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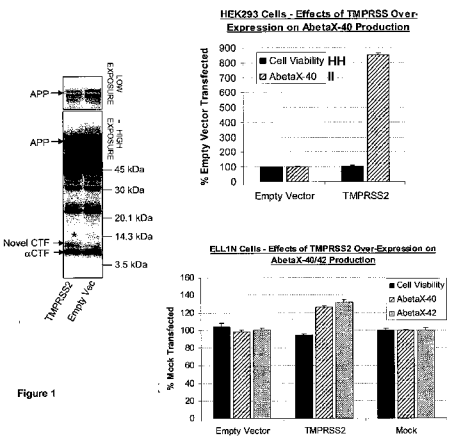

Figure 1

(57) Abstract: The invention relates to a method for identifying compounds that act as enhancers or inhibitors of transmembrane protease, serine 2 (TMPRS S2) protease activity. The invention also relates to a method for diagnosing whether or not a subject is at risk of developing, or has, a disease associated with pathogenic APP processing. The invention further relates to the use of TMPRSS2 antagonists for preventing or treating diseases associated with pathogenic APP processing, such as Alzheimer's disease.

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Field of the Invention

The invention relates to a method for identifying compounds that act as enhancers or inhibitors of transmembrane protease, serine 2 (TMPRSS2) protease activity. The invention also relates to a method for diagnosing whether or not a subject is at risk of developing, or has, a disease associated with pathogenic APP processing. The invention further relates to the use of TMPRSS2 antagonists for preventing or treating diseases associated with pathogenic APP processing, such as Alzheimer's disease.

Background to the Invention

Alzheimer's disease is a common form of dementia found mainly among older people. The disease is reviewed in Selkoe DJ, Physiological Reviews (2001) 81: 741-766, 2001; Cummings JL, New England Journal of Medicine (2004) 351:56-67 and Kalaria RN., *et al.*, Lancet Neurol. (2008) 9:812-26. A common symptom of the disease is the formation of abnormal amyloid plaques and/or neurofibrillary tangles in brain tissue. Amyloid plaques are formed by aggregation of proteolytic cleavage products of amyloid precursor protein (APP) termed amyloid β (Aβ) peptides. These peptides are toxic to neurons and may also cause aggregation of the microtubule associated protein tau, thereby forming neurofibrillary tangles. The amyloid hypothesis postulates that aberrant production or clearance of Aβ peptide underlies the neurodegeneration and consequent dementia observed in Alzheimer's disease.

β-secretase (BACE), γ-secretase and α-secretase proteases are known to be involved in the proteolytic cleavage of APP. BACE and γ-secretases are specifically linked to production of Aβ peptides. Cleavage by γ-secretase generates Aβ peptides of variable lengths, mostly between 37 and 42 amino acids. The 42 amino acid form of Aβ (Aβ 1-42) is known to be highly toxic, but N-terminally truncated Aβ peptides are also implicated in the pathology of amyloid plaques, and display enhanced aggregation and toxicity as compared to non-truncated versions.

In addition to the well characterised amyloid plaque pathology observed in brain tissue in Alzheimer's disease, other chronic diseases are associated with aberrant processing of APP. For example, cerebral amyloid angiopathy results from deposits of amyloid protein in small blood vessels in the brain which can cause stroke, brain haemorrhage or dementia. There is a need to identify new medicines for the treatment of

diseases associated with pathogenic APP processing, in particular those which may function by regulating aberrant production of Aβ peptides.

Summary of the Invention

The invention utilises substrate polypeptides having a consensus sequence from APP protein to identify enhancers or inhibitors of transmembrane protease, serine 2 (TMPRSS2) protease activity. The inventors have surprisingly shown that TMPRSS2 cleaves APP. This means that TMPRSS2 is involved in hitherto uncharacterised N-terminal processing events, which give rise to N-terminally truncated variants of Aβ. Identification of the involvement of TMPRSS2 in processing of APP provides a novel method of drug development. In particular, it allows for screening of drugs that can inhibit production of N-terminally truncated variants of Aβ, and thus prevent the aggregation of Aβ that underlies amyloid plaque formation.

The finding that TMPRSS2 mediates the formation of N-terminal truncation products of Aβ also allows TMPRSS2 to be used as a biomarker in the identification of diseases associated with pathogenic APP processing. In particular, the invention uses TMPRSS2 expression level and/or protease activity to determine whether or not a subject is at risk of, or has, a diseases associated with pathogenic APP processing.

Given that TMPRSS2 cleaves APP, the invention also concerns the use of TMPRSS2 antagonists in the prevention or treatment of diseases associated with pathogenic APP processing, in particular Alzheimer's disease.

Over-expression of TMPRSS2 may also be used to generate non-human animal models of diseases of pathogenic APP processing. Such animal models can not only be used to investigate the pathology of such diseases, but may also be used to screen for compounds which can prevent or treat them.

Accordingly, the invention provides a method for identifying a compound that enhances or inhibits transmembrane protease, serine 2 (TMPRSS2) protease activity, the method comprising: a) contacting a TMPRSS2 polypeptide with the compound; b) contacting the TMPRSS2 polypeptide with a substrate polypeptide; c) measuring TMPRSS2 protease activity; and d) comparing the TMPRSS2 protease activity measured in c) with a control value obtained for a TMPRSS2 polypeptide that has not been contacted with the compound, and thereby determining whether the compound is an enhancer or inhibitor of TMPRSS2 protease activity; wherein the substrate polypeptide comprises the

sequence DAEFRHDSG (SEQ ID NO: 17) or an equivalent thereof; wherein an increase in TMPRSS2 protease activity compared with said control value identifies the compound as being an enhancer of TMPRSS2 protease activity; and wherein a decrease in TMPRSS2 protease activity compared with said control value identifies the compound as being an inhibitor of TMPRSS2 protease activity.

The invention further provides a method for identifying a compound that enhances or inhibits amyloid precursor protein (APP) processing, the method comprising carrying out a method for identifying a compound that enhances or inhibits transmembrane protease, serine 2 (TMPRSS2) protease activity as defined above and thereby identifying a compound that enhances or inhibits APP processing, wherein an increase in TMPRSS2 protease activity in the presence of said compound compared with said control value identifies said compound as an enhancer of APP processing; and wherein a decrease in TMPRSS2 protease activity in the presence of said compound compared with said control value identifies said compound as an inhibitor of APP processing.

The invention additionally provides a method for identifying a compound that enhances or inhibits Aβ aggregation or formation of amyloid plaques and/or neurofibrillary tangles, the method comprising carrying out a method for identifying a compound that enhances or inhibits transmembrane protease, serine 2 (TMPRSS2) protease activity as defined above and thereby identifying a compound that enhances or inhibits Aβ aggregation or formation of amyloid plaques and/or neurofibrillary tangles, wherein an increase in TMPRSS2 protease activity in the presence of said compound compared with said control value identifies said compound as an enhancer of Aβ aggregation or formation of amyloid plaques and/or neurofibrillary tangles; and wherein a decrease in TMPRSS2 protease activity in the presence of said compound compared with said control value identifies said compound as an inhibitor of Aβ aggregation or formation of amyloid plaques and/or neurofibrillary tangles.

The invention also provides a method for identifying a compound suitable for the prevention or treatment of a disease associated with pathogenic APP processing, the method comprising carrying out a method for identifying a compound that enhances or inhibits transmembrane protease, serine 2 (TMPRSS2) protease activity as defined above and thereby identifying a compound suitable for the prevention or treatment of a disease associated with pathogenic APP processing, wherein a decrease in TMPRSS2 protease activity in the presence of said compound compared with said control value identifies said

compound as being suitable for the prevention or treatment of a disease associated with pathogenic APP processing.

In a related aspect to the above methods, the invention provides a kit comprising a TMPRSS2 polypeptide and a substrate polypeptide comprising the sequence DAEFRHDSG (SEQ ID NO: 17) or an equivalent thereof.

The invention further provides an enhancer or inhibitor of TMPRSS2 protease activity identified by the method for identifying a compound that enhances or inhibits transmembrane protease, serine 2 (TMPRSS2) protease activity as defined above.

The invention additionally provides a method for identifying whether or not a subject is at risk of developing, or has, a disease associated with pathogenic APP processing, said method comprising: a) measuring the expression level and/or protease activity of TMPRSS2 in a sample derived from said subject; b) comparing the TMPRSS2 expression level and/or protease activity measured in said sample to a normal level of TMPRSS2 expression and/or protease activity and thereby identifying whether or not a subject is at risk of developing, or has, a disease associated with pathogenic APP processing; wherein an increased level of TMPRSS2 expression and/or an increased level of TMPRSS2 protease activity in the sample compared with the normal level identifies the subject as being at risk of developing, or having, a disease associated with pathogenic APP processing.

The invention also provides a TMPRSS2 antagonist for use in a method of preventing or treating a disease associated with pathogenic APP processing. In a related aspect, the invention further provides a use of a TMPRSS2 antagonist in the manufacture of a medicament for preventing or treating a disease associated with pathogenic APP processing. The invention additionally provides a method of treating or preventing a disease associated with pathogenic APP processing in a subject, comprising administering to the subject an effective amount of a TMPRSS2 antagonist.

The invention further provides a non-human animal in which a disease of pathogenic APP processing has been established by over-expression of TMPRSS2. In a related aspect, the invention provides a method for establishing a disease of pathogenic APP processing in a non-human animal comprising over-expressing TMPRSS2 in said animal in an amount sufficient to cause a disease of pathogenic APP processing.

Additionally, the invention provides a method for identifying a compound which prevents or treats a disease of pathogenic APP processing, comprising administering said

compound to a non-human animal as defined above and assessing whether or not said compound prevents or treats the disease of pathogenic APP processing. Furthermore, the invention provides a compound identified by this method for use in a method of preventing or treating a disease associated with pathogenic APP processing. In a related aspect, the invention provides a use of a compound identified by this method in the manufacture of a medicament for prevention or treatment of a disease associated with pathogenic APP processing.

Brief Description of the Figures

Figure 1 shows APP cleavage and increase in AβX-40/42 production induced by TMPRSS2 over-expression. A) Generation of a novel C-terminal fragment of APP by TMPRSS2 over-expression in HEK293 cells. B) Increase in AβX-40 generation by TMPRSS2 over-expression in HEK293 cells. C) Increase in AβX-40 and AβX-42 production by TMPRSS2 over-expression in ELL1N cells.

Figure 2 shows immunoprecipitation/mass spectroscopy analysis of Aβ species produced by TMPRSS2 over-expression.

Figure 3 shows that TMPRSS3 and TMPRSS5 do not cleave human APP. A) APP C-terminal fragments generated after TMPRSS2 and TMPRSS3 over-expression in HEK293 cells. Only TMPRSS2 over-expression produces novel fragments. B) TMPRSS3 variant over-expression does not increase AβX-40 production in HEK293 cells. C) TMPRSS5 over-expression does not increase AβX-40 production in HEK293 cells.

Figure 4 shows cleavage of human APP by murine TMPRSS2. A) Murine TMPRSS2 cuts human APP more efficiently than human TMPRSS2, reducing the steady state levels of total APP in HEK293 cells. Similarly to human TMPRSS2, over-expression of murine TMRPSS2 in HEK293 cells generated a novel APP C-terminal fragment (arrow-heads). However, cleavage at or near the α-secretase site was also upregulated (arrow). B) Despite more efficient cleavage of human APP by murine TMPRSS2, the increase in AβX-40 was lower than for human TMPRSS2 and Aβ1-40 was not affected. This is perhaps a consequence of enhanced cleavage at or near the α-secretase site.

Figure 5 shows enhanced clearance/degradation of Aβ1-40 by TMPRSS2. A) Both murine and human TMPRSS2 expression enhances clearance of endogenous Aβ1-40 in HEK293 cell cultures in which new Aβ production is inhibited by a γ-secretase inhibitor.

This suggests that isolated Aβ1-40 is cleaved by TMPRSS2. B) Aβ1-40 decreases relative to Aβx-40 over 72hrs suggesting Aβ1-40 is converted to AβX-40 by TMPRSS2. C) Parallel control experiment demonstrating successful over-expression of TMPRSS2 and increases in AβX-40 in cells not treated with γ-secretase inhibitor.

Description of the Sequences

SEQ ID NO: 1 is the nucleic acid sequence of human TMPRSS2, transcript variant 2.

SEQ ID NO: 2 is the amino acid sequence of human TMPRSS2, transcript variant 2 encoded by SEQ ID NO 1.

SEQ ID NO: 3 is the nucleic acid sequence of murine TMPRSS2.

SEQ ID NO: 4 is the amino acid sequence of murine TMPRSS2 encoded by SEQ ID NO 3.

SEQ ID NO: 5 is the nucleic acid sequence of human APP, transcript variant 1.

SEQ ID NO: 6 is the amino acid sequence of human APP, transcript variant 1 encoded by SEQ ID NO 5.

SEQ ID NO: 7 is the nucleic acid sequence of human APP, transcript variant 2.

SEQ ID NO: 8 is the amino acid sequence of human APP, transcript variant 2 encoded by SEQ ID NO 7.

SEQ ID NO: 9 is the nucleic acid sequence of human APP, transcript variant 3.

SEQ ID NO: 10 is the amino acid sequence of human APP, transcript variant 3 encoded by SEQ ID NO 9.

SEQ ID NO: 11 is the nucleic acid sequence of rat TMPRSS2.

SEQ ID NO: 12 is the amino acid sequence of rat TMPRSS2 encoded by SEQ ID NO 11.

SEQ ID NO: 13 is the nucleic acid sequence of chicken TMPRSS2.

SEQ ID NO: 14 is the amino acid sequence of chicken TMPRSS2 encoded by SEQ ID NO 13.

SEQ ID NO: 15 is the nucleic acid sequence of chimpanzee TMPRSS2.

SEQ ID NO: 16 is the amino acid sequence of chimpanzee TMPRSS2 encoded by SEQ ID NO 15.

SEQ ID NO: 17 (DAEFRHDSG) is the minimum sequence of the substrate polypeptide.

SEQ ID NOS: 18, 19, 20, 21 and 22 are preferred minimum sequences of the substrate polypeptide.

SEQ ID NO:18 is the amino acid sequence DAEFRHDSGY.

SEQ ID NO:19 is the amino acid sequence DAEFRHDSGYE.

SEQ ID NO:20 is the amino acid sequence DAEFRHDSGYEV.

SEQ ID NO:21 is the amino acid sequence DAEFRHDSGYEVH.

SEQ ID NO:22 is the amino acid sequence DAEFRHDSGYEVHH.

Detailed Description of the Invention

It is to be understood that different applications of the disclosed methods may be tailored to the specific needs in the art. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting. In addition as used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a compound" includes "compounds", reference to "a polypeptide" includes two or more such polypeptides, and the like. All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

Method for identifying compounds that enhance or inhibit TMPRSS2 protease activity

The invention provides a method for identifying a compound that acts as an enhancer or inhibitor of TMPRSS2 protease activity. Such methods allow the screening of one or more compounds for their ability to act as enhancers or inhibitors of TMPRSS2 protease activity. The methods are preferably carried out *in vitro* or *ex vivo*. The method can also be used to confirm that a known enhancer or inhibitor of TMPRSS2 protease activity enhances or inhibits cleavage of a substrate polypeptide as defined herein by TMPRSS2. Thus, the method can be used to confirm the effects of an enhancer or inhibitor of TMPRSS2 protease activity identified by any other means.

Techniques for determining the effect of compound(s) on an enzyme-substrate reaction are well known in the art. Any of those techniques may be used in accordance with the invention.

An enhancer increases the protease activity and/or expression of TMPRSS2. An inhibitor decreases the protease activity and/or expression of TMPRSS2. The enhancer or

inhibitor preferably increases or decreases the protease activity of TMPRSS2 on the substrate polypeptide defined herein. An enhancer or inhibitor of TMPRSS2 protease activity may enhance or inhibit TMPRSS2 by any mechanism. For instance, an enhancer or inhibitor may act directly by binding to TMPRSS2 polypeptide. It may bind directly at the enzyme active site or may bind at another site and exert allosteric effects on enzyme function. The enhancer or inhibitor may act in a non-competitive or a competitive manner with respect to the substrate polypeptide.

An enhancer or inhibitor may also act indirectly on TMPRSS2 protease activity. It may have effects on activation of TMPRSS2 protease activity, for example by acting via secondary messenger systems, or on cleavage of TMPRSS2 polypeptide to release the protease domain. An enhancer or inhibitor of TMPRSS2 protease activity may also act at the level of TMPRSS2 expression so as to increase or decrease TMPRSS2 mRNA or protein levels. It may also act to regulate the stability of the expressed mRNA or protein.

An enhancer or inhibitor of TMPRSS2 protease activity may also act by altering substrate specificity. For example, inhibitors of TMPRSS2 protease activity may shift the substrate specificity from APP-type substrates (as discussed below) towards other substrate polypeptides.

The method can be carried out using any TMPRSS2 polypeptide in any form. Suitable TMPRSS2 polypeptides are discussed in more detail below. The TMPRSS2 polypeptide can be in solution. The solution may comprise a purified or substantially purified recombinant TMPRSS2 polypeptide in a suitable buffer. Such buffers are known in the art. Alternatively, the solution may be a culture medium or a cell lysate from a cell culture expressing a TMPRSS2 polypeptide. The TMPRSS2 may be anchored to a lipid-containing membrane. The membrane may be natural or artificial. Suitable membranes are known in the art. The TMPRSS2 polypeptide is preferably expressed in a cell or cell culture. Suitable cell types are discussed in more detail below. The cell or cell culture may additionally express one or more non-TMPRSS2 proteases that cleave APP and/or the substrate polypeptide. Alternatively, the TMPRSS2 polypeptide can be immobilised on a platform or surface. Suitable platforms or surfaces are known in the art. An example is a standard 96 or 384 well plate.

The substrate polypeptide can also be used in any form. The substrate polypeptide can be in solution. The solution may comprise a purified or substantially purified recombinant substrate polypeptide in a suitable buffer. The solution may also comprise a

synthetic substrate polypeptide in a suitable buffer. The solution may be a culture medium or a cell lysate from a cell culture expressing a substrate polypeptide. The substrate polypeptide may be anchored to a lipid-containing membrane. The membrane may be the same one to which the TMPRSS2 polypeptide is anchored. The substrate polypeptide is preferably expressed in a cell or cell culture. The cell or cell culture may be the same cell or cell culture expressing TMPRSS2 polypeptide or may be a different one. The cell or cell culture may additionally express the TMPRSS2 polypeptide and/or one or more additional proteases that cleave APP. Alternatively, the substrate polypeptide can be provided immobilised to a platform or surface. The substrate polypeptide may be immobilised such that cleavage products derived from it are retained or released from the surface.

The substrate polypeptide can also be provided in the form of conditioned medium isolated from cell lines expressing the substrate polypeptide. In particular, conditioned medium isolated from HEK293 or ELL1N cells (patent application WO2008084254) may be a good source of substrate polypeptide. The term "conditioned" is intended to refer to a difference in the chemical composition of the medium used in culture of the cells, as a result of substances secreted by the cells. In such embodiments, the cells providing the conditioned media may express one or more proteases which cleave APP, such as α-secretases, β-secretase (BACE), and γ-secretases. The conditioned medium may also be used to contact purified recombinant TMPRSS2 polypeptide.

