrophysiological  
15 screening, for compounds active at GPR39 GPCR, may be  
performed using conventional electrophysiological  
techniques and when they become available, novel high  
throughput methods currently under development.

20 Fluorescence - Calcium and sodium fluxes are measurable  
using several ion-sensitive fluorescent dyes, including  
fluo-3, fluo-4, fluo-5N, fura red, Sodium Green, SBFI and  
other similar probes from suppliers including Molecular  
Probes. Calcium and sodium influx as a result of GPR39  
25 GPCR can thus be characterised in real time, using  
fluorometric and fluorescence imaging techniques,  
including fluorescence microscopy with or without laser  
confocal methods combined with image analysis algorithms.

30 Another approach is a high throughput screening assay for  
compounds active as either agonists or modulators which  
affect calcium transients. This assay is based around an  
instrument called a **FLuorescence Imaging Plate Reader**  
(**FLIPR®**), Molecular Devices Corporation). In its most  
35 common configuration, it excites and measures fluorescence



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emitted by fluorescein-based dyes. It uses an argon-ion laser to produce high power excitation at 488 nm of a fluorophore, a system of optics to rapidly scan the over the bottom of a 96-/384-well plate and a sensitive, cooled  
5 CCD camera to capture the emitted fluorescence. It also contains a 96-/384-well pipetting head allowing the instrument to deliver solutions of test agents into the wells of a 96-/384-well plate. The FLIPR assay is designed to measure fluorescence signals from populations  
10 of cells before, during and after addition of compounds, in real time, from all 96-/384-wells simultaneously. The FLIPR assay may be used to screen for and characterise compounds functionally active at recombinant GPR39 GPCR, eg rat or human GPR39 GPCR, expressed in cell lines. As  
15 described below, calcium transients in cells transfected with GPR39 GPCR were measured using the FLIPR assay in order to measure activation of the receptors by various substrates in order to determine the natural ligand of the receptor.

20

Cyclic AMP (cAMP) assay - The receptor-mediated stimulation or inhibition of cyclic AMP (cAMP) formation may be assayed in cells expressing the receptor. An exemplary protocol for a cAMP assay is provided  
25 hereinafter.

In this protocol COS-7 cells are transiently transfected with the receptor gene using the DEAE-dextran method and plated in 96-well places. 48 hours after transfection, cells are washed twice with Dulbecco's phosphate buffered  
30 saline (FES) supplemented with 10 mM HEPES, 10 mM glucose and 5 mM theophylline and are incubated in the same buffer for 20 min at 37°C, in 5% CO<sub>2</sub>. Test compounds are added and cells are incubated for an additional 10 min at 37°C. The medium is then aspirated and the reaction stopped by  
35 the addition of 100 mM HCl. The plates are stored at -

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20°C for 2-5 days For cAMP measurement, plates are thawed and the cAMP content in each well is measured by cAMP Scintillation Proximity Assay (Amersham Pharmacia Biotech). Radioactivity is quantified using microbeta  
5 Trilux counter (Wallac).

Microphysiometric assay - Because cellular metabolism is intricately involved in a broad range of cellular events (including receptor activation of multiple messenger  
10 pathways), the use of microphysiometric measurements of cell metabolism can provide a generic assay of cellular activity arising from the activation of any orphan receptor regardless of the specifics of the receptor's signaling pathway.

15

General guidelines for transient receptor expression, cell preparation and microphysiometric recording are described elsewhere (Salon, J. A. and Cwicki, J. A., 1996). Typically cells expressing receptors are harvested and  
20 seeded at  $3 \times 10^5$  cells per microphysiometer capsule in complete media 24 hours prior to an experiment. The media is replaced with serum free media 16 hours prior to recording to minimize non-specific metabolic stimulation by assorted and ill-defined serum factors. On the day of  
25 the experiment the cell capsules are transferred to the microphysiometer and allowed to equilibrate in recording media (low buffer RPMI 1640, no bicarbonate, no serum (Molecular Devices Corporation, Sunnyvale, CA) containing 0.1-1% fatty acid free BSA), during which a vaseline  
30 measurement of basal metabolic activity is established.

A standard recording protocol specifies a 100  $\mu$ l/min flow rate, with a 2 min total pump cycle which includes a 30 sec flow interruption during which the acidification rare

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measurement is taken. Ligand challenges involve a 1 min 20 sec exposure to the sample just prior to the first post challenge rate measurement being taken, followed by two additional pump cycles for a total of 5 min 20 sec sample exposure. Typically, drugs in a primary screen are presented to the cells at 10  $\mu$ M final concentration.

Follow up experiments to examine dose-dependency of active compounds are then done by sequentially challenging the cells with a drug concentration range that exceeds the amount needed to generate responses ranging from threshold to maximal levels. Ligand samples are then washed out and the acidification rates reported are expressed as a percentage increase of the peak response over the baseline rate observed just prior to challenge.

Compounds found to modulate the activity of the polypeptides of the present invention have a number of therapeutic uses, based on the finding that GPR39 is involved in the regulation of gastrointestinal kinetics and cholesterol homeostasis. In specific, this includes diseases related to delayed gastric emptying such as for example, gastroparesis post-operative ileus, gastroparesis in diabetic, functional dyspepsia, post-vagotomy gastroparesis and idiopathic intestinal pseudo-obstruction. Furthermore, this includes diseases related to an increased gastric emptying such as for example, dumping syndrome or increased motility such as diarrhoea, diarrhoea-IBS and mixed-IBS. Further based on the observed effect on cholesterol homeostasis it also includes the metabolic syndrome with in particular cardiovascular diseases related to increased cholesterol levels such as atherosclerosis. In summary GPR39 GPCR and related receptors can be used as a valuable tool for drug

development in a variety of therapeutic areas.

#### Binding agents

5 Thus the invention further provides novel binding agents, including modulatory agents obtained by an assay according to the present invention, and compositions comprising such agents. Agents which bind to the receptor and which may have agonist or antagonist activity may be used in methods  
10 of treating diseases as characterized hereinbefore, whose pathology is characterised by action via the GPR39 GPCR receptor, and such use forms a further aspect of the invention.

15 The agents may be administered an effective amount of an agent of the invention. Since many of the above-mentioned conditions are chronic and often incurable, it will be understood that Atreatment $\equiv$  is intended to include achieving a reduction in the symptoms for a period of time  
20 such as a few hours, days or weeks, and to include slowing the progression of the course of the disease.

Such agents may be formulated into compositions comprising an agent together with a pharmaceutically acceptable  
25 carrier or diluent. The agent may in the form of a physiologically functional derivative, such as an ester or a salt, such as an acid addition salt or basic metal salt, or an N or S oxide. Compositions may be formulated for any suitable route and means of administration.

