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Detection of complement proteins

This application claims priority from GB 2006789.8 filed on 7 May 2020, the contents and elements of which are herein incorporated by reference for all purposes.

Field of the invention

- 5 The present invention relates to the fields of molecular biology and medicine. More specifically, the present invention relates to methods for detecting complement proteins and the use of such methods for diagnostic and therapeutic uses.

Background to the Invention

- 10 The complement system contributes to innate host immune defence by assisting in the rapid recognition and elimination of microbial intruders. However, dysregulation of complement can contribute to inflammatory, immune-related, and age-related conditions. As a result inappropriate regulation of the complement system has been implicated in a wide variety of diseases in humans e.g. diseases of the eye and kidney, as well as neurological diseases and cancer (Morgan, B.P., Semin Immunopathol, 2018. 40(1): p. 113-124; Halbgbauer, R., et al., Semin Immunol, 2018. 37: p. 12-20; Ma, Y., et al., Aging Dis, 2019. 10(2): p. 429-462; and Kleczko, E.K., et al., Front Immunol, 2019. 10: p. 954.

- 20 Complement pathway activation and control is regulated by a complex interplay between pathway activators and inhibitors. These activators and inhibitors are commonly enzymes which cleave and inactivate complement molecules on biological surfaces and/or in solution to maintain steady regulation of complement activating species. The complement pathways are in a constant state of flux and balance, and disturbances to this balance can lead to inappropriate activation and the consequences above.

- 25 One activating molecule is complement component 3 (C3), a member of the alternative complement pathway and amplification loop. C3 comprises a β chain and an α' chain which associate through interchain disulphide bonds. During complement activation, C3 is cleaved to generate two functional fragments, C3a and C3b. C3a is a potent anaphylatoxin. Deposition of C3b on biological surfaces, e.g. extracellular matrix and cell surfaces, is the central activating mechanism of the alternative pathway. C3b is a potent opsonin, targeting pathogens, antibody-antigen immune complexes and apoptotic cells for phagocytosis by phagocytes and NK cells. Surface-linked C3b also reacts with other complement proteins to form active convertase enzymes that are able to produce further (surface-attachable) C3b molecules, serving to activate and amplify complement responses (Clark, S.J., et al., J Immunol, 2014. 193(10): p. 4962-70). C3b associates with Factor B to form the C3bBb-type C3 convertase and with C3bBb to form the C3bBb3b-type C5 convertase. Proteolytic cleavage of C3 also produces C3a and C3b through the classical complement pathway and the lectin pathway.

Insufficient control of C3 convertases results in massive production of C3b and C3a molecules and a shift of the complement cascade to its terminal lytic pathway. This produces the potent anaphylatoxin, C5a, and the cell lytic protein complex termed the membrane attack complex; both providing strong inflammatory signals (Clark, S.J., et al., *supra*). This ultimately leads to cell/tissue destruction and a local inflammatory response.

C3b activation of complement is regulated by complement protein factor I (FI). FI prevents complement activation by cleaving C3b to a proteolytically-inactive form, designated iC3b, which is unable to participate in convertase assembly, and further to downstream products iC3dg and C3d. FI requires the presence of a cofactor, examples of which include the blood-borne Factor H (FH) protein and the membrane-bound surface co-factor 'complement factor 1' (CR1; CD35). FH and CR1 also help to exert decay-accelerating activity, which can assist in the deconstruction of already formed C3 convertases.

FH is encoded by the *CFH* gene on human chromosome 1q32 within the RCA (regulators of complement) gene cluster. There is a naturally-occurring truncated form of FH called FH-like protein 1 (FHL-1) which arises from alternative splicing of the *CFH* gene and has cofactor activity like FH. FH comprises 20 CCP domains. FHL-1 is identical to FH for the first seven CCP domains before terminating with a unique 4-aa C terminus.

Proteins encoded by the *CFHR1-5* genes at the RCA locus also exert complement regulatory functions. The *CFHR1-5* genes encode a group of five secreted plasma proteins (FHR-1 to FHR-5) synthesised primarily by hepatocytes. The FHR proteins retain some sequence homology with C3b binding domains of FH and are thought to enhance complement activation (Skerka et al., *Mol Immunol.* 2013, 56:170-180).

One complement-related disorder is macular degeneration, e.g. age-related macular degeneration (AMD). Macular degeneration is believed to be driven in part by complement-mediated attack on ocular tissues. A major driver of AMD risk is genetic variation at the RCA locus resulting in dysregulation of the complement cascade. AMD is the leading cause of blindness in the developed world: currently responsible for 8.7% of all global blind registrations. It is estimated that 196 million people will be affected by 2020, increasing to 288 million by 2040 (Wong et al. *Lancet Glob Heal* (2014) 2:e106-16). AMD manifests as the progressive destruction of the macula, the central part of the retina at the back of the eye, leading to loss of central visual acuity. Early stages of the disease see morphological changes in the macula such as the loss of blood vessels in the choriocapillaris (Whitmore et al., *Prog Retin Eye Res* (2015) 45:1-29); a layer of capillaries found in the choroid (a highly vascularized layer that supplies oxygen and nutrition to the outer retina). The choriocapillaris is separated from the metabolically active retinal pigment epithelium (RPE) by Bruch's membrane (BrM); a thin (2-4 μ m), acellular, five-layered sheet of extracellular matrix. The BrM serves two major functions: the substratum of the RPE and a blood vessel wall. The structure and function of BrM is

reviewed e.g. in Curcio and Johnson, Structure, Function and Pathology of Bruch's Membrane, In: Ryan et al. (2013), Retina, Vol. 1, Part 2: Basic Science and Translation to Therapy. 5th ed. London: Elsevier, pp466-481, which is hereby incorporated by reference in its entirety.

5 The role of complement in AMD is reviewed, for example, by Zipfel et al. Chapter 2, in Lambris and Adamis (eds.), Inflammation and Retinal Disease: Complement Biology and Pathology, Advances in Experimental Medicine and Biology 703, Springer Science+Business Media, LLC (2010), which is hereby incorporated by reference in its entirety. The key characteristics of AMD are indicative of over-active complement, including cell/tissue destruction and a local inflammatory response. Hallmark
10 lesions of early AMD, termed drusen, develop within BrM adjacent to the RPE layer (Bird et al, *Surv Ophthalmol* 1995, 39(5):367-374). Drusen are formed from the accumulation of lipids, proteins and cellular debris, and include a swathe of complement activation products (Anderson et al., *Prog Retin Eye Res* 2009, 29:95–112; Whitcup et al., *Int J Inflam* 2013, 1-10). The presence of drusen within BrM disrupts the flow of nutrients from the choroid across this extracellular matrix to the RPE cells, which
15 leads to cell dysfunction and eventual death, leading to the loss of visual acuity.

'Dry' AMD, also known as geographic atrophy, represents around 50% of late-stage AMD cases. In the remaining percentage of late-stage cases, choroidal neovascularisation (CNV) develops, in which the increased synthesis of vascular endothelial growth factor (VEGF) by RPE cells promotes new
20 blood vessel growth from the choroid/choriocapillaris that breaks through BrM into the retina. These new blood vessels leak and eventually form scar tissue; this is referred to as 'wet' (neovascular or exudative) AMD. 'Wet' AMD is the most virulent form of late-stage AMD and has different disease characteristics to 'dry' AMD. There are treatments for wet AMD, where for example the injection of anti-VEGF agents into the vitreous of the eye can slow or reverse the growth of these blood vessels,
25 although it cannot prevent their formation in the first place. Geographic atrophy ('dry' AMD) remains untreatable.

FHL-1 predominates at BrM, suggesting an important role for this variant in protection of retinal tissue from complement-mediated attack (Clark, S.J., et al., *supra*). FH is found in the blood at a higher
30 concentration than FHL-1. Both FH and FHL-1 protect against complement over-activation in the ECM of the choroid (the capillary network underlying BrM). The role of the five FHR proteins are less well understood, although there is some evidence that they may counter the inhibitory effects of FH and FHL-1 (Clark, S.J. and P.N. Bishop, *J Clin Med*, 2015. 4(1): p. 18-31).

35 WO2019/215330 describes that FHR-4 is a positive regulator of complement activation and prevents FH-mediated C3b breakdown, leading to the formation of C3 convertase and the progression of the complement activation loop. High levels of circulating FHR-4 indicate an increased risk of developing complement-related disorders.

Defining the exact molecular changes and activation state underpinning the dysregulation of complement processes in human disease tissue remains problematic, largely because it requires measurements at the protein level and an understanding of the relative quantities of the different regulators. It is critical to be able to accurately measure absolute levels of FH and related RCA locus proteins in plasma, as well as levels of FI and C3b itself, for effective diagnosis and treatment of complement-related disorders. Whilst assays have been developed for FH, distinct measurement of FHL-1 and FHR1-5 is difficult due to high sequence homology between all these proteins. This sequence similarity has meant that, with the exception of the full length FH protein, it has proven difficult to generate antibodies which are specific to only one of these family members in order to obtain useful immunoassays.

In another example, a recent study quantified levels of FH and FHR1-5 using mass spectrometry but the assay was unable to detect biologically important isoform FHL-1 that is found at significant levels in the blood and at key sites of AMD pathogenesis (Zhang, P., et al., *Proteomics*. 2017;17(6):10).

The present invention seeks to address these issues.

Summary of the invention

The present invention relates to detecting complement proteins using mass spectrometry. The present application describes methods that are capable of distinguishing between different Complement Factor H, FHL-1 and the five Complement Factor H related (FHR) proteins, despite their sequence similarity. The method can also distinguish between breakdown products derived from C3, and other complement-related proteins with high sequence similarity.

Provided is a method for detecting at least one complement protein in a sample, the method comprising digesting the protein(s) with endoproteinase GluC to obtain one or more peptides; and detecting the one or more peptides by mass spectrometry.

The invention also provides a method for determining the level of at least one complement protein in a sample, the method comprising digesting the protein(s) with endoproteinase GluC to obtain one or more peptides; and determining the level of the one or more peptides by mass spectrometry.

In some embodiments the step of detecting the one or more peptides, and/or determining the level of the one or more peptides, consists of measuring the one or more peptides by mass spectrometry. In some embodiments the step of detecting the one or more peptides, and/or determining the level of the one or more peptides, involves measuring the level of the one or more peptides by mass spectrometry alone.

In some embodiments the method comprises determining the concentration of the complement protein(s) in the sample.

In another aspect the invention provides a method for preparing at least one complement protein for analysis, the method comprising digesting the protein(s) with endoproteinase GluC to obtain one or more peptides.

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In some embodiments the complement protein(s) is one or more of FH, FHL-1, FHR1, FHR2, FHR3, FHR4 and/or FHR5. In some embodiments the complement protein(s) is FH and/or FHL-1. In some embodiments the complement protein(s) is involved in the complement amplification loop and/or C3 convertase activity. In some embodiments the complement protein(s) is a breakdown product of C3b.

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In some embodiments the complement protein(s) is one or more of C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d. In some embodiments the complement protein(s) is one or more of C3, C3a, C3f, C3c, and/or C3d. In some embodiments the complement protein(s) is C3b and/or iC3b. In some embodiments the complement protein is FI. All combinations of the above proteins are envisaged.

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The methods described herein may involve detecting/determining the level of two or more complement proteins. In some embodiments, the method comprises simultaneous detection/determination of the levels of the two or more complement proteins.

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In some embodiments the sample has been obtained from a subject. In some embodiments the method comprises a step of obtaining the sample from a subject. In some embodiments the sample comprises, or is derived from, blood, lymph, plasma, serum, tissue or cells.

The peptides may be any suitable peptide disclosed herein. The one or more peptides may be selected from the group consisting of:

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(a) VTYKCFE (SEQ ID NO:20);

(b) NGWSPTPRCIRVSFTL (SEQ ID NO:21);

(c) ATFCDFPKINHGILYDEE (SEQ ID NO:22);

(d) RGWSTPPKCRSTISAE (SEQ ID NO:23) or AMFCDFPKINHGILYDEE (SEQ ID NO:24);

(e) VACHPGYGLPKAQTTVTCTE (SEQ ID NO:25);

(f) YQCQSYYE (SEQ ID NO:26);

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(g) RGWSTPPICSFTKGE (SEQ ID NO:27);

(h) GTAFVIFGIQDGE (SEQ ID NO:28);

(i) LRRQHARASHLGLARSNLDE (SEQ ID NO:29);

(j) LRRQHARASHLGLAR (SEQ ID NO:30) and/or LRRQHARASHLGLA (SEQ ID NO:156);

(k) LNLDVSLQLPSRSSKITHRIHWE (SEQ ID NO:31);

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(l) LNLDVSLQLPSR (SEQ ID NO:32);

(m) RLGRE (SEQ ID NO:33);

(n) SSKITHRIHWE (SEQ ID NO:34);

(o) SASLLR (SEQ ID NO:35) and/or SASLL (SEQ ID NO:157);

(p) RLGR (SEQ ID NO:36);

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(q) HLIVTPSGCGE (SEQ ID NO:37); and/or

(r) any one or more of SEQ ID NO:38 to 60.

The invention also provides the use of endoproteinase GluC for preparing at least one complement protein for detection by mass spectrometry, optionally for preparing at least two complement proteins for detection by mass spectrometry, e.g. any combination of proteins described herein.

Also provided is a method for detecting turnover of C3b, comprising a method for detecting at least one complement protein in a sample e.g. as described herein.

Also provided is a method of determining the presence and/or level of a complement protein in a subject, the method comprising performing a method as described herein. In some embodiments the method is performed on a sample obtained from the subject.

The invention also comprises a method of determining whether a subject is at risk of developing a complement-related disorder, the method comprising:

- a) digesting at least one complement protein in a sample obtained from the subject with endoproteinase GluC to obtain one or more peptides;
- b) determining the presence and/or level of the one or more peptides by mass spectrometry; and
- c) using the results of (b) to determine the likelihood of the subject to develop a complement-related disorder.

Also provided is a method of identifying a subject having a complement-related disorder, the method comprising:

- a) digesting at least one complement protein in a sample obtained from the subject with endoproteinase GluC to obtain one or more peptides;
- b) determining the presence and/or level of the one or more peptides by mass spectrometry; and
- c) using the results of (b) to determine whether the subject has a complement-related disorder.

In some embodiments the methods comprise (d) treating a subject who has been determined to be at risk of developing, or to have, a complement-related disorder. Treating a subject may comprise administering a therapeutically effective amount of a complement-targeted therapeutic to the subject.

Also provided is a method of selecting a subject for treatment of a complement-related disorder with a complement-targeted therapeutic, the method comprising:

- a) digesting at least one complement protein in a sample obtained from the subject with endoproteinase GluC to obtain one or more peptides;
- b) determining the presence and/or level of the one or more peptides by mass spectrometry; and
- c) using the results of (b) to determine whether the subject is in need of a complement-targeted therapeutic.

Also provided is a method of treating a subject who is suspected to have a complement-related disorder, the method comprising:

- a) digesting at least one complement protein in a sample obtained from the subject with endoproteinase GluC to obtain one or more peptides;
- 5 b) determining the presence and/or level of the one or more peptides by mass spectrometry; and based on the results of (b), treating said disorder.

In some embodiments the methods comprise administering an effective amount of a complement-targeted therapeutic to the subject, e.g. a subject identified as having a disorder or selected for treatment as described herein.

Also provided is a complement-targeted therapeutic for use in a method of treating a complement-related disorder in a subject, the method comprising:

- a) digesting at least one complement protein in a sample obtained from the subject with endoproteinase GluC to obtain one or more peptides;
- 15 b) determining the presence and/or level of the one or more peptides by mass spectrometry; and
- c) based on the results of (b), administering an effective amount of the complement-targeted therapeutic.

In some embodiments the methods comprise obtaining a sample from a subject comprising at least one complement protein. The sample may comprise or be derived from blood, lymph, plasma, serum, tissue or cells.

In some embodiments the complement-related disorder is macular degeneration. In some embodiments the complement-related disorder is selected from AMD, geographic atrophy ('dry' (i.e. non-exudative) AMD), early AMD, EOMD, intermediate AMD, late/advanced AMD, 'wet' (neovascular or exudative) AMD, choroidal neovascularisation (CNV) and/or retinal dystrophy.

In some embodiments the complement-related disorder is selected from Haemolytic Uremic Syndrome (HUS), atypical Haemolytic Uremic Syndrome (aHUS), DEAP HUS (Deficiency of FHR plasma proteins and Autoantibody Positive form of Hemolytic Uremic Syndrome), autoimmune uveitis, Membranoproliferative Glomerulonephritis Type II (MPGN II), sepsis, Henoch-Schönlein purpura (HSP), IgA nephropathy, chronic kidney disease, paroxysmal nocturnal hemoglobinuria (PNH), autoimmune hemolytic anemia (AIHA), systemic lupus erythematosus (SLE), Sjogren's syndrome (SS), rheumatoid arthritis (RA), C3 glomerulopathy (C3G), dense deposit disease (DDD), C3 nephritic factor glomerulonephritis (C3 NF GN), FHR5 nephropathy, hereditary angioedema (HAE), acquired angioedema (AAE), encephalomyelitis, atherosclerosis, neurodegeneration/neurodegenerative disease, dementia, multiple sclerosis (MS), cancer, stroke, Parkinson's disease, and/or Alzheimer's disease.