Where the TMPRSS2 polypeptide and/or substrate polypeptide are derived from a cell or cell culture, a processing step will typically be required prior to measurement of TMPRSS2 protease activity. For example, cell medium may be processed by centrifugation or by passage through a membrane that filters out unwanted molecules or cells. A solution derived from a cell may be stored prior to measurement of TMPRSS2 protease activity, preferably below -70°C. Similarly, a cell culture expressing TMPRSS2 polypeptide or substrate polypeptide may be stored, prior to harvesting cell lysate, preferably below -70°C.

Where TMPRSS2 polypeptide or substrate polypeptide are expressed in a cell or cell culture, expression of one or both polypeptides can be transient or stable. Expression of one or both polypeptides may result from an endogenous gene or from an exogenous polynucleotide. Expression may be inducible or constitutive. Methods of providing a cell

or cell culture expressing a TMPRSS2 polypeptide or a substrate polypeptide are described below.

As will be appreciated, a cell-based method of the invention can be carried out in many ways. Both the TMPRSS2 polypeptide and substrate polypeptide may be expressed in the same cell or cell culture and the compound may be contacted therewith. Alternatively, the TMPRSS2 polypeptide alone may be expressed in the cell or cell culture and the compound and substrate may then be contacted therewith. Similarly, the substrate polypeptide alone may be expressed in a cell or cell culture and the compound and TMPRSS2 polypeptide may then be contacted therewith. Furthermore, the TMPRSS2 polypeptide, the substrate polypeptide and the compound may be expressed in a cell or cell culture.

Typically, only one TMPRSS2 polypeptide is used. However, in some embodiments, two or more, such as 3, 4 or 5 or more, different TMPRSS2 polypeptides are used. Typically, only one substrate polypeptide is used. However, two or more, such as 3, 5, 10, 15, 30 or more, different substrate polypeptides can be used. For example, two or more distinct amyloid precursor protein (APP) or amyloid β (Aβ) species may be used. Substrate polypeptides are discussed in more detail below.

If the contacting takes place in solution or on a surface or platform, the method is carried out under conditions that allow the enzyme to function. Suitable conditions include, but are not limited to room temperature (such as 25°C) - 37°C, a buffer comprising Tris-HCL, Hepes, or phosphate at pH in the range of pH 7-8 and containing moderate amounts of $CaCl_2$ (10 mM). If the TMPRSS2 polypeptide and/or the substrate polypeptide are expressed in a cell or cell culture, the method is carried out under conditions that maintain viability of the cell or the cell culture. Suitable conditions include, but are not limited to, a humidified atmosphere of 5% CO_2 at 37°C in appropriate culture media. Suitable conditions for culture of HEK293 and ELL1N cells are described in the materials and methods section.

The TMPRSS2 polypeptide, compound and substrate polypeptide can be contacted in any order. The TMPRSS2 polypeptide may be contacted first with compound and then with the substrate polypeptide. This type of pre-incubation may be necessary to allow sufficient time for a compound to have an effect on TMPRSS2 protease activity. The TMPRSS2 polypeptide may be contacted first with the substrate polypeptide and then with the compound. This order is useful for determining how quickly the compound can exert

its effect on protease activity. The TMPRSS2 polypeptide may be contacted with the substrate polypeptide and the compound at the same time. Contacting is carried out for a sufficient period to allow for TMPRSS2 protease activity on the substrate polypeptide to be measured by the methods described below. The TMPRSS2 polypeptide is preferably contacted with the substrate polypeptide in the presence of the compound.

The TMPRSS2 polypeptide and the substrate polypeptide are contacted in a manner that allows a physical interaction between the two polypeptides. This is necessary for the TMPRSS2 polypeptide to cleave the substrate polypeptide. However, it should be understood that, in some embodiments of the method, the TMPRSS2 polypeptide will not physically interact with the substrate polypeptide. For instance, the compound may abolish cleavage of the substrate polypeptide by irreversibly binding to the active site of the TMPRSS2 polypeptide or by effects on TMPRSS2 expression or stability. Under such circumstances, there may be no interaction between the TMPRSS2 polypeptide and the substrate.

The TMPRSS2 polypeptide and the compound are contacted in any manner that allows the compound to have an effect on TMPRSS2 protease activity. This may not necessarily involve a physical interaction between the compound and the TMPRSS2 polypeptide. For instance, the compound may affect expression of the TMPRSS2 polypeptide via RNA interference. A person skilled in the art will be able to determine appropriate techniques for contacting the TMPRSS2 polypeptide with the substrate polypeptide and the compound.

The method of the invention can further comprise contacting the substrate polypeptide with one or more, such as 2, 3 or 4, non-TMPRSS2 proteases which are able to cleave the substrate polypeptide. Such proteases include, but are not limited to, α-secretases, β-secretase (BACE), and γ-secretases. Inclusion of a γ-secretase is particularly preferred. Additional proteases may result in the formation of more than two, such as 3, 4, 5 or more, cleavage products. Where the substrate polypeptide is APP or a fragment thereof, this will allow for measurement of the effects of the compound on the pattern of Aβ species which can be generated by the simultaneous, concurrent or sequential action of the one or more proteases. The additional protease(s) may be used in any of the forms discussed above for the TMPRSS2 polypeptide and substrate polypeptide. In a preferred embodiment, the additional protease(s) are expressed in the same cell or cell culture as the TMPRSS2 polypeptide and/or substrate polypeptide.

The method of the invention can be carried out in a single reaction (i.e. one which contains a compound, a TMPRSS2 polypeptide and a substrate polypeptide). For instance, the method of the invention can be used to identify whether or not a single or an individual compound is an enhancer or inhibitor of TMPRSS2 protease activity. Alternatively, the method of the invention can be used to identify whether or not two or more compounds in combination are capable of enhancing or inhibiting TMPRSS2 protease activity.

As will be appreciated, the method of the invention is preferably carried out in multiple simultaneous or concurrent reactions, such as 5, 10, 15, 20, 30, 40, 50, 100, 150, 200 or more simultaneous or concurrent reactions. Each reaction contains at least one compound, at least one TMPRSS2 polypeptide and at least one substrate polypeptide. This allows a variety of aspects of TMPRSS2 protease activity to be investigated.

Preferably, the method of the invention involves simultaneously or concurrently identifying multiple compounds that enhance or inhibit TMPRSS2 protease activity. In other words, the method of the invention may involve high-throughput screening of more than one compound. High-throughput screening is typically carried out using 5, 10, 15, 20, 30, 40, 50, 100, 150, 200 or more different compounds. Typically, each compound is screened in a different reaction. However, two or more compounds may be assayed in the same reaction.

The method of the invention can be used to identify the concentration at which a compound optimally enhances or inhibits TMPRSS2 protease activity. In such an embodiment, multiple reactions are simultaneously or concurrently carried out using different concentrations of the compound in each reaction. The method of the invention can be used to identify whether a compound affects TMPRSS2 protease activity in a substrate specific manner. In such an embodiment, multiple reactions are simultaneously or concurrently carried out using different substrate polypeptides in each reaction. The method of the invention can be used to determine the extent to which the compound's effects may be saturated out by substrate concentration. In such an embodiment, multiple reactions are simultaneously or concurrently carried out using different concentrations of substrate polypeptides in each reaction.

Multiple reactions can be carried out in the wells of a flat plate. The wells typically have a capacity of from about 25µl to about 250µl, from about 30µl to about 200µl, from about 40µl to about 150µl or from about 50 to 100µl. 96 or 384 reactions may be simultaneously or concurrently carried out in the wells of a standard 96 or 384 well plate.

Such plates are commercially available for example from Greiner Labortechnik Ltd and Corning BV. Binding proteins or antibodies may be immobilised on a surface of one or more, preferably all, of the wells where required. These can be used to immobilise the TMPRSS2 polypeptide and/or the substrate polypeptide to the surface of the wells.

Where multiple reactions are performed, each reaction will typically be carried out under a set of similar conditions to allow for comparison of results obtained. Suitable conditions are set out above. As appropriate, each reaction is also typically carried out using the same molar concentration of the reaction constituents, namely the compound, the substrate polypeptide and/or the TMPRSS2 polypeptide, to allow for comparison of results obtained. As described above, the concentration of one or more of the constituents may vary between reactions depending on the purpose of the assay. Suitable enzyme and substrate concentrations may be approximately 0.5μM active enzyme (minimum of 1.25μg) and approximately 150μM substrate (Biochem. J. (2005) 388, 967–972). The amount of the TMPRSS2 polypeptide in each reaction may also be measured in enzyme units of TMPRSS2 protease activity. A suitable system for calculating enzyme units of TMPRSS2 protease activity is also described in the above publication. TMPRSS2 is a trypsin-like protease and so typical spectroscopic assays for trypsin-like serine proteases may be used to determine enzyme units of TMPRSS2 activity, using any number of commercially available substrates, such as, but not limited by: Bz-Arg-OEt (BAEE), Tos-Arg-OMe (TAME), Z-Gly-Pro-Arg-NHMec or Suc-Ala-Ala-Pro-Arg-NHPhNO_2. The concentration of the compound contacted with the TMPRSS2 polypeptide will vary depending on the nature of the compound. A person skilled in the art can determine an appropriate concentration. Typically, from about 0.01 to 100 nM concentrations of compound may be used, for example from 0.1 to 10 nM.

Where cells or cell cultures are used, each reaction typically involves the same number of cells. For instance, cells are typically seeded with approximately the same number of cells in each well of a plate, and each reaction is performed after the same time period. Typically 3-5x10^4 cells are seeded per well of a 96-well plate.

For each of the embodiments discussed above, the precise conditions used in the assay may vary. Experimental conditions may be optimised as a matter of routine by the person skilled in the art on the basis of their general knowledge to improve sensitivity and reliability of the method of the invention.

In order to allow for a determination of whether or not the compound is an enhancer or inhibitor of TMPRSS2 protease activity, a comparison is made with a control value. The protease activity value obtained following contacting of TMPRSS2 polypeptide with the compound and the substrate polypeptide is compared with the control value. The control value is the TMPRSS2 protease activity observed under conditions where the TMPRSS2 polypeptide has been contacted with the substrate polypeptide, but has not been contacted with the compound. Preferably, the conditions are otherwise identical to those used to obtain the protease activity value following contacting with the compound. Following the comparison with the control value, the effect of the compound may be identified in terms of an increase in TMPRSS2 protease activity or a decrease in TMPRSS2 protease activity with respect to the control value. An increase is indicative of an enhancer. A decrease is indicative of an inhibitor.

Preferably, the control value is obtained while carrying out the method of the invention. For example, a control reaction is performed at the same time as reaction(s) where the TMPRSS2 polypeptide is contacted with the substrate polypeptide and the compound. This ensures that the control value is obtained under the same conditions as the TMPRSS2 protease activity measured following contacting of TMPRSS2 polypeptide with the substrate polypeptide and the compound. The control value can also be obtained separately from the method of the invention. For instance, the control value may be obtained beforehand and recorded, for instance on a computer. The control value may be used for multiple repetitions of the method. The control value can be derived from more than one control reaction. For instance, the control value may be the arithmetic mean of the measurement obtained from several, such as 2, 5, 10, 15 or more, control reactions. In order to allow for an effective comparison, the control value has the same units as the measurement in the test sample with which it is being compared. A person skilled in the art is capable of obtaining such a value.

The type of control value referred to above is commonly known in the art as a "negative control". The method of the invention can also be carried out in conjunction with one or more positive controls for TMPRSS2 protease activity. This involves carrying out reactions using one or more compounds which are known enhancers or inhibitors of TMPRSS2 protease activity. A positive control allows for validation or measurement of the protease activity of TMPRSS2 polypeptide that is used in the method of the invention. For instance, this may be useful to allow comparison of results that have been obtained

using different sources of TMPRSS2 polypeptide. A positive control also allows the extent to which the compound enhances or inhibits TMPRSS2 protease activity to be determined. Suitable known enhancers of TMPRSS2 include, but are not limited to, fluorogenic peptide t-benzyloxycarbonyl(CBZ)-Gly-Gly-Arg-AMC. Suitable known inhibitors of TMPRSS2 include, but are not limited to, Cbz-Lys$(OPh)_2$, and serine protease inhibitors such as antitrypsin and antithrombin. Examples of TMPRSS2 inhibitors which effect a decrease in TMPRSS2 expression include TMPRSS2 antisense polynucleotides, transcriptional inhibitors that bind to the TMPRSS2 5' promoter/regulatory region and hammerhead ribozymes.

The incubation period of the reaction constituents prior to measurement of protease activity will be selected on the basis of the time required to generate a signal of appropriate strength. Measurement of TMPRSS2 protease activity can be performed at one or more timepoints following contacting of a TMPRSS2 polypeptide with the test compound. This may allow for a determination of the duration and stability of the effect of the compound. Similarly, TMPRSS2 protease activity can be measured at one more timepoints subsequent to addition of substrate polypeptide to allow for determination of the effects of the compound on the kinetics of protease activity. As discussed above, the substrate polypeptide can be contacted with the TMPRSS2 polypeptide prior to contacting with the compound. This may allow for a determination of how quickly the compound exerts its effect on pre-existing TMPRSS2 protease activity.

Techniques for measuring protease activity are well known in the art. Any technique may be used. The method preferably involves detecting of one or more specific cleavage products derived from the substrate polypeptide. Preferred methods of measuring TMPRSS2 protease activity involve fluorescence, an immunoassay or mass spectrometry. Measuring substrate cleavage using fluorescence is well known in the art. For example, a substrate polypeptide may be labelled with a fluorescent moiety and cleavage can be monitored by a change in the fluorescence spectrum or a decay in the fluorescent signal. A preferred fluorescence-based method that may be used to measure TMPRSS2 protease activity is fluorescence resonance energy transfer (FRET). This uses two fluorophores, a donor and an acceptor. Excitation of the donor by an energy source (e.g. flash lamp or fluorometer laser) triggers an energy transfer to the acceptor if they are within a given proximity to each other. The acceptor in turn emits light at its given wavelength. The use of long-lived fluorophores combined with time-resolved detection (a delay between

excitation and emission detection) is preferred to minimize interference. A particularly preferred fluorescence-based method is homologous time resolved fluorescence (HTRF). This uses lanthanides which have large Stoke's shifts and extremely long emission half-lives (from μsec to msec) when compared to more traditional fluorophores (Mathis G J Biomol Screen. 1999; 4(6):309-314).

Measuring substrate cleavage using an immunoassay is also well known in the art. The immunoassay can involve specific detection of one or more cleavage products derived from the substrate polypeptide. Conversely, an immunoassay can be used to detect clearance or degradation of the substrate polypeptide by measuring the amount of uncleaved substrate polypeptide remaining after the action of TMPRSS2 on the substrate polypeptide. Any suitable immunoassay which allows for detection of cleavage products or uncleaved substrate polypeptide by an antibody may be used. Any suitable commercially available antibody for a given target may be used.

A preferred immunoassay is Enzyme-Linked ImmunoSorbent Assay (ELISA). For example, production of specific cleavage products of APP such as Aβ1-40, Aβ6-40 can be measured using antibodies specific to those peptides. Suitable ELISA assays for detection of Aβ species are commercially available, for example from WAKO. In some embodiments, the ELISA assay may be performed in flat plates where wells are coated with binding proteins or antibodies which can bind and allow for detection of the cleavage product or uncleaved substrate polypeptide. Other types of immunoassay include immunoprecipitation and Western blotting.

Whilst immunoassays are preferred, any other high-affinity ligand-receptor interaction, such as streptavidin-biotin, could be used to measure TMPRSS2 protease activity.

Measuring substrate cleavage using mass spectrometry is also well known in the art. Cleavage products derived by the action of TMPRSS2 protease on the substrate polypeptide can be separated on the basis of their mass and charge to allow for a determination of the relative proportions of each specific cleavage product in the reaction mixture. In such embodiments, the reaction mixture may be concentrated prior to analysis by use of a suitable antibody which precipitates all cleavage products.

Preferred cell based assays include reporter assays for cleavage of a protein substrate. For example, APP can be N-terminally tagged with secreted alkaline phosphatase (SEAP) or a similar enzymatically active protein tag such as luciferase or

beta-galactosidase. Shedding of APP ectodomain can be measured by accumulation of enzyme activity in conditioned media. Alternatively, the C-terminus of APP can be tagged with a Gal4 reporter element. On cleavage of APP by TMPRSS2 the APP C-terminal fragment/Gal4 chimera migrates to the nucleus where it activates an artificial reporter gene by binding to a UAS promoter element upstream of a reporter gene expressing luciferase/SEAP/β-galactosidase.

TMPRSS2 polypeptide

The TMPRSS2 gene encodes a protein that belongs to the serine protease family. The human gene is shown in SEQ ID NO: 1 and encodes the protein shown in SEQ ID NO: 2. SEQ ID NO:1 is known as transcript variant 2, as a longer transcript variant has also been reported. The mouse gene is shown in SEQ ID NO: 3 and encodes a protein of SEQ ID NO: 4. The rat gene is shown in SEQ ID NO 11 and encodes a protein of SEQ ID NO: 12. The chicken gene is shown in SEQ ID NO 13 and encodes a protein of SEQ ID NO: 14. The chimpanzee gene is shown in SEQ ID NO 15 and encodes a protein of SEQ ID NO: 16.

The human protein of SEQ ID NO: 2 contains a type II transmembrane domain, a Low Density Lipoprotein (LDL) receptor class A domain, a scavenger receptor cysteine-rich domain and a protease domain. The domain positions in the sequence of the human protein of SEQ ID NO: 2 are approximately as follows: Low Density Lipoprotein (LDL) receptor class A domain: aa112-150; Scavenger receptor cysteine-rich domain: aa157-192; Trypsin protease domain: aa256-489; Transmembrane domain: aa86-104. The domain positions in the sequence of the mouse protein of SEQ ID NO:4 are approximately as follows: Low Density Lipoprotein (LDL) receptor class A domain: aa110-147; Scavenger receptor cysteine-rich domain: aa156-191; Trypsin protease domain: aa254-487; Transmembrane domain: aa86-104.

The full-length TMPRSS2 protein is expressed as a 70 kDa polypeptide, which contains a 32 kDa serine protease domain. Activation of the serine protease requires its cleavage, which is autocatalytic. The active serine protease with trypsin-like specificity is then shed into the extracellular space, where it is predicted to interact with other proteins on the cell surface, as well as soluble proteins, matrix components and proteins on adjacent cells.

TMPRSS2 is expressed predominantly in the prostate with lower levels of expression found in the pancreas, liver, lung, kidney and colon. TMPRSS2 is also expressed in vascular endothelial cells (Aimes RT *et al.*, Thromb Haemost. 2003 89(3):561-72. In malignancies, TMPRSS2 is over-expressed in neoplastic prostate epithelium and to a lesser extent in colon cancer tissue (Vaarala, M *et al.*, (2001) Int. J. Cancer 94, 705–710).

The method of the invention uses a TMPRSS2 polypeptide. A TMPRSS2 polypeptide is any polypeptide which cleaves a substrate polypeptide within the sequence DAEFRHDSG. A TMPRSS2 polypeptide is preferably a polypeptide which cleaves a substrate polypeptide between amino acids 5 and 6 of the sequence DAEFRHDSG.

The ability of a polypeptide to cleave a substrate polypeptide within the sequence DAEFRHDSG may be routinely determined by a person skilled in the art. The ability of a polypeptide to cleave a substrate polypeptide within the sequence DAEFRHDSG may be determined by any of the methods disclosed above for measuring TMPRSS2 protease activity. The ability of a polypeptide to cleave a substrate polypeptide within the sequence DAEFRHDSG is preferably determined as described in the Example.