30 Pharmaceutically acceptable carriers or diluents include those used in formulations suitable for oral, rectal, nasal, inhalable, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal,

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intrathecal and epidural) administration. The choice of carrier or diluent will of course depend on the proposed route of administration, which, may depend on the agent and its therapeutic purpose. The formulations may

5 conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general

10 the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

15 For solid compositions, conventional non-toxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, cellulose, cellulose derivatives, starch, magnesium stearate, sodium saccharin, talcum, glucose, sucrose, magnesium carbonate, and the like may be

20 used. The active compound as defined above may be formulated as suppositories using, for example, polyalkylene glycols, acetylated triglycerides and the like, as the carrier. Liquid pharmaceutically

administrable compositions can, for example, be prepared

25 by dissolving, dispersing, etc, an active compound as defined above and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical

30 composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, sorbitan monolaurate,

35 triethanolamine oleate, etc. Actual methods of preparing

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such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington=s Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 15th Edition, 1975.

5

The composition or formulation to be administered will, in any event, contain a quantity of the active compound(s) in an amount effective to alleviate the symptoms of the subject being treated.

10

Dosage forms or compositions containing active ingredient in the range of 0.25 to 95% with the balance made up from non-toxic carrier may be prepared.

15

For oral administration, a pharmaceutically acceptable non-toxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example, pharmaceutical grades of mannitol, lactose, cellulose, cellulose derivatives, sodium crosscarmellose, starch, magnesium stearate, sodium saccharin, talcum, glucose, sucrose, magnesium, carbonate, and the like. Such compositions take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained release formulations and the like. Such compositions may contain 20 1%-95% active ingredient, more preferably 2-50%, most 25 preferably 5-8%.

Parenteral administration is generally characterized by injection, either subcutaneously, intramuscularly or 30 intravenously. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions.

Suitable excipients are, for example, water, saline,

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dextrose, glycerol, ethanol or the like. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, triethanolamine sodium acetate, etc.

10 The percentage of active compound contained in such parental compositions is highly dependent on the specific nature thereof, as well as the activity of the compound and the needs of the subject. However, percentages of active ingredient of 0.1% to 10% in solution are employable, and will be higher if the composition is a solid which will be subsequently diluted to the above percentages. Preferably, the composition will comprise 0.2-2% of the active agent in solution.

20 It is accordingly an object of the present invention to provide a pharmaceutical composition for the treatment of delayed gastric emptying, diseases related to delayed gastric emptying such as for example, gastroparesis post-operative ileus, gastroparesis in diabetic, functional dyspepsia, post-vagotomy gastroparesis and idiopathic intestinal pseudo-obstruction, said composition comprising a GPR39 receptor antagonist.

Another object of the invention is a pharmaceutical composition for the treatment of increased cholesterol levels, increased gastric emptying, diseases related to increased gastric emptying such as for example dumping syndrome or increased intestinal motility such as diarrhoea, diarrhoea-IBS and mixed-IBS, said composition comprising a GPR39 agonist.

It is accordingly an object of the present invention to provide the use of a GPR39 antagonist in the manufacture  
5 of a medicament for the treatment of a disease condition related to delayed gastric emptying. In particular in the treatment of gastroparesis post-operative ileus, gastroparesis in diabetic, functional dyspepsia, post-vagotomy gastroparesis and idiopathic intestinal pseudo-  
10 obstruction. In another embodiment the present invention provides the use of a GPR39 agonist in the manufacture of a medicament for the treatment of a disease condition related to increased gastric emptying. In particular in the treatment of dumping syndrome, diarrhoea, diarrhoea-  
15 IBS and mixed-IBS.

This invention will be better understood by reference to the Experimental Details that follow, but those skilled in the art will readily appreciate that these are only  
20 illustrative of the invention as described more fully in the claims that follow thereafter. Additionally, throughout this application, various publications are cited. The disclosure of these publications is hereby incorporated by reference into this application to describe  
25 more fully the state of the art to which this invention pertains.

#### EXAMPLES

30 The following examples illustrate the invention. Other embodiments will occur to the person skilled in the art in light of these examples.

#### *Materials and Methods*



GPR39 knock-out mouse

GPR39 knock-out mice were obtained from Lexicon Genetics Inc. Disruption of the open reading frame of GPR39 was performed by replacing the coding region of the first  
5 coding exon of the GPR39 gene with an IRES LacZ/MC1-Neo reporter gene/selection cassette. Animals were maintained in an SPF facility that meets all Belgian and European requirements for animal care. Mice were group-housed in a climate-controlled animal colony with a 12h dark-light  
10 cycle (light on 7:00 EST) with free access to food and water, unless indicated differently. Adequate measures were taken to minimize pain or discomfort. All experiments were carried out in accordance with the European Communities Council Directives (86/609/EEC) and were  
15 approved by the local ethical committee.

Real-Time Quantitative Reverse Transcription PCR analysis of GPR39

Total RNA from different tissues dissected from wild type (brain, liver, spinal cord, stomach, kidney, spleen,  
20 colon, lung, heart, oesophagus, pancreas, ileum, jejunum) and GPR39 knock-out (liver, stomach, jejunum, colon) mouse were analysed using real-time quantitative reverse transcription PCR to study the tissue distribution of GPR39 and confirm the GPR39 knock-out.  
25 First strand cDNA synthesis was performed on 1,0 mg total RNA using random hexamer primers and Superscript II RT (Invitrogen Life Technologies). Quantitative PCR was performed on an ABIPrism 7000 cycler (Applied Biosystems) using a Taqman PCR kit. Serial dilutions of cDNA were  
30 used to generate standard curves of threshold cycles versus the logarithms of concentrations for  $\beta$ -actin. The RTQ specific primer pairs and probes are enlisted below.

Mouse IMPA2 Selection of RTQ primers and probe:

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GPR39\_FW 5'-CCA CTC ACA AGG GAC TCA ACT G-3' (SEQ ID NO:5)

GPR39\_REV 5'-TGG AGT TTC CAG GTT CAT CGT-3' (SEQ ID NO:6)

GPR39\_Probe 5'-AAC CTC TCT CGC ACC CGC CA-3' [5' FAM] [3' TAMRA] (SEQ ID NO:7)

5

Samples were run in triplicate and results are displayed only when complying with quality standards. Expression levels in the different mouse tissues are expressed as relative levels after normalisation to mouse  $\beta$ -actin

#### 10 Staining for $\beta$ -galactosidase activity in GPR39 knock out mouse

Tissues (pancreas, stomach, ileum, jejunum and colon) were dissected from homozygous GPR39 knock-out mice that express the bacterial  $\beta$ -galactosidase gene (LacZ) under the regulatory control of the endogenous mouse GPR39 promoter. Whole-mount  
15 staining on these tissues was performed according to described protocols (InvitroGen), tissues were next paraffin imbedded and sectioned.

### PHENOTYPICAL ANALYSIS

#### Gastric emptying

20 A panel of 6 wild type and 6 homozygous male GPR39 knock-out mice were housed in cages at 20-22°C, with a 14h -10h light-dark cycle. Standard commercial mouse chow (4352 Muracon G, Nutreco Belgium NV, Gent, Belgium) (4.5% fat, 4% cellulose, 21% proteins, 1.404 kcal/g) was available ad  
25 libitum until the evening before the test. At 4 p.m. the food was removed and the test was started at 11 a.m. the following day. A wire-mesh plate was inserted into the bottom of the cage to avoid coprophagia during the period of fasting. Tap water was freely available at all times,  
30 except during the test.