In any of the diagnostic or therapeutic methods described herein the complement protein may be any one or more of the proteins provided herein. i.e. any one or more, or any combination of, FH, FHL-1, FHR1, FHR2, FHR3, FHR4, FHR5, FI, C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d. The protein(s) may be involved in the complement amplification loop and/or C3 convertase activity. The complement protein may be a breakdown product of C3b. In some embodiments the complement protein(s) is one or more of FH, FHL-1, FHR1, FHR2, FHR3, FHR4 and/or FHR5. In some embodiments the complement protein is FH and/or FHL-1. In some embodiments the complement protein(s) is one or more of C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d. In some embodiments the complement protein(s) is one or more of C3, C3a, C3f, C3c, and/or C3d. In some embodiments the complement protein(s) is C3b and/or iC3b. In some embodiments the complement protein is FI. The one or more peptides may be any suitable peptide such as those disclosed herein, e.g. any one of SEQ ID NO:20 to 60.

In some embodiments an increase in the level of one or more of C3, C3b, C3a, iC3b, FHR1, FHR2, FHR3, FHR4 and/or FHR5; and/or a decrease in the level of one or more of iC3b, C3f, C3c, C3dg, C3d, C3g, FI, FH, FHL-1, FHR1, FHR2, FHR3, FHR4 and/or FHR5 as compared to reference value(s), indicates that the subject is at risk of developing or has a complement-related disorder.

Also provided is a kit for use in a method of detecting and/or determining the level of one or more complement protein(s) in a sample, the kit comprising endoproteinase GluC.

Description

Detection, differentiation and quantitation of highly similar proteins can be achieved using mass spectrometry (MS). In order to achieve good sensitivity by MS, proteins e.g. in a sample are routinely digested into peptides using a specific protease. The industry standard protease for this purpose is trypsin. Other enzymes that are commonly used to digest proteins for MS analysis include elastase, chymotrypsin or LysN.

Trypsin cleaves C-terminal to all K and R residues, provided they are not followed by a proline residue, and yields peptides which retain a basic group at their C-terminus which subsequently helps ionisation and transmission of peptides into the gas phase in a mass spectrometer. Peptides digested by trypsin tend to be ionised more efficiently during MS and thus produce a larger signal than peptides digested by non-trypsin enzymes. Using MS, individual peptides in the sample digest can be detected with a signal proportional to its abundance. The concentration of the parent protein can be derived from the relative abundance (signal) of endogenous peptide compared to an exogenous 'standard' peptide e.g. containing a stable isotope.

However, trypsin digestion of complement proteins FH and FHL-1 does not produce peptides that can be detected individually using MS alone. The only FHL-1 specific tryptic peptide is a 4-amino acid C-terminal sequence which is too small to be detected reliably by MS techniques. The FHR proteins

also share substantial sequence identity, meaning that it is hard to distinguish between them and measure them specifically using e.g. antibody-based assays.

The inventors have developed a unique targeted mass spectrometry assay using a non-standard proteolytic enzyme, GluC (V8 protease), to produce distinct proteotypic peptides for all of the FHR proteins, as well as proteotypic peptides that can be used to distinguish between FHL-1 and FH, which can be used for the simultaneous detection and accurate measurement in plasma of all seven key regulatory proteins encoded from the *CFH* gene cluster using a single MS assay, i.e. simultaneous detection of FH, FHL-1, and FHR1, FHR2, FHR3, FHR4 and FHR5.

FHL-1 is a distinct biological entity from FH. The proteins have a similar action but the size of FHL-1 means that its distribution in the body is likely to be distinct from FH. This is apparent in the eye where FHL-1 can cross to the retinal side of Bruch's membrane, e.g. where drusen form, but the larger FH protein cannot, see e.g. Clark et al., J Immunol 2014, 193(10) 4962-4970 and Clark et al., Frontiers in Immunology 2017 8:177, which are hereby incorporated by reference in their entirety. In this respect, there is evidence that FHL-1 is the prime driver of complement C3b turnover in the eye, meaning that levels of FHL-1 are likely to better inform disease risk than levels of FH.

GluC is also able to produce proteotypic peptides for C3b and FI, enabling direct measurement of C3b itself as well as levels of its proteolytic enzyme and required fluid-phase cofactors. Thus, the methods described herein mean that all these complement proteins can be measured using a single assay. Furthermore, breakdown of C3b occurs via trypsin-like cleavages at basic residues (K and R) so trypsin digestion of C3b breakdown products is unable to produce useful peptides for analysis. In contrast, C3 turnover can be measured using the MS approach of the present invention because GluC digestion also produces proteotypic neopeptides from many C3 inactivation and breakdown products generated during inactivating cleavages. The inventors demonstrate herein that a series of products produced as a result of C3/C3b cleavage can be detected and quantified using the same single GluC/MS assay. This allows the concentrations of all known C3 fragments e.g. iC3b, C3c, C3dg and C3d to be determined accurately. Thus, the methods described herein can not only measure absolute levels of regulatory complement proteins, but can also track protein products resulting from C3 inactivation and thus assess complement activation and the progression of the amplification loop.

This is advantageous because the measurement of C3 breakdown products is analytically challenging. The pattern by which C3 is broken down is complex: first into C3a and C3b, followed by cleavage of C3b into iC3b (which cannot drive formation of the membrane attack complex (MAC) but can still act as an opsonin), and then subsequent inactivating cleavage of iC3b into C3c via the release of a C3dg fragment. To approach detection of these products with antibodies is problematic. While each sequential cleavage step in this cascade generates a new proteoform (a distinct form of a protein encoded from the same gene, including cleaved forms and splice variants), they share

sequence homology and likely only undergo minor structural changes. Directing antibodies at each form is likely to be unsuccessful and while there are methods which can measure single components following some form of separation, e.g. C3dg following polyethylene glycol-based enrichment, simultaneous measurement of all fragments in the same sample is not currently possible.

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Thus, the present invention relates to a single methodology for concurrent determination of the presence, absolute levels and relative molar ratios of seven individual complement-related proteins from the *CFH* family plus C3b-inactivating enzyme FI, central complement component C3, and seven proteins derived from C3 breakdown, which may be referred to herein as the “complementome”. The ability to detect absolute levels of so many complement-related proteins in one assay is critical for the successful detection, diagnosis and treatment of complement-related diseases.

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Complement proteins

Complement is a central part of the innate immunity that serves as a first line of defence against foreign and altered host cells. Complement is activated upon infection with microorganisms to induce inflammation and promote elimination of the pathogens. The complement system is composed of plasma proteins produced mainly by the liver or membrane proteins expressed on cell surface. Complement operates in plasma, in tissues, or within cells. For a review of the complement system, see e.g. Merle NS et al., Front Immunol. 2015 Jun 2;6:262, which is hereby incorporated by reference in its entirety.

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The complement system can be activated via three distinct pathways: the classical pathway (CP), alternative pathway (AP) and lectin binding pathway (LP). In a healthy individual, the AP is permanently active at low levels to survey for presence of pathogens but host cells are protected against complement attack and are resistant to persistent low-level activation. C3b molecules bound to host cells are inactivated rapidly by a group of membrane-bound or plasma complement regulators.

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In response to the recognition of molecular components of microorganisms, complement proteins become sequentially activated in an enzyme cascade: the activation of one protein enzymatically cleaves and activates the next protein in the cascade.

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The three pathways converge into the generation of a C3 convertase, which cleaves the central complement component C3 into activation products C3b, a large fragment that acts as an opsonin (binds to foreign microorganisms to increase their susceptibility to phagocytosis), and C3a, an anaphylatoxin that promotes inflammation. Along with factor B (FB), C3b forms the C3 convertase (C3bBb) which cleaves further C3 molecules, generates more C3b and C3a, and amplifies C3b deposition on cell surfaces. This is the complement amplification loop. C3b deposition and activation of complement may occur on acellular structures (i.e. on extracellular matrix), such as Bruch's membrane (BrM) and the intercapillary septa of the choriocapillaris in the eye.

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Activated C3 can trigger the lytic pathway, which can damage the plasma membranes of cells and some bacteria. C5a, another anaphylatoxin produced by this process, attracts macrophages and neutrophils and also activates mast cells.

Once activated, the complement system needs tight control, as newly generated complement activation products, e.g. C3b, can induce severe inflammation and cell damage to the host. A number of soluble as well as membrane bound complement regulators ensure regulation of complement activation at the surface of host cells and control different activation phases and sites of action (Skerka et al., Mol Immunol 2013, 56:170-180). Complement regulators are described further herein.

"Complement protein" may be used interchangeably herein with "complement regulator", "a regulator of complement", or "protein of the complement system" and refers to a protein component of the complement system or complement cascade, e.g. as described in Merle et al., Front. Immunol., 2015, 6:262 and Merle et al., Front. Immunol., 2015, 6:257, which are hereby incorporated by reference in their entirety. A "complement protein" referred to herein may be involved in any of the three complement pathways and/or in the amplification loop.

In some embodiments a "complement protein" referred to herein is involved in the alternative pathway and/or the complement activation loop. In some embodiments, a "complement protein" referred to herein is involved in the breakdown, turnover and/or inactivation of C3 or C3b, or is a product of said breakdown, turnover and/or inactivation.

In some embodiments, a "complement protein" as used herein may refer to one or more of FH, FHL-1, FHR1, FHR2, FHR3, FHR4, FHR5, FI, C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d.

Complement Factor H (FH) protein family

Factor H (FH) regulates the alternative complement pathway and the amplification loop. It inhibits C3 convertase formation by competing with FB binding to C3b and also acts as a cofactor for C3b inactivation to iC3b by Factor I (FI), thus preventing inappropriate complement activation and inflammation. FH also exerts decay-accelerating activity, which can assist in the deconstruction of already formed C3 convertases, see e.g. Clark et al., J Immunol 2014, 193(10) 4962-4970, which is hereby incorporated by reference in its entirety.

The sequence of human FH (Uniprot P08603-1) is provided herein as SEQ ID NO:1. For a review of FH structure and function see e.g. Merle NS et al., Front Immunol. 2015 Jun 2;6:262, which is hereby incorporated by reference in its entirety.

Human FH comprises 20 CCP domains. The *CFH* gene also produces a truncated form of FH, called FHL-1, comprising only the first seven CCP domains before terminating with a unique 4-amino acid C

terminus (Clark et al, 2014 *supra*). The sequence of human FHL-1 (Uniprot: P08603-2) is provided herein as SEQ ID NO:2.

In the eye, full-length FH protein is found on the choroidal side of Bruch's membrane (BrM), with particular accumulation in the choriocapillaris (capillary layer in the choroid). Small amounts have also been found in patches on the RPE side of the BrM, but no FH was observed in the BrM itself. FHL-1 on the other hand has been observed throughout BrM and other ECM structures e.g. drusen (Clark et al, 2014 *supra*). It is likely that FHL-1 confers greater complement protection to BrM than does FH, whereas FH provides the main protection for the ECM of the choroid. It is thought that FHL-1 is therefore a major regulator of complement in the BrM (a key site in AMD pathogenesis). The methods described herein allow for the individual detection and quantitation of FH and FHL-1.

The *CFHR1-5* genes encode a group of five secreted plasma proteins (FHR-1 to FHR-5) synthesised primarily by hepatocytes. FH, FHL-1 and FHR1-FHR5 are described in e.g. Clark et al., J Clin Med, 2015. 4(1): 18-31, which is hereby incorporated by reference in its entirety.

The FHR proteins retain some sequence homology with C3b binding domains of FH and are thought to enhance complement activation, see e.g. Skerka et al., Mol Immunol 2013, 56:170-180, which is hereby incorporated by reference in its entirety. These proteins are highly related and share a high degree of sequence identity. The N termini share 36-94% sequence identity, whilst the C-terminal domains are very similar to the FH C-terminus (36-100%). The high amino acid identity among family members is demonstrated by the fact that antibodies raised against FH can detect multiple FHR proteins in plasma and that antibodies generated against FHR proteins cross-react with the other FHRs. This cross-reactivity presents a challenge for purification of FHR proteins from plasma, as well as determining their concentration.

FHR proteins are divided into two groups depending on their conserved domains. FHR1 (SEQ ID NO:3), FHR2 (SEQ ID NO:3, 4), and FHR5 (SEQ ID NO:10) form Group I and are characterised by their conserved N-termini. They exist in plasma as homo- and heterodimers, mediated by the conserved N-terminal domains. Group II contains FHR3 (SEQ ID NO:6, 7) and FHR4 (SEQ ID NO:8, 9) which lack the N-terminal dimerisation domains, but which show a high degree of sequence similarity to portions of FH. All five FHR proteins comprise C-termini sequences that act to recognise and bind C3b, and which are very similar to the C-terminus of FH.

FHR1 is known to compete with FH and FHL-1 for binding to C3b. It is also reported to bind to C3b components of the C5 convertase and interfere with the assembly of the MAC (see e.g. Heinen S et al., Blood (2009) 114 (12): 2439-2447 and Hannan JP et al., PLoS One. 2016; 11(11):e0166200, which are hereby incorporated by reference in their entirety). As used herein, the term "FHR1" includes at least one of FHR1 (SEQ ID NO:3; FHRA) and a second FHR1 isoform (FHRB) with 3 point mutations, and preferably includes both FHR1 isoforms. "FHR1" refers to FHR1 from any species and

includes isoforms, fragments, variants or homologues of FHR1 from any species. In preferred embodiments, "FHR1" refers to human FHR1.

FHR2 may inhibit C3 convertase activity, acting to inhibit the amplification loop, but may also activate the amplification loop. There are two FHR2 isoforms (SEQ ID NO:4 and 5). The protein has two glycosylated forms, a single glycosylated form (24 kDa) and a double glycosylated form (28 kDa). As used herein, the term "FHR2" includes at least one of the two isoforms or at least one of the glycosylated forms, and preferably includes both isoforms and any glycosylated forms. "FHR2" refers to FHR2 from any species and includes isoforms, fragments, variants or homologues of FHR2 from any species. In preferred embodiments, "FHR2" refers to human FHR2.

FHR3 binds to C3b and C3d and may have low cofactor activity for FI-mediated cleavage of C3b. FHR3 may also upregulate complement. There are two FHR3 isoforms (SEQ ID NO:6 and 7). FHR3 is detected in plasma in multiple variants (ranging from 35 to 56 kDa), reflecting the existence of four different glycosylated variants of FHR3. As used herein, the term "FHR3" includes at least one of the two isoforms or at least one of the glycosylated variants of FHR3, and preferably includes both isoforms and any glycosylated forms. "FHR3" refers to FHR3 from any species and includes isoforms, fragments, variants or homologues of FHR3 from any species. In preferred embodiments, "FHR3" refers to human FHR3.

The human *CFHR4* gene encodes two proteins: FHR4A (SEQ ID NO:8) and FHR4B (SEQ ID NO:9), an alternative splice variant. WO 2019/215330, hereby incorporated by reference in its entirety, describes that FHR4 is a positive regulator of complement activation and prevents FH-mediated C3b breakdown. High levels of FHR-4 in tissues are likely to promote local inflammatory responses and cell lysis, leading to disorders associated with complement activation, and circulating FHR4 levels can be used as an indicator of risk of developing complement-related disorders, see e.g. Cipriani et al., *Nat Commun* 11, 778 (2020), hereby incorporated by reference in its entirety. As used herein, the term "FHR4" includes at least one of FHR4A isoform 1, FHR4A isoform 2 (G20 point deletion from isoform 1) or FHR4B, and preferably includes FHR4A isoforms 1 and 2 as well as FHR4B. "FHR4" refers to FHR4 from any species and includes isoforms, fragments, variants or homologues of FHR4 from any species. In preferred embodiments, "FHR4" refers to human FHR4.

FHR5 also recognises and binds to C3b on self surfaces. FHR5 appears as a glycosylated protein of 62 kDa. As used herein, the term "FHR5" includes any glycosylated variants of FHR5, and preferably includes all isoforms and any glycosylated forms. As used herein, "FHR5" refers to FHR5 from any species and includes isoforms, fragments, variants or homologues of FHR5 from any species. In preferred embodiments, "FHR5" refers to human FHR5.

Given the different roles of the different members of the *CFH* family in activation and amplification of complement and pathogenesis of complement-related disorders, it is important to be able to

distinguish between the presence and levels of all seven *CFH* family members. *CFH* family members, particularly FHR1-5, can also be used as biomarkers for diagnosing or predicting disorders in which dysregulation of complement is pathologically implicated.

5 *C3, C3b and breakdown products*

C3 is the central complement component. The pathways by which C3 is processed into various downstream products can lead to activation of complement, e.g. including inflammation and immune responses, or to the inactivation and regulation of complement. It is therefore important in terms of complement pathogenesis and treatment of complement-related disorders to be able to detect and measure the levels, including relative levels, of C3, C3b and their downstream components/processing products.

Processing of C3 is described, for example, in Foley et al. J Thromb Haemostasis (2015) 13: 610-618, which is hereby incorporated by reference in its entirety. Human C3 (UniProt: P01024; SEQ ID NO:12) comprises a 1,663 amino acid sequence (including an N-terminal, 22 amino acid signal peptide). Amino acids 23 to 667 encode C3 β chain (SEQ ID NO:13), and amino acids 749 to 1,663 encode C3b α' chain (SEQ ID NO:14). C3 β chain and C3 α' chain associate through interchain disulphide bonds (formed between cysteine 559 of C3 β chain, and cysteine 816 of the C3 α' chain) to form C3b. C3a is a 77 amino acid fragment corresponding to amino acid positions 672 to 748 of C3 (SEQ ID NO:15), generated by proteolytic cleavage of C3 to form C3b.