The TMPRSS2 polypeptide preferably comprises the amino acid sequence of SEQ ID NO: 2 or 4 or a variant thereof. The TMPRSS2 polypeptide more preferably consists of the amino acid sequence of SEQ ID NO: 2 or 4. The TMPRSS2 polypeptide may also comprise or consist of the amino acid sequence of SEQ ID NO: 12, 14 or 16, or a variant thereof.

A variant of SEQ ID NO: 2 or 4 may comprise truncations, mutants or homologues thereof. A variant of SEQ ID NO: 2 or 4 also includes any transcript variants thereof. A variant of SEQ ID NO: 2 or 4 must cleave a substrate polypeptide within the sequence DAEFRHDSG or preferably between amino acids 5 and 6 of the sequence DAEFRHDSG as described above.

Any homologues mentioned herein are typically at least 40% homologous to the relevant region of SEQ ID NO: 2 or 4. Homology can be measured using known methods. For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux *et al* (1984) Nucleic Acids Research 12, 387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (typically on their default settings), for example as described in Altschul S. F. (1993) J Mol Evol 36:290-300; Altschul, S, F *et al* (1990) J

Mol Biol 215:403-10. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) Proc. Natl. Acad. Sci. USA 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.comprises (or consists of) sequence which has at least 40% identity to a given sequence.

In preferred embodiments, a variant sequence may be at least 55%, 65%, 70%, 75%, 80%, 85%, 90% and more preferably at least 95%, 97% or 99% homologous to a particular region of SEQ ID NO: 2 or 4 over at least 20, preferably at least 30, for instance at least 40, 60, 100, 200, 300, 400 or more contiguous amino acids, or even over the entire sequence of the variant. Alternatively, the variant sequence may be at least 55%, 65%, 70%, 75%, 80%, 85%, 90% and more preferably at least 95%, 97% or 99% homologous to

full-length SEQ ID NO: 2 or 4. Typically the variant sequence differs from the relevant region of SEQ ID NO: 2 or 4 by at least, or less than, 2, 5, 10, 20, 40, 50 or 60 mutations (each of which can be substitutions, insertions or deletions). A variant TMPRSS2 polypeptide of the invention may have a percentage identity with a particular region of SEQ ID NO: 2 or 4 which is the same as any of the specific percentage homology values (i.e. it may have at least 40%, 55%, 80% or 90% and more preferably at least 95%, 97% or 99% identity) across any of the lengths of sequence mentioned above.

Variants of SEQ ID NO: 2 or 4 also include truncations. Any truncation may be used so long as the variant is still able to cleave a substrate polypeptide within the sequence DAEFRHDSG. Truncations will typically be made to remove sequences that are non-essential for protease activity and/or do not affect conformation of the folded protein, in particular folding of the active site. Truncations may also be selected to improve solubility of the TMPRSS2 polypeptide. Appropriate truncations can routinely be identified by systematic truncation of sequences of varying length from the N- or C-terminus. Preferred truncations are N-terminal and may remove all other sequences except for the protease domain. Such truncations are particularly preferred where the assay is carried *in vitro*. Other preferred truncations remove all other sequences except for the protease domain, and the LDL receptor Class A domain. Such truncations are preferred where full-length APP is used as a substrate in a cell-based assay. In such assays, the truncated variant may additionally comprise the transmembrane domain. Domain positions for the protease, LDL receptor Class A and transmembrane domains in SEQ ID NO:2 and 4 are described above.

Variants of SEQ ID NO: 2 or 4 further include mutants which have one or more, for example, 2, 3, 4, 5 to 10, 10 to 20, 20 to 40 or more, amino acid insertions, substitutions or deletions with respect to a particular region of SEQ ID NO: 2 or 4. Deletions and insertions are made preferably outside of the protease domain as described below. Insertions are typically made at the N- or C-terminal ends of a sequence derived from SEQ ID NO: 2 or 4, for example for the purposes of recombinant expression as detailed below. Another common N-terminal insertion is a signal peptide to assist secretion in a cell system where the variant sequence derived from SEQ ID NO: 2 or 4 does not contain one. Substitutions are also typically made in regions that are non-essential for protease activity and/or do not affect conformation of the folded protein. Such substitutions may be made to improve solubility or other characteristics of the

enzyme. Although not generally preferred, substitutions may also be made in the active site or in the second sphere, i.e. residues which affect or contact the position or orientation of one or more of the amino acids in the active site. These substitutions may be made to improve turnover of substrate polypeptide or to map the binding site of a test compound. This is discussed in more detail below.

Substitutions preferably introduce one or more conservative changes, which replace amino acids with other amino acids of similar chemical structure, similar chemical properties or similar side-chain volume. The amino acids introduced may have similar polarity, hydrophilicity, hydrophobicity, basicity, acidity, neutrality or charge to the amino acids they replace. Alternatively, the conservative change may introduce another amino acid that is aromatic or aliphatic in the place of a pre-existing aromatic or aliphatic amino acid. Conservative amino acid changes are well known in the art and may be selected in accordance with the properties of the 20 main amino acids as defined in Table A. Where amino acids have similar polarity, this can also be determined by reference to the hydropathy scale for amino acid side chains in Table B.

Any of the variants described above for SEQ ID NOs: 2 and 4 are also described as variants for SEQ ID NOs: 12, 14 or 16.

Table A – Chemical properties of amino acids

Ala	aliphatic, hydrophobic, neutral	Met	hydrophobic, neutral
Cys	polar, hydrophobic, neutral	Asn	polar, hydrophilic, neutral
Asp	polar, hydrophilic, charged (-)	Pro	hydrophobic, neutral
Glu	polar, hydrophilic, charged (-)	Gln	polar, hydrophilic, neutral
Phe	aromatic, hydrophobic, neutral	Arg	polar, hydrophilic, charged (+)
Gly	aliphatic, neutral	Ser	polar, hydrophilic, neutral
His	aromatic, polar, hydrophilic, charged (+)	Thr	polar, hydrophilic, neutral
Ile	aliphatic, hydrophobic, neutral	Val	aliphatic, hydrophobic, neutral
Lys	polar, hydrophilic, charged(+)	Trp	aromatic, hydrophobic, neutral
Leu	aliphatic, hydrophobic, neutral	Tyr	aromatic, polar, hydrophobic

Table B. Hydropathy scale

Side Chain	Hydropathy
Ile	4.5
Val	4.2
Leu	3.8
Phe	2.8
Cys	2.5
Met	1.9
Ala	1.8
Gly	-0.4
Thr	-0.7
Ser	-0.8
Trp	-0.9
Tyr	-1.3
Pro	-1.6
His	-3.2
Glu	-3.5
Gln	-3.5
Asp	-3.5
Asn	-3.5
Lys	-3.9
Arg	-4.5

It is preferred that a variant of SEQ ID NO: 2 comprises residues 256 to 489 of SEQ ID NO: 2 or residues 254 to 487 of SEQ ID NO: 4. These regions correspond to the protease domains of SEQ ID NOs: 2 and 4 respectively. However, a variant can comprise amino acid insertions, substitutions or deletions in the protease domains as long as the variant is still able to cleave a substrate polypeptide within the sequence DAEFRHDSG (SEQ ID NO:17). For instance, a variant may contain conservative amino acid substitutions in residues 256 to 489 of SEQ ID NO: 2 or residues 254 to 487 of SEQ ID NO: 4 as long as the variant is still able to cleave a substrate polypeptide within the sequence DAEFRHDSG. Suitable conservative substitutions are discussed above. If the variant does comprise amino acid insertions, substitutions or deletions in the protease domains, it is preferred that the variant is able to cleave a substrate polypeptide within the sequence DAEFRHDSG with an efficiency that is comparable to, or the same as, SEQ ID NO: 2 or 4. In particular, it is preferred that a variant comprises a sequence that is at least

90%, at least 95%, at least 97% or at least 99% homologous to residues 256 to 489 of SEQ ID NO: 2 or residues residues 254 to 487of SEQ ID NO: 4.

Substrate polypeptide

The inventors have surprisingly identified a site in human APP where TMPRSS2-mediated proteolytic cleavage occurs. Human APP, transcript variant 1 is shown in SEQ ID NO: 6. TMPRSS2 cleaves APP between residues 5 and 6 (arginine and histidine) of the Aβ peptide within human APP, for example TMPRSS2 cleaves between residues 676 and 677 of the APP variant shown in SEQ ID NO: 6.. Typical cleavage products derived from human APP including the action of human TMPRSS2 are Aβ6-40 and Aβ6-42. The inventors have also surprisingly shown that isolated Aβ1-40 peptide acts as a substrate for TMPRSS2.

The substrate polypeptide comprises, consists or consists essentially of the sequence DAEFRHDSG or an equivalent thereof. An equivalent is any sequence that is cleaved by SEQ ID NO: 2 or 4. Cleavage by SEQ ID NO: 2 or 4 may be determined using any method disclosed herein. An equivalent thereby allows the effect of a compound on TMPRSS2 protease activity to be determined. An equivalent is preferably cleaved by SEQ ID NO: 2 or 4 with similar or comparable efficiency to a substrate polypeptide comprising DAEFRHDSG. A reduced cleavage efficiency for the equivalent is acceptable, so long as a detectable signal is generated.

The equivalent is preferably DAEFRHDSG comprising 1, 2 or 3 conservative substitutions such that the equivalent has 66.6% identity, preferably 77.7% or 88.8% identity to DAEFRHDSG. The 1, 2 or 3 conservative substitutions are preferably made at the residues surrounding or adjacent to the cleavage site. However, it is particularly preferred that R is unchanged i.e that conservative substitutions are made for any of E, F and H. These types of equivalents may be used to improve catalytic activity of TMPRSS2 by enhancing binding contacts at the active site. Conservative substitutions may be made at other positions than those described above so long as the equivalent has 66.6% identity, preferably 77.7% or 88.8% identity to DAEFRHDSG. Conservative substitutions may be selected according to the options presented above, including those in Tables A and B.

It is straightforward to identify which residues in DAEFRHDSG can be conservatively substituted without loss of cleavage by SEQ ID NO: 2 or 4. For instance, the residues in DAEFRHDSG which are involved in binding with TMPRSS2 could be

identified. Structural studies or chemical cross-linking experiments can be carried out to identify enzyme-substrate contacts. Substitution of contact residues would typically result in a loss of cleavage by SEQ ID NO: 2 or 4. Alternatively, each residue of DAEFRHDSG may be substituted in a systematic manner and cleavage by SEQ ID NO: 2 or 4 may be determined for each mutant.

Other preferred substrate polypeptides comprise, consist or consist essentially of sequences comprising SEQ ID NO: 17 and additional C-terminal sequence from human APP of SEQ ID NO: 6. Particularly preferred substrate polypeptides comprise, consist or consist essentially of the sequences DAEFRHDSGY (SEQ ID NO:18), DAEFRHDSGYE (SEQ ID NO:19), DAEFRHDSGYEV (SEQ ID NO:20), DAEFRHDSGYEVH (SEQ ID NO:21), and DAEFRHDSGYEVHH (SEQ ID NO:22).

In addition to comprising DAEFRHDSG or an equivalent thereof, the substrate polypeptide may further comprise any other amino acid sequence, so long as cleavage by SEQ ID NO: 2 or 4 is still observed. Preferably, the DAEFRHDSG sequence (corresponding to residues 672 to 680 of SEQ ID NO: 6) may be extended N-terminally and/or C-terminally by one or more residues along the human APP sequence, preferably along the sequence shown in SEQ ID NO: 6. Even more preferred substrate polypeptides are those which comprise cleavage sites for α-secretases, β-secretases (BACE) and/or γ-secretases. Cleavage sites for these proteases are well known in the art.

Most preferred substrate polypeptides are those which comprise, consist, or consist essentially of any of SEQ ID NOs: 6, 8 or 10. Use of such substrates is particularly preferred where the method comprises contacting the substrate polypeptide with a TMPRSS2 polypeptide and one or more additional APP proteases.

Preparation of reagents

The TMPRSS2 polypeptide can be produced by recombinant expression in a suitable host system. It is preferred that the recombinant polypeptide be produced by prokaryotic expression. For example, a bacterial expression vector may be prepared containing a polynucleotide sequence encoding a TMPRSS2 polypeptide as defined above. The polynucleotide sequence may further comprise a protein tag at the C- or N-terminus which is suitable for purification (such as a His tag, HA tag, V5 tag, VSVG tag, GST or similar). The TMPRSS2 polypeptide may be fused at the N- or C- terminus to another protein to increase stability. Multiple suitable bacterial expression vectors may be used.

The pET vector series is an example of such a vector, where recombinant protein expression is induced by the addition of IPTG (isopropyl β-Dthiogalactoside).

The construct may typically be transformed into a suitable bacterial host such as BL21 (DE3) bacterial cells (or equivalent) and recombinant protein expression induced as described. Soluble, tagged TMPRSS2 will be column-purified as per the purification protocol appropriate to the protein tag. For example, with a His tag, the protein would be purified on a nickel resin column.

Alternatively, purified TMPRSS2 polypeptide may be produced recombinantly in a eukaryotic system, for example in insect cells or mammalian cells. For example, a human stable cell line expressing the TMPRSS2 polypeptide may be used. LNCaP cells are a preferred host cell line. Alternatively, a cell line transfected with a TMPRSS2 expression construct and transiently expressing a TMPRSS2 polypeptide may be used. The TMPRSS2 polypeptide may be purified by immunoprecipitation from the conditioned media of transfected or stable TMPRSS2-expressing cells using an anti-TMPRSS2 antibody. Such antibodies are commercially available.

Purity of the TMPRSS2 polypeptide may be assessed by SDS/PAGE and protein staining by colloidal blue or a similar method. The amount of purified protein and total enzyme activity may be determined by routine procedures known in the art. This will then allow for similar or identical molar amounts or enzyme units of recombinantly expressed TMPRSS2 polypeptide to be provided in each reaction.

The same protein production methodology outlined above may be applied to preparation of the substrate polypeptide. A bacterial expression vector may be prepared containing a polynucleotide sequence encoding a substrate polypeptide. The substrate polypeptide will then typically be purified, for example as described above for the TMPRSS2 polypeptide. The amount of purified substrate polypeptide may then be determined by routine procedures known in the art. This will then allow for similar or identical molar amounts of recombinantly expressed substrate polypeptide to be provided in each reaction.

The method of the invention may use cells or cell cultures expressing TMPRSS2 polypeptide and/or substrate polypeptide. In such embodiments, the cells will generally harbour a polynucleotide sequence encoding a TMPRSS2 polypeptide and/or a substrate polypeptide. Additional polynucleotide sequences encoding other APP proteases may also be provided. The discussion below applies to provision of any of these polynucleotide

sequences. The polynucleotide sequences may be provided transiently in the cell, for example in the form of a cDNA housed in a vector that has been transfected into the cell by methods known in the art. Examples of such transfection methods include the use of cationic lipids or liposomes and calcium phosphate. Alternatively, the polynucleotide sequence may be stably expressed in the cell. Techniques for generating stable cell lines are also well known in the art. For example, cells may be transiently transfected with a linearised vector comprising the polynucleotide sequence. The linearised vector can integrate into the genome of the cell, providing for stable expression. Alternatively, the cells may be infected with a virus comprising the polynucleotide sequence, where the virus provides for integration of the sequence into the genome.

Efficient transfection and generation of stable cell lines will typically require selection of the cell population for uptake of vector or virus comprising the polynucleotide sequence. In these situations, the vector or virus will further comprise a selectable marker that expresses a protein conferring resistance to a compound which is toxic to mammalian cells. Cells that have taken up the vector or virus will be resistant to the compound, whilst other cells will be eliminated from cell culture by the toxic effects of the compound. Suitable selectable markers are known in the art.

The polynucleotide sequence will be operably linked to a promoter allowing for expression in mammalian cells. A variety of suitable promoters are known in the art, and may be selected according to the specific cell system used to express the TMPRSS2 polypeptide, and according to the level of expression that is required in the cell. Examples of suitable promoters include CMV, SV40, and RSV. The promoter may be inducible in response to presence of an inducer compound in the cell culture, allowing for timed regulation of expression of the TMPRSS2 polypeptide.

In further embodiments, the TMPRSS2 polypeptide may be expressed from an endogenous TMPRSS2-encoding gene in a suitable cell line, such as (LNCaP+ cells) prepared from LNCaP-FGC cells (ECACC #89110211) (Vaarala MH., *et al.*, Lab Invest 2000, 80:1259–1268). Suitable cell lines expressing substrate polypeptide from an endogenous gene include, but are not limited to, HEK293 and ELL1N (patent application WO2008084254) neuroblastoma cell lines. Examples of suitable cells expressing other APP proteases include, but are not limited to HeLa, CHO. Suitable cell lines for use in exogenous expression of substrate and/or TMPRSS2 polypeptides include HeLa, HEK293 and CHO.

Compound(s)

Any compound(s) can be used in the method of the invention. The compound(s) are preferably ones that are suspected of enhancing or inhibiting TMPRSS2 protease activity.

The compound(s) can be in any suitable form. It is typically in solution. The solution typically comprises a suitable buffer. The solution may be cell medium or cell lysate from a cell culture expressing the compound(s). A polynucleotide encoding the compound may be provided in the cell in the same way as described above for TMPRSS2 polypeptide and substrate polypeptide. The compound may be expressed in a cell together with the TMPRSS2 polypeptide and/or the substrate polypeptide. The compound may be expressed in an inducible manner.

The compound(s) may be any chemical compound(s) used in drug screening programmes. They may be natural or synthetic. Extracts of plants which contain several characterised or uncharacterised components may also be used. Typically, organic molecules will be screened, preferably small organic molecules which have a molecular weight of from 50 to 2500 Daltons. Compounds can be biomolecules including peptide and peptide mimetics, oligonucleotides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Candidate compounds may be obtained from a wide variety of sources including libraries of synthetic or natural substances. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs. The compound(s) may be the product(s) of a combinatorial library such as are now well known in the art (see e.g. Newton (1997) Expert Opinion Therapeutic Patents, 7(10): 1183-1194). Natural product libraries, such as display libraries (e.g. phage display libraries), may also be used.

Antibodies directed to the site of interaction between the TMPRSS2 polypeptide and the substrate polypeptide are another class of suitable compounds. For example, monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, CDR-grafted antibodies and humanized antibodies may be used. The antibody may be an intact immunoglobulin molecule or a fragment thereof such as a Fab, $F(ab')_2$ or Fv fragment. Candidate inhibitor antibodies may be characterised and their binding regions determined to provide single chain antibodies and fragments thereof which are responsible for disrupting the interaction between the TMPRSS2 polypeptide and the substrate

polypeptide. A suitable antibody may bind to either the TMPRSS2 polypeptide or the substrate polypeptide, and thereby prevent or block the interaction between these molecules. Antibodies may be raised against specific epitopes of the TMPRSS2 polypeptide or the substrate polypeptide. For example, antibodies may be raised specifically against those regions which are involved in the interaction between the TMPRSS2 polypeptide and the substrate polypeptide.

Further classes of compounds include oligonucleotides which act at the level of transcription of the TMPRSS2 polypeptide. In one aspect, decreased functional expression of the TMPRSS2 polypeptide may be achieved by inhibiting the expression from the TMPRSS2 gene. For example, down-regulation of expression of TMPRSS2 may be achieved using anti-sense technology or RNA interference.