### Test Meal

The solid test meal, consisted of 0.1 g baked scrambled egg yolk, doped with 0.01  $\mu\text{Ci}$   $^{14}\text{C}$ -octanoic acid sodium salt (American Radiolabeled chemicals Inc, St Louis, MO, USA) and mixed with 0.1g standard mice chow. Tap water was added and mixed to form a homogenous paste. Before the start of the experiments, fasted mice (19h) were trained twice weekly, for 2 weeks, on a fixed time schedule, to eat spontaneously the test meal, without the radioactive marker. After this training the test meal was ingested within 60s by most of the animals.

### Breath Test Procedure: CO<sub>2</sub> sampling

Six airtight plastic tubes were adapted with an inlet valve through which a continuous airflow (80% N<sub>2</sub>, 20% O<sub>2</sub>, 360 ml/min) passed, while the air outflow was bubbled through a vial containing the CO<sub>2</sub> trapper, tetramethylammoniumhydroxide (1.3 mmol) (Acros, Geel, Belgium), and the pH indicator thymolphthaleine (0.006%). With the sampling time used no decoloration was observed indicating that all CO<sub>2</sub> expired during the experiments was captured. The dimensions of the tube (diameter 50mm, length 150mm) allowed free movement of the mice. Each mouse was placed in an airtight tube and before giving the test meal a baseline breath sample (5 min) was taken. The tubes were opened, the food was given to the mouse and the tube was closed again. Sampling occurred every 5 minutes during the first half hour and every 15 min during the next 4h. Scintillation liquid was added and the samples were counted in a  $\beta$ -counter.

### Data analysis

Using least-square analysis,  $^{14}\text{CO}_2$  excreted in breath was

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fitted by two mathematical formulae to derive the curve of the best fit (Ghoos *et al.*, 1993 ; Maes *et al.*, 1994):

$$\text{Formula 1 : } y = mk\beta e^{-kt} (1 - e^{-kt})^{\beta-1}$$

$$\text{Formula 2 : } y = at^b e^{-ct}$$

- 5 y is the percentage of  $^{14}\text{CO}_2$  excretion in breath per hour, t is the time in hours; m, k,  $\beta$ , a, b and c are regression estimated constants, with m the total amount of  $^{14}\text{CO}_2$  recovered when time is infinite.

From the best fit the following parameters were  
10 calculated:

- Gastric half excretion time ( $t_{1/2}$ ), time at which 50% of the total amount of  $^{14}\text{CO}_2$  was excreted. The  $t_{1/2}$ -value therefore not only contains the gastric half emptying time but also includes the time necessary for postgastric  
15 processes such as absorption and metabolism of the  $^{14}\text{C}$  octanoic acid and the excretion of  $^{14}\text{CO}_2$  in the breath.

$$\text{Formula 1: } t_{1/2} = (-1/k) * \ln(1 - 2^{-1/\beta})$$

$$\text{Formula 2: } t_{1/2} = 60 * (b/c)$$

- 20 The lag phase ( $t_{lag}$ ), delay in gastric emptying due to the time required for the stomach to grind the meal in fine particles.

$$\text{Formula 1: } t_{lag} = \ln(\beta) / k$$

$$\text{Formula 2: } t_{lag} = 60 * \text{gammainv}(0.5; 1+b; 1/c)$$

- 25 In order to ensure that the measured  $^{14}\text{CO}_2$  production did not diverge too much from the fitted mathematical curve, measured values were correlated with values mathematically fitted to the theoretical curve. If the correlation coefficient was less than 0.95 ( $r \leq 0.95$ ) the mathematical  
30 fitting was not considered as acceptable and the data were rejected from analysis.

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GASTRIC SECRETION

A panel of 6 wild type (WT) and 6 homozygous GPR39 knock-out (GPR 39<sup>-/-</sup>) female mice were used to investigate the gastric secretion.

5 Mice were anesthetized using isoflurane. Under anesthesia the pylorus was closed by means of a suture (vicryl 4-0, Ethicon) then the abdominal cavity was sutured. The animal was replaced individually in its cage for 4h.

Four hours after the pylorus closing mice were sacrificed  
10 and the esophagus was closed just above the cardia. The stomach was withdraw from the abdominal cavity. The stomach was weighed and opened to take the gastric secretion. The gastric secretion was measured and then diluted in 5 ml of water (Water Molecular Biology Grade,  
15 Eppendorf). The pH was measured using a pH electrode (pH 597, Profi Lab).

Data analysis

Data are expressed as means  $\pm$  SD. Mice and stomach weight  
20 and gastric secretion were compared between wild type (WT) and GPR39<sup>-/-</sup> mice using a non parametric Wilcoxon test. The pH of water (5 ml) was firstly measured then added to the gastric secretion and the pH was again measured. The difference between the pH of water and the pH of solution  
25 with gastric secretion was calculated and then reported to 1 ml of the solution and then compared between both groups using a non parametric Wilcoxon test.

FAECAL PELLETS PROPULSION

30 A panel of 6 wild type (WT) and 6 homozygous GPR39 knock-out (GPR 39<sup>-/-</sup>) male mice were used to investigate the fecal pellets propulsion.

Mice were asphyxiated by CO<sub>2</sub> followed by decapitation. The

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colon was cut out from the caecum till the rectum, and freed of adhering tissues. The tissue was transferred to a glass beaker containing 100 ml of Krebs-Henseleit solution, gassed with a mixture of 95 % O<sub>2</sub> and 5 % C O<sub>2</sub> and maintained at 37° C. Prior to the start of the experiment, the length of the colon was measured. The pellets in the colon were counted and their distance to the rectum was measured. Then the time to expulsion of each pellet was recorded for 20 min.

10

#### Data analysis

The distribution of the pellets over the length of the colon at t=0 and 20 min was determined by binning the position of each pellet relative the length of the colon (bins of 15% of colon length: 0%-15%, >15%-30%, >30%-45%, >60%-75%, >75%-90% and >90%-100%, where 0% represented the rectum and 100% the IC-valve). Bins of 15% of total colon length were considered, and the number of pellets in each bin and expelled from each bin was counted during a 20 min period. Statistical analysis was performed using a 2-sided Sign Test

#### 25 Mouse behavioural tests

A panel of 14 wild type and 14 homozygous male GPR39 knock-out mice and 12 wild type and 12 homozygous female GPR39 knock-out mice, were subjected to two behavioural tests: Open Field Test (an art known method to evaluate locomotor activity) and Elevated Zero Maze (an art known method to evaluate anxiety-related behaviour)

The Open Field Test (OFT) was performed during the light phase of a normal light/dark cycle. Each mouse was

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subjected to a 30-min testing session in an automated open field system. Locomotion in the horizontal and vertical pane was recorded. During this session, the following parameters were recorded: duration of moves, number of moves, total distance traveled, distance traveled in the centre, distance traveled in the margin, relative distance traveled in the centre, time spent in the centre, time spent in the margin, relative time spent in the centre, distance traveled in centre vs. distance traveled in margin, time spent in the centre vs. time spent in the margin, number of rearings, and duration of rearing. A Single Factor ANOVA was used as statistical analysis of the results obtained.