Processing of C3b to the inactive form iC3b, which cannot itself promote further complement amplification, involves proteolytic cleavage of the C3b α' chain at amino acid positions 1303 and 1320 to form an α' chain fragment 1 (corresponding to amino acid positions 749-1663 of C3; SEQ ID NO:16), and an α' chain fragment 2 (corresponding to amino acid positions 1321 to 1,663 of C3; SEQ ID NO:17). Thus, iC3b comprises the C3 β chain, C3 α' chain fragment 1 and C3 α' chain fragment 2 (associated via disulphide bonds). Cleavage of the α' chain also liberates C3f, which corresponds to amino acid positions 1304 to 1320 of C3 (SEQ ID NO:18).

iC3b is processed further to C3c comprising the C3 β chain, C3 α' chain fragment 2 and C3c α' chain fragment 1 (corresponding to amino acid positions 749-954 of C3; SEQ ID NO:19). This cleavage event produces fragment C3dg (corresponding to amino acid positions 955-1303 of C3; SEQ ID NO:142), which is itself broken down into fragments C3g (corresponding to amino acid positions 955-1001 of C3; SEQ ID NO:143) and C3d (corresponding to amino acid positions 1002-1303 of C3; SEQ ID NO:144).

Processing of C3b to iC3b is performed by Complement Factor I (FI; encoded in humans by the gene *CFI*). Human Complement Factor I (UniProt: P05156; SEQ ID NO:11) has a 583 amino acid sequence (including an N-terminal, 18 amino acid signal peptide). Amino acids 340 to 574 of the light

chain encode the proteolytic domain of FI, which is a serine protease containing the catalytic triad responsible for cleaving C3b to produce iC3b (Ekdahl et al., J Immunol (1990) 144 (11): 4269–74). Proteolytic cleavage of C3b by FI to yield iC3b is facilitated by co-factors, including FH, CR1 and possibly some of the FHR proteins. Co-factors for FI typically bind to C3b and/or FI, and potentiate processing of C3b to iC3b by FI.

As used herein, any reference to a complement protein, e.g. C3, C3b, C3a, FH, FI etc, refers to said protein from any species and include isoforms, fragments, variants or homologues of said protein from any species. In some embodiments, the protein is a mammalian protein (e.g. cynomolgous, human and/or rodent (e.g. rat and/or murine) protein). Isoforms, fragments, variants or homologues of the complement proteins described herein may optionally be characterised as having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of the immature or mature protein from a given species, e.g. human protein sequences provided herein. Isoforms, fragments, variants or homologues of complement proteins described herein may optionally be functional isoforms, fragments, variants or homologues, e.g. having a functional property/activity of the reference protein, as determined by analysis by a suitable assay for the functional property/activity.

Methods for detecting complement proteins

The present invention relates to detecting the presence of, and/or determining the level of, one or more complement proteins using suitable analytical techniques, e.g. as described herein.

In some aspects the present invention provides a method for detecting a complement protein, the method comprising contacting the protein with endoproteinase GluC to obtain one or more peptides, and detecting the one or more peptides by mass spectrometry.

In some aspects the present invention provides a method for determining the level of a complement protein, the method comprising contacting, e.g. digesting, the protein with GluC to obtain one or more peptides, and determining the level of the one or more peptides by mass spectrometry. In some cases, the methods involves both detecting a complement protein and determining the level of a complement protein. The protein may be the same protein, or the methods may involve detection of a first complement protein and determining the level of a second complement protein.

In any and all methods described herein, the step of detecting/determining the level of the one or more peptides consists of detecting/determining the level of/measuring the peptide(s) by mass spectrometry. That is, the step of detecting/determining the level of/measuring the peptide(s) is performed by mass spectrometry only. Measuring the peptide(s) may include detecting the presence or absence of the one or more peptides, and/or determining the level, amount and/or concentration of each peptide in the sample.

The term “digesting” as used herein refers to placing the protein in contact with GluC under suitable conditions, e.g. temperature, pH etc, and for a suitable time such that the protein is digested, i.e. cleaved, into two or more fragments. In some cases, the digesting involves incubating the protein with GluC under suitable conditions, e.g. as described herein.

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In some aspects the present invention provides a method for preparing a complement protein for analysis, the method comprising contacting/digesting the protein with endoproteinase GluC to obtain one or more peptides. In some cases, the method comprises preparing a complement protein for subsequent analysis. The one or more peptides may then be subjected to an analytical technique, e.g. mass spectrometry or any other suitable analytical technique. In some cases the method comprises preparing a complement protein for analysis by mass spectrometry. The analytical technique may be used to detect the presence and/or level of the one or more peptides.

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It will be appreciated that where “complement protein” is referred to herein in the singular (i.e. “a/the complement protein”), pluralities/groups/populations of different complement proteins are also contemplated. For example, any disclosure herein comprising a complement protein also comprises more than one complement protein, i.e. at least one protein, or one or more proteins. In all aspects and embodiments described herein, “a/the complement protein” may refer to “at least one complement protein”.

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“Detecting” a protein as used herein refers to identifying/observing the presence or existence of the protein, e.g. in a sample, cell, tissue or subject.

The “level” of a complement protein used herein refers to the level, amount or concentration of said protein, e.g. in a sample, cell, tissue or subject. The term “determining the level”, e.g. of a protein, used herein refers to the measurement and/or quantification of the level, amount or concentration of a protein. In some cases, “determining the level” includes calculating the level, amount or concentration of a protein in a sample. The sample may be from a subject. In some cases, “determining the level” includes calculating the level, amount or concentration of a protein in a subject, e.g. using a sample taken from the subject. “Determining the level” of a protein may include digesting the protein with GluC to obtain one or more peptides, detecting the one or more peptides as described herein and then calculating the level, amount or concentration of the protein/peptide, e.g. in a sample.

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In some cases, “determining the level” comprises quantifying, i.e. measuring the quantity of, the level, amount or concentration of a protein e.g. in a sample or in a subject. “Determining the level” may include determining the concentration of a protein. Quantification/measuring may include comparing the level, amount or concentration of a protein with a reference value, and/or comparing the level, amount or concentration of a protein with that in a control sample e.g. taken from the subject at a different time point, or taken from a healthy subject, e.g. one known not to have a complement-related disorder.

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In methods described herein the level of the complement protein(s) is compared to the level of a reference value or level, sometimes called a control. In some cases the level of the complement protein(s) is compared to the level of the same complement protein in a control subject that does not have a complement-related disorder. A reference value may be obtained from a control sample, which itself may be obtained from a control subject. Data or values obtained from the individual to be tested, e.g. from a sample, can be compared to data or values obtained from the control sample. In some cases, the control is a spouse, partner, or friend of the subject.

As used herein the term "reference value" refers to a known measurement value used for comparison during analysis. In some cases, the reference value is one or a set of test values obtained from an individual or group in a defined state of health. The reference value may be one or a set of test values obtained from a control. In some cases, the reference value is/has been obtained from determining the level of complement proteins in subjects known not to have a complement-related disorder. In some cases, the reference value is set by determining the level or amount of a complement protein previously from the individual to be tested e.g. at an earlier stage of disease progression, or prior to onset of the disease. The reference value may be taken from a sample obtained from the same subject, or a different subject or subject(s). The sample may be derived from the same tissue/cells/bodily fluid as the sample used by the present invention. The reference value may be a standard value, standard curve or standard data set. Values/levels which deviate significantly from reference values may be described as atypical values/levels.

In some cases the control may be a reference sample or reference dataset, or one or more values from said sample or dataset. The reference value may be derived from a reference sample or reference dataset. The reference value may be derived from one or more samples that have previously been obtained from one or more subjects that are known not to have a complement-related disorder and/or known or expected not to be at risk of developing a complement-related disorder. The reference value may be derived from one or more samples that have previously been obtained from one or more subjects that are known to have a complement-related disorder. The reference value may be derived from one or more samples that have previously been obtained from one or more subjects that are known to be at risk of developing a complement-related disorder. The reference value may be an average, or mean, value calculated from a reference dataset, e.g. a mean protein level. The reference dataset/value may be obtained from a large-scale study of subjects known to have a complement-related disorder.

The reference value may be derived from one or more samples that have previously been obtained from one or more subjects that are in the same family as the subject of interest, or from one or more subjects that are not in the same family as the subject of interest.

The reference value may be derived from one or more samples that have previously been obtained and/or analysed from the individual/subject/patient to be tested, e.g. a sample was obtained from the individual when they were at an earlier stage of a complement-related disorder, or a sample was obtained from the individual before the onset of a complement-related disorder.

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The reference value may be obtained by performing analysis of the sample taken from a control subject in parallel with a sample from the individual to be tested. Alternatively, the control value may be obtained from a database or other previously obtained value. The reference value may be determined concurrently with the methods disclosed herein, or may have been determined previously.

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Control subjects from which samples are/have been obtained may have undergone treatment for a complement-related disorder and/or received a complement-related therapy/therapeutic agent.

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Controls may be positive controls in which the target molecule is known to be present, or expressed at high level, or negative controls in which the target molecule is known to be absent or expressed at low level.

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Samples, e.g. from one or more control subjects, may comprise any one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen or sixteen of FHR1, FHR2, FHR3, FHR4, FHR5, FH, FHL-1, FI, C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d, or any combination thereof. In some cases each complement protein is in a separate control sample. In some cases a control sample contains multiple complement proteins. In some cases the methods described herein comprise comparing the level of one or more complement proteins determined as described herein to different, e.g. one or more, samples, each sample containing one or more complement proteins. In some cases the methods described herein comprise comparing the level of one or more complement proteins determined as described herein to a single sample, wherein the sample contains one or more complement proteins.

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In some cases control samples are obtained from the same tissue(s) as the sample obtained from the individual to be tested. In some cases control samples are obtained from different tissue(s) as the sample obtained from the individual to be tested. Control samples may be obtained from control subjects at certain time(s) of day, or on certain days. Sample(s) obtained from the individual to be tested are preferably obtained at the same time(s) of day and/or day(s) as the control samples.

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In some embodiments the methods comprise detecting/determining the level of a complement protein in a sample. The sample may be *in vitro* or *ex vivo*. A sample may have been taken from a subject. A sample may be taken from any tissue or bodily fluid. In preferred arrangements the sample is taken from a bodily fluid, more preferably one that circulates through the body. Accordingly, the sample may be a blood sample or lymph sample. In a particularly preferred arrangement the sample is a blood sample or blood-derived sample. The blood-derived sample may be a selected fraction of a

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patient or subject's blood, e.g. a selected cell-containing fraction or a plasma or serum fraction. A selected serum fraction may comprise the fluid portion of the blood obtained after removal of the fibrin clot and blood cells. Alternatively the sample may comprise or may be derived from a tissue sample, biopsy or isolated cells from said individual. The sample may be taken from the eye, kidney, brain or liver, e.g. comprising cells from the eye, kidney, brain or liver. The sample may comprise retinal tissue. The sample may comprise RPE cells or tissue from Bruch's membrane or the choroid. The sample may comprise drusen or other deposits of complement-related components.

In some embodiments the methods described herein comprise taking or obtaining a sample from a subject, e.g. blood, tissue etc. In some embodiments the methods described herein are performed on a sample that has been obtained/was obtained from a subject. In some cases the sample is a blood sample. The blood sample may undergo/have undergone processing to obtain a plasma sample or a serum sample. In some cases, the methods comprise obtaining a blood-derived sample from a subject. In some cases, the methods comprise obtaining a plasma or serum sample from a subject. In some embodiments the methods comprise isolating protein, e.g. total protein, from the sample. Suitable techniques to isolate protein from biological samples are well known in the field. In some embodiments the methods do not comprise isolating protein from the sample, e.g. the methods are performed on the unprocessed sample.

In some embodiments, the methods are performed *in vitro*. For example, the presence, level, amount and/or concentration of the complement protein(s) may be detected/determined *in vitro*.

In some cases the methods involve determining the presence, level, amount and/or concentration of the complement protein(s) in a subject. This may involve performing the methods described herein *in vitro*, and using the results to calculate the presence, level, amount and/or concentration of the protein(s) in the subject.

Also provided is a method for detecting at least one complement protein in a sample, the method comprising digesting the protein(s) in the sample with endoproteinase GluC to obtain one or more peptides; and using mass spectrometry to detect the one or more peptides in the sample.

Also provided is a method for determining the level of at least one complement protein in a sample, the method comprising digesting the protein(s) in the sample with endoproteinase GluC to obtain one or more peptides and using mass spectrometry to determine the level of the one or more peptides in the sample.

Using mass spectrometry to detect one or more peptides in a sample, or detecting and/or determining the level of one or more peptides by mass spectrometry, e.g. by the methods described herein, may include applying a mass spectrometry technique to the sample, e.g. by putting the sample in a mass

spectrometer, and instructing the mass spectrometer to analyse the sample. Various suitable mass spectrometry techniques are disclosed herein and are within the routine tasks of a skilled person.

In any aspect provided herein, the methods described herein may comprise both detecting at least one complement protein and determining the level of at least one complement protein. The complement protein may be the same protein, and/or the methods may comprise detecting at least a first complement protein and determining the level of at least a second complement protein.

In some embodiments, the methods described herein comprise detecting/determining the level of one complement protein. In some embodiments, the methods described herein comprise detecting/determining the level of at least one complement protein, one or more complement proteins, and/or groups or complement proteins e.g. as provided herein.

In some embodiments the complement protein is encoded from the RCA (regulators of complement) gene cluster, or RCA locus, on human chromosome 1. The RCA cluster is located on chromosome 1q32 and includes the *CFH* and *CFHR1-5* genes. The gene cluster also includes the membrane bound proteins CR1 (CD35), CR2 (CD21), decay-accelerating factor (DAF; CD55), and membrane cofactor protein (MCP; CD46), as well as soluble C4b-binding protein (C4bp).

The methods described herein are suitable for detecting/determining the level of multiple complement proteins via a single assay: i.e. using a single enzyme, GluC, to obtain analysable peptides and then using a single analytical technique, mass spectrometry, to detect and/or determine the levels of said peptides. In this way, the complementome of a sample or a subject can be determined via a single assay.

In some embodiments the methods described herein comprise detecting/determining the level of any one or more, e.g. any or all combinations, of FH, FHL-1, FHR1, FHR2, FHR3, FHR4, and/or FHR5. In some embodiments the complement protein(s) is/are selected from the group consisting of FH, FHL-1, FHR1, FHR2, FHR3, FHR4, and/or FHR5. In some cases the methods comprise detecting/determining the level of any one, two, three, four, five, six and/or seven of FH, FHL-1, FHR1, FHR2, FHR3, FHR4, and FHR5, alone or in combination. In some cases the methods described herein are able to differentiate (i.e. distinguish, discriminate, separate) between the presence of (or levels of) each of FH, FHL-1, FHR1, FHR2, FHR3, FHR4 and/or FHR5.

In some cases the complement protein to be detected/determine the level of is FH and/or FHL-1. In some cases the methods described herein comprise detecting/determining the level of both FH and FHL-1. In some cases the methods described herein differentiate (i.e. distinguish, discriminate, separate) between the presence of FH and the presence of FHL-1 and/or between the level/concentration of FH and the level/concentration of FHL-1. In some cases the methods described herein permit or allow the detection of FH alone, i.e. without detecting FHL-1. In some

cases the methods described herein permit or allow the detection of FHL-1 alone, i.e. without detecting FH.

In some cases the complement protein is any one or more, e.g. any or all combinations, of FHR1, FHR2, FHR3, FHR4 and/or FHR5. In some cases the complement protein is FHR4. In some cases the methods described herein are able to differentiate (i.e. distinguish, discriminate, separate) between the presence of (or levels of) each of FHR1, FHR2, FHR3, FHR4 and/or FHR5. In some cases, the methods described herein permit or allow the detection of FHR1 alone, i.e. without detecting FHR2-FHR5. In some cases, the methods described herein permit or allow the detection of FHR2 alone, i.e. without detecting FHR1 or FHR3-FHR5. In some cases, the methods described herein permit or allow the detection of FHR3 alone, i.e. without detecting FHR1, FHR2, FHR4, or FHR5. In some cases, the methods described herein permit or allow the detection of FHR4 alone, i.e. without detecting FHR1-FHR3 or FHR5. In some cases, the methods described herein permit or allow the detection of FHR5 alone, i.e. without detecting FHR1-FHR4.

In some embodiments the complement protein to be detected/the level of which is determined is involved with breakdown, turnover and/or inactivation of C3/C3b. In some embodiments, the complement protein is produced by the breakdown and/or inactivation of C3/C3b, i.e. is a product of C3b inactivation/breakdown. In some embodiments the methods described herein include determining the presence, rate and/or progression of C3b turnover. In some embodiments the methods described herein involve detecting/determining the level of a protein involved in, or produced as a result of, the complement amplification loop. In some embodiments the methods described herein involve detecting/determining the level of a protein involved in the generation or breakdown of C3 convertase. In some cases the protein is a cofactor for FI, e.g. FH, CR1, some of the FHR proteins. Any method disclosed herein, e.g. a method for detecting at least one complement protein in a sample comprising digesting proteins with GluC and detecting the resulting peptides by mass spectrometry, may be described in the alternative as a method for detecting C3 turnover, a method for detecting C3 breakdown, a method for measuring C3b turnover or C3b breakdown, or a method for measuring the progress of C3b turnover or C3b breakdown.

Thus, in some aspects the present invention provides a method for detecting turnover or breakdown of C3b, comprising the steps described herein, e.g. digesting at least one complement protein with endoproteinase GluC to obtain one or more peptides and detecting the peptide(s) by mass spectrometry. In some cases the method comprises digesting and then detecting at least two, three, four or more, up to 16, of the 16 complement proteins described herein.

In some embodiments the methods described herein comprise/further comprise detecting/determining the level of FI, either alone or in combination with other complement proteins such as those described herein.