When using anti-sense genes or partial gene sequences to downregulate gene expression, a nucleotide sequence is placed under the control of a promoter in a "reverse orientation" such that transcription yields RNA which is complementary to normal mRNA transcribed from the "sense" strand of the target gene (see, for example, Smith *et al*, (1988) Nature 334, 724-726). Such methods would use a nucleotide sequence which is complementary to the coding sequence. Further options for down regulation of gene expression include the use of transcriptional inhibitors that bind to the TMPRSS2 5' promoter/regulatory region and ribozymes, e.g. hammerhead ribozymes, which can catalyse the site-specific cleavage of RNA, such as mRNA (see e.g. Jaeger (1997) Curr Opin Struct Biol 7:324-335, or Gibson & Shillitoe (1997) Mol Biotechnol 7: 242-251).

RNA interference is based on the use of small double stranded RNA (dsRNA) duplexes known as small interfering or silencing RNAs (siRNAs). Such molecules are capable of inhibiting the expression of a target gene that they share sequence identity or homology to. Typically, the dsRNA may be introduced into cells by techniques such as microinjection or transfection. Methods of RNA interference are described in, for example, Hannon (2002) Nature 418: 244-251 and Elbashir *et al* (2001) Nature 411: 494-498; Aigner A., J Biotechnol. 2006 Jun 124(1):12-25.

Methods for identifying potential therapeutic agents

The inventors have surprisingly shown that TMPRSS2 cleaves APP. In particular, TMPRSS2 protease activity is specifically involved in generation of N-terminally truncated forms of Aβ. It is known that N-terminally truncated Aβ peptides have enhanced

toxicity and aggregate more quickly compared to non-truncated forms (Pike CJ., *et al.*, J Biol Chem. (1995) 270(41): 23895-23898). Also, analysis of amyloid plaques from the brains of humans affected with Alzheimer's disease has shown that these plaques are a complex mixture of N-terminally truncated Aβ peptides (Kalback W *et al.*, Biochemistry. (2002) 41(3):922-928).

Interestingly, N-terminally truncated Aβ species are not found in amyloid plaques extracted from the brains of Alzheimer's disease transgenic mice (Pike CJ *et al.*, J Biol Chem. (2001) 276(16):12991-12998). It is possible that the mouse lifespan is too short for the amyloid plaques to be degraded in the same way as that seen in humans. In any case, the absence of N-terminally truncated Aβ species in animal models of Alzheimer's disease can also be explained by the inventors' finding that murine APP is not cleaved by TMPRSS2.

Given that TMPRSS2 is capable of cleaving APP to form N-terminally truncated Aβ species, the method of the invention described above can be used for identifying compounds that enhance or inhibit APP processing, that enhance or inhibit Aβ aggregation or formation of amyloid plaques and/or neurofibrillary tangles or that are suitable for the prevention or treatment of a disease associated with pathogenic APP processing.

In one embodiment, the invention provides a method for identifying a compound that enhances or inhibits Amyloid Precursor Protein (APP) processing. "Processing" is intended to refer to the proteolytic cleavage of APP into peptide fragments. In this embodiment, any method described above for identifying an enhancer of inhibitor of TMPRSS2 protease activity is carried out. An increase in TMPRSS2 protease activity in the presence of the compound compared with said control value identifies said compound as an enhancer of APP processing. A decrease in TMPRSS2 protease activity in the presence of the compound compared with said control value identifies said compound as an inhibitor of APP processing.

Compounds that enhance or inhibit APP processing will typically enhance or inhibit the formation of N-terminally truncated Aβ species. N-terminally truncated Aβ species include any peptides derived from APP which lack one or more residues normally present at the N-terminus of APP. Typically, such species lack the first 5 N-terminal residues of APP. Preferred N-terminally truncated Aβ species include A*β*6-40 and A*β*6-42.

In another embodiment, the invention provides a method for identifying a compound that enhances or inhibits Aβ aggregation or the formation of amyloid plaques and/or neurofibrillary tangles. In this embodiment, any method described above for identifying an enhancer of inhibitor of TMPRSS2 protease activity is carried out. An increase in TMPRSS2 protease activity in the presence of the compound compared with said control value identifies said compound as an enhancer of Aβ aggregation or the formation of amyloid plaques and/or neurofibrillary tangles. A decrease in TMPRSS2 protease activity in the presence of the compound compared with said control value identifies said compound as an inhibitor of Aβ aggregation or the formation of amyloid plaques and/or neurofibrillary tangles. The ability of the compound to enhance or inhibit Aβ aggregation or the formation of amyloid plaques and/or neurofibrillary tangles may be subsequently tested using known assays for Aβ aggregation and the formation of amyloid plaques and/or neurofibrillary tangles. Any assays of this type known in the art may be used.

Examples of *in vitro* Aβ aggregation assays include LeVine H., Arch Biochem Biophys. 1997 342(2):306-16, Okuno H *et al.*, Chem Biol Drug Des. 2006 Nov;68(5):273-5 , and Curr Alzheimer Res. 2007 Dec;4(5):544-6. *In vivo* animal models are reviewed in Götz J., *et al.*, (2008) Nature Reviews Neuroscience 9, 532-544. Specific examples of *in vivo* models include Taconic model 001349 (which overexpresses APP containing the Swedish familial Alzheimer's disease mutation, where the APP BACE cleavage site is mutated from KMDA to NLDA), and Taconic model 001638 (which overexpresses the P301L tau mutant). These two types of mutations may be used alone or in combination. Mutations of presenilin genes 1 and 2 may also be included.

In a further embodiment, the invention provides a method for identifying a compound suitable for the prevention or treatment of a disease associated with pathogenic APP processing. In this embodiment, any method described above for identifying an enhancer of inhibitor of TMPRSS2 protease activity is carried out. A decrease in TMPRSS2 protease activity in the presence of the compound compared with the control value identifies the compound as being suitable for treating a disease associated with pathogenic APP processing.

Compounds which inhibit TMPRSS2 protease activity are likely to be inhibitors of TMPRSS2-associated APP cleavage *in vivo*. They therefore have the ability to inhibit pathogenic APP processing and Aβ aggregation *in vivo* and thus prevent or treat a disease

associated with pathogenic APP processing. The prevention or treatment of diseases associated with pathogenic APP processing is discussed in more detail below. Animal models in which a disease associated with pathogenic APP processing has been established can also be used to identify such compounds. Suitable animal models are described below.

Diseases of pathogenic APP processing involve altered APP processing. APP processing can be increased or decreased. Such diseases typically result from the toxic effects of peptide species derived from APP, commonly known as Aβ species. These peptides have a propensity to aggregate and form pathogenic deposits in tissue and/or blood vessels. Aβ peptides may be toxic to cells *per se*, and/or have additional toxicity or pathogenicity resulting from formation of deposits or aggregates. Preferred diseases associated with pathogenic APP processing include, but are not limited to, Alzheimer's disease cerebral amyloid angiopathy, and Parkinson's disease. The disease is preferably Alzheimer's disease.

Diagnostic method

As discussed above, the inventors have surprisingly demonstrated the involvement of TMPRSS2 in the cleavage of APP to form toxic N-terminally truncated Aβ peptides. Thus, altered TMPRSS2 function may be used as a biomarker for diseases associated with pathogenic APP processing, particularly those involving the formation of toxic N-terminally truncated Aβ peptides.

The invention therefore provides a method for identifying whether or not a subject is at risk of developing, or has, a disease associated with pathogenic APP processing. The method involves measuring the expression level and/or protease activity of TMPRSS2 in a subject. The method may involve measuring the expression level of TMPRSS2, the protease activity of TMPRSS2 or both. TMPRSS2 may be that shown in SEQ ID NO: 2 or 4 or a variant thereof as described above.

The expression level and/or protease activity of TMPRSS2 is then compared with a normal value for TMPRSS2 expression or protease activity. An increased level of TMPRSS2 expression and/or an increased level of TMPRSS2 protease activity in the sample compared with the normal level identifies the subject as being at risk of developing, or having, a disease associated with pathogenic APP processing. The disease associated with pathogenic APP processing can be any of those discussed above. It is preferably Alzheimer's disease.

In one embodiment, the invention relates to identifying whether or not the subject is at risk of developing the disease. The invention therefore relates to the diagnosis of susceptibility of a subject to the disease. This may allow for an early prophylactic or palliative treatment to prevent development of the disease. The invention may be used to confirm susceptibility in subjects already suspected as being at risk or selected as being predisposed to developing the disease. Risk factors that increase susceptibility to developing diseases associated with pathogenic APP processing include, but are not limited to, aging, lifestyle risk factors, genetic risk factors and environmental risk factors. The major genetic risk factors for early onset Alzheimer's disease are APP and presenilin mutations, and APP gene dosage (such as in Downs' syndrome). The main genetic risk factor for late onset Alzheimer's disease is the presence of the ApoE4 allele. Genetic risk factors are reviewed in Nat Genet. 2007 Jan;39(1):17-23. Lifestyle risk factors are reviewed in Am J Epidemiol. 2002 Sep 1;156(5):445-53. Biomarkers that may be used to identify susceptibility include: phospho tau in CSF (cerebrospinal fluid) (Hansson O., *et al.*, Lancet Neurol. 2006 Mar;5(3):228-34), Aβ40:Aβ42 CSF ratio (Shoji M, Kanai M. J Alzheimers Dis. (2001) (3):313-321), brain shrinkage as measured by MRI reference scanning (Sluimer JD., *et al.*, Neurology. 2008 May 6;70(19 Pt 2):1836-41), and PIB compound *in vivo* amyloid imaging (Forsberg A., Neurobiol Aging. 2008 Oct; 29(10):1456-65).

In another embodiment, the invention relates to identifying whether or not the subject has the disease. The invention therefore relates to the diagnosis of the disease. Typically, the subject has the disease or displays symptoms of the disease. The method may therefore be carried out on subjects who display preliminary symptoms of the disease.

The method of the invention is carried out *in vitro* or *ex vivo* on a sample derived from the subject. The sample is preferably a fluid sample. The sample typically comprises a body fluid. The sample may be urine, lymph, saliva, cerebrospinal fluid, peritoneal fluid, pericardial fluid, vitreous or other ocular sample, pleural fluid, vaginal fluid, mucus, pus or amniotic fluid but is preferably blood, plasma or serum. The sample can be a cell or tissue sample, such as lung, brain, liver, skin or nails. The sample is preferably a brain tissue or cell sample. The sample is typically processed prior to its use in measurement of TMPRSS2 expression level or protease activity.

Typically, the subject is human. However, it may be non-human. For instance, the subject can be a commercially farmed animal, such as a horse, cow, sheep or pig, or may

be a pet such as a cat or a dog. Preferred non-human animals include, but are not limited to, primates, such as a marmoset or monkey. The subject can be a human or non-human animal undergoing treatment for a disease associated with pathogenic APP processing.

The protease activity of TMPRSS2 in the sample may be measured by any method known in the art or described herein. Measurement of TMPRSS2 expression level is typically performed at the mRNA or protein level, but TMPRSS2 gene copy number may also be measured. Standard mRNA detection methodology is based on a quantitative or semi-quantitative measurement of the presence of a specific RNA molecule in the sample by a PCR technique, using one or more primers comprising a sequence derived from the molecule of interest i.e. TMPRSS2. Standard protein detection methodology may comprise use of an antibody specific to TMPRSS2 in an immunological assay where binding of the antibody to TMPRSS2 polypeptide generates a quantitative or semi-quantitative signal, for example ELISA.

A person skilled in the art will be able to determine a normal level of expression and/or protease activity for TMPRSS2. It will typically be the average level of TMPRSS2 expression and/or protease activity observed in a representative sample of a healthy population. Specifically, the population does not have a disease associated with pathogenic APP processing or any other disease or condition that is likely to result in altered TMPRSS2 expression or protease activity. This will allow for a statistically significant diagnosis to be performed on the basis of comparison with the normal level i.e. one which takes into account natural variation in TMPRSS2 expression level or activity that is observed in the sample population.

The expression level and/or protease activity of TMPRSS2 in a sample from a subject can also be used to monitor the progression of a disease associated with APP processing in a subject or the suitability of a treatment. The expression level and/or protease activity may be measured at suitable time intervals after diagnosis as described above. An increase in TMPRSS2 expression level and/or protease activity with time is indicative of a worsening of the disease. A decrease in TMPRSS2 expression level and/or protease activity with time is indicative of successful treatment of the disease.

Method of treatment and medical use

The invention also provides a method of preventing or treating a disease associated with pathogenic APP processing by administering an effective amount of a TMPRSS2

antagonist. The invention also provides a TMPRSS2 antagonist for use in a method of preventing or treating of a disease associated with pathogenic APP processing. The invention further provides use of a TMPRSS2 antagonist in the manufacture of a medicament for preventing or treating a disease associated with pathogenic APP processing.

In all these embodiments, the TMPRSS2 antagonist may be administered in order to prevent the onset of one or more symptoms of the disease. In this embodiment, the subject can be asymptomatic. The subject may have a predisposition to the disease as described above. A prophylactically effective amount of the antagonist is administered to such a subject. A prophylactically effective amount is an amount which prevents the onset of one or more symptoms of the disease.

Alternatively, the TMPRSS2 antagonist may be administered once the symptoms of the disease have appeared in a subject i.e. to cure existing symptoms of the disease. A therapeutically effective amount of the antagonist is administered to such a subject. A therapeutically effective amount is an amount which is effective to ameliorate one or more symptoms of the disease. Typically, such an amount reduces the production of N-terminally truncated Aβ peptides in the subject. This can be confirmed as described above.

The subject may be any of those discussed above in relation to the diagnostic method. The subject is preferably identified as being at risk of, or having, the disease using the method of the invention described above.

The disease associated with pathogenic APP processing can be any of those discussed above. It is preferably Alzheimer's disease. As outlined above, Alzheimer's disease is a common form of dementia found mainly among older people. A common symptom of the disease is the formation of abnormal amyloid plaques and neurofibrillary tangles in brain tissue. This underlies the neurodegenerative phenotype. TMPRSS2 antagonists may be used to prevent or delay the neurodegeneration observed in Alzheimer's disease or to ameliorate symptoms of dementia. TMPRSS2 antagonists may also be used to prevent or slow growth of existing amyloid plaques and neurofibrillary tangles, or prevent or slow growth of new instances of these lesions, thereby stabilising an existing condition. In these embodiments, TMPRSS2 antagonists may primarily exert their effects through enhancing clearance of Aβ peptides.

As outlined above, cerebral amyloid angiopathy results from deposits of amyloid protein in small blood vessels in the brain which can cause stroke, brain haemorrhage or

dementia. According to the invention, TMPRSS2 antagonists may be used to prevent or slow formation of such deposits, thereby treating or preventing cerebral amyloid angiopathy prior to an instance of a stroke or brain haemorrhage. It is known that TMPRSS2 is expressed in vascular endothelial cells (Aimes RT *et al.*, Thromb Haemost. 2003 89(3):561-72), and thus TMPRSS2 antagonists could directly inhibit formation of amyloid deposits in blood vessels.

TMPRSS2 "antagonists" include all compounds which are inhibitors of TMPRSS2 expression and/or protease activity. TMPRSS2 may be that shown in SEQ ID NO: 2 or 4 or a variant thereof as described above. The effect of a compound on the expression and/or protease activity of TMPRSS2 may be measured as described above. The antagonist is preferably an inhibitor identified in accordance with the invention.

TMPRSS2 antagonists also specifically include any compound previously known in the art to act as an inhibitor of TMPRSS2 protease activity or TMPRSS2 expression. Preferred TMPRSS2 antagonists for use in accordance with the invention include, but are not limited to small organic molecules which have a molecular weight of from 50 to 2500 Daltons, antibodies directed to the site of interaction between the TMPRSS2 polypeptide and the substrate polypeptide, and oligonucleotides which act to reduce transcription of the TMPRSS2 polypeptide e.g. siRNAs.

TMPRSS2 antagonists may be provided isolated and/or purified from their natural environment, in substantially pure or homogeneous form, or free or substantially free of other materials from their source or origin. Where used herein, the term "isolated" encompasses all of these possibilities. They may optionally be labelled or conjugated to other compounds.

TMPRSS2 antagonists can be formulated into pharmaceutical compositions. These compositions may comprise, in addition to one of the above substances, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water,

petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

For delayed release, the TMPRSS2 antagonist may be included in a pharmaceutical composition which is formulated for slow release, such as in microcapsules formed from biocompatible polymers or in liposomal carrier systems according to methods known in the art. For continuous release of peptides, the peptide may be covalently conjugated to a water soluble polymer, such as a polylactide or biodegradable hydrogel derived from an amphipathic block copolymer, as described in U.S. Pat. No. 5,320,840. Collagen-based matrix implants, such as described in U.S. Pat. No. 5,024,841, are also useful for sustained delivery of peptide therapeutics. Also useful, particularly for subdermal slow-release delivery to perineural regions, is a composition that includes a biodegradable polymer that is self-curing and that forms an implant *in situ*, after delivery in liquid form. Such a composition is described, for example in U.S. Pat. No. 5,278,202.

The dose of a TMPRSS2 antagonist may be determined according to various parameters, especially according to the substance used; the age, weight and condition of the patient to be treated; the route of administration; and the required regimen. Again, a physician will be able to determine the required route of administration and dosage for any particular patient. A typical daily dose is from about 0.1 to 50 mg per kg of body weight, according to the activity of the specific antagonist, the age, weight and conditions of the subject to be treated and the frequency and route of administration. Preferably, daily dosage levels are from 5 mg to 2 g. That dose may be provided as a single dose or may be provided as multiple doses, for example taken at regular intervals, for example 2, 3 or 4 doses administered daily.

Non-human animal and model of diseases of pathogenic APP processing

The inventors have surprisingly shown that TMPRSS2 plays a role in the formation of N-terminally truncated Aβ peptides, which are strongly implicated in the pathology of diseases of APP processing. TMPRSS2 over-expression may therefore be used to generate an animal that displays symptoms similar to those displayed by a human subject that has been diagnosed with a disease of pathogenic APP processing. Such an animal is suitable for use as a model for studying diseases of pathogenic APP processing. The animal will also be suitable for identifying compounds that prevent or treat diseases of pathogenic APP processing.

The invention provides a non-human animal in which a disease of pathogenic APP processing has been established by over-expression of TMPRSS2 and a method of generating such an animal. TMPRSS2 may be that shown in SEQ ID NO: 2 or 4 or a variant thereof as described above. TMPRSS2 is preferably that shown in SEQ ID NO: 2 (human TMPRSS2) or a variant thereof as described above. The disease of pathogenic APP processing may be any of those discussed above.

The non-human animal can comprise further mutations or modifications to establish the disease. The disease may also be established by the coordinate expression of other genes or mutant forms thereof which impact on diseases of APP processing. Preferred mutants include APP, presenilin and tau mutants either alone or in combination. Examples of suitable animal models in which TMPRSS2 overexpression may be established include Taconic model 001349 (which overexpresses APP containing the Swedish familial Alzhimer's disease mutation, where the APP BACE cleavage site is mutated from KMDA to NLDA), and Taconic model 001638 (which overexpresses the P301L tau mutant). These two types of mutations may be present alone or in combination. Mutations of presenilin genes 1 and 2 may also be included. Other suitable animal models are described in Gotz J, *et al* cited above.

In one embodiment, an APP-type substrate of TMPRSS2 which is not normally present in the animal is also expressed. A preferred example of such a substrate is human APP. Murine APP is not cleaved by TMPRSS2, which may explain why N-terminally cleaved toxic Aβ species are not prevalent in mouse models of Alzheimer's disease. Providing human APP in the context of TMPRSS2 over-expression may allow for generation of a mouse disease model that more faithfully replicates the pathology of human diseases of APP processing.