The Elevated Zero Maze (O-maze) was performed during the light phase of a normal light/dark cycle. Each mouse was subjected to a 5-min testing session in the elevated zero maze. During this session, the following parameters were recorded: move time in closed and open arms, the relative time spent in closed and open arms, total distance traveled, distance traveled in closed and open arms. A Single Factor ANOVA was used as statistical analysis of the results obtained.

#### 25 Respiratory rate

A panel of six wild type and six homozygous male GPR39 knock-out mice were habituated to AIN-93G Rodent Purified Diet (Dyets, Inc., USA) prior to putting them into the metabolic cages. Mice were individually housed in type-2 cages under the same conditions as for the metabolic cages (see below) for a minimum of 1 week prior to measurement. For two consecutive days, all mice were housed individually in metabolic cages under controlled temperature (28.8°C), humidity (55-60 %) and light (12:12

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h light/dark cycle, lights on 06:00 h). The animals had free access to rodent chow and water. The following parameters were measured and expressed as mean: respiratory quotient (RER), VO<sub>2</sub> and heat production.

5

#### BLOOD ANALYSIS

Plasmatic level of cholesterol and triglycerides were determined in 6 month old male and female GPR39<sup>-/-</sup> mice versus GPR39<sup>+/+</sup> littermates after 12h food deprivation after a ad libitum availability of a standard chow diet. Cholesterol and triglyceride levels were determined by means a Hitachi Modular random access analyzer (Roche Diagnostics, Basel, Switzerland) using reagents and test methods from the same supplier.

15

#### FOOD INTAKE

Cumulative food intake analysis during 6h in ad libitum fed and fasted young (17 weeks) and old (56 weeks) GPR39<sup>-/-</sup> mice and GPR39<sup>+/+</sup> littermates (n=6 per group) was started at 10 a.m. Each mouse was put in an individual cage, placed in a dark room (unless stated otherwise), where it received a pre-weighed amount of food. Cumulative food intake was measured by subtracting the uneaten food at 30 min, 1, 2, 3, 4, 5 and 6 hours.

25

#### RESULTS

##### 30 Real-Time Quantitative Reverse Transcription PCR analysis of GPR39.

Real-Time Quantitative Reverse Transcription PCR of GPR39 in multiple mouse tissues derived from wild type mice showed a wide tissue distribution (Fig. 1A). Real-Time Quantitative Reverse Transcription PCR of GPR39 in four

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different tissues (liver, stomach, jejunum, colon) derived from wild type mice and heterozygote and homozygote GPR39 knock-out mice confirmed the absence of the GPR39 transcript in the GPR39 knock-out mouse. Intermediate  
5 levels were obtained in the heterozygote mice (Fig. 1B).

#### Staining for $\beta$ -galactosidase activity in GPR39 knock-out mouse

Expression of GPR39 was detected in the exocrine (Islets of Langerhans) and endocrine portion of the pancreas, the  
10 parietal cells located in the upper region of the mucosal glandular tissue in the pylorus and in the mucous neck cells in the corpus of the stomach, the enterocytes in the small intestine and the colon as well as in the neurons that innervate the muscle layers of the intestine.

15

#### Gastric emptying

The gastric emptying of a solid meal in GPCR 39 knock-out mice is significantly accelerated in comparison with the wild type mice. Indeed the half emptying time ( $t_{1/2}$ ) in  
20 GPCR 39 knock-out mice ( $48.5 \pm 2$  min,  $n=6$ ) is significantly decreased in comparison with wild type mice ( $89.9 \pm 6$  min,  $n=6$ ) (Fig. 2.A). The  $T_{lag}$  is also significantly decreased in GPCR 39 knock-out mice vs. wild type mice ( $22.5 \pm 1.3$  min vs.  $39.2 \pm 3.4$  min,  $n=6$ ) confirming the acceleration of  
25 gastric emptying of a solid meal in GPR39 knock-out mice (Fig. 2.B). These results are mean of three consecutive tests in 6 mice per group at 3 days interval.

#### GASTRIC SECRETION

30 No difference in mice weight was observed between WT and GPR39<sup>-/-</sup> mice ( $30.4 \pm 6.6$  g vs.  $26.9 \pm 4.0$  g, respectively). A trend in stomach weight between both groups was measured (WT:  $978 \pm 262$  mg vs. GPR39<sup>-/-</sup>:  $1395 \pm 343$  mg;  $P=0.0547$ ).

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Gastric secretion ( $\mu\text{L}$ ) was significantly increased in GPR39<sup>-/-</sup> mice in comparison with WT mice ( $638 \pm 336 \mu\text{L}$  vs.  $225 \pm 170 \mu\text{L}$ , respectively;  $P=0.0242$ ). The pH decrease between water pH and gastric secretion solution pH in GPR39<sup>-/-</sup> was significantly smaller than in WT ( $0.9 \pm 0.2$  vs.  $1.1 \pm 0.2$ , respectively;  $P=0.0374$ ). These data suggest that the deletion of GPR39 receptor induces a significant increase of stomach secretion but this increase is not due to an increase of acid secretion.

10

#### FAECAL PELLETS PROPULSION

The results show that distribution of pellets over the colon at the start of the experiment was not different between GPR39<sup>-/-</sup> and WT ( $p=0.5637$  by 2-sided Sign Test; blue bars) (Fig. 3). Pellets most proximal to the rectum (0-30% of total length) were expelled in both WT and GPR39<sup>-/-</sup>; the majority of distally located pellets (30-75% of length) were expelled from colon of GPR39<sup>-/-</sup>-animals, but not from WT-animals ( $p=0.0253$ ; yellow bars) (Fig 3). These results indicate a more effective expulsion of faecal pellets from colon of GPR39<sup>-/-</sup> animals.

20

#### Mouse behavioural tests

Since the observed differences in gastric emptying could be confounded by a difference in general activity or emotional status of the GPR39 knock-out animals, we performed a neurological examination existing of an open field test (OFT) and zero maze test (O-maze).

30

#### Open Field test

No difference in motor activity was observed between female GPR39 knock-out and wild type littermates. Thus the

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observed difference in the gastric emptying is no artefact due to an increased general activity in the female GPR39 knock-out mice. No significant difference was observed in duration of moves, number of moves, total distance traveled, total time traveled, distance traveled in the centre, distance traveled in the margin, relative distance traveled in the centre, time spent in the centre, time spent in the margin, relative time spent in the centre, distance traveled in centre vs. distance traveled in margin, time spent in the centre vs. time spent in the margin, velocity, number of rearings, and duration of rearing. When comparing male GPR39 knock-out and wild type littermates no increase in general activity that can confound the observed difference in gastric emptying was observed. If anything a small but significant decreased general activity was observed in the male GPR39 knock-out mice as evident from a significant decrease in the total move time and the total distance traveled (ANOVA,  $p < 0.001$ ) in male GPR39 knock-out mice compared to wild type controls. In addition the distance traveled in the centre, the relative distance traveled in the centre and the centre vs. margin distance is significantly lower in GPR39 knock-out mice compared to wild type littermates. The latter differences may be due to the reduced locomotor activity or, alternatively, may reflect an anxiogenic phenotype. Therefore the O-maze test was performed.