In some embodiments the methods described herein comprise detecting/determining the level of any one or more, e.g. any or all combinations, of C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d. In some embodiments the complement protein(s) is/are selected from the group consisting of C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d. In some cases the methods comprise detecting/determining the level of any one, two, three, four, five, six, seven and/or eight of C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d, in any combination. In some embodiments the methods described herein comprise detecting/determining the level of one or more of C3, C3a, C3f, C3c, and/or C3d. In some cases the methods described herein comprise determining the presence and/or level of C3b, iC3b, and/or C3dg, e.g. via the methodology in Table 3. In some cases the methods described herein comprise detecting/determining the level of C3, C3b and/or iC3b. In some cases the methods described herein are able to differentiate (i.e. distinguish, discriminate, separate) between the presence of (or levels of) two or more, or all, of C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d.

The methods described herein can detect multiple complement proteins, and distinguish between said complement proteins, using one enzyme e.g. GluC and one analytical method e.g. mass spectrometry. The methods described herein may be used to detect/determine the level of any one of the individual proteins described herein, as well as any and all combinations of FH, FHL-1, FHR1, FHR2, FHR3, FHR4, FHR5, FI, C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d, i.e. any one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen and/or sixteen of these proteins in any combination. In some embodiments the complement protein(s) is/are selected from the group consisting of FH, FHL-1, FHR1, FHR2, FHR3, FHR4, FHR5, FI, C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d. In some cases the methods described herein may be used to detect/determine the level of FHL-1 and to detect/determine the level of any one or more of FH, FHR1, FHR2, FHR3, FHR4, FHR5, FI, C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d. In some cases the method comprises distinguishing (i.e. differentiating, discriminating, separating) between the presence/level of FHL-1 and the presence/level of any one or more of FH, FHR1, FHR2, FHR3, FHR4, FHR5, FI, C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d. The terms "distinguishing", "differentiating", "discriminating", and "separating" are used interchangeably herein.

In some cases the methods provided herein allow for simultaneous detection of one or more of FH, FHL-1, FHR1, FHR2, FHR3, FHR4, FHR5, FI, C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d, including any combination thereof. In some cases the methods provided herein allow for detection/determination of the level of one or more of FH, FHL-1, FHR1, FHR2, FHR3, FHR4, FHR5, FI, C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d, including any combination thereof, in a single assay. The methods provided herein allow for distinct, separable and detectable peptides to be produced from every protein listed above such that the presence and/or level of each protein can be distinguished from the others.

In some cases, the present invention provides a method for detecting and/or determining the level of at least two complement proteins in a sample simultaneously and/or in one assay, the method comprising:

digesting the proteins with endoproteinase GluC to obtain one or more peptides; and

detecting and/or determining the level of the one or more peptides by mass spectrometry.

In any method described herein, the complement protein may be any protein involved in one or more of the complement system pathways. For example, the complement protein may be one or more of C1, C2, C4b2a C4, C4a, C5, C5a, FB, FD, C3Bb, MASP1, MASP2, C1q, C1r, C1s, C6, C7, C8, C9, CD59, Clusterin, Properdin, and/or Compstatin. In any embodiment described herein, the complement protein to be detected (or the protein whose level is determined) is not one or more of C1, C2, C4b2a C4, C4a, C5, C5a, FB, FD, C3Bb, MASP1, MASP2, C1q, C1r, C1s, C6, C7, C8, C9, CD59, Clusterin, Properdin, and/or Compstatin.

In some cases, the present invention uses endoproteinase GluC for preparing at least one complement protein for detection by mass spectrometry. In some cases the invention uses endoproteinase GluC for preparing at least two, i.e. multiple or a plurality of, complement proteins for detection by mass spectrometry. The at least two complement proteins may be any two, three, four or more, up to 16, of FH, FHL-1, FHR1, FHR2, FHR3, FHR4, FHR5, FI, C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d, in any combination, as described herein.

Endoproteinase GluC, also known as glutamyl endopeptidase, is a serine proteinase which preferentially cleaves peptide bonds C-terminal to glutamic acid residues. It also cleaves at aspartic acid residues at a rate 100-300 times slower than at glutamic acid residues. The specificity of GluC depends on the pH and the buffer composition. At pH 4, the enzyme preferentially cleaves at the C terminus of E, whereas at pH 8 it additionally cleaves at D residues. The sequence of GluC is provided in SEQ ID NO:153 and 154.

In preferred embodiments, the methods described herein use GluC alone (i.e. only GluC) to digest the one or more complement proteins. In preferred embodiments, a step of digesting the protein(s) in the described methods consists of digesting the protein(s) with GluC. In preferred embodiments, any method described herein does not employ/use any other protease alone or in combination with GluC. For example, in some embodiments the digestion step of any method described herein does not use, or is not performed by, any one or more of the following enzymes or agents: trypsin, chymotrypsin (high specificity or low specificity), Lys-C, Lys-N, Arg-C, Asp-N, elastase, LysargiNase, pepsin, Sap9, OmpT, BNPS-skatole, any caspase, clostripain (clostridiopeptidase B), CNBr, enterokinase, factor Xa, granzymeB, neutrophil elastase, proteinase K, thermolysin, non-GluC glutamyl endopeptidase e.g. GluBI or GluSGB, proline endopeptidase, TEV protease, thrombin, formic acid, hydroxylamine, iodosobenzoic acid, and/or NTCB (or any combination thereof).

GluC is obtainable from standard reagent providers e.g. Sigma Aldrich, NEB etc, and may be used according to the accompanying instructions or according to protocols well known in the field. An example protocol is described herein. Obtaining proteins from biological samples and suitable buffers to prepare samples/proteins for GluC digestion will also be known to the skilled person. An example cell lysis buffer comprises: 8 M urea (4.8 g per 10 ml) in 50 mM NH_4HCO_3 and 20mM methylamine, diluted to a urea concentration of <2 M, pH 8 (40 mg per 10 ml), containing 1 tablet of cOmplete™ Mini EDTA-free protease inhibitor cocktail per 10 ml of lysis buffer.

In some cases, a complement protein is contacted/incubated/digested with GluC enzyme for at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, or at least 24 hours. In some cases a complement protein is contacted/incubated/digested with GluC enzyme for at least 12 hours. In some cases a complement protein is contacted/incubated/digested with GluC enzyme for about 12 hours, e.g. 12 hours. In some cases a complement protein is contacted/incubated/digested with GluC enzyme for about 16 hours, e.g. 16 hours. The terms contacted, incubated and digested are used interchangeably herein.

In some cases a complement protein is contacted/incubated/digested with GluC enzyme at a temperature of at least 20°C, at least 21°C, at least 22°C, at least 23°C, at least 24°C, at least 25°C, at least 26°C, at least 27°C, at least 28°C, at least 29°C, or at least 30°C. In some cases a complement protein is contacted/incubated/digested with GluC enzyme at a temperature of at least 25°C. In some cases a complement protein is contacted/incubated/digested with GluC enzyme at a temperature of about 25°C, e.g. 25°C.

In some cases a complement protein is contacted/incubated/digested with GluC enzyme at a pH of at least 7.0, at least 7.1, at least 7.2, at least 7.3, at least 7.4, at least 7.5, at least 7.6, at least 7.7, at least 7.8, at least 7.9, at least 8.0, at least 8.1, at least 8.2, at least 8.3, at least 8.4, at least 8.5, at least 8.6, at least 8.7, at least 8.8, at least 8.9, or at least 9.0. In some cases a complement protein is contacted/incubated/digested with GluC enzyme at a pH of at least 8.0. In some cases a complement protein is contacted/incubated/digested with GluC enzyme at a pH of about 8.0, e.g. a pH of 8.0.

In some cases the GluC enzyme and complement protein are contacted/incubated at a wt/wt ratio of 1/75. The incubation step may comprise gentle shaking, e.g. at 400 rpm.

The methods described herein may comprise a contacting/incubation/digestion step comprising any combination of temperature, pH, and/or time as described above. In some cases, contacting/incubating/digesting is performed at 25°C at pH8 for 12 hours.

In some embodiments the invention provides a method for detecting and/or determining the level of at least one complement protein e.g. in a sample, the method comprising:

digesting the protein(s) with endoproteinase GluC to obtain one or more peptides, the digesting comprising incubating the protein(s) with GluC at 25°C at pH8 for up to 12 hours; and detecting the one or more peptides by mass spectrometry.

- 5 In some embodiments the invention provides a method for detecting and/or determining the level of at least one complement protein e.g. in a sample, the method comprising:
digesting the protein(s) with endoproteinase GluC to obtain one or more peptides, the digesting comprising incubating the protein(s) with GluC at 25°C at pH8 for up to 16 hours; and
detecting the one or more peptides by mass spectrometry.

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The following peptides may be produced by GluC digestion of complement proteins, e.g. as described herein. In some embodiments, the methods described herein comprise detecting/determining the level of any one or more of these peptides, i.e. any one or more of SEQ ID NO:20 to 141, or 155, 156 or 157, in any combination. All combinations of peptides are envisaged. The mass of peptides
15 represented by SEQ ID NOs 20-27 can be found in Table 1.

In some embodiments, the FH peptide is VTYKCFE (SEQ ID NO:20).

- In some embodiments the FH peptide is any one or more of SNTGSTTGSIVCGYNGWSDLPICYE
20 (SEQ ID NO:112; mass 2623.1206), NGWSPTPRCIRVKTCSSSIDIE (SEQ ID NO:113; mass 2576.2839), LPKIDVHLVPDRKKDQYKVG (SEQ ID NO:114; mass 2476.3801),
YYCNPRFLMKGPNIQCVDGE (SEQ ID NO:115; mass 2474.1545), NYNIALRWTAQKQLYSRTGE
(SEQ ID NO:116; mass 2411.2709), KWSHPPSCIKTDCSLPSFE (SEQ ID NO:117; mass 2274.0813), HGWAQLSSPPYYYGDSVE (SEQ ID NO:118; mass 2054.9010),
25 ISHGVAHMSDSYQYGEE (SEQ ID NO:119; mass 2007.8632), FDHNSNIRYRCRGKE (SEQ ID NO:120; mass 1893.9016), ITCKDGRWQSIPLCVE (SEQ ID NO:121; mass 1846.9069),
GWIHTVCINGRWDPE (SEQ ID NO:122; mass 1781.8307), KAKYQCKLGYVTADGE (SEQ ID NO:123; mass 1772.8767), TTCYMGKWSSPPQCE (SEQ ID NO:124; mass 1716.6946),
SYAHGTKLSYTCE (SEQ ID NO:125; mass 1458.6449), RVRVYQCRSPYE (SEQ ID NO:126; mass
30 1455.7041), GFGIDGPAIAKCLGE (SEQ ID NO:127; mass 1446.7176), HGTINSSRSSQE (SEQ ID NO:128; mass 1301.5960), YQCQONLYQLE (SEQ ID NO:129; mass 1300.5758), WTTLPVCIVEE
(SEQ ID NO:130; mass 1288.6373), KIPCSQPPQIE (SEQ ID NO:131; mass 1238.6329),
SQYTYALKE (SEQ ID NO:132; mass 1101.5342), QVQSCGPPPE (SEQ ID NO:133; mass 1040.4597), KKDVYKAGE (SEQ ID NO:134; mass 1036.5553), GLPCKSPPE (SEQ ID NO:135; mass
35 926.4531), KVSVLCQE (SEQ ID NO:136; mass 904.4688), HLKNKKE (SEQ ID NO:137; mass 895.5239), GGFRISSE (SEQ ID NO:138; mass 893.4243), LLNGNVKE (SEQ ID NO: 139; mass 885.4920), YPTCAKR (SEQ ID NO:140; mass 837.4167), or STCGDIPE (SEQ ID NO:141; mass 820.3273).

- 40 In some embodiments, the FHL-1 peptide is NGWSPTPRCIRVSFTL (SEQ ID NO:21).

In some embodiments, the FHR1 peptide is ATFCDFPKINHGILYGEE (SEQ ID NO:22).

In some embodiments the FHR1 peptide is NYNIALRWTAQKQKLYLRTGE (SEQ ID NO:91; mass 2437.3230).

In some embodiments the FHR2 peptide is RGWSTPPKCRSTISAE (SEQ ID NO:23).

In some embodiments the FHR2 peptide is AMFCDFPKINHGILYDEE (SEQ ID NO:24). In some embodiments the FHR2 peptide is YNFVSPSKSFWTRITCAEE (SEQ ID NO:92; mass 2264.0572).

In some embodiments the FHR3 peptide is VACHPGYGLPKAQTTVTCTE (SEQ ID NO:25).

In some embodiments the FHR3 peptide is any one or more of KGWSPTPRCIRVRTCSKSDIE (SEQ ID NO:93; mass 2418.2260), NGYNQNYGRKFVQGNSTE (SEQ ID NO:94; mass 2074.9457), QVKPCDFPDIKHGGLFHE (SEQ ID NO:95; mass 2066.0043), FMCKLGYNANTSILSFQAVCRE (SEQ ID NO:96; mass 2494.1807), or YQCQPYYE (SEQ ID NO:97; mass 1092.4222).

In some embodiments the FHR4 peptide is YQCQSYYE (SEQ ID NO:26).

In some embodiments the FHR4 peptide is any one or more of NSRAKSNGMRFKLHDTLDYE (SEQ ID NO: 98; mass 2381.1546), DGWSHFPTCYNSSSE (SEQ ID NO:99; mass 1628.6202), ISYGNTTGSIVCGE (SEQ ID NO:100; mass 1399.6289), or FMCKLGYNANTSVLSFQAVCRE (SEQ ID NO:101; mass 2480.1650).

In some embodiments the FHR5 peptide is RGWSTPPICSFTKGE (SEQ ID NO:27).

In some embodiments the FHR5 peptide is any one or more of GTLCDFPKIHGFLYDEE (SEQ ID NO:102; mass 2119.9673), YAMIGNNMITCINGIWTE (SEQ ID NO:103; mass 2042.9264), YGYVQPSVPPYQHGVSVSE (SEQ ID NO:104; mass 2004.9581), GDTVQIICNTGYSLQNE (SEQ ID NO:105; mass 1967.8895), IVCKDGRWQSLPRCVE (SEQ ID NO:106; mass 1887.9447), DYNPFSQVPTGE (SEQ ID NO:107; mass 1352.5884), QVKTCGYIPE (SEQ ID NO:108; mass 1136.5536), ANVDAQPKKE (SEQ ID NO:109; mass 1098.5669), WTLPTCVE (SEQ ID NO:110; mass 1048.4899), or KVAVLCKE (SEQ ID NO:111; mass 888.5102).

In some cases, the methods described herein comprise detecting/determining the level of one or more of SEQ ID NOs 21-27, in any combination.

In some cases, any method described herein may comprise detecting/determining the level of one or more of SEQ ID NOs 28-37, 156 or 157, in any combination. In some cases, the methods provided

herein are used to detect C3, C3b and breakdown products using one or more or all of the peptides in Table 2 in any combination, plus optionally SEQ ID NO:156 and/or 157, for example according to the methodology in Table 3.

- 5 In some embodiments the FI peptide is any one or more of VKLVDQDKTMFICKSSWSMRE (SEQ ID NO:45; mass 2531.2455), VKLISNCSKFYGNRFYE (SEQ ID NO:46; mass 2068.0320), CLHPGKFLNNGTCTAE (SEQ ID NO:47; mass 1805.8309), NYNAGTYQNDIALIE (SEQ ID NO:48; mass 1698.7969), GKFSVSLKHGNTDSE (SEQ ID NO:49; mass 1605.7867), VGCAGFASVTQEE (SEQ ID NO:50; mass 1297.5729), VGCAGFASVTQE (SEQ ID NO:155; mass 1168.272),
- 10 MKKDGNGKKDCE (SEQ ID NO:51; mass 1295.6082), YVDRIIFHE (SEQ ID NO:52; mass 1191.6156), CLHVHCRGLE (SEQ ID NO:53; mass 1166.5557), RVFSLQWGE (SEQ ID NO:54; mass 1121.5738), ILTADMDAE (SEQ ID NO:55; mass 978.4448), or KVTYTSQE (SEQ ID NO:56; mass 955.4731).

In some embodiments the FI peptide is any one or more of

- 15 CAGTYDGSIDACKGDSGGPLVCM DANNVTYVWGVVSWGE (SEQ ID NO:38; mass 3996.7183), GTCVCKLPYQCPKNGTAVCATNRRSFPTYCQKSLE (SEQ ID NO:39; mass 3994.8853), FPGVYTKVANYFDWISYHVGRPFISQYNV (SEQ ID NO:40; mass 3467.7211), ANVACL DLGFQQGADTQRRFKLS DLSINSTE (SEQ ID NO:41; mass 3397.6804), LPRSIPACVPWSPYLFQPNDCIVSGWGRE (SEQ ID NO:42; mass 3388.6605),
- 20 KKCLAKKYTHLSCDKVFCQPWQRCIE (SEQ ID NO:43; mass 3155.5773), LCKKACQKGKGFHCKSGVCIPSQYQCNGE (SEQ ID NO:44; mass 2991.2861), VKLVDQDKTMFICKSSWSMRE (SEQ ID NO:45; mass 2531.2455), VKLISNCSKFYGNRFYE (SEQ ID NO:46; mass 2068.0320), CLHPGKFLNNGTCTAE (SEQ ID NO:47; mass 1805.8309), NYNAGTYQNDIALIE (SEQ ID NO:48; mass 1698.7969), GKFSVSLKHGNTDSE (SEQ ID NO:49; mass 1605.7867), VGCAGFASVTQEE (SEQ ID NO:50; mass 1297.5729), MKKDGNGKKDCE (SEQ ID NO:51; mass 1295.6082), YVDRIIFHE (SEQ ID NO:52; mass 1191.6156), CLHVHCRGLE (SEQ ID NO:53; mass 1166.5557), RVFSLQWGE (SEQ ID NO:54; mass 1121.5738), ILTADMDAE (SEQ ID NO:55; mass 978.4448), KVTYTSQE (SEQ ID NO:56; mass 955.4731), VDCITGE (SEQ ID NO:57; mass 736.3182), NCGKPE (SEQ ID NO:58; mass 647.2817), TSLAE (SEQ ID NO:59; mass
- 25 520.2613), or KDNE (SEQ ID NO:60; mass 505.2252).