TMPRSS2 is expressed in a sufficient amount to cause or generate symptoms of pathogenic APP processing in the animals. The sufficient amount typically varies between animals and will depend on a number of factors, for example the age of the animal, and whether or not additional genes contributing to the disease of pathogenic APP processing are also present. The animal is non-human. The non-human animal is typically of a species commonly used in biomedical research, for example a mammal, and is preferably a laboratory strain. Suitable animals include non-human primates, dogs, cats, sheep and rodents. It is preferred that the animal is a rodent, particularly a mouse, rat, guinea pig, ferret, gerbil or hamster. Most preferably the animal is a mouse.

The animal over-expresses TMPRSS2. Techniques for generating transgenic non-human animals are well known in the art. Any such technique may be used. For instance, a polynucleotide construct comprising a promoter operably linked to a coding sequence for TMPRSS2, for example SEQ ID NO: 1 or SEQ ID NO: 3 or a variant thereof, is produced. The polynucleotide construct may be randomly integrated in the genome of the animal or may be targeted to a particular site. Targeting may be achieved by flanking the promoter and coding sequence with genomic sequences, which correspond to genomic sequences at the locus where insertion is required. Thus, if the polynucleotide construct is contacted with the locus of interest, homologous recombination events may lead to replacement of the chromosomal sequence with the promoter operably linked to the coding sequence for TMPRSS2. Targeting may take place to swap an endogenous TMPRSS2 gene with a polynucleotide construct that allows for over-expression of the exogenous TMPRSS2 gene. Alternatively, both an endogenous and an exogenous TMPRSS2 gene may be present in the animal.

The polynucleotide construct is typically transferred into a fertilized egg of a mammalian animal by pronuclear microinjection. Alternative approaches may also be used. For example, embryonic stem cells or retroviral mediated gene transfer into germ lines may be used. Whichever approach is taken, transgenic animals are then generated. For example, microinjected eggs may be implanted into a host female and the progeny may be screened for the expression of the marker gene. The success of targeting may be monitored by use of an appropriate selection marker. The founder animals that are obtained may be bred. Preferred animals are mice in which the endogenous TMPRSS2 gene has been replaced with a polynucleotide driving high level expression of SEQ ID NO: 2 or SEQ ID NO: 4or a variant thereof as defined above.

The invention provides a method of establishing a disease of pathogenic APP processing in a non-human animal comprising over-expressing TMPRSS2 in said animal in an amount sufficient to cause a disease of pathogenic APP processing. The method may involve the use of a transgenic technology as described above. The method may further comprise expression or over-expression in the non-human animal of one or more of genes that also contribute to onset and progression of the disease. Such genes are discussed above. The disease may be established in varying levels of severity by regulation of the expression levels of TMPRSS2 and the other genes mentioned above.

Known therapeutic compounds which are used to treat diseases of pathogenic APP processing, in particular Alzheimer's disease may be administered to the animal to investigate their impact on the disease model. In a related aspect, the invention provides a method for identifying a compound which prevents or treats a disease of pathogenic APP processing. It is preferred that the method identifies a compound which prevents or treats Alzheimer's disease. The method comprises administering a compound to a non-human animal of the invention and assessing whether or not the compound prevents or treats the disease of pathogenic APP processing. Compounds which prevent a disease of pathogenic APP processing reduce, prevent or delay the appearance of any symptoms of the disease. For example, where the disease is Alzheimer's disease, symptoms of dementia or appearance of amyloid plaques may be prevented or delayed. Substances which treat diseases of pathogenic APP processing may alleviate or abolish the symptoms of the disease in the animal.

The method of identifying compounds is typically carried out before or after the symptoms of the disease have developed in the animal. The method of identifying substances that prevent the disease is typically carried out before its symptoms have developed in the animal. The method of identifying substances that treat the disease is typically carried out after the symptoms have developed in the animal. Suitable compounds that can be tested in the above method include any of those described above.

Where a compound has been identified by the above method as being able to prevent or treat a disease of pathogenic APP processing in a non-human animal of the invention, it may then be used in medical applications. Thus, in one aspect, the invention provides a compound identified by the above method for use in a method of preventing or treating a disease associated with pathogenic APP processing. In a related aspect, the invention also provides for use of a compound identified by the above method in the

manufacture of a medicament for prevention or treatment of a disease associated with pathogenic APP processing. In both of these aspects, it is preferred that the disease is Alzheimer's disease. A more detailed discussion of treating diseases associated with pathogenic APP processing can be found above.

Kit

The invention also provides a kit that may be used to carry out the screening method of the invention. The kit comprises a TMPRSS2 polypeptide and a substrate polypeptide. Any of the TMPRSS2 polypeptides and substrate polypeptides discussed above may be used.

The kit may additionally comprise one or more other reagents or instruments which enable any of the embodiments of the method mentioned above to be carried out. Such reagents or instruments include one or more of the following: suitable buffer(s) (aqueous solutions), antibodies conjugated to detection moieties, substrates for enzymatically active tags, means to obtain a sample from a subject (such as a vessel or an instrument comprising a needle), means to measure TMPRSS2 protease activity and/or expression or cell culture apparatus. Reagents may be present in the kit in a dry state such that a fluid sample resuspends the reagents. The kit may also, optionally, comprise instructions to enable the kit to be used in the method of the invention.

The following Example illustrates the invention.

Example

1. Materials and methods

1.1 Sources of cDNAs

All human cDNAs were purchased from Origene in the pCMV6-XL4 mammalian expression vector:

Human TMPRSS2 (Accession #NM_005656.2, Origene #SC116619)

Human TMPRSS3 transcript variant A (Accession #NM_024022.1, Origene #SC111838)

Human TMPRSS3 transcript variant C (Accession #NM_032404.1, Origene #SC110611)

Human TMPRSS3 transcript variant D (Accession #NM_032405.1, Origene #SC316502)

Human TMPRSS5 (Accession # M_030770.1 Origene #RC223774)

Mouse cDNAs were purchased from Invitrogen in the pCMV-SPORT6 vector:

Mouse TMPRSS2 (Accession # BC054348, Invitrogen #2648889).

pCMV6-XL4 lacking any gene transcript was used as the empty-vector control.

1.2 HEK293 cell growth conditions

HEK293 cells were cultured in a humidified atmosphere of 5% CO_2 at 37°C in Dulbecco's Modified Eagle's Medium with 4500 mg/L glucose (Sigma #D6546) supplemented with 5% (v/v) bovine foetal calf serum (PAA laboratories #A15-003), 100 units/ml penicillin and 100 mg/ml streptomycin (Invitrogen #15140-114) and 2 mM L-glutamine (Invitrogen #25030-032).

1.3 ELL1N cell growth conditions

ELL1N cells were cultured in a humidified atmosphere of 5% CO_2 at 37°C in a 1:1 mix of Minimum Essential Medium (Sigma-Aldrich #M2279) and HAM'S F12 medium (Invitrogen #21765-029) supplemented with 15% (v/v) bovine foetal calf serum (PAA laboratories #A15-003), a 1:100 dilution of 100X Non-Essential Amino Acids (Sigma-Aldrich #M7145), 100 units/ml penicillin and 100 mg/ml streptomycin (Invitrogen #15140-114).

1.4 cDNA transfection protocol

HEK293 cells or ELL1N cells were transfected using Lipofectamine 2000 (Invitrogen #11668-027) using the standard manufacturer's protocol. 48 hours after transfection cell media were harvested for analysis of Aβ levels. Aβ1-40 levels were determined using an HTRF kit purchased from CisBio (#62B40PEB). Aβx-40 and Aβx-42 ELISAs were carried out using ELISA kits purchased from WAKO (#294-62501 and #290-62601 respectively). Cell viability was determined using Alamar Blue (Biosource #DAL1025).

Cell lysates were also harvested and both full-length APP and APP C-terminal fragment levels analysed by Western blotting using an anti-APP antibody (1:2000 Invitrogen #51-2700).

1.5 Immunoprecipitation Mass-Spec Protocol

HEK293 cells were transfected with cDNAs as described. Media samples were harvested after 48hrs and Aβ species then immunoprecipitated as follows. A slurry of G-Plus/Protein A agarose beads (Calbiochem #IP05) was prepared and 5ml of beads per sample were combined with 5ml of 1mg/ml 4G8 anti-human Aβ monoclonal antibody (Signet labs #9220-02). 10ml of conditioned media taken from the transfected cells were supplemented with protease inhibitors (Roche #11697498001) and then spiked with 10pmoles of Aβ12-28 (Bachem #H-7865.1000) as an internal control for success of the immunoprecipitation. The cell media were then mixed with the beads/antibody mixture and CHAPS detergent added to a final concentration of 1% v/v. Media samples were rotated overnight at 4°C.

The following day the beads were collected by centrifugation and washed twice with 500ml ice cold 10mM TrisHCl pH8, 140mM NaCl, 0.01% w/v (N-Octyl Glucosamine) NOG (Anatrace, Inc. #O331). The beads were then washed once with 500ml ice cold 10mM TrisHCl pH8 and once with distilled water. Aβ peptides were eluted with 50% acetonitrile, 2.5% TFA, 0.2% NOG and spotted onto a MALDI target. MALDI-TOF analysis was then carried out.

1.6 Aβ1-40 Clearance Assay

HEK293 cells were transfected with cDNAs as described. 24hrs later transfected cells were treated with conditioned media from ELL1N neuroblastoma cells containing Aβ species accumulated over 48hrs. The HEK293 cells were also treated with 10nM γ-secretase inhibitor (such as LY411,575) to prevent any further Aβ production. Aβ1-40 levels were tracked over 72hrs by HTRF assay (Cis-Bio # 62B40PEB). Cell viability was determined at 72hrs using Alamar Blue (Biosource #DAL1025).

2 Results

2.1 Effects of TMPRSS2 over-expression on APP cleavage

TMPRSS2 was over-expressed in HEK293 cells by transient transfection. Cell lysate was taken from control cells transfected with empty vector and cells over-expressing TMPRSS2. The cell lysate was processed by SDS-PAGE and Western blotting was performed with an anti-APP antibody (Invitrogen # 1-7300). The results are shown in Fig. 1A. The Western blot indicates that TMPRSS2 cleaves APP upstream of the α-secretase cleavage site, close to the BACE cleavage site. A novel C-terminal fragment of APP is generated.

An ELISA assay (WAKO # 294-62501) was then used to investigate further the effects of TMPRSS2 over-expression on APP cleavage in HEK293 cells. The results are shown in Fig. 1B. TMPRSS2 over-expression in parental HEK293 cells significantly increased secretion of N-terminally truncated AβX-40. No effects were seen on cell viability.

The effects of TMPRSS2 over-expression were also investigated in ELL1N neuroblastoma cells. These cells secrete relatively high levels of endogenous Aβ. The results shown in Fig. 1C indicate that TMPRSS2 upregulates production of AβX-42 and AβX-40 in ELL1N cells. The increase in AβX-40/42 production was comparatively lower in this cell line compared to HEK293 due to the low efficiency of cDNA transfection. Production of Aβ1-40, as measured by HTRF assay (Cis-Bio # 62B40PEB), was not affected. Thus, TMPRSS2 over-expression specifically results in formation of N-terminally truncated Aβ species.

2.2 Identification of a cleavage site for TMPRSS2 in human APP

The nature of the Aβ species produced by TMPRSS2 transfected HEK293 cells was further investigated by mass spectrometry. Lysates from empty vector control cells and cells over-expressing TMPRSS2 were immunoprecipitated using the anti-Aβ antibody 4G8 (Kim KS, *et al.* Neurosci Res. (1988) Comm 2:121-130). The immunoprecipitated species were then analysed by mass spectroscopy. The results are shown in Fig. 2. The mass spectrum demonstrates that TMPRSS2 cleaves human APP after amino acid 5 in the Aβ sequence increasing production of Aβ6-40 and related variants 6-37, 6-38 and 6-39.

2.3 Analysis of other TMPRSS family members

The TMPRSS2 knockout mouse does not display any phenotype indicating that functional compensation by other TMPRSS family members may occur (Kim TS., *et al.*, Mol Cell Biol. (2006) 26(3): 965–975). Three variants of the most closely related isoform, TMPRSS3, were hence tested in similar experiments to those carried out to analyse the effects of TMPRSS2 over-expression. The results are shown in Fig. 3A and 3B.

In contrast to the effects of TMPRSS2, no effect was observed on Aβ production on over-expression of any of the transcript variants of TMPRSS3. Immunoblotting with anti-APP antibody (Invitrogen # 1-7300) in Fig. 3A shows no novel C-terminal fragments of APP are formed on over-expression of TMPRSS3 family members. ELISA for AβX-40 (Fig. 3B) also showed no increase in AβX-40 levels on over-expression of TMPRSS3 family members.

Additionally the effects of over-expressing the neuronally expressed family member TMPRSS5 were also tested. The results are shown in Fig. 3C. The ELISA results show no increase in AβX-40 was observed on over-expression of TMPRSS5. These data indicate that APP cleavage is a specific function of TMPRSS2 which is not shared by other family members.

2.4 Investigation of APP cleavage by murine TMPRSS2

The TMPRSS2 cleavage site in human APP does not exist in rodent APP due to the substitution of an arginine residue for a glycine (human cleavage site sequence: DAEFR↓HDSG, rodent sequence: DAEFGHDSG). The cleavage of APP by TMPRSS2 may hence reflect a species-specific difference which could partially explain the increased quantities of N-terminally truncated Aβ in human disease.

Although the TMPRSS2 cleavage site in human APP does not exist in rodent APP, studies have been carried out on Alzheimer's disease transgenic mice expressing human APP with the Swedish familial Alzheimer's disease mutation in conjunction with a knock-out of BACE. These mice have virtually no residual Aβ production (Roberds, S., Human Mol. Genetics (2001) 10(12): 1317-1324, Cai H., Nature Neurosci.(2001) 4(3): 233-234, Luo Y., Nature Neurosci.(2001) 4(3): 231-232).

It was therefore tested whether murine TMPRSS2 can cleave human APP. The results obtained on over-expression of murine TMPRSS2 in HEK293 cells are shown in Fig. 4A and Fig. 4B. Fig. 4A shows immunoblotting of cell lysates with anti-APP

antibody (Invitrogen # 1-7300). Similarly to human TMPRSS2, over-expression of murine TMRPSS2 in HEK293 cells generated a novel APP C-terminal fragment (arrow-heads). Cleavage at or near the α -secretase site was also upregulated (arrow). The results show that murine TMPRSS2 does indeed cut human APP but does so both between amino acids 5 and 6 and also near the α -secretase cleavage site. Murine TMPRSS2 also cuts human APP more efficiently than human TMPRSS2, reducing the steady state levels of total APP in HEK293 cells.

Fig. 4B shows the results of an ELISA for AβX-40 and Aβ1-40 in HEK293 cells.

The increase in AβX-40 observed on over-expression of murine TMPRSS2 is lower than that seen with human TMPRSS2 over-expression, despite the enhanced cleavage of human APP by murine TMPRSS2. This is perhaps a consequence of enhanced cleavage of human APP by murine TMPRSS2 at or near the α -secretase site. These data suggest species-specific differences in the protease activity of murine and human TMPRSS2 which may partly explain the results in BACE knockout mice.

2.5 Effects of TMPRSS2 over-expression on clearance of Aβ peptide

It was tested whether TMPRSS2 is capable of cleaving isolated Aβ peptide. The results are shown in Fig. 5. Expression of both human and murine TMPRSS2 increased clearance/degradation of endogenous Aβ1-40 from conditioned cell media derived from HEK293 cells indicating that it is possible for this protease to cleave isolated Aβ. TMPRSS2 could hence play a potentially pathogenic role in Alzheimer's disease by cleaving Aβ1-40 /42 in blood vessels to produce the more toxic Aβ6-40/42 species. Inhibition of TMPRSS2 may hence alleviate cerebral amyloid angiopathy in Alzheimer's disease.

Sequences of the Invention

Table C

Homo sapiens TMPRSS2 transcript variant 2, nucleic acid sequence (SEQ ID NO: 1), Accession number NM_005656.3

```
gagtaggcgc gagctaagca ggaggcggag gcggaggcgg agggcgaggg gcggggagcg      60
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aaaaccatgg ataccaaccg gaaaacccct atcccgcaca gcccactgtg gtccccactg     240
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tgtcacactg ccccggcggg gaggacgaga atcggtgtgt tcgcctctac ggaccaaact     600
tcatccttca ggtgtactca tctcagagga agtcctggca ccctgtgtgc caagacgact     660
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Human TMPRSS2, transcript variant 2 amino acid sequence (SEQ ID NO:2),
Accession number NM_005647

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DSSGTCINPS NWCDGVSHCP GGEDENRCVR LYGPNFILQM YSSQRKSWHP VCQDDWNENY     180
GRAACRDMGY KNNFYSSQGI VDDSGSTSFM KLNTSAGNVD IYKKLYHSDA CSSKAVVSLR     240
CIACGVNLNS SRQSRIVGGE SALPGAWPWQ VSLHVQNVHV CGGSIITPEW IVTAAHCVEK     300
PLNNPWHWTA FAGILRQSFM FYGAGYQVEK VISHPNYDSK TKNNDIALMK LQKPLTFNDL     360
VKPVCLPNPG MMLQPEQLCW ISGWGATEEK GKTSEVLNAA KVLLIETQRC NSRYVYDNLI     420
TPAMICAGFL QGNVDSCQGD SGGPLVTSKN NIWWLIGDTS WGSGCAKAYR PGVYGNVMVF     480
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TDWIYRQMRA DG	492

Table D

Mus musculus TMPRSS2 nucleic acid sequence (SEQ ID NO:3), Accession number BC054348.1

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tccttgtctt tgctgttggt tggttatttc ctgctttgtg tctgttctga gctgtgagat    3120
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Mus musculus TMPRSS2 amino acid sequence (SEQ ID NO:4), Accession number
NP_056590

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STSVIHTHPK SSGALCTSKS KKSLCLALAL GTVLTGAAVA AVLLWRFWDS NCSTSEMECG     120
SSGTCISSSL WCDGVAHCPN GEDENRCVRL YGQSFILQVY SSQRKAWYPV CQDDWSESYG     180
RAACKDMGYK NNFYSSQGIP DQSGATSFMK LNVSSGNVDL YKKLYHSDSC SSRMVVSLRC     240
IECGVRSVKR QSRIVGGLNA SPGDWPWQVS LHVQGVHVCG GSIITPEWIV TAAHCVEEPL     300
SSPRYWTAFA GILRQSLMFY GSRHQVEKVI SHPNYDSKTK NNDIALMKLQ TPLAFNDLVK     360
PVCLPNPGMM LDLDQECWIS GWGATYEKGK TSDVLNAAMV PLIEPSKCNS KYIYNNLITP     420
AMICAGFLQG SVDSCQGDSG GPLVTLKNGI WWLIGDTSWG SGCAKALRPG VYGNVTVFTD     480
WIYQQMRANS                                                            490
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Table E

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Homo sapiens APP, transcript variant 1 nucleic acid sequence (SEQ ID
NO:5), Accession number NM_000484.2

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tgcctggaca aacccttctt ttaagatgtg tcttcaattt gtataaaatg gtgttttcat    3600
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Homo sapiens APP, transcript variant 1 amino acid sequence (SEQ ID NO:6),
Accession number NP_000475.1

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TCIDTKEGIL QYCQEVYPEL QITNVVEANQ PVTIQNWCKR GRKQCKTHPH FVIPYRCLVG     120
EFVSDALLVP DKCKFLHQER MDVCETHLHW HTVAKETCSE KSTNLHDYGM LLPCGIDKFR     180
GVEFVCCPLA EESDNVDSAD AEEDDSDVWW GGADTDYADG SEDKVVEVAE EEEVAEVEEE     240
EADDDEDDED GDEVEEEAEE PYEEATERTT SIATTTTTTT ESVEEVVREV CSEQAETGPC     300
RAMISRWYFD VTEGKCAPFF YGGCGGNRNN FDTEEYCMAV CGSAMSQSLL KTTQEPLARD     360
PVKLPTTAAS TPDAVDKYLE TPGDENEHAH FQKAKERLEA KHRERMSQVM REWEEAERQA     420
KNLPKADKKA VIQHFQEKVE SLEQEAANER QQLVETHMAR VEAMLNDRRR LALENYITAL     480
QAVPPRPRHV FNMLKKYVRA EQKDRQHTLK HFEHVRMVDP KKAAQIRSQV MTHLRVIYER     540
MNQSLSLLYN VPAVAEEIQD EVDELLQKEQ NYSDDVLANM ISEPRISYGN DALMPSLTET     600
KTTVELLPVN GEFSLDDLQP WHSFGADSVP ANTENEVEPV DARPAADRGL TTRPGSGLTN     660
IKTEEISEVK MDAEFRHDSG YEVHHQKLVF FAEDVGSNKG AIIGLMVGGV VIATVIVITL     720
VMLKKKQYTS IHHGVVEVDA AVTPEERHLS KMQQNGYENP TYKFFEQMQN                770
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Table F

Homo sapiens APP, transcript variant 2 nucleic acid sequence (SEQ ID NO:7), Accession number NM_201413.1