#### Zero maze test

In the O-maze the GPR39 knock-out mice did not display an anxiogenic nor an anxiolytic phenotype. Both the relative time spend in the closed versus open arm as well as the relative distance traveled in the open versus closed arms was similar in the GPR39 knock-out and wild type control mice. Thus the increased rate in gastric emptying in the GPR39 knock-out mice is not due to changed response to

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anxiety inducing conditions.

#### Respiratory rate

During the two recording days no difference in respiratory  
5 rate was observed between male GPR39 knock-out and wild  
type littermates. Thus the observed difference in the  
gastric emptying is no artefact due to an respiratory rate  
in the female GPR39 knock-out mice. The respiratory  
quotient remained around 1.0 for both wild type and GPR39  
10 knock-out mice and the VO<sub>2</sub> together with heat production  
did not change with genotype either.

#### Blood analysis

Blood chemistry data were collected in 6 month old male  
15 and female GPR39<sup>-/-</sup> mice versus GPR39<sup>+/+</sup> littermates after  
12h of food deprivation after ad libitum availability of a  
standard chow diet. Cholesterol levels (average in mg/dl  
± SD) were significantly increased in both male (142.2 ±  
29.0) and female (118.7 ± 16.2) GPR39<sup>-/-</sup> mice compared to  
20 GPR39<sup>+/+</sup> male (107.3 ± 37.8) and female (83.5 ± 24.2)  
littermates (p<0.05 and p<0.001 for males and females  
respectively). Triglyceride levels (average in mg/dl ±  
SD) appeared increased in both male (162.5 ± 14.7) and  
female (84.6 ± 7.2) GPR39<sup>-/-</sup> mice compared to GPR39<sup>+/+</sup> male  
25 (123.9 ± 20.8) and female (71.4 ± 12.0) littermates, but  
the differences did not reach significance.

#### Food intake

Cumulative food intake in mice (17 weeks, n=6) that had  
30 free access to food before the start of the experiment was  
not significantly (P=0.47) different in GPR39 knock-out  
mice compared with wild type mice. The same observation  
was made in older mice (56 weeks, n=6, P=0.78). In  
contrast in fasted mice (19 h) cumulative food intake was

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significantly ( $P < 0.0001$ ) lower in GPR39 mice compared with wild type mice. This effect was even more pronounced in older mice (56 weeks). Indeed, after 6h, cumulative food intake was 0.62g lower in young and 1.25g in old GPR39 mice compared with age matched wild type mice.

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CLAIMS

1. A method to identify compounds that modulate gastrointestinal kinetics and/or cholesterol metabolism, said method comprising the use of all or part of the GPR39 receptor protein.
2. A method according to claim 1 wherein the GPR39 receptor protein is being selected from the group consisting of SEQ ID No:2, SEQ ID No:4, a splice variant of the proteins having the aforementioned SEQ ID's, and an amino acid sequence having at least 50% and preferably at least 80%, 90%, 95% or 98% sequence identity to the amino acid sequence of SEQ ID No:2 or SEQ ID No:4.
3. A method according to claim 1 wherein a part of the GPR39 receptor protein consist of a fragment of at least 10 amino acids of the polypeptide of SEQ ID No:2 or SEQ ID No:4.
4. A method for identifying a compound that modulates gastrointestinal kinetics and/or cholesterol metabolism, which method comprises:
  - (i) contacting host cells expressing all or part of the GPR39 protein with said compounds, and
  - (ii) detecting binding of the compound to said receptor protein
5. A method for identifying a compound that modulates gastrointestinal kinetics and/or cholesterol metabolism, which method comprises:
  - (i) contacting membrane preparations from host cells expressing all or part of the GPR39 protein with



said compounds, and

- (ii) detecting binding of the compound to said receptor protein

6. A method according to claims 4 or 5 wherein the binding of the compound to the GPR39 receptor protein is being detected by means of a label directly or indirectly associated with the compound to be tested or in an assay involving competition with a labelled competitor.

7. A method according to claim 6 wherein the labelled competitor is labelled obestatin.

8. A method to identify compounds that modulate gastrointestinal kinetics and cholesterol metabolism, said method comprising:

- (i) contacting all or parts of the GPR39 protein receptor with the compound to be tested, and
- (ii) measuring the activity of the GPR39 receptor protein, wherein the change of activity in the presence of the compound is an indication of the compounds capability to modulate gastrointestinal kinetics.

9. A method according to claim 8 wherein in step i), the compounds are contacted with host cells expressing all or part of the GPR39 protein as defined in claims 2 and 3.

10. A method according to claims 8 or 9 wherein the activity of the GPR39 receptor protein is being measured as the modulation of an intracellular messenger.

11. The method according to claim 10, wherein the intracellular second messenger is cAMP, calcium or a

reporter gene product.

12. Use of an isolated nucleic acid sequence selected from the group consisting of;

- (i) a nucleic acid sequence encoding all or part of the polypeptides of SEQ ID NO:2 or SEQ ID No:4,
  - (ii) a nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3, or
  - (iii) a nucleic acid sequence having at least 80% sequence identity to the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3,
- in a method according to any one of claims 1 to 11.

13. Use of a vector comprising a nucleic acid sequence as defined in claim 12, in a method according to any one of claims 1, 4, 5, 8 or 9.

14. Use of a host cell comprising a nucleic acid sequence as defined in claim 12 or a vector as defined in claim 13, in a method according to any one of claims 1, 4, 5, 8 or 9.

15. A pharmaceutical composition for the treatment of delayed gastric emptying and delayed colonic motility in a human or animal comprising a GPR39 receptor antagonist.

16. A pharmaceutical composition for the treatment of increased gastric emptying and increased colonic motility in a human or animal comprising a GPR39 agonist.

17. Use of a GPR39 antagonist in the manufacture of a medicament for the treatment of a disease condition related to delayed gastric emptying and delayed colonic motility.