Peptides detected by the methods described herein may optionally have at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequences of the peptides described herein, e.g. any one of SEQ ID NOS

35 21-141. Other suitable peptides may be readily determined by a skilled person and may be employed in the methods described herein. In preferred embodiments, the peptides used according to the methods herein permit mass spectrometry techniques to distinguish or differentiate between two or more complement proteins in a sample.

Mass spectrometry

Methods provided herein comprising detecting and/or determining the levels of proteins may involve using mass spectrometry to detect and/or determine the levels of proteins in a sample.

- 5 In preferred embodiments, any method described herein involves using only mass spectrometry (i.e. mass spectrometry alone) to detect/determine the level of the one or more peptides. That is, in some embodiments, the methods provided herein do not employ multiple analytical techniques and the peptide(s) are detected/determined/measured using a single assay. In preferred embodiments, the methods described herein do not detect/determine the level of/measure the peptide(s) using mass
- 10 spectrometry in combination with another analytical technique suitable for detecting proteins/peptides. In preferred embodiments, detection/determination of the level of the one or more peptides is not performed at any stage using a non-mass spectrometry technique, e.g. detection/determination of the level of the peptide(s) is not performed using high performance liquid chromatography (HPLC), immunological-based methods such as quantitative enzyme-linked immunosorbent assays (ELISA),
- 15 Western blotting, protein immunoprecipitation, dot blotting or immunoelectrophoresis, electrophoresis or autoradiography. In some cases, liquid chromatography-mass spectrometry (LC/MS) is not used.

As used herein, "detecting and/or determining the level of" e.g. a complement protein or peptide "by mass spectrometry" is the same as "using mass spectrometry to detect and/or determine the level of"

20 e.g. a complement protein or peptide.

Mass spectrometry is a well-known analytical technique for analysing a sample that typically comprises generating ions from the sample, optionally fragmenting the ions, separating the ions according to their mass/charge ratio (in time and/or space), and detecting the ions to provide

25 information regarding the content of the sample.

For the purpose of detecting and/or determining the levels of proteins, at least one fragmentation step may be included.

- 30 Mass spectrometry techniques are well-known in the field and any suitable mass spectrometry technique may be employed for detecting and/or determining the levels of proteins in a sample, e.g. LC/MS, GC/MS, tandem mass spectrometry (MS/MS), quadrupole MS e.g. triple quadrupole MS (TQMS), time-of-flight MS e.g. MALDI-TOF, targeted MS e.g. selected reaction monitoring MS (SRM-MS)/multiple reaction monitoring (MRM-MS), parallel-reaction monitoring (PRM-MS), trapped-ion
- 35 based methods e.g. three-dimensional quadrupole ion traps ("dynamic" traps) and ion cyclotron resonance mass spectrometers ("static" traps), quadrupole trap MS, hybrid linear trap orbitrap MS, quadrupole-Orbitrap MS, electrospray ionization mass spectrometry (ESI-MS), or electron transfer dissociation MS (ETD).

In some embodiments, the mass spectrometry technique may be a liquid chromatography-selected reaction monitoring mass spectrometry (LC-SRM-MS)-based assay.

Fragmenting the ions may be achieved using any suitable fragmentation technique, e.g. collision-induced dissociation (CID)/collisionally activated dissociation (CAD), electron-capture dissociation (ECD), electron transfer dissociation (ETD), in-source decay (ISD), infrared multiple photon dissociation (IRMPD) etc. Again, such techniques are well known.

The mass spectrometry techniques useful in the present invention may comprise quantitative analysis. Mass spectrometry methods comprising quantitative analysis may comprise a targeted approach to detect and measure peptides of interest and their corresponding fragments. This may allow for greater specificity and sensitivity for quantification. Quantitative mass spectrometry in proteomics is reviewed in e.g. Bantscheff, M., et al. *Anal Bioanal Chem* 2007, 389, 1017–1031, which is hereby incorporated by reference in its entirety.

For example, input peptides may undergo fragmentation in a collision cell, thus generating product ions exclusive to the peptides. Both the intact peptide mass and one or more specific fragment ions of that peptides can be monitored over the course of an MS experiment e.g. using SRM/MRM, PRM etc.

The observed m/z ratio of a peptide and its corresponding product ion m/z ratio are referred to as a “transition”, i.e. a mass pair representing the m/z of an analyte (the parent ion) and the m/z of one of its product ions which is formed upon fragmentation of the parent ion.

It is well within the routine work of a skilled person to develop suitable transitions for quantitative mass spectrometry techniques, e.g. SRM/MRM-MS and PRM-MS. Mead et al., *Mol Cell Proteomics*. 2009 Apr; 8(4): 696–705, hereby incorporated by reference in its entirety, describes one such technique for designing transitions.

Tables 7 and 8 provide examples of transitions for the complement proteins described herein, based on fragmentation of synthetic versions of each peptide of interest. Suitable alternative transitions may also be used, the identification of which is well within the routine remit of a skilled person.

Quantitation can be achieved by ‘spiking’ the sample with known quantities of labelled synthetic peptides. The combination of retention time, peptide mass, and fragment mass practically eliminates ambiguities in peptide assignments and extends the quantification range to 4–5 orders of magnitude. In some cases, the methods provided herein comprise a step of determining optimised MS settings and/or quantitation reference values using stable isotopic standards.

Mass spectrometry techniques that may be used in the present invention may comprise targeted or semi-targeted MS workflows and/or data-dependent acquisition (DDA) or data-independent acquisition (DIA) techniques.

- 5 DDA uses knowledge obtained during the acquisition to decide which MS1 peptide precursors to subject for fragmentation (MS/MS) in the collision cell. DIA, in contrast, performs predefined MS/MS fragmentation and data collection regardless of sample content, which allows for more sensitive and accurate protein quantification compared to DDA. DIA strategies can be further segregated into targeted or untargeted acquisitions. Targeted DIA methods fragment predefined precursor ions that
- 10 correspond to the peptide analytes, usually at known (measured or predicted) retention times. Targeted DIA has become widely used in academic, pharmaceutical, and biotechnology research for quantification of small molecules (metabolites), peptides, and post-translational modifications (PTMs). For example, selected-reaction monitoring (SRM), a type of targeted DIA, is currently considered the gold standard method for mass spectrometric quantification due to its high accuracy and precision.
- 15 For a review of DIA techniques, see e.g. Meyer and Schilling, Expert Rev Proteomics. 2017 May; 14(5): 419–429, hereby incorporated in its entirety.

- Other suitable DIA methods include e.g. Sequential Window Acquisition of All Theoretical mass spectrometry (SWATH MS; see e.g. Ludwig et al., Mol Syst Biol (2018)14:e8126), SONAR
- 20 (Waters.com), or Online Parallel Accumulation–Serial Fragmentation (PASEF; see e.g. Meier et al., J Proteome Res. 2015 Dec 4;14(12):5378-87 and Meier et al., Mol Cell Proteomics. 2018 Dec; 17(12): 2534–2545).

Applications of the methods

- In some aspects the invention provides methods for assessing the risk of onset or risk of progression
- 25 of a complement-related disorder using the detection/determination methods described herein.

- The methods for assessing risk may be diagnostic, prognostic and/or predictive of the risk of onset or progression of a complement-related disorder. Diagnostic methods can be used to determine the diagnosis or severity of a disease, prognostic methods help to predict the likely course of disease in a
- 30 defined clinical population under standard treatment conditions, and predictive methods predict the likely response to a treatment in terms of efficacy and/or safety, thus supporting clinical decision-making. The methods described herein may be useful in monitoring the success of treatment, including past or ongoing treatment, for complement-related disorders.

- 35 The terms “disorder”, “disease” and “condition” may be used interchangeably and refer to a pathological issue of a body part, organ or system which may be characterised by an identifiable group of signs or symptoms. The term “complement-related disorder” refers to disorders, diseases or conditions that comprise or arise from deficiencies or abnormalities in the complement system. In some embodiments, the complement-related disorder is a disorder driven by complement activation

or complement over-activation. The terms “develop”, “developing”, and “development”, e.g. of a disorder, as used herein refer both to the onset of a disease as well as the progression, exacerbation or worsening of a disease state.

- 5 In some embodiments the disorder is one in which the complement system, or activation/over-activation/dysregulation thereof, is pathologically implicated. The complement related disorder may be any disorder described herein. “Pathologically implicated” as used herein may refer to a protein level which is raised or lowered in the disorder compared with a reference value, and/or where the protein contributes towards the pathology of the disorder. The selection or combination of
10 complement protein(s) detected may depend on the complement-related disorder of interest and the complement protein(s) that are useful biomarkers for said disorder.

- The complement-related disorder may comprise disruption of the classical, alternative and/or lectin complement pathways. In some cases, the disorder may be associated with deficiencies in,
15 abnormalities in, or absence of regulatory components of the complement system. In some embodiments, the disorder may be a disorder associated with the alternative complement pathway, disruption of the alternative complement pathway and/or associated with deficiencies in, abnormalities in, or absence of regulatory components of the alternative complement pathway. In some cases the disorder is associated with the complement amplification loop. In some cases the disorder is
20 associated with inappropriate activation, over-activation, or dysregulation of the complement system, in whole or in part, e.g. C3 convertase assembly, C3b production, C3b deposition, and/or the amplification loop.

- In some cases, the disorder is associated with any one or more of C3, C3b, iC3b, FI, FH, FHL-1, or
25 FHR1-FHR5. In some cases, the disorder is associated with deficiencies or abnormalities in the activity of any one or more of C3, C3b, iC3b, FI, FH, FHL-1, or FHR1-FHR5. In some cases one or more of these proteins are pathologically implicated in the disorder, e.g. have raised or lower levels compared with a reference value.

- 30 In some cases, the disorder is associated with one or more of CR1, CD46, CD55, C4BP, Factor B (FB), Factor D (FD), SPICE, VCP (or VICE) and/or MOPICE. In some cases, the disorder is associated with deficiencies or abnormalities in the activity of one or more of CR1, CD46, CD55, C4BP, Factor B, Factor D, SPICE, VCP (or VICE) and/or MOPICE, or where one or more of these proteins are pathologically implicated.

- 35 In some embodiments, the disorder may be a disorder associated with C3 or a C3-containing complex, an activity/response associated with C3 or a C3-containing complex, or a product of an activity/response associated with C3 or a C3-containing complex. That is, in some embodiments, the disorder is a disorder in which C3, a C3-containing complex, an activity/response associated with C3
40 or a C3-containing complex, or the product of said activity/response is pathologically implicated. In

some embodiments, the disorder may be associated with an increased level of C3 or a C3-containing complex, an increased level of an activity/response associated with C3 or a C3-containing complex, or an increased level of a product of an activity/response associated with C3 or a C3-containing complex as compared to the control state. In some embodiments, the disorder may be associated with a decreased level of C3 or a C3-containing complex, a decreased level of an activity/response associated with C3 or a C3-containing complex, or a decreased level of a product of an activity/response associated with C3 or a C3-containing complex as compared to the control state.

In some embodiments, the disorder may be a disorder associated with C3b or a C3b-containing complex, an activity/response associated with C3b or a C3b-containing complex, or a product of an activity/response associated with C3b or a C3b-containing complex. That is, in some embodiments, the disorder is a disorder in which C3b, a C3b-containing complex, an activity/response associated with C3b or a C3b-containing complex, or the product of said activity/response is pathologically implicated. In some embodiments, the disorder may be associated with an increased level of C3b or a C3b-containing complex, an increased level of an activity/response associated with C3b or a C3b-containing complex, or increased level of a product of an activity/response associated with C3b or a C3b-containing complex as compared to the control state. In some embodiments, the disorder may be associated with a decreased level of C3b or a C3b-containing complex, a decreased level of an activity/response associated with C3b or a C3b-containing complex, or a decreased level of a product of an activity/response associated with C3b or a C3b-containing complex as compared to the control state.

In some embodiments, the disorder may be a disorder associated with any one or more of FH, FHL-1, FI, FHR1-FHR5, FB, FD, CR1 and/or CD46, an activity/response associated with any one or more of FH, FHL-1, FI, FHR1-FHR5, FB, FD, CR1 and/or CD46 or a product of an activity/response associated with any one or more of FH, FHL-1, FI, FHR1-FHR5, FB, FD, CR1 and/or CD46. In some embodiments, the disorder is a disorder in which any one or more of FH, FHL-1, FI, FHR1-FHR5, FB, FD, CR1 and/or CD46, an activity/response associated with any one or more of FH, FHL-1, FI, FHR1-FHR5, FB, FD, CR1 and/or CD46, or the product of said activity/response is pathologically implicated. In some embodiments, the disorder may be associated with a decreased level of any one or more of FH, FHL-1, FI, FHR1-FHR5, FB, FD, CR1 and/or CD46, a decreased level of an activity/response associated with any one or more of FH, FHL-1, FI, FHR1-FHR5, FB, FD, CR1 and/or CD46, or a decreased level of a product of an activity/response associated with any one or more of FH, FHL-1, FI, FHR1-FHR5, FB, FD, CR1 and/or CD46 as compared to a control state.

In some embodiments, the disorder may be associated with an increased level of any one or more of FHR1-FHR5, an increased level of an activity/response associated with any one or more of FHR1-FHR5, or an increased level of a product of an activity/response associated with any one or more of FHR1-FHR5 as compared to a control state, see e.g. Zhu et al., *Kidney Int.* 2018 Jul;94(1):150-158; Pouw et al., *Front Immunol.* 2018 Apr 24;9:848; both hereby incorporated by reference in their

entirety. In some embodiments the disorder may be associated with an increased level of FHR4, an increased level of an activity/response associated with FHR4, or an increased level of a product of an activity/response associated with FHR4 as compared to a control state, see e.g. WO 2019/215330 and Cipriani et al., *Nat Commun* 11, 778 (2020), both hereby incorporated by reference in their entirety. The methods may comprise determining the systemic level of FHR4.

In some embodiments the disorder is associated with increased levels of any one or more of C3, C3b, C3 convertase and/or C3bBb as compared to a control state. In some embodiments the disorder is associated with decreased levels of any one or more of C3, C3b, C3 convertase and/or C3bBb as compared to a control state. In some embodiments, the disorder is associated with increased levels of iC3b as compared to a control state. In some embodiments, the disorder is associated with decreased levels of iC3b as compared to a control state. In some embodiments the disorder is associated with increased levels of any one or more of C3a, C3f, C3c, C3dg, C3d, and/or C3g as compared to a control state. In some embodiments the disorder is associated with decreased levels of any one or more of C3a, C3f, C3c, C3dg, C3d, and/or C3g as compared to a control state.

In some cases, methods provided herein are useful for determining whether a complement-related disorder is associated with over-activation of the complement system. In some cases, the methods are capable of determining whether elevated levels of a complement protein, e.g. those described herein, are contributing to complement over-activation and/or complement-related disorders, e.g. as compared to a control subject that does not have a complement-related disorder.

The disorder may be an ocular disorder. In some embodiments, a disease or condition to be assessed, diagnosed, treated or prevented as described herein is a complement-related ocular disease. In some embodiments, the disorder is macular degeneration. In some embodiments, the disorder may be selected from, i.e. is one or more of, age-related macular degeneration (AMD), choroidal neovascularisation (CNV), macular dystrophy, and diabetic maculopathy. As used herein, the term "AMD" includes early AMD, intermediate AMD, late/advanced AMD, geographic atrophy ('dry' (i.e. non-exudative) AMD), and 'wet' (i.e. exudative or neovascular) AMD, each of which may be a disorder in its own right that is detected, treated and/or prevented as described herein. In some embodiments the disease or condition to be treated or prevented is a combination of the diseases/conditions above, e.g. 'dry' and 'wet' AMD. In some embodiments the disease or condition to be treated or prevented is not 'wet' AMD or choroidal neovascularisation. AMD is commonly-defined as causing vision loss in subjects age 50 and older. In some embodiments a subject to be treated is age 50 or older, i.e. is at least 50 years old.

As used herein "early AMD" refers to a stage of AMD characterised by the presence of medium-sized drusen, commonly having a diameter of up to ~200 μm , within Bruch's membrane adjacent to the RPE layer. Subjects with early AMD typically do not present with significant vision loss. As used herein "intermediate AMD" refers to a stage of AMD characterised by large drusen and/or pigment

changes in the retina. Intermediate AMD may be accompanied by some vision loss. As used herein “late AMD” refers to a stage of AMD characterised by the presence of drusen and vision loss, e.g. severe central vision loss, due to damage to the macula. In all stages of AMD, ‘reticular

pseudodrusen’ (RPD) or ‘reticular drusen’ (also referred to as subretinal drusenoid deposits (SDD))

may be present, referring to the accumulation of extracellular material in the subretinal space between the neurosensory retina and RPE. “Late AMD” encompasses ‘dry’ and ‘wet’ AMD. In ‘dry’ AMD (also known as geographic atrophy), there is a gradual breakdown of the light-sensitive cells in the macula that convey visual information to the brain and of the supporting tissue beneath the macula. In ‘wet’ AMD (also known as choroidal neovascularization, neovascular and exudative AMD), abnormal blood vessels grow underneath and into the retina. These vessels can leak fluid and blood which can lead to swelling and damage of the macula and subsequent scar formation. The damage may be rapid and severe.