```
gctgactcgc ctggctctga gccccgccgc cgcgctcggg ctccgtcagt ttcctcggca      60
gcggtaggcg agagcacgcg gaggagcgtg cgcgggggcc ccgggagacg gcggcggtgg     120
cggcgcgggc agagcaagga cgcggcggat cccactcgca cagcagcgca ctcggtgccc     180
cgcgcagggt cgcgatgctg cccggtttgg cactgctcct gctggccgcc tggacggctc     240
gggcgctgga ggtacccact gatggtaatg ctggcctgct ggctgaaccc cagattgcca     300
tgttctgtgg cagactgaac atgcacatga atgtccagaa tgggaagtgg gattcagatc     360
catcagggac caaaacctgc attgatacca aggaaggcat cctgcagtat tgccaagaag     420
tctaccctga actgcagatc accaatgtgg tagaagccaa ccaaccagtg accatccaga     480
actggtgcaa gcggggccgc aagcagtgca agacccatcc ccactttgtg attccctacc     540
gctgcttagt tggtgagttt gtaagtgatg cccttctcgt tcctgacaag tgcaaattct     600
tacaccagga gaggatggat gtttgcgaaa ctcatcttca ctggcacacc gtcgccaaag     660
agacatgcag tgagaagagt accaacttgc atgactacgg catgttgctg ccctgcggaa     720
ttgacaagtt ccgaggggta gagtttgtgt gttgcccact ggctgaagaa agtgacaatg     780
tggattctgc tgatgcggag gaggatgact cggatgtctg gtggggcgga gcagacacag     840
actatgcaga tgggagtgaa gacaaagtag tagaagtagc agaggaggaa gaagtggctg     900
aggtggaaga agaagaagcc gatgatgacg aggacgatga ggatggtgat gaggtagagg     960
aagaggctga ggaaccctac gaagaagcca cagagagaac caccagcatt gccaccacca    1020
ccaccaccac cacagagtct gtggaagagg tggttcgaga ggtgtgctct gaacaagccg    1080
agacggggcc gtgccgagca atgatctccc gctggtactt tgatgtgact gaagggaagt    1140
gtgccccatt cttttacggc ggatgtggcg gcaaccggaa caactttgac acagaagagt    1200
actgcatggc cgtgtgtggc agcgccattc ctacaacagc agccagtacc cctgatgccg    1260
ttgacaagta tctcgagaca cctggggatg agaatgaaca tgcccatttc cagaaagcca    1320
aagagaggct tgaggccaag caccgagaga gaatgtccca ggtcatgaga gaatgggaag    1380
aggcagaacg tcaagcaaag aacttgccta aagctgataa gaaggcagtt atccagcatt    1440
tccaggagaa agtggaatct ttggaacagg aagcagccaa cgagagacag cagctggtgg    1500
agacacacat ggccagagtg gaagccatgc tcaatgaccg ccgccgcctg gccctggaga    1560
actacatcac cgctctgcag gctgttcctc ctcggcctcg tcacgtgttc aatatgctaa    1620
agaagtatgt ccgcgcagaa cagaaggaca gacagcacac cctaaagcat ttcgagcatg    1680
tgcgcatggt ggatcccaag aaagccgctc agatccggtc ccaggttatg acacacctcc    1740
gtgtgattta tgagcgcatg aatcagtctc tctccctgct ctacaacgtg cctgcagtgg    1800
ccgaggagat tcaggatgaa gttgatgagc tgcttcagaa agagcaaaac tattcagatg    1860
acgtcttggc caacatgatt agtgaaccaa ggatcagtta cggaaacgat gctctcatgc    1920
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catctttgac cgaaacgaaa accaccgtgg agctccttcc cgtgaatgga gagttcagcc    1980
tggacgatct ccagccgtgg cattcttttg gggctgactc tgtgccagcc aacacagaaa    2040
acgaagttga gcctgttgat gcccgccctg ctgccgaccg aggactgacc actcgaccag    2100
gttctgggtt gacaaatatc aagacggagg agatctctga agtgaagatg gatgcagaat    2160
tccgacatga ctcaggatat gaagttcatc atcaaaaatt ggtgttcttt gcagaagatg    2220
tgggttcaaa caaaggtgca atcattggac tcatggtggg cggtgttgtc atagcgacag    2280
tgatcgtcat caccttggtg atgctgaaga agaaacagta cacatccatt catcatggtg    2340
tggtggaggt tgacgccgct gtcaccccag aggagcgcca cctgtccaag atgcagcaga    2400
acggctacga aaatccaacc tacaagttct ttgagcagat gcagaactag acccccgcca    2460
cagcagcctc tgaagttgga cagcaaaacc attgcttcac tacccatcgg tgtccattta    2520
tagaataatg tgggaagaaa caaaccccgtt ttatgattta ctcattatcg ccttttgaca    2580
gctgtgctgt aacacaagta gatgcctgaa cttgaattaa tccacacatc agtaatgtat    2640
tctatctctc tttacatttt ggtctctata ctacattatt aatgggtttt gtgtactgta    2700
aagaatttag ctgtatcaaa ctagtgcatg aatagattct ctcctgatta tttatcacat    2760
agccccttag ccagttgtat attattcttg tggtttgtga cccaattaag tcctacttta    2820
catatgcttt aagaatcgat gggggatgct tcatgtgaac gtgggagttc agctgcttct    2880
cttgcctaag tattcctttc ctgatcacta tgcattttaa agttaaacat ttttaagtat    2940
ttcagatgct ttagagagat ttttttcca tgactgcatt ttactgtaca gattgctgct    3000
tctgctatat ttgtgatata ggaattaaga ggatacacac gtttgtttct tcgtgcctgt    3060
tttatgtgca cacattaggc attgagactt caagctttc ttttttgtc cacgtatctt    3120
tgggtctttg ataaagaaaa gaatccctgt tcattgtaag cacttttacg gggcgggtgg    3180
ggaggggtgc tctgctggtc ttcaattacc aagaattctc caaaacaatt ttctgcagga    3240
tgattgtaca gaatcattgc ttatgacatg atcgctttct acactgtatt acataaataa    3300
attaaataaa ataaccccgg gcaagacttt tctttgaagg atgactacag acattaaata    3360
atcgaagtaa ttttgggtgg ggagaagagg cagattcaat tttctttaac cagtctgaag    3420
tttcatttat gatacaaaag aagatgaaaa tggaagtggc aatataaggg gatgaggaag    3480
gcatgcctgg acaaaccctt cttttaagat gtgtcttcaa tttgtataaa atggtgtttt    3540
catgtaaata aatacattct tggaggagca aaaaaaaaaa aaaa                     3584

Homo sapiens APP, transcript variant 2 amino acid sequence (SEQ ID NO:8),
Accession number NP_958816.1

MLPGLALLLL AAWTARALEV PTDGNAGLLA EPQIAMFCGR LNMHMNVQNG KWDSDPSGTK      60
TCIDTKEGIL QYCQEVYPEL QITNVVEANQ PVTIQNWCKR GRKQCKTHPH FVIPYRCLVG     120
EFVSDALLVP DKCKFLHQER MDVCETHLHW HTVAKETCSE KSTNLHDYGM LLPCGIDKFR     180
GVEFVCCPLA EESDNVDSAD AEEDDSDVWW GGADTDYADG SEDKVVEVAE EEEVAEVEEE     240
EADDDEDDED GDEVEEEAEE PYEEATERTT SIATTTTTTT ESVEEVVREV CSEQAETGPC     300
RAMISRWYFD VTEGKCAPFF YGGCGGNRNN FDTEEYCMAV CGSAIPTTAA STPDAVDKYL     360
ETPGDENEHA HFQKAKERLE AKHRERMSQV MREWEEAERQ AKNLPKADKK AVIQHFQEKV     420
ESLEQEAANE RQQLVETHMA RVEAMLNDRR RLALENYITA LQAVPPRPRH VFNMLKKYVR     480
AEQKDRQHTL KHFEHVRMVD PKKAAQIRSQ VMTHLRVIYE RMNQSLSLLY NVPAVAEEIQ     540
DEVDELLQKE QNYSDDVLAN MISEPRISYG NDALMPSLTE TKTTVELLPV NGEFSLDDLQ     600
PWHSFGADSV PANTENEVEP VDARPAADRG LTTRPGSGLT NIKTEEISEV KMDAEFRHDS     660
GYEVHHQKLV FFAEDVGSNK GAIIGLMVGG VVIATVIVIT LVMLKKKQYT SIHHGVVEVD     720
AAVTPEERHL SKMQQNGYEN PTYKFFEQMQ N                                    751
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Table G

Homo sapiens APP, transcript variant 3 nucleic acid sequence (SEQ ID NO: 9), Accession number NM_201414.1

```
gctgactcgc ctggctctga gccccgccgc cgcgctcggg ctccgtcagt ttcctcggca      60
gcggtaggcg agagcacgcg gaggagcgtg cgcgggggcc ccgggagacg gcggcggtgg     120
cggcgcgggc agagcaagga cgcggcggat cccactcgca cagcagcgca ctcggtgccc     180
cgcgcagggt cgcgatgctg cccggtttgg cactgctcct gctggccgcc tggacggctc     240
gggcgctgga ggtacccact gatggtaatg ctggcctgct ggctgaaccc cagattgcca     300
tgttctgtgg cagactgaac atgcacatga atgtccagaa tgggaagtgg gattcagatc     360
catcagggac caaaacctgc attgatacca aggaaggcat cctgcagtat tgccaagaag     420
tctaccctga actgcagatc accaatgtgg tagaagccaa ccaaccagtg accatccaga     480
actggtgcaa gcggggccgc aagcagtgca agacccatcc ccactttgtg attccctacc     540
gctgcttagt tggtgagttt gtaagtgatg cccttctcgt tcctgacaag tgcaaattct     600
tacaccagga gaggatggat gtttgcgaaa ctcatcttca ctggcacacc gtcgccaaag     660
agacatgcag tgagaagagt accaacttgc atgactacgg catgttgctg ccctgcggaa     720
ttgacaagtt ccgaggggta gagtttgtgt gttgcccact ggctgaagaa agtgacaatg     780
tggattctgc tgatgcggag gaggatgact cggatgtctg gtggggcgga gcagacacag     840
actatgcaga tgggagtgaa gacaaagtag tagaagtagc agaggaggaa gaagtggctg     900
aggtggaaga agaagaagcc gatgatgacg aggacgatga ggatggtgat gaggtagagg     960
aagaggctga ggaaccctac gaagaagcca cagagagaac caccagcatt gccaccacca    1020
ccaccaccac cacagagtct gtggaagagg tggttcgagt tcctacaaca gcagccagta    1080
cccctgatgc cgttgacaag tatctcgaga cacctgggga tgagaatgaa catgcccatt    1140
tccagaaagc caaagagagg cttgaggcca agcaccgaga gagaatgtcc caggtcatga    1200
gagaatggga agaggcagaa cgtcaagcaa agaacttgcc taaagctgat aagaaggcag    1260
ttatccagca tttccaggag aaagtggaat ctttggaaca ggaagcagcc aacgagagac    1320
agcagctggt ggagacacac atggccagag tggaagccat gctcaatgac cgccgccgcc    1380
tggccctgga gaactacatc accgctctgc aggctgttcc tcctcggcct cgtcacgtgt    1440
tcaatatgct aaagaagtat gtccgcgcag aacagaagga cagacagcac accctaaagc    1500
atttcgagca tgtgcgcatg gtggatccca agaaagccgc tcagatccgg tcccaggtta    1560
tgacacacct ccgtgtgatt tatgagcgca tgaatcagtc tctctccctg ctctacaacg    1620
tgcctgcagt ggccgaggag attcaggatg aagttgatga gctgcttcag aaagagcaaa    1680
actattcaga tgacgtcttg gccaacatga ttagtgaacc aaggatcagt tacggaaacg    1740
atgctctcat gccatctttg accgaaacga aaaccaccgt ggagctcctt cccgtgaatg    1800
gagagttcag cctggacgat ctccagccgt ggcattcttt tggggctgac tctgtgccag    1860
ccaacacaga aaacgaagtt gagcctgttg atgcccgccc tgctgccgac cgaggactga    1920
ccactcgacc aggttctggg ttgacaaata tcaagacgga ggagatctct gaagtgaaga    1980
tggatgcaga attccgacat gactcaggat atgaagttca tcatcaaaaa ttggtgttct    2040
ttgcagaaga tgtgggttca aacaaaggtg caatcattgg actcatggtg ggcggtgttg    2100
tcatagcgac agtgatcgtc atcaccttgg tgatgctgaa gaagaaacag tacacatcca    2160
ttcatcatgg tgtggtggag gttgacgccg ctgtcacccc agaggagcgc cacctgtcca    2220
agatgcagca gaacggctac gaaaatccaa cctacaagtt ctttgagcag atgcagaact    2280
agacccccgc cacagcagcc tctgaagttg gacagcaaaa ccattgcttc actacccatc    2340
ggtgtccatt tatagaataa tgtgggaaga aacaaacccg ttttatgatt tactcattat    2400
cgccttttga cagctgtgct gtaacacaag tagatgcctg aacttgaatt aatccacaca    2460
tcagtaatgt attctatctc tctttacatt ttggtctcta tactacatta ttaatgggtt    2520
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agtcctactt tacatatgct ttaagaatcg atgggggatg cttcatgtga acgtgggagt    2700
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atttttaagt atttcagatg ctttagagag attttttttc catgactgca ttttactgta    2820
cagattgctg cttctgctat atttgtgata taggaattaa gaggatacac acgtttgttt    2880
cttcgtgcct gttttatgtg cacacattag gcattgagac ttcaagcttt tctttttttg    2940
tccacgtatc tttgggtctt tgataaagaa aagaatccct gttcattgta agcactttta    3000
cggggcgggt ggggaggggt gctctgctgg tcttcaatta ccaagaattc tccaaaacaa    3060
ttttctgcag gatgattgta cagaatcatt gcttatgaca tgatcgcttt ctacactgta    3120
ttacataaat aaattaaata aaataacccc gggcaagact tttctttgaa ggatgactac    3180
agacattaaa taatcgaagt aattttgggt ggggagaaga ggcagattca attttcttta    3240
accagtctga agtttcattt atgatacaaa agaagatgaa aatggaagtg gcaatataag    3300
gggatgagga aggcatgcct ggacaaaccc ttcttttaag atgtgtcttc aatttgtata    3360
aaatggtgtt ttcatgtaaa taaatacatt cttggaggag caaaaaaaaa aaaaaa         3416
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Homo sapiens APP, transcript variant 3 amino acid sequence (SEQ ID NO: 10), Accession number NP_958817.1

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MLPGLALLLL AAWTARALEV PTDGNAGLLA EPQIAMFCGR LNMHMNVQNG KWDSDPSGTK      60
TCIDTKEGIL QYCQEVYPEL QITNVVEANQ PVTIQNWCKR GRKQCKTHPH FVIPYRCLVG     120
EFVSDALLVP DKCKFLHQER MDVCETHLHW HTVAKETCSE KSTNLHDYGM LLPCGIDKFR     180
GVEFVCCPLA EESDNVDSAD AEEDDSDVWW GGADTDYADG SEDKVVEVAE EEEVAEVEEE     240
EADDDEDDED GDEVEEEAEE PYEEATERTT SIATTTTTTT ESVEEVVRVP TTAASTPDAV     300
DKYLETPGDE NEHAHFQKAK ERLEAKHRER MSQVMREWEE AERQAKNLPK ADKKAVIQHF     360
QEKVESLEQE AANERQQLVE THMARVEAML NDRRRLALEN YITALQAVPP RPRHVFNMLK     420
KYVRAEQKDR QHTLKHFEHV RMVDPKKAAQ IRSQVMTHLR VIYERMNQSL SLLYNVPAVA     480
EEIQDEVDEL LQKEQNYSDD VLANMISEPR ISYGNDALMP SLTETKTTVE LLPVNGEFSL     540
DDLQPWHSFG ADSVPANTEN EVEPVDARPA ADRGLTTRPG SGLTNIKTEE ISEVKMDAEF     600
RHDSGYEVHH QKLVFFAEDV GSNKGAIIGL MVGGVVIATV IVITLVMLKK KQYTSIHHGV     660
VEVDAAVTPE ERHLSKMQQN GYENPTYKFF EQMQN                                695
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Table H

Rattus norvegicus TMPRSS2 nucleic acid sequence (SEQ ID NO: 11), Accession number NM_130424

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ggaaaccgag agccccggcg gaggcggaga ggggcgggga gcacagcagg tcacctggag      60
gaagccacat actgacctcc tcatgctgct gacacaggca ggatggcatt gaactcaggg     120
tcacctccag gaattggacc ttactatgag aaccacgggt atcagtctga gcacgtctat     180
tccccgaggc cacctgtgtc tcccagtggc tacaacttgt atccagccca gagctgccca     240
tctccagtgc cccagtatgc tccgagagtc acaactcaag cctcaacacc tgccatccac     300
atacagcccc gatcctcagg aacactgtgc acctcaaaat ctaagaaatc catgcttgtc     360
gccctggccc tgggcactgt cctcgccggt gctgctgtgg ctgctggctt gctttggaag     420
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agtgactcgt gctcatcccg catggtggtt tctttgcgct gtatagaatg cggggttcgc     840
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gagtggattg tgacagccgc ccactgtgtg gaagaacccc tcagcagccc taggtactgg    1020
acggcatttg cgggaatttt gaaacagtct ctcatgttct atggaagtag acaccaggta    1080
gaaaaagtga tttcccatcc aaattacgac tctaagacca agaataatga cattgctctc    1140
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ccaggcatga tgctggacct agcccaggag tgctggattt cagggtgggg ggccacctat    1260
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ttcctccagg gctctgtcga ctcttgtcag ggagacagtg gagggcccct ggttactttg    1440
aagaatgaaa tctggtggct gattggggac acgagctggg gctcgggctg tgccaaggca    1500
tacagacctg gagtatacgg gaatgtgaca gtatttacag attggatcta ccagcaaatg    1560
agggcgaaca gctaatccac gtggctttgt ccccgacttc ctttgtcttc aacaaccttc    1620
tgcaagaaaa ccaaggggcg gattttcaac ttcctgtgca caatgtacct tttgagatga    1680
ttcaaagggc ctttcacttt tattaaacag tgacttatct gactgtgctc cctggtcctg    1740
tgagggcttc agtgccccac cctccaggct acttcagcag ctcctaccag aaaggatgac    1800
cagatcctgt tgggtttggg cacatagggc caaatacaga agaaggtggc actctcatgt    1860
tgagacttct ttttggctta tgctcaggcc tcttttggat gagtaaggac tatgacctct    1920
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ggtacagaga gagaaagaga gagagagaga gagagagaga gagagagaga gagagagaga    2040
gagaacacta cccattgcct gtggcctagc tctgctgtgt agttttgggc tggtcatact    2100
ataatgactt catgaacaac agcccaggag taaaggactt attctcctgc atgttgacat    2160
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tggttcttgg ctccccaccg ctatttctgt cagcctttca cagcctgaaa cttacctctt    2340
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gtgtttattc ccttactacc tcactgcacc tgcacactag tctggatgaa tgtctgtagt    2520
tcacctgcat ttggactgtg tgcttgtgcc tcagatatta gacctcttcc agatggttag    2580
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caatcaccaa atatgaagta tgaatgtctt acctatggtg aggctctgcc tcctcctctg    2760
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ggtctcccac ccaggaaata gttgccactc catgtaaaga gttccctcat gcttcctctg    2880
ggaacaacac tctagggacc ttcttagatg ccataaggta cctacttaca agactaaatg    2940
tacaagcacc ttgaagtgtg agaacatgtc ccctccttag ctctccctgt ctttgctgtt    3000
ggttggttat ttcctgtttt gtgtctgttc tgagctgtga gattccactg tgaaatgtat    3060
gaataaagta tgtaattctg tccattgttc aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa    3120
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa    3180
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Rattus norvegicus TMPRSS2 amino acid sequence (SEQ ID NO: 12), Accession number NP_569108

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MALNSGSPPG IGPYYENHGY QSEHVYSPRP PVSPSGYNLY PAQSCPSPVP QYAPRVTTQA      60
STPAIHIQPR SSGTLCTSKS KKSMLVALAL GTVLAGAAVA AGLLWKFWDS KCSSSEMECG     120
SSGTCISSSL WCDGVSQCPN GEDENRCVRL YGTSFTLQVY SSQRKAWYPV CQDDWNESYG     180
RAACKDMGYK NSFYSSQGIP DQSGATSFMK LNVSAGNVDL YKKLYHSDSC SSRMVVSLRC     240
IECGVRSVRR QSRIVGGSTA SPGDWPWQVS LHVQGIHVCG GSIITPEWIV TAAHCVEEPL     300
SSPRYWTAFA GILKQSLMFY GSRHQVEKVI SHPNYDSKTK NNDIALMKLQ TPLAFNDVVK     360
PVCLPNPGMM LDLAQECWIS GWGATYEKGK TSDVLNAAMV PLIEPSKCNS KYIYNNLITP     420
AMICAGFLQG SVDSCQGDSG GPLVTLKNEI WWLIGDTSWG SGCAKAYRPG VYGNVTVFTD     480
WIYQQMRANS                                                            490
```