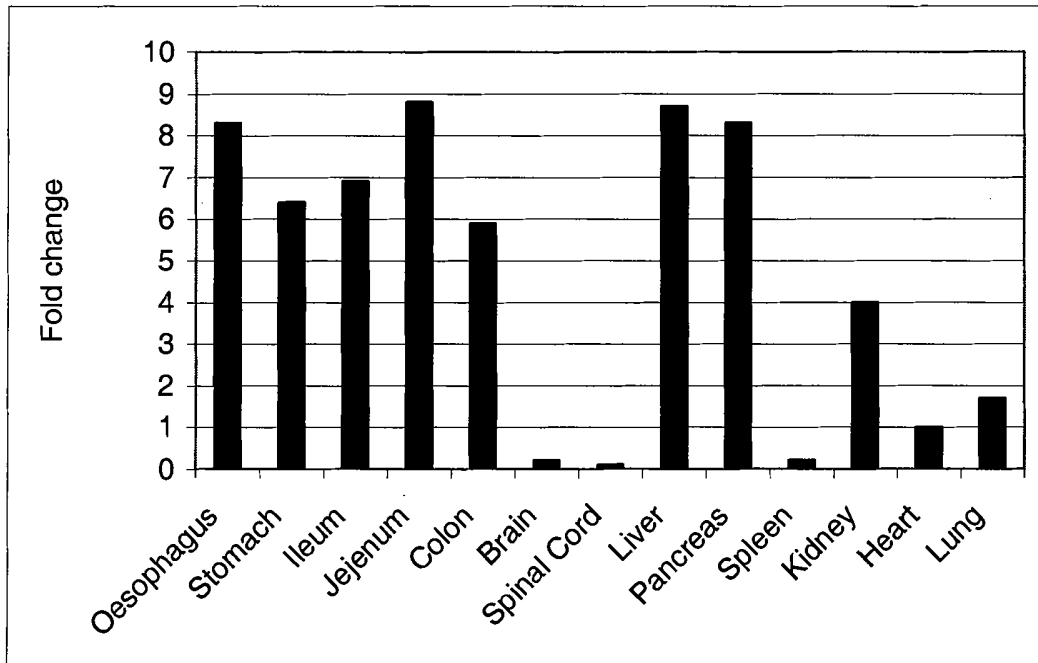
18. Use of a GPR39 agonist in the manufacture of a medicament for the treatment of a disease condition related to increased gastric emptying and increased colonic motility.

19. A pharmaceutical composition for the treatment of increased cholesterol levels in a human or animal comprising a GPR39 receptor agonist.

20. Use of a GPR39 agonist in the manufacture of a medicament for the treatment of a disease condition related to increased cholesterol levels.

Fig. 1

A



B

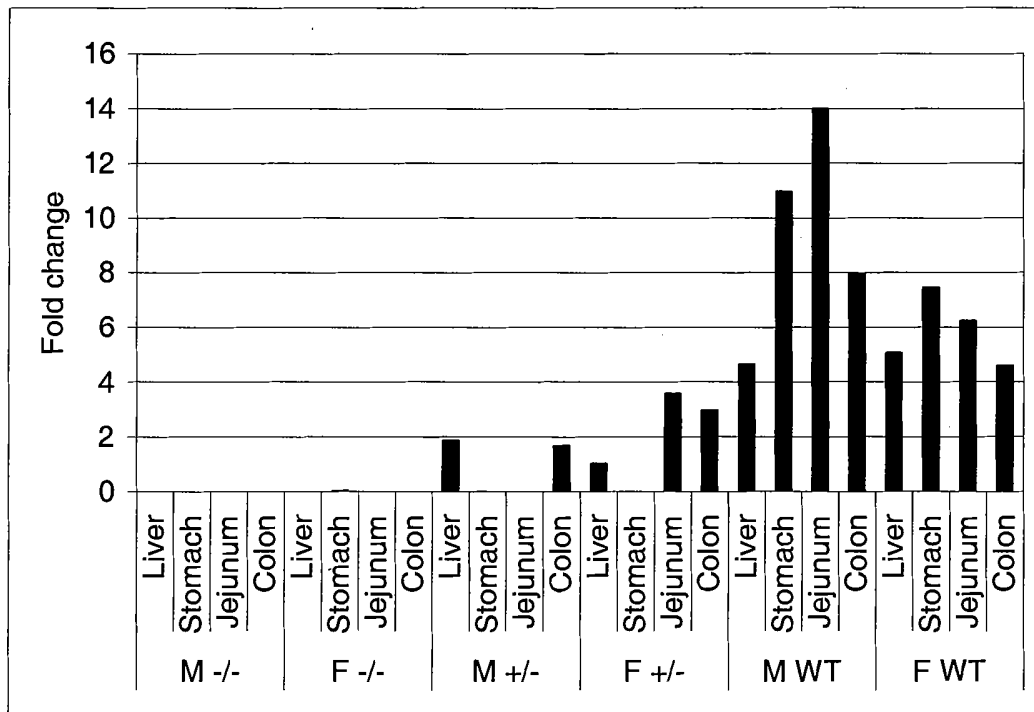
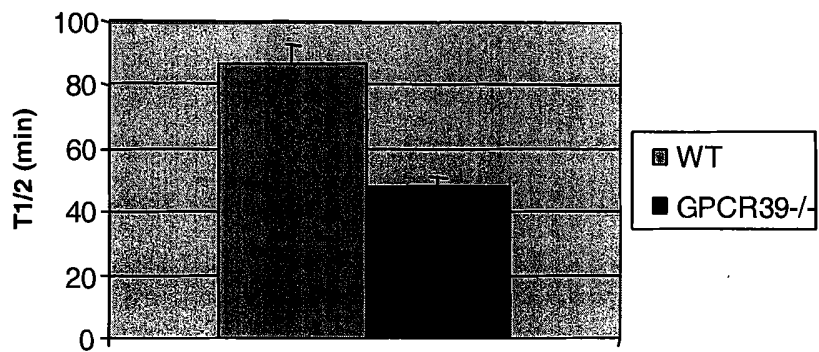