In some embodiments the disorder is early-onset macular degeneration (EOMD). As used herein

“EOMD” refers to a phenotypically severe sub-type of macular degeneration that demonstrates a much earlier age of onset than classical AMD and results in many more years of substantial visual loss. Sufferers may show an early-onset drusen phenotype comprising uniform small, slightly raised, yellow subretinal nodules randomly scattered in the macular, also known as ‘basal laminar drusen’ or ‘cuticular drusen’. EOMD may also be referred to as “middle-onset macular degeneration”. The EOMD subset is described in e.g. Boon CJ et al. Am J Hum Genet 2008; 82(2):516-23, van de Ven JP, et al. Arch Ophthalmol 2012;130(8):1038-47, and Taylor, R.L. et al., Ophthalmol. 2019, 126, 1410-1421, all of which are hereby incorporated by reference in their entirety. As with other types of macular degeneration, EOMD is related to complement dysregulation and disrupted Factor H activity. In some embodiments a subject to be treated is age 49 or younger. In some embodiments a subject to be treated is between ages 15 and 49, i.e. is between 15 and 49 years old. In some embodiments the disease or condition to be treated is a macular dystrophy. A macular dystrophy can be a genetic condition, usually caused by a mutation in a single gene, that results in degeneration of the macula.

In some embodiments the disorder is one associated with the kidney, e.g. nephropathy/a

nephropathic disorder. In some cases, the disorder is a neurological and/or neurodegenerative disorder. In some cases, the disorder is associated with autoimmunity, e.g. an autoimmune disease. In some cases, the disorder is associated with inflammation, e.g. an inflammatory disease. In some cases the disorder is characterised by the deposition of C3, e.g. the glomerular pathologies (see e.g. Skerka et al 2013, *supra*).

In some embodiments the disorder may be selected from Haemolytic Uremic Syndrome (HUS), atypical Haemolytic Uremic Syndrome (aHUS), DEAP HUS (Deficiency of FHR plasma proteins and Autoantibody Positive form of Hemolytic Uremic Syndrome), autoimmune uveitis,

Membranoproliferative Glomerulonephritis Type II (MPGN II), sepsis, Henoch-Schönlein purpura (HSP), IgA nephropathy, chronic kidney disease, paroxysmal nocturnal hemoglobinuria (PNH),

autoimmune hemolytic anemia (AIHA), systemic lupus erythematosus (SLE), Sjogren's syndrome (SS), rheumatoid arthritis (RA), glomerular diseases, C3 glomerulopathy (C3G), dense deposit disease (DDD), C3 nephritic factor glomerulonephritis (C3 NF GN), FHR5 nephropathy, hereditary angioedema (HAE), acquired angioedema (AAE), encephalomyelitis, atherosclerosis, multiple sclerosis (MS), stroke, Parkinson's disease, and Alzheimer's disease.

In some cases, the disorder is cancer. The cancer may be a liquid or blood cancer, such as leukemia, lymphoma or myeloma. In other cases, the cancer is a solid cancer, such as breast cancer, lung cancer, liver cancer, colorectal cancer, nasopharyngeal cancer, kidney cancer or glioma. In some cases, the cancer is located in the liver, bone marrow, lung, spleen, brain, pancreas, stomach or intestine. In some cases the cancer is lung cancer. In some cases the cancer is glioblastoma (glioblastoma multiforme (GBM)).

In some cases the disorder is neurodegeneration or neurodegenerative disease. The disorder may comprise progressive atrophy and loss of function of neurons. The disorder may be selected from Parkinson's disease, Alzheimer's disease, dementia, stroke, Lewy body disease, Amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Huntington's disease and prion diseases.

The role of complement in various diseases is described in e.g. Morgan, B.P., *Complement in the pathogenesis of Alzheimer's disease*. Semin Immunopathol, 2018. 40(1): p. 113-124; Halbgebauer, R., et al., *Janus face of complement-driven neutrophil activation during sepsis*. Semin Immunol, 2018. 37: p. 12-20; Ma, Y., et al., *Significance of Complement System in Ischemic Stroke: A Comprehensive Review*. Aging Dis, 2019. 10(2): p. 429-462; Bonifati and Kishore, *Role of complement in neurodegeneration and neuroinflammation*. Mol Immunol. 2007 Feb;44(5):999-1010; Kleczko, E.K., et al., *Targeting the Complement Pathway as a Therapeutic Strategy in Lung Cancer*. Front Immunol, 2019. 10: p. 954; and Schafer N. et al., *Complement Regulator FHR-3 Is Elevated either Locally or Systemically in a Selection of Autoimmune Diseases*, Front Immunol. 2016; 7: 542, which are all hereby incorporated by reference in their entirety. For example, FHL-1 is expressed more in certain tumour cell lines than FH (Junnikkala et al (2000) J. Immunol. 164: 6075-81) and glioblastoma tumours have been shown to express FHR proteins (DeCordova et al. (2019) Immunobiology 224: 625-631), both references hereby incorporated in their entirety. Being able to measure and differentiate between FH family proteins is advantageous.

In some aspects, the present invention provides methods of predicting, based on the analysis described herein of a sample from a subject, whether a subject is at risk of developing a complement-related disorder, has a complement-related disorder, is in need of treatment for a complement-related disorder, will respond to treatment for a complement-related disorder, and/or is responding/has responded to treatment for a complement-related disorder. The methods may be used for determining whether a subject is at risk of onset of the disorder, and/or is at risk of progression, exacerbation or worsening of the disorder.

In one aspect, the present invention provides a method for determining whether a subject is at risk of developing a complement-related disorder, the method comprising detecting/determining the level of at least one complement protein, e.g. in a sample from the subject, digesting the protein(s) with endoproteinase GluC to obtain one or more peptides; and detecting the one or more peptides by mass spectrometry. The method may comprise an initial step of obtaining a sample and/or at least one protein from the subject. Suitable sources of samples are described herein. The method may include using the results of the mass spectrometry step to determine the likelihood of the subject to develop a complement-related disorder.

In one aspect, provided is the use of endoproteinase GluC in a method for determining the presence and/or level of a complement protein, e.g. in a sample or a subject, e.g. according to the methods described herein. Also provided is the use of endoproteinase GluC in a method of identifying a subject having a complement-related disorder or at risk of developing a complement-related disorder, the method comprising:

- a) digesting at least one complement protein in a sample obtained from the subject with endoproteinase GluC to obtain one or more peptides;
- b) determining the presence and/or level of the one or more peptides by mass spectrometry; and
- c) using the results of (b) to determine whether the subject has or is likely to develop a complement-related disorder.

Also provided is the use of GluC in a method of selecting a subject for treatment of a complement-related disorder with a complement-targeted therapeutic, the method comprising:

- a) digesting at least one complement protein in a sample obtained from the subject with endoproteinase GluC to obtain one or more peptides;
- b) determining the presence and/or level of the one or more peptides by mass spectrometry; and
- c) using the results of (b) to determine whether the subject is in need of a complement-targeted therapeutic.

The methods described herein may be used for determining whether a subject is at risk of onset of macular degeneration, e.g. EOMD and/or AMD, and/or is at risk of EOMD and/or AMD progression. In some cases, the disorder is selected from EOMD, AMD, geographic atrophy ('dry' (i.e. non-exudative) AMD), early AMD, intermediate AMD, late/advanced AMD, 'wet' (neovascular or exudative) AMD, choroidal neovascularisation (CNV) and retinal dystrophy. In some cases, the subject has or is suspected to have a complement-related disorder. In some cases the disorder is AMD. In some cases the disorder is EOMD.

Thus the present invention also provides a method for determining whether a subject is at risk of developing macular degeneration, e.g. EOMD and/or AMD, the method comprising detecting/determining the level of at least one complement protein, e.g. in a sample from the subject,

digesting the protein(s) with endoproteinase GluC to obtain one or more peptides; and detecting the one or more peptides by mass spectrometry. The method may comprise an initial step of obtaining a sample and/or at least one protein from the subject. The method may include using the results of the mass spectrometry step to determine the likelihood of the subject to develop macular degeneration.

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Also provided herein is a method for assessing the propensity or predisposition of a subject to develop a complement-related disorder, comprising:

(a) providing a blood sample from the subject;

(b) assessing the presence or level of at least one complement protein in the sample, as

10 described herein;

(c) using the results of (b) to determine the likelihood of the subject to develop a complement-related disorder.

15 In other aspects, the present invention provides a method for identifying a subject at risk of developing, or having, a complement-related disorder, the method comprising detecting/determining the level of a complement protein as described herein. The disorder may be EOMD and/or AMD, or related disorders e.g. as described herein.

20 The present invention provides a method of identifying a subject having a complement-related disorder, the method comprising:

a) digesting at least one complement protein in a sample obtained from the subject with endoproteinase GluC to obtain one or more peptides;

b) determining the presence and/or level of the one or more peptides by mass spectrometry; and

c) using the results of (b) to determine whether the subject has a complement-related disorder.

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Methods described herein may also be useful for assessing whether treatment for a complement-related disorder is/has been effective or successful.

30 In some aspects, the present invention provides methods employing the techniques described herein for determining whether a subject is likely to respond or not respond to a therapeutic treatment, or whether a subject is responding to a therapeutic treatment. Such methods should enable patients to receive the most effective therapy for their particular pathological requirements.

35 In some cases, an increase/decrease of a complement protein, e.g. as described herein, as compared to a reference value indicates an increased risk of developing a complement-related disorder. In some cases, an increase/decrease of a complement protein, e.g. as described herein, indicates an increased risk of developing the disorder as compared to a reference value taken from the same subject/a sample from the same subject at an earlier stage of the disorder.

In some embodiments, a method described herein may comprise determining the level of two or more complement proteins and comparing their values e.g. concentrations. The values may be compared to each other, as well as to reference values, e.g. increased levels of C3 and C3b compared to stationary or decreased levels of iC3b and further C3b breakdown products may be indicative of a higher risk of development of a complement-related disorder and/or the need to treat a subject for a complement-related disorder. Decreased levels of C3 and C3b compared to stationary or increased levels of iC3b and further C3b breakdown products may be indicative of a lower risk of development of a complement-related disorder and/or that treatment for a complement-related disorder is effective.

In some embodiments, a method provided herein comprises a step of correlating the presence of an atypical amount/level of a complement protein with an increased risk of the subject developing or having a complement-related disorder.

As used herein the term "reference value" refers to a known measurement value used for comparison during analysis. In some cases, the reference value is one or a set of test values obtained from an individual or group in a defined state of health. In some cases, the reference value is/has been obtained from determining the level of complement proteins in subjects known not to have a complement-related disorder. In some cases, the reference value is set by determining the level or amount of a complement protein previously from the same subject e.g. at an earlier stage of disease progression. The reference value may be taken from a sample obtained from the same subject, or a different subject or subject(s). The sample may be derived from the same tissue/cells/bodily fluid as the sample used by the present invention. The reference value may be a standard value, standard curve or standard data set. Values/levels which deviate significantly from reference values may be described as atypical values/levels.

In some cases the control may be a reference sample or reference dataset, or one or more values from said sample or dataset. The reference value may be derived from a reference sample or reference dataset. The reference value may be derived from one or more samples that have previously been obtained from one or more subjects that are known not to have a complement-related disorder and/or known or expected not to be at risk of developing a complement-related disorder. The reference value may be derived from one or more samples that have previously been obtained from one or more subjects that are known to have a complement-related disorder. The reference value may be derived from one or more samples that have previously been obtained from one or more subjects that are known to be at risk of developing a complement-related disorder. The reference value may be consensus level or an average, or mean, value calculated from a reference dataset, e.g. a mean protein level. The reference dataset/value may be obtained from a large-scale study of subjects known to have a complement-related disorder, such as AMD.

Examples of reference values for complement proteins in human subjects known not to have a complement-related disorder include:

- a) FH: ~150 to 500 µg/ml in human blood (Clark et al., J Immunol 2014. 193(10):4962-70 and unpublished data);
- b) FHL-1: ~0.5 to 50 µg/ml in human blood (Clark et al., J Immunol 2014. 193(10):4962-70 and unpublished data);
- 5 c) FHR1: ~70 to 100 µg/ml in human plasma (Heinen, S et al., Blood 114, 2439–2447);
- d) FHR2: ~15-50 µg/ml in human plasma, or about 1/10 of FH concentration (Skerka et al., Mol Immunol 2013, 56:170-180);
- e) FHR3: ~70 to 100 µg/ml in human plasma (Fritsche, L.G. et al., Hum. Mol. Genet. 2010.19, 4694-4704);
- 10 f) FHR4: ≤5 µg/ml in human blood (WO 2019/215330);
- g) FHR5: ~1.5 µg/ml in human plasma (van Beek, AE et al., Front Immunol. 2017 Oct 18;8:1328);
- h) FI: ~35 µg/ml in human plasma;
- i) C3: ~0.5-16 mg/ml in human plasma (Engström, G. et al., J Hum Hypertens. 2007
- 15 Apr;21(4):276-82; Lee SH et al., Am J Respir Crit Care Med. 2006 Feb 15;173(4):370-8);
- j) C3a: 46-157 ng/ml (Lee SH et al., Am J Respir Crit Care Med. 2006 Feb 15;173(4):370-8);
- k) iC3b: ~0.7-5 µg/ml (Kim AHJ et al., Arthritis Rheumatol. 2019 Mar;71(3):420-430).

In some cases, mean reference values for circulating FH, FHL-1 and FHR1-5 in human subjects known not to have a complement-related disorder, e.g. AMD, include the following (95% CI in parentheses):

- a) FH, nM: 737.3 (718.2 – 756.5)
- b) FHL-1, nM: 10.4 (10.1 – 10.8)
- c) FHR-1, nM: 31.2 (29.4 – 32.9)
- 25 d) FHR-2, nM: 45.3 (43.1 – 47.6)
- e) FHR-3, nM: 24.1 (21.7 – 26.5)
- f) FHR-4, nM: 46.1 (42.7 – 49.6)
- g) FHR-5, nM: 25.5 (24.5 – 26.5).

30 The relative concentrations of one complement protein to another can be determined using their reference values. For example, the ratio of the level of one complement protein to the level of another, or others, can be inferred from the concentrations provided above, e.g. FH:FHL-1, C3:iC3b, C3:C3b etc. The relative concentrations and/or ratios of the level of one complement protein to another, or others, may be altered in complement-related disorders. In some embodiments the

35 methods provided herein involve detecting two or more complement proteins and determining how the levels of the complement proteins change with respect to one another as compared to a reference value(s). For example, the level of a first complement protein may increase as compared to the level of a second complement protein, or vice versa, e.g. FH vs FHL-1, C3 vs iC3b, C3 vs C3b.

Methods provided herein for assessing the risk of development, i.e. the onset or risk of progression of, or for identifying subjects having/at risk of, a complement-related disorder may be performed in conjunction with additional diagnostic methods and/or tests for such disorders that will be known to one skilled in the art. In some cases, methods for assessing the risk of development of a complement-related disorder comprise further techniques selected from: CH50 or AH50 measurement via haemolytic assay, measurement of neoantigen formation during MAC complex (C5b, C6, C7, C8, C9) generation, C3 deficiency screening, mannose-binding lectin assays, immunochemical assays to quantify individual complement components, flow cytometry to assess cell-bound regulatory proteins e.g. CD55, CD59 and CD35, and/or renal function tests, see e.g. Shih AR and Murali MR, *Am. J. Hematol.* 2015, 90(12):1180-1186, Ogedegbe HO, *Laboratory Medicine*, 2007, 38(5):295-304, and Gowda S et al., *N Am J Med Sci.* 2010, 2(4): 170-173, which are herein incorporated by reference in their entirety.

In some cases, methods provided herein for assessing the risk of development of AMD and/or EOMD comprise further assessment techniques selected from: dark adaptation testing, contrast sensitivity testing e.g. Pelli Robson, visual acuity testing using e.g. a Snellen chart and/or Amsler grid, Farnsworth-Munsell 100 hue test and Maximum Color Contrast Sensitivity test (MCCS) for assessing colour acuity and colour contrast sensitivity, preferential hyperacuity perimetry (PHP), fundus photography of the back of the eye, fundus examination, fundus autofluorescence, optical coherence tomography, angiography e.g. fluorescence angiography, fundus fluorescein angiography, indocyanine green angiography, optical coherence tomography angiography, adaptive optics retinal imaging, deep learning analysis of fundus images, electroretinogram methods, and/or methods to measure histological changes such as atrophy, retinal pigment changes, exudative changes e.g. hemorrhages in the eye, hard exudates, subretinal/sub-RPE/intraretinal fluid, and/or the presence of drusen.

Treating complement-related disorders

In some aspects, the methods of the present invention include treating a subject who is at risk of developing, predicted to be at risk of developing, who has been determined to be at risk of developing, or who has, has been identified as having, has been determined to have, or has been diagnosed as having a complement-related disorder, e.g. as described herein.

Any method provided herein for determining whether a subject is at risk of developing a complement-related disorder may additionally comprise a treatment step to treat said disorder. For example, a method provided herein for determining whether a subject is at risk of developing a complement-related disorder may comprise a treatment step to treat or prevent said disorder, wherein the subject has been determined to have atypical presence or levels of one or more complement proteins, e.g. detected/determined as described herein, as compared to a reference value(s).