Table I

Gallus gallus TMPRSS2 nucleic acid sequence (SEQ ID NO: 13), Accession number XM_416737

```
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gcacctccat ccagttcgtc caggatgtgt tcatcaagca taaagaaaat cgtaataatt     240
acattatcca ttttactagt catctgttgt gcaattgctg ctttcctcat ctggtatttt     300
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ccctcagtgt ggtgtgatgg agtgactgac tgcccaaatg gggaggatga aaaccggtgt     420
gttagacttt atggaccaaa cttcattctc gaagtttatt cgcctgtcag ccaaacgtgg     480
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agctttatga agctaaacac cagtgcgggg aatacagact tgtacaaaag gctgcaaagc     660
agtgattact gtgcatcagg aaatgtggtt tctctgcgct gcatagagtg cggcctgccc     720
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tgtagcagga tgctgagaca gttggttttg tagggtttta aattaactgt taagtaatgt    1860
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tgtatattca ggtagatata aatcctcagg atcgtgacag ccatcatcca actcagattg    2040
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gtgttgagga tgaggagaca caaacccgtg tttctgccta accagatgaa ctacctcatt    2340
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ctttgcctgt taaaatctcc ctgtgaaaga tgttacaatt atgatgtgca gtattatgtt    3180
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aaatctcagg cattagaaaa aca                                            3383
```

Gallus gallus TMPRSS2 amino acid sequence (SEQ ID NO: 14), Accession number XP_416737

```
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PSVWCDGVTD CPNGEDENRC VRLYGPNFIL EVYSPVSQTW YPVCQDDWTD DFGKIACEDM     180
GYNVDTYYYS QGVAAEVSFK SFMKLNTSAG NTDLYKRLQS SDYCASGNVV SLRCIECGLP     240
TKSTAVMSRI VGGSMASLGQ WPWQVSLHVQ DTHVCGGSII TREWLVTAAH CVEGLFSDPY     300
IWSVYAGILS QNEMHSRPGY RVQKIISHPN YDTDSKDNDV ALMKLETPLS FTNTIRPVCL     360
PNPGMMFQPN QQCWISGWGA EYQGGKTAND LNYVMVPLIE RSTCNSVYVY DGMVLPTMVC     420
AGYLQGGIDS CQGDSGGPLV TNKNSVWWLV GDTSWGTGCA SPNRPGVYGN MTVFTDWIYK     480
NMQANR                                                                486
```

Table J

Pan troglodytes TMPRSS2 nucleic acid sequence (SEQ ID NO: 15), Accession Number XM_514911

```
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cctgcatcag cccctctaac tggtgtgatg gcgtgtcaca ctgccccagc ggggaggacg     540
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gcagggacat gggctataag aataattttt actctagcca aggaatagtg gatgacagcg     720
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Pan troglodytes TMPRSS2 amino acid sequence (SEQ ID NO: 16), Accession Number XP_514911