Fig. 2

A



B

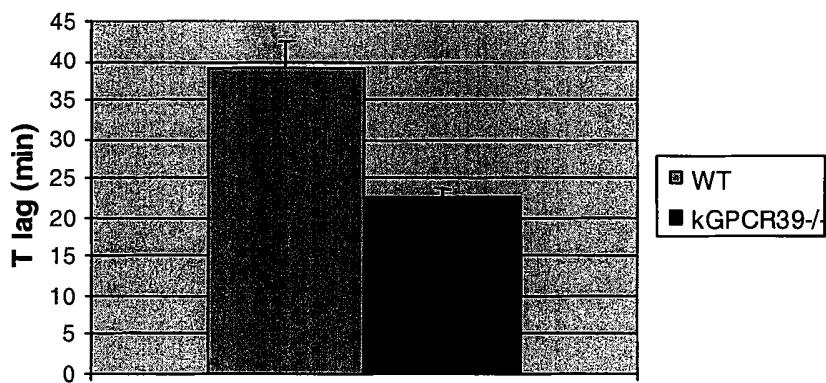


Fig. 3

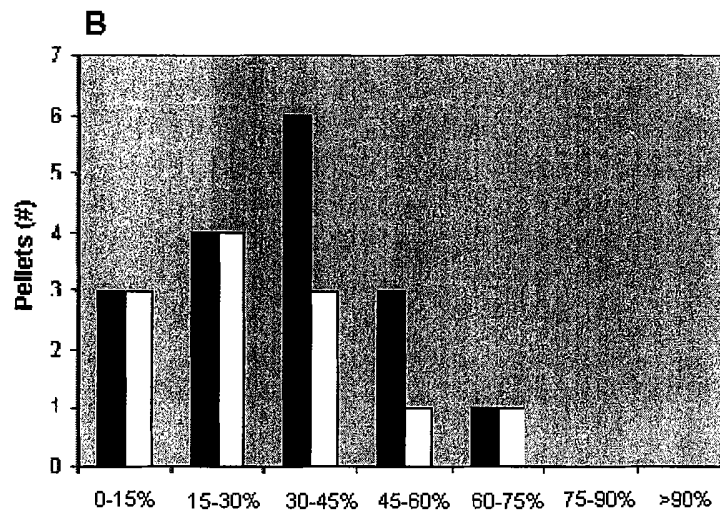
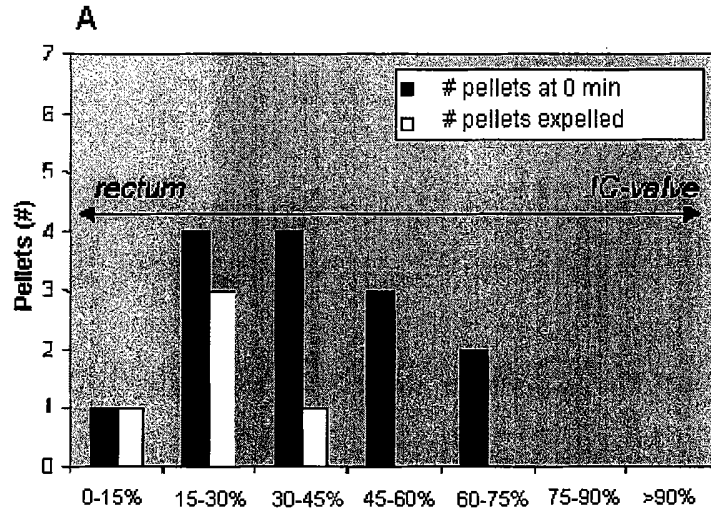


Fig. 4

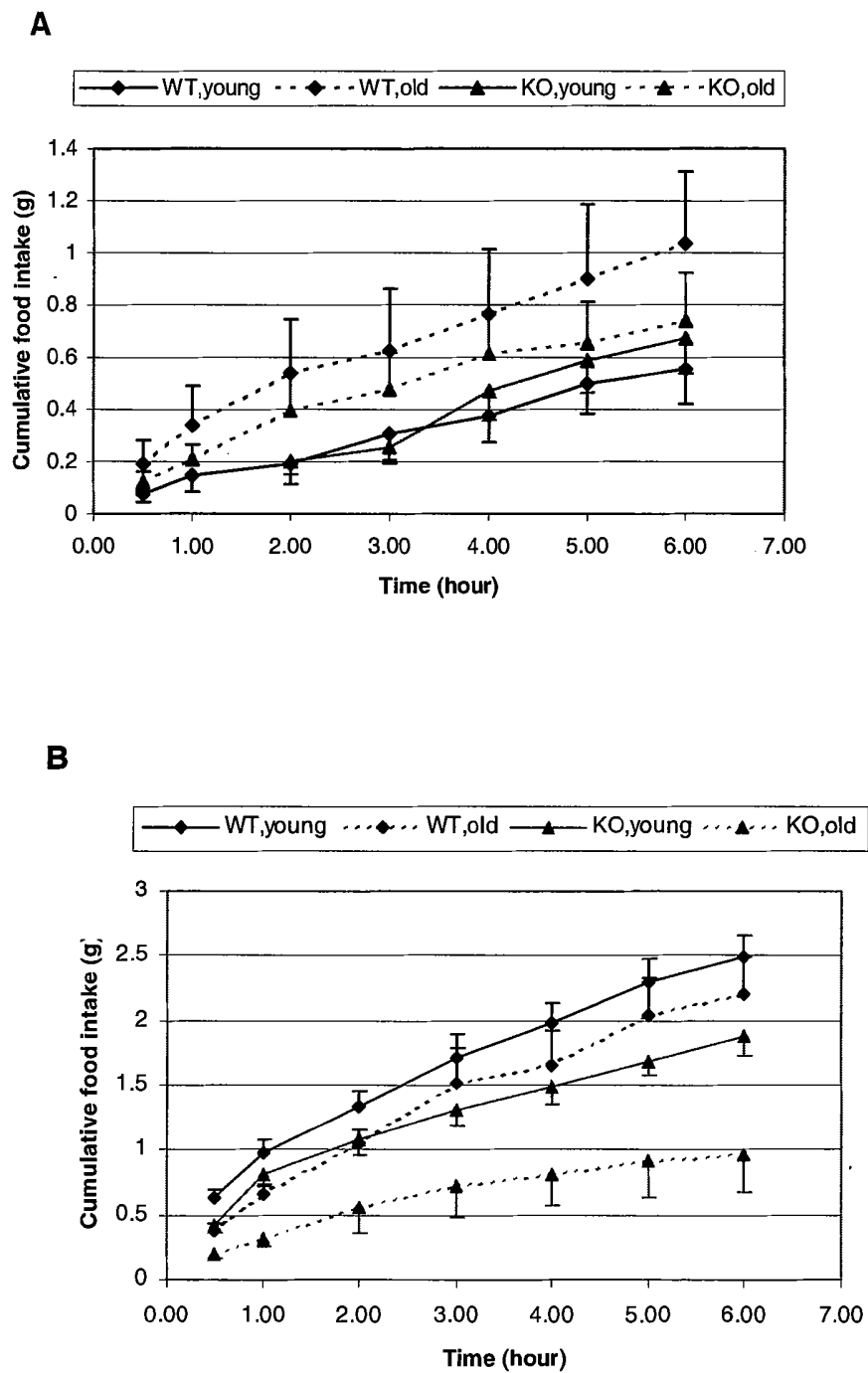


Fig. 5

Human	<b>MPSPGTVC</b> SLLLLG- <b>MLNLDL</b> AMAGSSFLSPEHDKVQORKESSKPPAKLQPRALAGWLR
Monkey	<b>NPSPGTVC</b> SLLLLG- <b>MLNLDL</b> AMAGSSFLSPEHDKVQORKESSKPPAKLQPRALGGWLR
Mouse	<b>MLSSGTVC</b> SLLLLS- <b>MLNNDV</b> AMAGSSFLSPEHDKVQORKESSKPPAKLQPRALEGWLH
Rat	<b>NVSSATVC</b> SLLLLS- <b>MLNMDV</b> AMAGSSFLSPEHDKVQORKESSKPPAKLQPRALEGWLH
Gerbil	<b>MSSSGTVC</b> SLLLLG- <b>VLNNDV</b> AMAGSSFLSPEHDKVQORKESSKPPAKLQPRALEGWLH
Pig	<b>MPSTGTVC</b> SLLLLS <b>VLLMADL</b> AMAGSSFLSPEHDKVQORKESSKPPAKLQPRALEGWLG
Cat	<b>NPSPGTVC</b> SLLLFS- <b>MLNADL</b> AMAGSSFLSPEHDKVQORKESSKPPAKLQPRALEGLIH
Dog	<b>MPSLGTVC</b> SLLLFS- <b>VLNVDL</b> AMAGSSFLSPEHDKVQORKESSKPPAKLQPRALEGSLG
Goat	<b>MPAPRTVC</b> SLLLLS- <b>MLNNDL</b> AMAGSSFLSPEHDKVQORKESSKPPAKLQPRALEGQFD
Sheep	<b>MPAPRTVC</b> SLLLLS- <b>LLNNDL</b> AMAGSSFLSPEHDKVQORKESSKPPAKLQPRALEGQFD
Cattle	<b>MPAPWTV</b> CSLLLS- <b>VLCMDL</b> AMAGSSFLSPEHDKVQORKESSKPPAKLQPRALEGQFD
consensus	<b>NpspgTic</b> SLLLLS <b>MLNMDL</b> AMAGSSFLSPEHDKVQORKESSKPPAKLQPRALeGwlr