A treatment step may comprise administering to a subject a therapeutically or prophylactically effective amount of one or more complement-targeted therapeutics, for example, one or more C1 inhibitors, C5 inhibitors, C5a inhibitors, C5aR antagonists, C3 inhibitors, C3a inhibitors, C3b inhibitors, C3aR antagonists, classical pathway inhibitors, alternative pathway inhibitors, FH-supplementation therapy and/or MBL pathway inhibitors. Specific complement-targeted therapeutics include without limitation one or more of human C1 esterase inhibitor (C1-INH), eculizumab (Soliris®, Alexion; a humanized monoclonal IgG2/4-antibody targeting C5), APL-2 (Apellis), mubodina (Adienne Pharma and Biotech), ergidina (Adienne Pharma and Biotech), POT-4 (a cyclic peptide inhibitor of C3; Alcon), rituximab (Biogen Idec, Genentech/Roche), ofatumumab (Genmab, GSK), compstatin analogues, soluble and targeted forms of CD59, PMX53 and PMX205, (Cephalon/Teva), JPE-1375 (Jerini), CCX168 (ChemoCentryx), NGD-2000-1 (former Neurogen), Cinryze (Shire), Berinert (CSL Behring), Cetor (Sanquin), Ruconest/Conestat alfa (Pharming), TNT009 (True North), OMS721 (Omeros), CLG561 (Novartis), AMY-101 (Amyndas), APL-1 (Apellis), APL-2 (Apellis), Mirococept (MRC), Lampalizumab (FCD4514S, Genentech/Roche), ACH-4471 (Achillion), ALXN1210 (Alexion), Tesidolumab/LFG316 (Novartis/Morphosys), Coversin (Akari), RA101495 (Ra Pharma), Zimura (ARC1905, Ophthotech), ALN-CC5 (Alnylam), IFX-1 (InflaRx), ALXN1007 (Alexion), Avacopan/CCX168 (Chemocentryx) and/or one or more therapeutic agents as described in e.g. Ricklin et al., *Mol Immunol.* 2017, 89:10-21; Ricklin and Lambris, *Adv Exp Med Biol.* 2013, 734: 1–22; Ricklin and Lambris, *Semin Immunol.* 2016, 28(3):208-22; Melis JPM et al., *Mol Immunol.* 2015 67(2):117-130; Thurman JM, *Nephrol Dial Transplant*, 2017 32: i57-i64, Cashman SM et al., *PLoS One.* 2011, 6(4):e19078; Bora NS et al., *J Biol Chem.* 2010, 285(44):33826-33; and Clark et al., *J Clin Med* 2015, 4(1):18-31 which are herein incorporated by reference in their entirety.

In some cases a treatment step comprises administering to a subject a therapeutically or prophylactically effective amount of one or more complement-targeted therapeutics described in WO 2018/224663 and/or WO 2019/138137, both hereby incorporated by reference in their entirety.

In some cases a complement-targeted therapeutic for use in the methods provided herein comprises a polypeptide which is capable of binding C3b, e.g. comprising an amino acid sequence having at least 85% identity to SEQ ID NO:145, 146, 147 or 148 and wherein the polypeptide has a total length of 450 amino acids or fewer, as described in WO 2019/138137. SEQ ID NO:145 to 148 described herein correspond to SEQ ID NO:4, 2, 3 and 13, respectively, described in WO 2019/138137. In some cases the complement-targeted therapeutic may have one or more of the following properties: binds to C3b, binds to C3b in the region of C3b bound by Complement Receptor 1, acts as a cofactor for FI, enables FI-mediated inactivation of C3b, reduces the amount of C3b via FI, increases the amount of C3b breakdown products e.g. iC3b, C3dg, C3d, C3f, e.g. via FI, and/or diffuses through BrM.

In some cases a complement-targeted therapeutic for use in the methods provided herein comprises a polypeptide comprising a C3b binding region and a C3b inactivating region, e.g. as described in WO 2018/224663, e.g. wherein the C3b inactivating region comprises, or consists of, an amino acid

sequence having at least 65% sequence identity to the amino acid sequence of SEQ ID NO:149 and/or wherein the C3b binding region comprises, or consists of, an amino acid sequence having at least 65% sequence identity to the amino acid sequence of SEQ ID NO:150, 151 or 152. SEQ ID NO:149 to 152 described herein correspond to SEQ ID NO:9, 11, 13 and 14, respectively, described in WO 2018/224663. The polypeptide may comprise a linker between the C3b binding region and the C3b inactivating region. In some cases the polypeptide comprises a sequence comprising or consisting of an amino acid sequence having at least 65% sequence identity to the amino acid sequence of SEQ ID NO:32, 33 or 34 disclosed in WO 2018/224663. In some cases the complement-targeted therapeutic may have one or more of the following properties: binds to C3b, binds to C3b in the region of C3b bound by a cofactor for FI, acts as a cofactor for FI, enables FI-mediated inactivation of C3b, reduces the amount of C3b via FI, increases the amount of C3b breakdown products e.g. iC3b, C3dg, C3d, C3f, e.g. via FI, and/or diffuses through BrM.

In some cases, the subject has or is suspected to have a complement-related disorder. In some cases the disorder is AMD. In some cases the disorder is EOMD.

In some aspects, the present invention provides a method for treating or preventing a complement-related disorder in a subject, the method comprising administering an effective amount of a complement-targeted therapeutic, wherein the subject to be treated has been determined to have atypical presence or levels of one or more complement proteins, e.g. detected/determined as described herein, as compared to a reference value(s). In some aspects the subject has been determined to be at risk of developing a complement-related disorder, and/or identified as having a complement-related disorder.

In other aspects, the present invention provides a complement-targeted therapeutic for use in a method of treating or preventing a complement-related disorder in a subject, wherein the subject has/has been determined to have atypical presence or levels of one or more complement proteins, e.g. detected/determined as described herein, as compared to a reference value(s). In some aspects the subject has been determined to be at risk of developing a complement-related disorder, and/or identified as having a complement-related disorder.

In some aspects, provided is the use of a complement-targeted therapeutic in the manufacture of a medicament for treating or preventing a complement-related disorder in a subject, wherein the subject has/has been determined to have atypical presence or levels of one or more complement proteins, e.g. detected/determined as described herein, as compared to a reference value(s). In some aspects the subject has been determined to be at risk of developing a complement-related disorder, and/or identified as having a complement-related disorder.

Also provided is a method of treating or preventing a complement-related disorder in a subject, or a complement-targeted therapeutic for use in a method of treating or preventing a complement-related

disorder in a subject, the method comprising administering an effective amount of a complement-targeted therapeutic wherein the subject is selected for treatment if the subject has/has been determined to have atypical presence or levels of one or more complement proteins, e.g. detected/determined as described herein, as compared to a reference value(s). In some aspects the subject has been determined to be at risk of developing a complement-related disorder, and/or identified as having a complement-related disorder.

The present invention also provides a method for selecting a subject for treatment with a complement-targeted therapeutic, comprising determining the presence and/or level of at least one complement protein, e.g. by detection/determining methods provided herein. The subject may have, or have been determined to have, a complement-related disorder, e.g. by methods provided herein.

In various aspects provided herein, the subject to be treated has atypical presence or levels of at least one complement protein, preferably one or more of FH, FHL-1, FHR1, FHR2, FHR3, FHR4, FHR5, FI, C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d. The subject may benefit from treatment to reduce the level of any complement proteins that are increased as compared to a reference value(s) and/or increase the level of any complement proteins that are decreased as compared to a reference value(s).

Methods described herein may comprise determining the level of one or more of FH, FHL-1, FHR1, FHR2, FHR3, FHR4, FHR5, FI, C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d and determining that the subject has or is likely to develop a complement-related disorder if the level of the measured protein(s) is altered, e.g. elevated or reduced, as compared to the level of that complement protein(s) in blood of a control subject that does not have a complement-related disorder, or the level of that complement protein(s) previously in blood of the subject of interest.

As used herein the term "reference value" refers to a known measurement value used for comparison during analysis. In some cases, the reference value is one or a set of test values obtained from an individual or group in a defined state of health. In some cases, the reference value is/has been obtained from determining the level of complement proteins in subjects known not to have a complement-related disorder. In some cases, the reference value is set by determining the level or amount of a complement protein previously from the same subject e.g. at an earlier stage of disease progression. The reference value may be taken from a sample obtained from the same subject, or a different subject or subject(s). The sample may be derived from the same tissue/cells/bodily fluid as the sample used by the present invention. The reference value may be a standard value, standard curve or standard data set. Values/levels which deviate significantly from reference values may be described as atypical values/levels.

In some cases, the methods described herein find use in diagnosing, treating or preventing, or selecting a subject for treatment or prevention of, a disorder which would benefit from one or more of:

a reduction in the level or activity of one or more of C3bBb-type C3 convertase, C3bBb3b-type C5 convertase and/or C4b2a3b-type C5 convertase; a reduction in the level of one or more of C3, C3b, C3a, iC3b, FHR1, FHR2, FHR3, FHR4, FHR5, C5b and/or C5a; or an increase in the level of one or more of iC3b, C3f, C3c, C3dg, C3d, C3g, FH, FHL-1, FI, FH, FHL-1, FHR1, FHR2, FHR3, FHR4 and/or FHR5 as compared to reference value(s).

As used herein, 'treatment' may, for example, be reduction in the development or progression of a disease/condition, alleviation of the symptoms of a disease/condition or reduction in the pathology of a disease/condition. Treatment or alleviation of a disease/condition may be effective to prevent progression of the disease/condition, e.g. to prevent worsening of the condition or to slow the rate of development. In some embodiments treatment or alleviation may lead to an improvement in the disease/condition, e.g. a reduction in the symptoms of the disease/condition or reduction in some other correlate of the severity/activity of the disease/condition. Prevention/prophylaxis of a disease/condition may refer to prevention of a worsening of the condition or prevention of the development of the disease/condition, e.g. preventing an early stage disease/condition developing to a later, chronic, stage.

The methods provided herein may comprise determining in a subject the presence or absence of a genetic profile characterised by polymorphisms in the subject's genome associated with complement dysregulation. The polymorphisms may be found within or near genes such as CCL28, FBN2, ADAM12, PTPRC, IGLC1, HS3ST4, PRELP, PPID, SPOCK, APOB, SLC2A2, COL4A1, MYOC, ADAM19, FGFR2, C8A, FCN1, IFNAR2, C1NH, C7 and ITGA4. A genetic profile associated with complement dysregulation may comprise one or more, often multiple, single nucleotide polymorphisms, e.g. as set out in Tables I and II of US 2010/0303832, which is hereby incorporated by reference in its entirety.

Genetic factors are thought to play a role in the development of AMD and EOMD. Thus, any of the assessment or therapeutic methods described herein may be performed in conjunction with methods to assess AMD-associated and/or EOMD-associated and/or macular dystrophy-associated genetic variants. In some cases a complement-related disorder described herein may comprise a genetic element and/or a genetic risk factor.

In some cases, the methods provided herein further comprise determining in a subject the presence or absence of one or more genetic factors associated with AMD, e.g. one or more AMD-associated genetic variants. In some cases, the methods comprise screening (directly or indirectly) for the presence or absence of the one or more genetic factors. In some embodiments, the genetic factor(s) are genetic risk factor(s). In some embodiments, the subject has been determined to have one or more such risk factors. In some embodiments, the methods of the present invention involve determining whether a subject possesses one or more such risk factors.

In some embodiments, the one or more genetic factors may be located on chromosome 1 at or near the RCA locus, e.g. in the *CFH/CFHR* genes.

The one or more genetic factors may be located in one or more of: *CFH* e.g. selected from Y402H (i.e. rs1061170^C), rs1410996^C, I62V (rs800292), A473A (rs2274700), R53C, D90G, D936E (rs1065489), R1210C, IVS1 (rs529825), IVS2 insTT, IVS6 (rs3766404), A307A (rs1061147), IVS10 (rs203674), rs3753396, R1210C, rs148553336, rs191281603, rs35292876, and rs800292; *CFHR4* e.g. selected from rs6685931, and rs1409153; *CFI* e.g. selected from G119R, and rs141853578; *CFB* e.g. rs4151667, *C2* e.g. rs9332739, *C9* e.g. P167S; and/or *C3* e.g. K155Q. In some embodiments, a genetic factor is Y402H (i.e. rs1061170^C). In some embodiments, a genetic factor is rs3753396. In some embodiments, a genetic factor is rs6685931 and/or rs1409153. In some embodiments, a genetic factor is not rs6685931.

Suitable genetic risk factors and genetic variants will be known in the art and may be as described in e.g. Edwards AO et al., *Science* 2005, 308(5720):421-4; Hageman GS et al., *Proc Natl Acad Sci U S A*. 2005, 102(20):7227-7232; Haines JL et al., *Science* 2005, 308(5720):419-21, Klein RJ et al., *Science* 2005, 308(5720):385-389; Fritsche et al., *Nat Genet.* 2016, 48(2):134-43; US 2010/0303832; Clark et al., *J Clin Med.* 2015, 4(1):18-31; Cipriani, V. et al., *Nat Commun.* 2020, 11, 778; and Hageman GS et al, *Hum Genomics.* 2011, 5, 420 (2011), each hereby incorporated by reference in its entirety.

In some cases, the methods provided herein further comprise determining in a subject the presence or absence of one or more genetic factors associated with EOMD, e.g. one or more EOMD-associated genetic variants. In some cases, the methods comprise screening (directly or indirectly) for the presence or absence of the one or more genetic factors. In some embodiments, the genetic factor(s) are genetic risk factor(s). In some embodiments, the subject has been determined to have one or more such risk factors. In some embodiments, the methods of the present invention involve determining whether a subject possesses one or more such risk factors. In some embodiments the subject may possess one or more risk factors for early-onset macular degeneration (EOMD).

EOMD is thought to be caused by monogenic inheritance of rare variants of the *CFH* gene (see e.g. Boon CJ et al. *Am J Hum Genet* 2008; 82(2):516-23; van de Ven JP, et al. *Arch Ophthalmol* 2012;130(8):1038-47; Yu Y et al. *Hum Mol Genet* 2014; 23(19):5283-93; Duvvari MR, et al. *Mol Vis* 2015; 21:285-92; Hughes AE, et al. *Acta Ophthalmol* 2016; 94(3):e247-8; Wagner et al. *Sci Rep* 2016;6:31531; Taylor RL et al, *Ophthalmology.* 2019 Mar 21. pii: S0161-6420(18):33171-3). In some embodiments, the subject may possess one or more of EOMD-associated genetic variants. EOMD-associated genetic variants are described in e.g. Servais A et al. *Kidney Int*, 2012; 82(4):454-64 and Dragon-Durey MA, et al. *J Am Soc Nephrol* 2004; 15(3):787-95; which are hereby incorporated by reference in their entirety. In some embodiments, the subject may possess one or more of the following EOMD-associated genetic variants: *CFH* c.1243del, p.(Ala415Profs*39) het; *CFH*

c.350+1G>T het; *CFH* c.619+1G>A het; *CFH* c.380G>A, p.(Arg127His); *CFH* c.694C>T, p.(Arg232Ter); or *CFH* c.1291T>A, p.(Cys431Ser).

In some cases, the methods provided herein comprise screening for deletions within the *RCA* locus (a region of DNA sequence located on chromosome one that extends from the *CFH* gene through to the *CD46* (*MCP*) gene) that are associated with AMD risk or protection.

Methods for determining the presence or absence of genetic factors include restriction fragment length polymorphism identification (RFLPI) of genomic DNA, random amplified polymorphic detection (RAPD) of genomic DNA, amplified fragment length polymorphism detection (AFLPD), multiple locus variable number tandem repeat (VNTR) analysis (MLVA), SNP genotyping, multilocus sequence typing, PCR, DNA sequencing e.g. Sanger sequencing or Next-Generation sequencing, allele specific oligonucleotide (ASO) probes, and oligonucleotide microarrays or beads. Other suitable methods are described in e.g. Edenberg HJ and Liu Y, *Cold Spring Harb Protoc*; 2009; doi:10.1101/pdb.top62, and Tsuchihashi Z and Dracopoli NC, *Pharmacogenomics J.*, 2002, 2:103–110.

In some embodiments, the subject is selected for therapeutic or prophylactic treatment with a complement-targeted therapeutic based on their being determined to possess one or more genetic factors for AMD and/or EOMD, e.g. one or more AMD-associated and/or EOMD-associated genetic variants or a macular dystrophy. In some embodiments, the subject has been determined to have one or more such genetic factors. In some embodiments, the methods provided herein comprise determining whether a subject possesses one or more such genetic factors. Such methods and genetic factors are described herein. Thus, provided herein is a method of diagnosing, treating or preventing a complement-related disorder in a subject, wherein the subject has/has been determined/is determined to possess one or more genetic factors for AMD and/or EOMD, and wherein the subject has/has been determined/is determined to have atypical presence or levels of one or more complement proteins, e.g. detected/determined as described herein, as compared to a reference value(s); optionally wherein the method comprises administering a complement-targeted therapy/therapeutic agent.

The term “subject” refers to a subject, patient or individual and may be any animal or human. The subject is preferably mammalian, more preferably human. The subject may be a non-human mammal, but is more preferably human. The subject may be male or female. The subject may be a patient. Therapeutic uses may be in human or animals (veterinary use). The subject to be treated with a therapeutic substance described herein may be a subject in need thereof.

A subject described herein may belong to a patient subpopulation i.e. the subject may be part of an identifiable, specific portion or subdivision of a population. The population and/or subpopulation may have or be suspected to have a complement-related disorder. The subpopulation may display atypical presence or levels of one or more complement proteins, e.g. detected/determined as described

herein, as compared to the population as a whole. The population and/or subpopulation may have or be suspected to have AMD, EOMD or a macular dystrophy.