```
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DSSGTCISPS NWCDGVSHCP SGEDENRCVR LYGPNFILQV YSSQRKSWHP VCQDDWNENY     180
GRAACRDMGY KNNFYSSQGI VDDSGSTSFM KLNTSAGNVD IYKKLYHSDA CSSKAVVSLR     240
CIACGVNLNS SRQSRIVGGE SALPGAWPWQ VSLHVQNVHV CGGSIITPEW IVTAAHCVEK     300
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VKPVCLPNPG MMLEPEQLCW ISGWGATEEK EVQDLLCCTH CEFRAALEIP PRPAWLSDRG     420
LGEDGRPGPM VATSKPVLCP DNHRERLSPP PSVAVCISL                             459
```

CLAIMS

1. A method for identifying a compound that enhances or inhibits transmembrane protease, serine 2 (TMPRSS2) protease activity, the method comprising:

a) contacting a TMPRSS2 polypeptide with the compound;

b) contacting the TMPRSS2 polypeptide with a substrate polypeptide;

c) measuring TMPRSS2 protease activity; and

d) comparing the TMPRSS2 protease activity measured in c) with a control value obtained for a TMPRSS2 polypeptide that has not been contacted with the compound, and thereby determining whether the compound is an enhancer or inhibitor of TMPRSS2 protease activity;

wherein the substrate polypeptide comprises the sequence DAEFRHDSG (SEQ ID NO: 17) or an equivalent thereof;

wherein an increase in TMPRSS2 protease activity compared with said control value identifies the compound as being an enhancer of TMPRSS2 protease activity; and

wherein a decrease in TMPRSS2 protease activity compared with said control value identifies the compound as being an inhibitor of TMPRSS2 protease activity.

2. A method according to claim 1, wherein the TMPRSS2 polypeptide is contacted with the compound in the presence of the substrate polypeptide.

3. A method according to claim 1 or 2, wherein the TMPRSS2 polypeptide is expressed in a cell or cell culture.

4. A method according to any one of the preceding claims, wherein said TMPRSS2 polypeptide comprises the amino acid sequence of SEQ ID NO: 2 or 4 or a variant of either thereof.

5. A method according to any one of the preceding claims, wherein said substrate polypeptide comprises the amino acid sequence of human APP of any of SEQ ID NO:s 6, 8 and 10 or a fragment of any thereof.

6. A method according to any one of the preceding claims, wherein said substrate polypeptide comprises residues 681-713 of human APP of SEQ ID NO: 6.

7. A method according to any one of the preceding claims, wherein measuring TMPRSS2 protease activity involves fluorescence, an immunoassay or mass spectrometry.

8. A method according to any one of the preceding claims, wherein measuring TMPRSS2 protease activity involves detecting one or more specific cleavage products derived from said substrate polypeptide.

9. A method according to claim 8, wherein the specific cleavage products are N-terminally truncated Aβ peptides or variants thereof.

10. A method for identifying a compound that enhances or inhibits amyloid precursor protein (APP) processing, the method comprising carrying out a method according to any one of the preceding claims and thereby identifying a compound that enhances or inhibits APP processing,

wherein an increase in TMPRSS2 protease activity in the presence of said compound compared with said control value identifies said compound as an enhancer of APP processing; and

wherein a decrease in TMPRSS2 protease activity in the presence of said compound compared with said control value identifies said compound as an inhibitor of APP processing.

11. A method for identifying a compound that enhances or inhibits Aβ aggregation or formation of amyloid plaques and/or neurofibrillary tangles, the method comprising carrying out a method according to any one of the preceding claims and thereby identifying a compound that enhances or inhibits Aβ aggregation or formation of amyloid plaques and/or neurofibrillary tangles,

wherein an increase in TMPRSS2 protease activity in the presence of said compound compared with said control value identifies said compound as an enhancer of Aβ aggregation or formation of amyloid plaques and/or neurofibrillary tangles; and

wherein a decrease in TMPRSS2 protease activity in the presence of said compound compared with said control value identifies said compound as an inhibitor of Aβ aggregation or formation of amyloid plaques and/or neurofibrillary tangles.

12. A method for identifying a compound suitable for the prevention or treatment of a disease associated with pathogenic APP processing, the method comprising carrying out a method according to any one of the preceding claims and thereby identifying a compound suitable for the prevention or treatment of a disease associated with pathogenic APP processing,

wherein a decrease in TMPRSS2 protease activity in the presence of said compound compared with said control value identifies said compound as being suitable for the prevention or treatment of a disease associated with pathogenic APP processing.

13. An enhancer or inhibitor of TMPRSS2 protease activity identified by the method of any one of the preceding claims.

14. A method for identifying whether or not a subject is at risk of developing, or has, a disease associated with pathogenic APP processing, said method comprising:

a) measuring the expression level and/or protease activity of TMPRSS2 in a sample derived from said subject;

b) comparing the TMPRSS2 expression level and/or protease activity measured in said sample to a normal level of TMPRSS2 expression and/or protease activity and thereby identifying whether or not a subject is at risk of developing, or has, a disease associated with pathogenic APP processing;

wherein an increased level of TMPRSS2 expression and/or an increased level of TMPRSS2 protease activity in the sample compared with the normal level identifies the subject as being at risk of developing, or having, a disease associated with pathogenic APP processing.

15. A method according to claim 14, wherein step a) comprises measuring the levels of N-terminally truncated peptides derived from APP, preferably Aβ6-40 or Aβ6-42.

16. A TMPRSS2 antagonist for use in a method of preventing or treating a disease associated with pathogenic APP processing.

17. A TMPRSS2 antagonist according to claim 16 wherein said disease is Alzheimer's disease.

18. Use of a TMPRSS2 antagonist in the manufacture of a medicament for preventing or treating of a disease associated with pathogenic APP processing.

19. Use according to claim 17 wherein said disease is Alzheimer's disease.

20. A method of treating or preventing a disease associated with pathogenic APP processing in a subject, comprising administering to the subject an effective amount of a TMPRSS2 antagonist.

21. A method according to claim 20, wherein said subject has been identified as being at risk of developing, or having, a disease associated with pathogenic APP processing using a method according to claim 14 or 15.

22. A method according to any one of claims 12, 14, 15, 20 and 21, wherein said disease is Alzheimer's disease.

23. A non-human animal in which a disease of pathogenic APP processing has been established by over-expression of TMPRSS2.

24. A non-human animal according to claim 23, wherein said disease is Alzheimer's disease.

25. A method for establishing a disease of pathogenic APP processing in a non-human animal comprising over-expressing TMPRSS2 in said animal in an amount sufficient to cause a disease of pathogenic APP processing.

26. A method for identifying a compound which prevents or treats a disease of pathogenic APP processing, comprising administering said compound to a non-human animal as defined in claim 23 or 24 and assessing whether or not said compound prevents or treats the disease of pathogenic APP processing.

27. A method according to claim 25 or 26, wherein said disease is Alzheimer's disease.

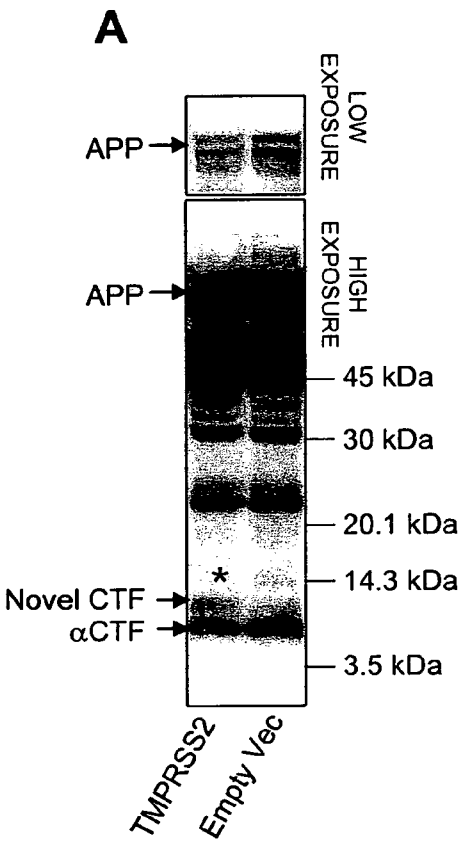
28. A compound identified by the method of claim 26 for use in a method of preventing or treating a disease associated with pathogenic APP processing.

29. A compound according to claim 28, wherein said disease is Alzheimer's disease.

30. Use of a compound identified by the method of claim 26 in the manufacture of a medicament for prevention or treatment of a disease associated with pathogenic APP processing.

31. Use according to claim 30, wherein said disease is Alzheimer's disease.

32. A kit comprising a TMPRSS2 polypeptide and a substrate polypeptide comprising the sequence DAEFRHDSG (SEQ ID NO: 17) or an equivalent thereof.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αCTF
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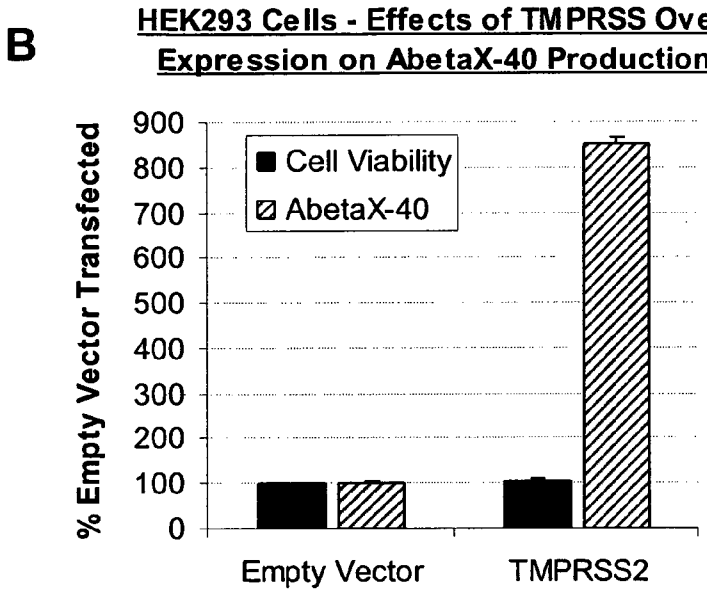
Empty Vec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Cell Viability
AbetaX-40
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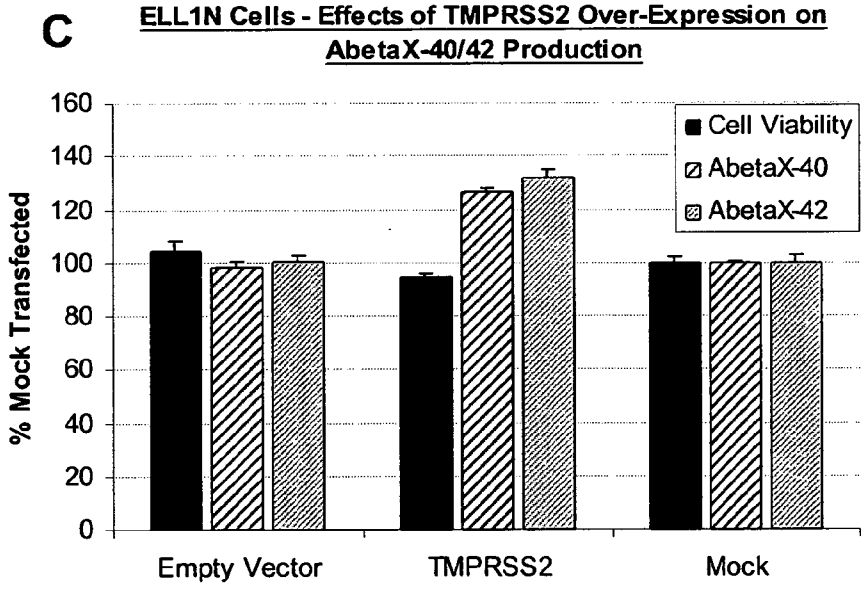
TMPRSS2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AbetaX-42
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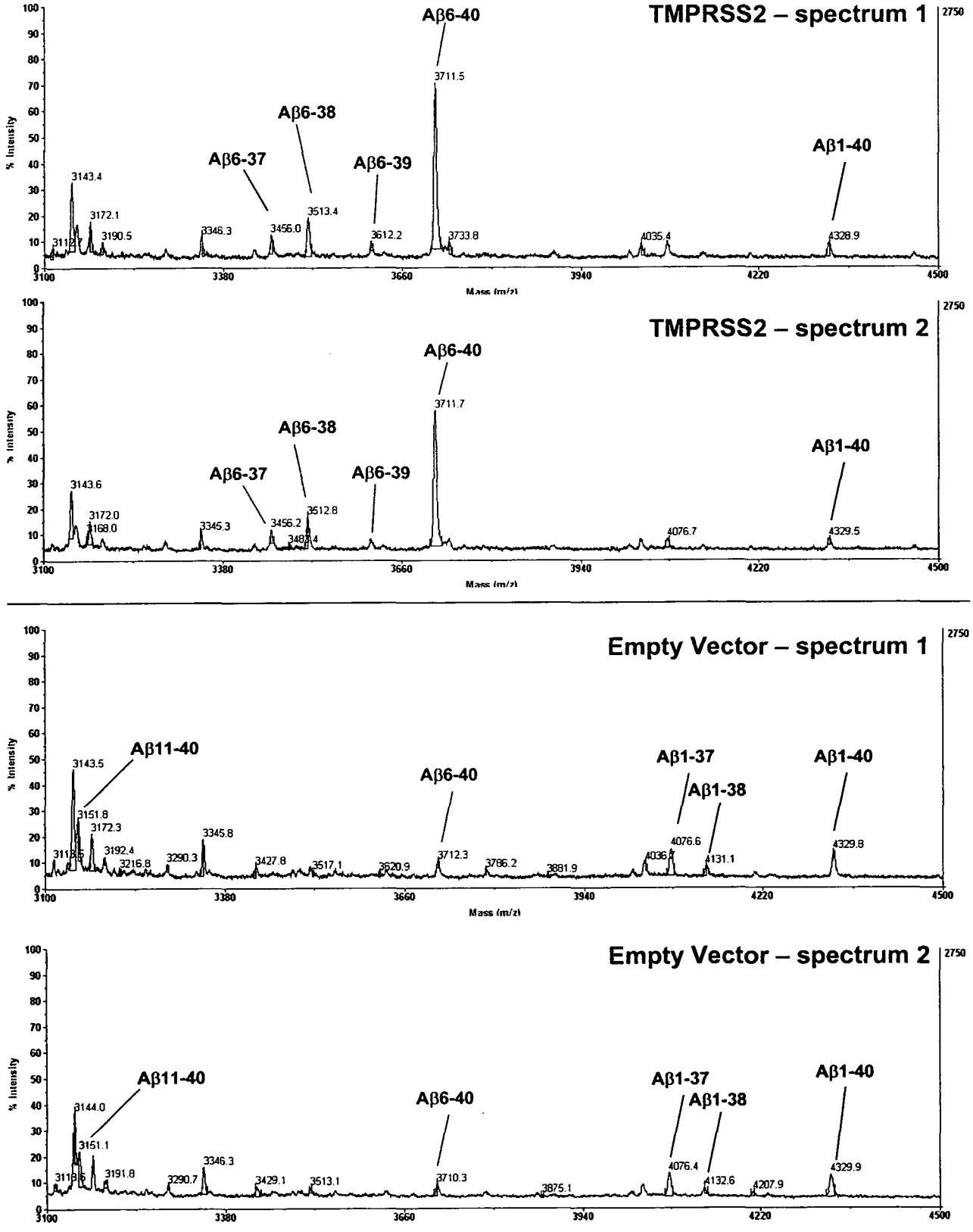
TMPRSS2
Mock

Figure 1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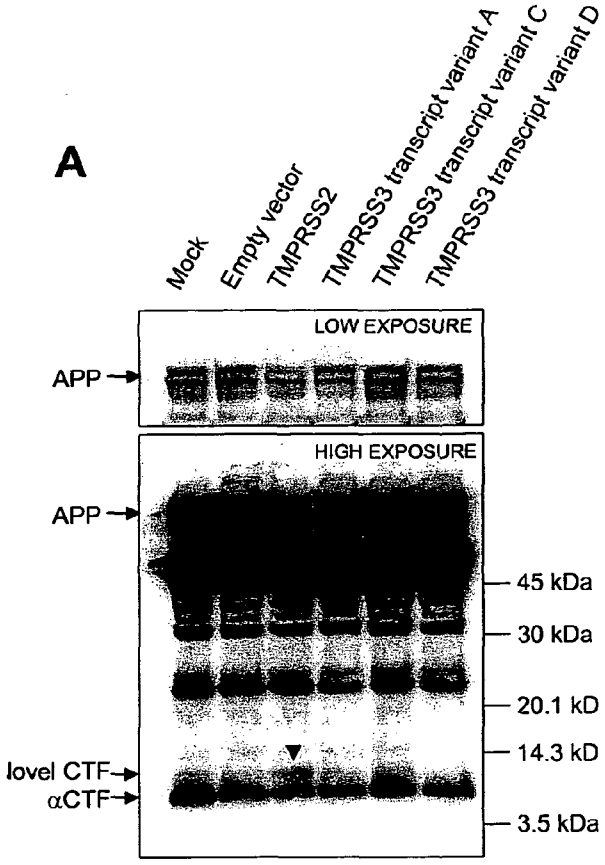
Aβ1-38
Empty Vector – spectrum 2
% Intensity
Mass (m/z)

Figure 2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APP
HIGH EXPOSURE
APP
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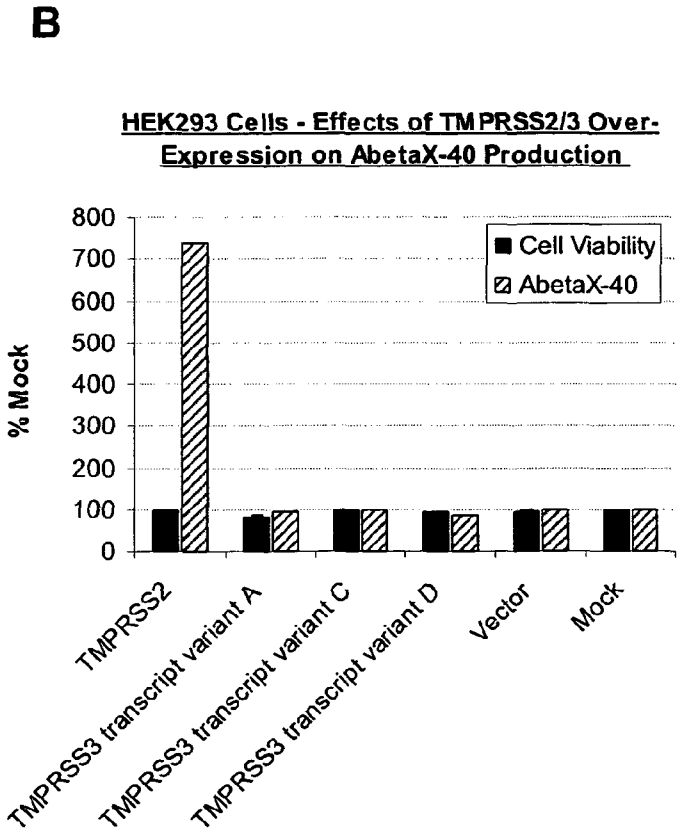
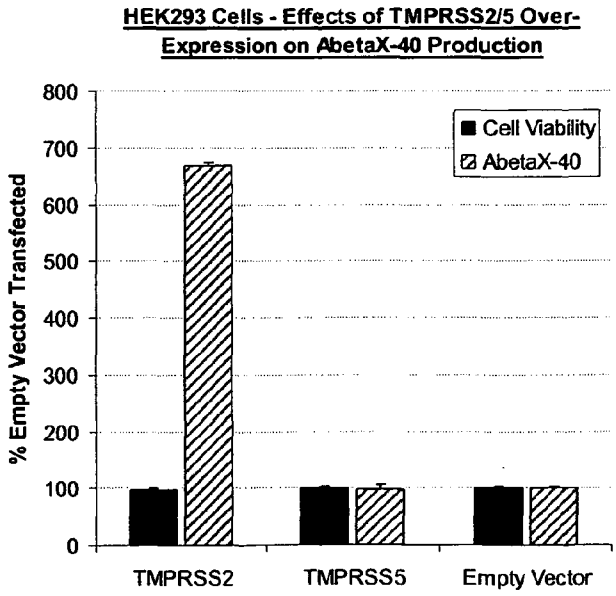
30 kDa
20.1 kD
14.3 kD
3.5 kDa
lovel CTF
αCTF


B
HEK293 Cells - Effects of TMPRSS2/3 Over-Expression on AbetaX-40 Production
% Mock
800
700
600
500
400
300
200
100
0
Cell Viability
AbetaX-40
TMPRSS2
TMPRSS3 transcript variant A
TMPRSS3 transcript variant C
TMPRSS3 transcript variant D
Vector
Mock


C
HEK293 Cells - Effects of TMPRSS2/5 Over-Expression on AbetaX-40 Production
% Empty Vector Transfected
800
700
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400
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200
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Cell Viability
AbetaX-40
TMPRSS2
TMPRSS5
Empty Vector

Figure 3

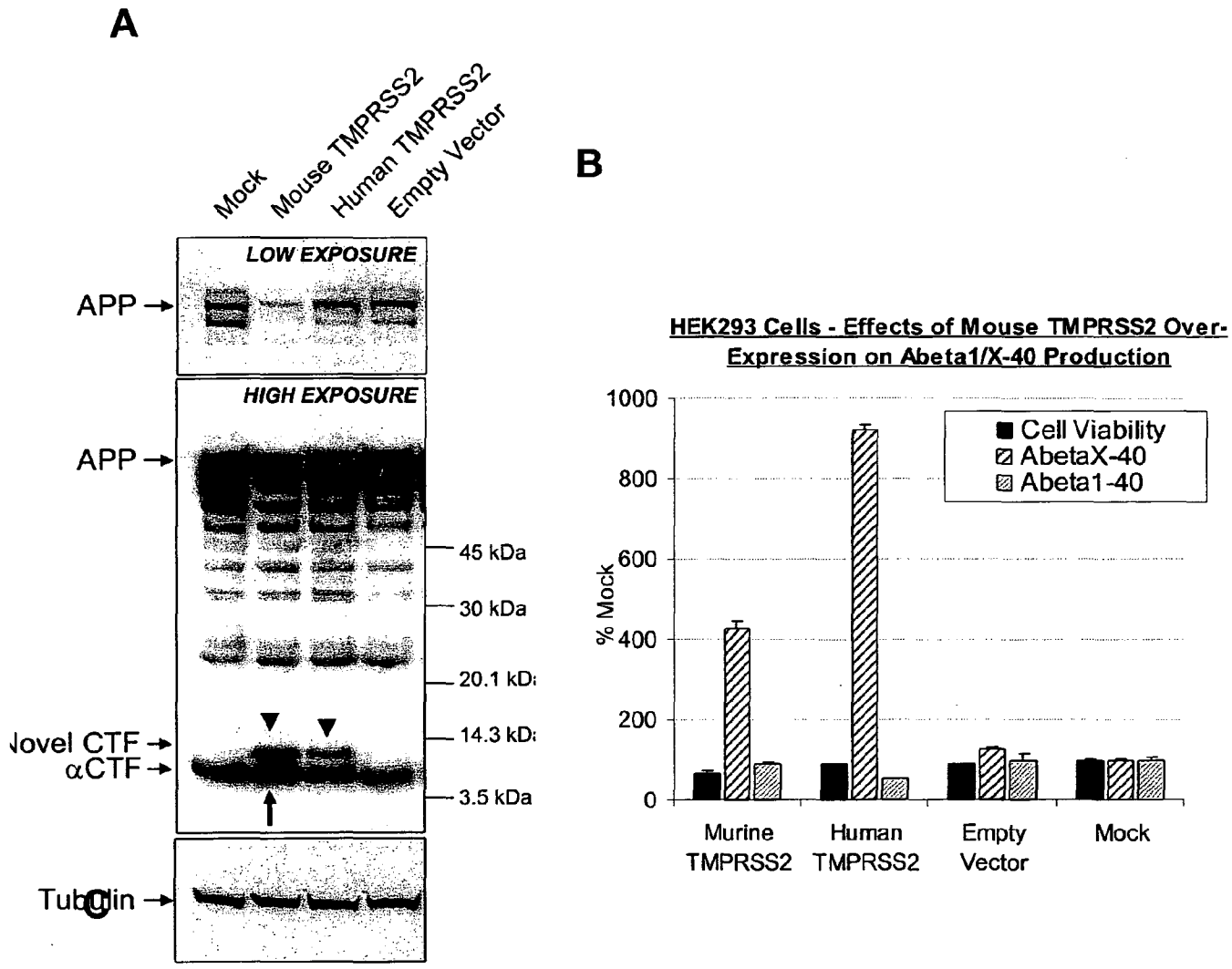
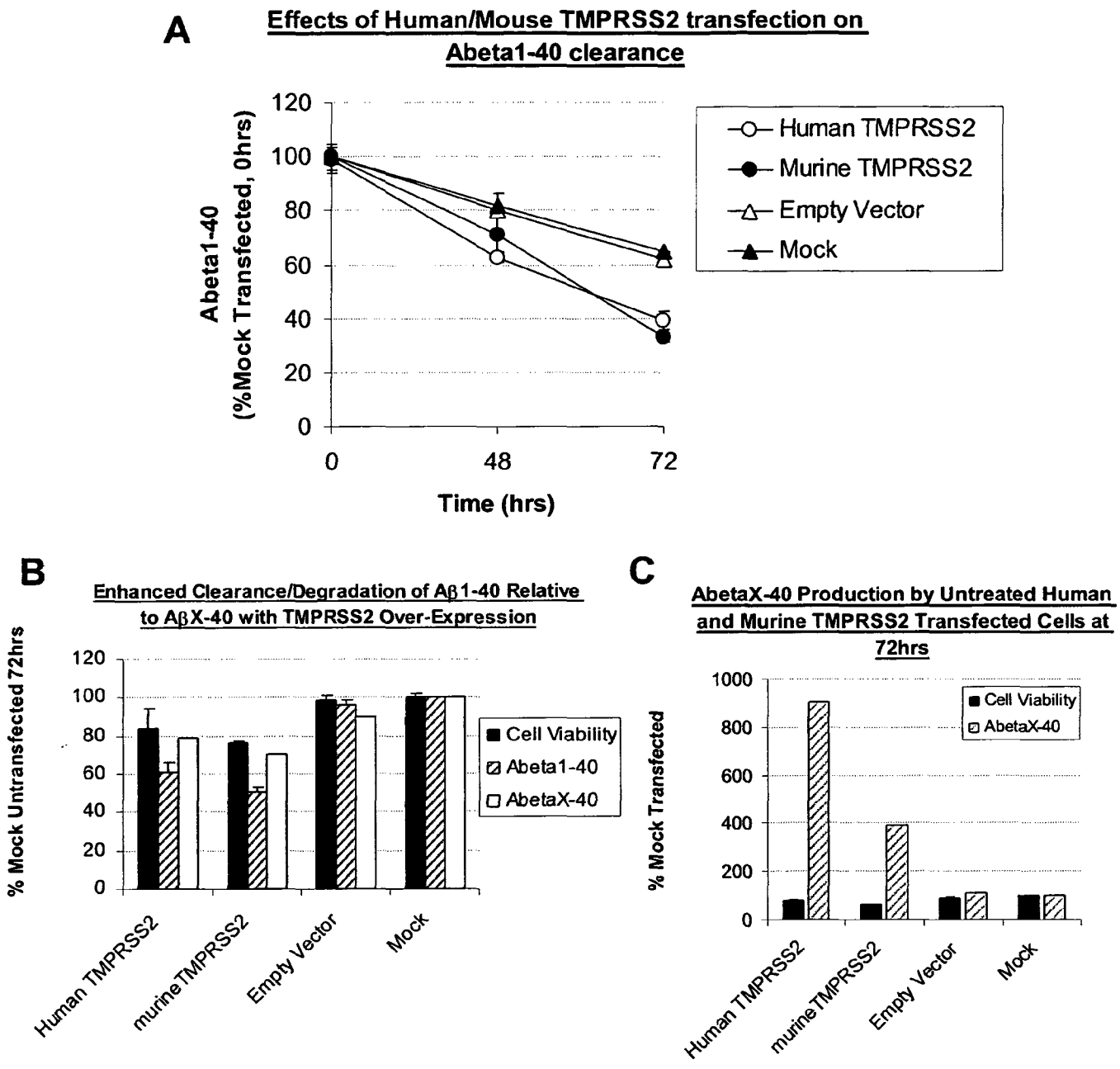

A
Mock
Mouse TMPRSS2
Human TMPRSS2
Empty Vector
LOW EXPOSURE
APP
HIGH EXPOSURE
APP
45 kDa
30 kDa
20.1 kDa
14.3 kDa
3.5 kDa
Novel CTF
αCTF
Tubulin
B
HEK293 Cells - Effects of Mouse TMPRSS2 Over-Expression on Abeta1/X-40 Production
1000
800
600
400
200
0
% Mock
Cell Viability
AbetaX-40
Abeta1-40
Murine TMPRSS2
Human TMPRSS2
Empty Vector
Mock

Figure 4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Abeta1-40 (%Mock Transfected, 0hrs)
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100
80
60
40
20
0
0
48
72
Time (hrs)
Human TMPRSS2
Murine TMPRSS2
Empty Vector
Mock
B
Enhanced Clearance/Degradation of Aβ1-40 Relative to AβX-40 with TMPRSS2 Over-Expression
% Mock Untransfected 72hrs
120
100
80
60
40
20
0
Cell Viability
Abeta1-40
AbetaX-40
Human TMPRSS2
murineTMPRSS2
Empty Vector
Mock
C
AbetaX-40 Production by Untreated Human and Murine TMPRSS2 Transfected Cells at 72hrs
% Mock Transfected
1000
800
600
400
200
0
Cell Viability
AbetaX-40
Human TMPRSS2
murineTMPRSS2
Empty Vector
Mock

Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2009/002779

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/37 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)
C12Q 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, WPI Data, Sequence Search, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005/108949 A2 (GALAPAGOS GENOMICS N V [BE]; SPITTAELS KOENRAAD FREDERIK FL [BE]; HOFF) 17 November 2005 (2005-11-17) page 3, column 4, line 1 - line 4; claims 1-30; examples 1-6	1-12, 14-15, 23-27,32
A	WO 92/03474 A1 (HARVARD COLLEGE [US]) 5 March 1992 (1992-03-05) claims 1-9	1-12, 14-15, 23-27,32
A	WO 2008/084254 A2 (EISAI LONDON RES LAB LTD [GB]; TAYLOR JOANNE [GB]; KLINE ADAM DAVID [G) 17 July 2008 (2008-07-17) claims 1-7; figure 1	1-12, 14-15, 23-27,32

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 2010	02/03/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Schmidt, Harald

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2009/002779

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00/00605 A1 (MYRIAD GENETICS INC [US]) 6 January 2000 (2000-01-06) page 37 - page 41; claim 28	1-12, 14-15, 23-27,32
A	WILSON S ET AL: "The membrane-anchored serine protease, TMPRSS2, activates PAR-2 in prostate cancer cells" BIOCHEMICAL JOURNAL, vol. 388, 10 November 2004 (2004-11-10), pages 967-972, XP002569756 abstract page 969	1-12, 14-15, 23-27,32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2009/002779

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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

2. ☒ Claims Nos.: 13, 16-22, 28-31
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 13, 16-22, 28-31

Present claims 13,16 to 22 and 28 to 31 encompass compounds defined only by their desired function, contrary to the requirements of clarity of Article 6 PCT, because the result-to-be-achieved type of definition does not allow the scope of the claims to be ascertained. The fact that any compound could be screened does not overcome this objection, as the skilled person would not have knowledge beforehand as to whether it would fall within the scope claimed. No working example is provided in the application that could plausibly show that a modulator of TMPRSS2 is useful in the treatment of diseases associated with pathogenic APP processing. Undue experimentation would be required to screen compounds randomly. The non-compliance with the substantive provisions is to such an extent that no meaningful search of claims 13,16 to 22 and 28 to 31 could be carried out at all (Article 17(2) PCT).

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.2), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2009/002779

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005108949 A2	17-11-2005	WO 2005109000 A2	17-11-2005
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WO 9203474 A1	05-03-1992	EP 0546101 A1	16-06-1993
		JP 6500560 T	20-01-1994
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