Human	<b>PEDGGQ</b> AEGAEDELEI <b>RENAP</b> FDVGIKLSGVQYQ <b>QHSQ</b> ALG <b>FLQD</b> ILWEEAKEAPADK
Monkey	<b>PEDGDQ</b> AEGAEDELEI <b>RENAP</b> FDVGIKLSGVQYQ <b>QHSQ</b> ALG <b>FLQD</b> ILWEEAKEAPADK
Mouse	<b>PEDRGQ</b> AEE <b>TEELE</b> I <b>RENAP</b> FDVGIKLS <b>GAQYQ</b> <b>QHGR</b> ALG <b>FLQD</b> ILWEEVKEAPADK
Rat	<b>PEDRGQ</b> AEE <b>HEELE</b> I <b>RENAP</b> FDVGIKLS <b>GAQYQ</b> <b>QHGR</b> ALG <b>FLQD</b> ILWEEVKEAPANK
Gerbil	<b>PDGRGQ</b> AEGAEDELEI <b>RENAP</b> FDVGIKLS <b>GAQYQ</b> <b>QHGR</b> ALG <b>FLQD</b> ILWEEVKEEATDK
Pig	<b>PEDSGE</b> VEGTEDKLEI <b>RENAP</b> CDVGIKLS <b>GAQSD</b> <b>QHGP</b> PLG <b>FLQD</b> ILWEEVTEAPADK
Cat	<b>PEDTQ</b> VEGAEDELEI <b>RENAP</b> FDVGIKLS <b>GAQYH</b> <b>QHGR</b> ALG <b>FLQD</b> VLWEEAEVLADE
Dog	<b>PEDTQ</b> VEGAEDELEI <b>RENAP</b> FDVGIKLS <b>GPQYH</b> <b>QHGR</b> ALG <b>FLQD</b> EVLMEDTNEALADE
Goat	<b>PDVGSQ</b> EEGAEDELEI <b>RENAP</b> FNIGIKLS <b>GAQSL</b> <b>QHGT</b> PLG <b>FLQD</b> ILWEEAEETLADE
Sheep	<b>PDVGSQ</b> EEGAEDELEI <b>RENAP</b> FNIGIKLS <b>GAQSL</b> <b>QHGT</b> PLG <b>FLQD</b> ILWEEAEETLADE
Cattle	<b>PEVGSQ</b> AEGAEDELEI <b>RENAP</b> FNIGIKL <b>AGAQSL</b> <b>QHGT</b> PLG <b>FLQD</b> ILWEEAEETLANE
consensus	<b>Ped</b> <b>qaEgaEdeLEi</b> <b>RENAP</b> <b>fdvGIKLS</b> <b>GaQyq</b> <b>QHqyaLG</b> <b>FLQd</b> ILWEEa <b>Ea</b> adk



**INTERNATIONAL SEARCH REPORT**

International application No  
PCT/EP2005/056350

**A. CLASSIFICATION OF SUBJECT MATTER**  
G01N33/74      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)  
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)

EPO-Internal, Sequence Search, WPI Data, PAJ, EMBASE, BIOSIS, INSPEC, FSTA, COMPENDEX

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/81634 A (MILLENNIUM PHARMACEUTICALS, INC; GALVIN, KATHERINE, A; RUDOLPH-OWEN, L) 1 November 2001 (2001-11-01) page 46, line 1 - page 58, line 4 page 56, line 27 - page 58, line 4 page 59, line 1 - page 65, line 13 page 65, line 16 - line 20 claims 10-12,25,28,30-32,35,37-49; example 2 example 4	1-6, 12-16, 19,20
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Further documents are listed in the continuation of Box C.

See patent family annex.

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Date of the actual completion of the international search

21 March 2006

Date of mailing of the international search report

04/04/2006

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## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2005/056350

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/232769 A1 (MONIA BRETT P ET AL) 18 December 2003 (2003-12-18) claims 1-13	15
A	WO 2004/099782 A (BAYER HEALTHCARE AG; GOLZ, STEFAN; BRUEGGEMEIER, ULF; SUMMER, HOLGER;) 18 November 2004 (2004-11-18) page 39, line 1 - page 53, line 27 page 56, line 1 - page 58, line 27 page 60, line 7 - line 27 page 61, line 16 - line 26 page 75, line 1 - page 81, line 11 page 97 - page 100; table 1 examples 10,11,14 claims 1-17	1-20
A	WO 2004/040000 A (PRIMAL, INC; GAITANARIS, GEORGE, A; BERGMANN, JOHN, E; GRAGEROV, ALEXA) 13 May 2004 (2004-05-13) table 16 page 228, line 19 table 18 page 232, lines 12,14,20 table 28 page 258, lines 8,21 claims 265-270,277-282,337-342,400,402,412	1-20
A	US 2002/192711 A1 (NESTOR JOHN J ET AL) 19 December 2002 (2002-12-19) page 1, paragraph 2 page 2, paragraph 12 page 4, paragraph 41 page 5, paragraph 48 claims 1,14,16	1-20
A	MCKEE KAREN KULJU ET AL: "Cloning and characterization of two human G protein-coupled receptor genes (GPR38 and GPR39) related to the growth hormone secretagogue and neurotensin receptors" GENOMICS, vol. 46, no. 3, 15 December 1997 (1997-12-15), pages 426-434, XP000889913 ISSN: 0888-7543 figure 4 page 433, column 1, paragraph 3	1-20
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International application No  
PCT/EP2005/056350

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DAVIES R ET AL: "Multiple roles for the Wilms' tumor suppressor, WT1." CANCER RESEARCH. 1 APR 1999, vol. 59, no. 7 Suppl, 1 April 1999 (1999-04-01), pages 1747s-1750s ; d, XP008046412 ISSN: 0008-5472 abstract	1-20
A	WO 2004/039837 A (JANSSEN PHARMACEUTICA N.V; PEETERS, PIETER, JOHAN; GOEHLMANN, HINRICH,) 13 May 2004 (2004-05-13) page 49 - page 50	1-15
P,X	ZHANG JIAN V ET AL: "Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake" SCIENCE (WASHINGTON D C), vol. 310, no. 5750, November 2005 (2005-11), pages 996-999, XP002373204 ISSN: 0036-8075 the whole document	1-20

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Information on patent family members

International application No

PCT/EP2005/056350

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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US 2003232769	A1	18-12-2003	NONE	
WO 2004099782	A	18-11-2004	NONE	
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