5 The subject may be identified, or may have been identified, as having a complement-related disorder or being at risk of developing a complement-related disorder, e.g. by a method described herein.

In some aspects provided herein, the subject is characterised as having an atypical presence or level of one or more complement proteins, e.g. detected/determined/measured as described herein.

10 Provided is a method of treating or preventing a complement-related disorder in a subject, wherein is characterised as having an atypical presence or levels of one or more complement proteins, e.g. detected/determined as described herein.

15 Also provided is a complement-targeted therapeutic for use in a method of treating or preventing a complement-related disease in a subject, wherein the subject is characterised as having an atypical presence or levels of one or more complement proteins, e.g. detected/determined as described herein.

20 Methods according to the present invention may be performed outside the human or animal body. Methods according to the present invention may be performed, or products may be present, *in vitro*, *ex vivo*, or *in vivo*. The term "*in vitro*" is intended to encompass experiments with materials, biological substances, cells and/or tissues in laboratory conditions or in culture whereas the term "*in vivo*" is intended to encompass experiments and procedures with intact multi-cellular organisms. "*Ex vivo*" refers to something present or taking place outside an organism, e.g. outside the human or animal
25 body, which may be on tissue (e.g. whole organs) or cells taken from the organism. In some embodiments, the determining, detecting, measuring, quantifying, predicting and/or diagnosing steps of the methods provided herein are performed *in vitro*.

30 Complement-targeted therapeutics described herein may be administered, or formulated for administration, by a number of routes, including but not limited to systemic, intratumoral, intraperitoneal, parenteral, intravenous, intra-arterial, intradermal, subcutaneous, intramuscular, oral and nasal. Preferably, the therapeutics are administered by a route selected from intratumoral, intraperitoneal or intravenous. The medicaments and compositions may be formulated in fluid or solid form. Fluid formulations may be formulated for administration by injection to a selected region of the
35 human or animal body.

Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment, e.g.
40 decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors,

and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.

5 Kits

Aspects of the disclosure include *in vitro* diagnostic methods and *in vitro* kits for performing such methods. In some embodiments, the present invention provides a kit comprising endoproteinase GluC for use in a method of detecting and/or determining the level of one or more complement protein(s) e.g. in a sample. The kit may be used for any of the methods described herein and/or for
 10 detecting/determining the level of any one or combination of proteins described herein. The kit may be suitable for, used for, or intended/sold/distributed for detecting at least one complement protein in a sample, determining the level of at least one complement protein in a sample, preparing at least one complement protein for analysis and/or detection, determining the presence and/or level of a complement protein in a subject, determining whether a subject is at risk of developing a complement-
 15 related disorder, identifying a subject having a complement-related disorder, selecting a patient/subject for treatment of a complement-related disorder, and/or treating a subject who is suspected to have a complement-related disorder. The kit and components thereof may be suitable for use with MS techniques.

20 A kit provided herein comprises one, two, or more components suitable for performing the methods described herein, in whole or in part. The kit may comprise standards or controls, e.g. labelled peptide standard(s) for each protein to be detected using the kit. The kit may comprise a predetermined quantity of labelled peptide standards. The kit may comprise a predetermined quantity of GluC enzyme, optionally with the necessary buffers and reagents for enzyme digestion. The
 25 components of the kit may be provided in a single composition, or may be provided as plural compositions.

The kit may be suitable for a point-of-care *in vitro* diagnostic test. It may be a kit for laboratory-based testing. The kit may include instructions for use, such as an instruction booklet or leaflet. The
 30 instructions may include a protocol for performing any one or more of the methods described herein e.g. for enzyme digestion, recommended MS settings, and/or data analysis templates. The kit may comprise components for separating proteins in a sample and/or performing MS techniques e.g. liquid chromatography columns.

35 The kit may be adapted for use with dry samples, wet samples, frozen samples, fixed samples, urine samples, saliva samples, tissue samples, blood samples, or any other type of sample, including any of the sample types disclosed herein. The kit may comprise a device for obtaining or processing a blood, serum, plasma, cell or tissue sample.

Sequence identity

As used herein, an amino acid sequence which corresponds to a reference amino acid sequence may comprise at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the reference sequence.

Pairwise and multiple sequence alignment for the purposes of determining percent identity between two or more amino acid or nucleic acid sequences can be achieved in various ways known to a person of skill in the art, for instance, using publicly available computer software such as ClustalOmega (Söding, J. 2005, Bioinformatics 21, 951-960), T-coffee (Notredame et al. 2000, J. Mol. Biol. (2000) 302, 205-217), Kalign (Lassmann and Sonnhammer 2005, BMC Bioinformatics, 6(298)) and MAFFT (Kato and Standley 2013, Molecular Biology and Evolution, 30(4) 772-780) software. When using such software, the default parameters, e.g. for gap penalty and extension penalty, are preferably used.

The features disclosed in the foregoing description, or in the following claims, or in the accompanying drawings, expressed in their specific forms or in terms of a means for performing the disclosed function, or a method or process for obtaining the disclosed results, as appropriate, may, separately, or in any combination of such features, be utilised for realising the invention in diverse forms thereof.

The invention includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

While the invention has been described in conjunction with the exemplary embodiments described above, many equivalent modifications and variations will be apparent to those skilled in the art when given this disclosure. Accordingly, the exemplary embodiments of the invention set forth above are considered to be illustrative and not limiting. Various changes to the described embodiments may be made without departing from the spirit and scope of the invention.

For the avoidance of any doubt, any theoretical explanations provided herein are provided for the purposes of improving the understanding of a reader. The inventors do not wish to be bound by any of these theoretical explanations.

Any section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Throughout this specification, including the claims which follow, unless the context requires otherwise, the word "comprise" and "include", and variations such as "comprises", "comprising", and "including" will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from “about” one particular value, and/or to “about” another particular value.

When such a range is expressed, another embodiment includes from the one particular value and/or

- 5 to the other particular value. Similarly, when values are expressed as approximations, by the use of the antecedent “about,” it will be understood that the particular value forms another embodiment. The term “about” in relation to a numerical value is optional and means for example +/- 10%.

The phrase “and/or” as used herein encompasses each member of the list individually, as well as any

- 10 combination of one or members of the list up to and including every member of the list.

For standard molecular biology techniques, see Sambrook, J., Russel, D.W. *Molecular Cloning, A Laboratory Manual*. 3 ed. 2001, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Sequences

SEQ ID No:	Description	Sequence
1	Human Complement Factor H (FH) Uniprot: P08603-1 Entry version 229 (11 Dec 2019) Sequence version 4 (11 Sep 2007)	MRLLAKIICMLWAICVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPG YRSLGNVIMVCRKGEWVALNPLRKQCQKRPCGHPGDTFPGTFTLTGGNVFEYGV KAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENGKIVSSAME PDREYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWWSKEPKKPCVEISCKSPDVIN GSPISQKIIYKENERFQYKCNMGYYSERGDVCTESGWRPLPSCEEKSCDNPY IPNGDYSPLRIKHRTGDEITYQCRNGFYPATRGNTAKCTSTGWIPAPRCTLKPCD YDIIKHGGLYHENMRRPYFPVAVGKYYSYYCDEHFETPSGSYWDHIHCTQDQGW SPAVPCLRKCYFPYLENGYNQNYGRKFVQGKSIDVACHPGYALPKAQTTVTCME NGWSPTPRCIRVKTCSSSIDIENGFISESQYTYALKEKAKYQCKLGYVTADGET SGSITCGKDGWSAQPTCIKSCDIPVFMNARTKNDFTWFKLNDTLDYECHEGYES NTGSTTGSIVCGYNGWSDLPICYERECELPKIDVHLVPDRKKDQYKVGELVKFSC KPGFTIVGPNSVQCYHFGLSPLPICKEQVQSCGPPPELLNGNVKEKTKEEYGH SEVVEYYCNPRLMKGPNIQCVDEWTTLPVCIVEESTCGDIPELEHGWAQLS SPPPYYGDSVEFNCSESFTMIGHRSITCIHGVTQLPQCVAIDKLKCKSSNLIILE EHLKNKKEFDHNSNIRYRCRGKEGWIHTVCINGRWDPEVNCMAIQCLCPPPPQ IPNSHNMTTTLNRYDGEKVSVLCQENYLIQEGEEITCKDGRWQSIPLCVEKIPCS QPPQIEHGTINSSRSSQESYAHGTKLSYTCGGFRISEENETTCYMGKWSSPPQ CEGLPCKSPPEISHGVVAHMSDSYQYGEEVYKCFEGFGIDGPAIAKCLGEKWS HPPSCIKTDCLSLPSFENAIPMGEEKDVYKAGEQVYTCATYKMDGASNVTCIN SRWTGRPTCRDTSVNPPTVQNAIVSRQMSKYPSEGERVRYQCRSPYEMFGD EEVMCLNGNWTEPPQCKDSTGKCGPPPPIDNGDITSFPLSVYAPASSVEYQCQ NLYQLEGNKRITCRNGQWSEPPKCLHPCVISREIMENYIALRWTAQKLYSRTG ESVEFVCKRGYRLSSRSHTLRTTCWDGKLEYPTCAKR
2	Human FH-like protein 1 (FHL-1) Uniprot: P08603-2 Entry version 229 (11 Dec 2019) Sequence version 4 (11 Sep 2007)	MRLLAKIICMLWAICVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPG YRSLGNVIMVCRKGEWVALNPLRKQCQKRPCGHPGDTFPGTFTLTGGNVFEYGV KAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENGKIVSSAME PDREYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWWSKEPKKPCVEISCKSPDVIN GSPISQKIIYKENERFQYKCNMGYYSERGDVCTESGWRPLPSCEEKSCDNPY IPNGDYSPLRIKHRTGDEITYQCRNGFYPATRGNTAKCTSTGWIPAPRCTLKPCD YDIIKHGGLYHENMRRPYFPVAVGKYYSYYCDEHFETPSGSYWDHIHCTQDQGW SPAVPCLRKCYFPYLENGYNQNYGRKFVQGKSIDVACHPGYALPKAQTTVTCME NGWSPTPRCIRVSFTL
3	Complement Factor H-related 1 (FHR-1) Uniprot: Q03591 Entry version 166 (11 Dec 2019) Sequence version 2 (16 Dec 2008)	MWLLVSVILISRISSVGGEATFCDFPKINHILYDEEKYPFSQVPTGEVFYYSCE YNFVSPSKSFWRITCTEEGWSPTPKCLRLCFFPFVENGHSESSGQTHLEGDTV QIICNTGYRLQNNENNISCVERGWSTPPKCRSTDTSCVNPPTVQNAHILSRQMS KYPSEGERVRYECRSPYEMFGDEEVMCLNGNWTEPPQCKDSTGKCGPPPPIDN GDITSFPLSVYAPASSVEYQCQNLQYQLEGNKRITCRNGQWSEPPKCLHPCVISR EIMENYIALRWTAQKLYLRTGESAEFVCKRGYRLSSRSHTLRTTCWDGKLEY PTCAKR

4	Complement Factor H-related 2 (FHR-2), isoform 1 Uniprot: P36980-1 Entry version 168 (11 Dec 2019) Sequence version 1 (01 Jun 1994)	MWLLVSVILISRISSVGGEAMFCDFPKINHGILYDEEKYPFSQVPTGEVFFYSSCE YNFVSPSKSFWTRITCAEEGWSPTPKCLRLCFFPFVENGHSESSGQTHLEGDTV QIICNTGYRLQNNENNISCVERGWSTPPKCRSTISAEKCGPPPIDNGDITSFLLS VYAPGSSVEYQCQNLYQLEGNNQITCRNGQWSEPPKCLDPCVISQEIMEKYNIK LKWTNQQKLYSRTGDIVEFVCKSGYHPTKSHSFRAMCQNGKLVYPSCEEK
5	Complement Factor H-related 2 (FHR-2), isoform 2 Uniprot: P36980-2 Entry version 168 (11 Dec 2019) Sequence version 1 (01 Jun 1994)	MWLLVSVILISRISSVGGEAMFCDFPKINHGILYDEEKYPFSQVPTGEVFFYSSCE YNFVSPSKSFWTRITCAEEGWSPTPKCLRLCFFPFVENGHSESSGQTHLEGDTV QIICNTGYRLQNNENNISCVERGWSTPPKCRSTSSSVEYQCQNLYQLEGNNQIT CRNGQWSEPPKCLDPCVISQEIMEKYNIKLKWTNQQKLYSRTGDIVEFVCKSGY HPTKSHSFRAMCQNGKLVYPSCEEK
6	Complement Factor H-related 3 (FHR-3), isoform 1 Uniprot: Q02985-1 Entry version 161 (11 Dec 2019) Sequence version 2 (21 Feb 2001)	MLLLINVILTLWVSCANGQVKPCDFPDIKHGGLFHENMRRPYFPVAVGKYYSYYC DEHFETPSGSYWDYIHCTQNGWSPA VPCLRKCYFPYLENGYNQNYGRKFVQG NSTEVACHPGYGLPKAQT TVTCTEKGWSPTPRCIRVTRCSKDIEIENGFISSSS SIYILNKEIQYKCKPGYATADGNSSGSITCLQNGWSAQPICINSSEKCGPPPPISN GDTTSFLLKVYVPQSRVEYQCQPYELQGSNYVTCSNGEWSEPPRCIHPCIITEE NMNKNNIKLGKRSRDKYYAKTGDTIEFMCKLGYNANTSILSFQAVCREGIVEYPR CE
7	Complement Factor H-related 3 (FHR-3), isoform 2 Uniprot: Q02985-2 Entry version 161 (11 Dec 2019) Sequence version 2 (21 Feb 2001)	MLLLINVILTLWVSCANGQVKPCDFPDIKHGGLFHENMRRPYFPVAVGKYYSYYC DEHFETPSGSYWDYIHCTQNGWSPA VPCLRKCYFPYLENGYNQNYGRKFVQG NSTEVACHPGYGLPKAQT TVTCTEKGWSPTPRCIRVNSSEKCGPPPPISNGDTT SFLLKVYVPQSRVEYQCQPYELQGSNYVTCSNGEWSEPPRCIHPCIITEENMN KNNIKLGKRSRDKYYAKTGDTIEFMCKLGYNANTSILSFQAVCREGIVEYPRCE
8	Complement Factor H-related 4 (FHR-4A) Uniprot: Q92496-1 Entry version 157 (11 Dec 2019) Sequence version 3 (22 Jan 2014)	MLLLINVILTLWVSCANGQEVKPCDFPEIQHGGLYKSLRRLYFPAAAGQSYSSYY CDQNFVTPSGSYWDYIHCTQDGWSPVPLRTRCSKSDVEIENGFISSSSSIYILN EETQYNCKPGYATAEGNSSGSITCLQNGWSTQPICIKFCDMVPFENSRAKSNGM WFKLHDTLDYECYDGYESSYGNTTDSIVCGEDGWSHLPTCYNSSENCPPPPIS NGDTTSFPQKVYLPWSRVEYQCQSYELQGSKYVTCSNGDWSEPPRCISMKPC EFPEIQHGHLYYENTRRPYFPVATGQSYSSYYCDQNFVTPSGSYWDYIHCTQDG WLPTVPLRTRCSKSDIEIENGFISSSSSIYILNKEIQYKCKPGYATADGNSSGSITC LQNGWSAQPICIKFCDMVPFENSRAKSNGMRFLHDTLDYECYDGYEISYDNTT GSIVCGEDGWSHFPTCYNSSSEKCGPPPPISNGDTTSFLLKVYVPQSRVEYQCQS YYELQGSNYVTCSNGEWSEPPRCIHPCIITEENMNKNNIQLKGKSDIKYYA KTGDTIEFMCKLGYNANTSVL SFQAVCREGIVEYPRCE
9	Complement Factor H-related 4 (FHR-4B) Uniprot: Q92496-3 Entry version 157 (11 Dec 2019) Sequence version 3 (22 Jan 2014)	MLLLINVILTLWVSCANGQEVKPCDFPEIQHGGLYKSLRRLYFPAAAGQSYSSYY CDQNFVTPSGSYWDYIHCTQDGWSPVPLRTRCSKSDIEIENGFISSSSSIYILNK EIYKCKPGYATADGNSSGSITCLQNGWSAQPICIKFCDMVPFENSRAKSNGMR FKLHDTLDYECYDGYEISYGNTTGSIVCGEDGWSHFPTCYNSSSEKCGPPPPISN GDTTSFLLKVYVPQSRVEYQCQSYELQGSNYVTCSNGEWSEPPRCIHPCIITEE NMNKNNIQLKGKSDIKYYAKTGDTIEFMCKLGYNANTSVL SFQAVCREGIVEYPR CE
10	Complement Factor H-related 5 (FHR-5) Uniprot: Q9BXR6	MLLLFSVILISWVSTVGEGTLCDFPKIHGFLYDEEDYNPFSQVPTGEVFFYSSC EYNFVSPSKSFWTRITCTEEGWSPTPKCLRMCSFFPVKNHSESSGLIHLEGDT VQIICNTGYSLQNNENKISCVERGWSTPPICSFTKGECHVPILEANVDAQPKKESY KVGDLVKFSCRKNLIRVGSDSVQCYQFGWSPNFPCTCKGQVRSCGPPPPQLSNGE VKEIRKEEYGHNEVVEYDCNPNFIINGPKKIQCVDGEWTTLPCTCVEQVKTCGYIP ELEYGYVQPSVPPYQHGSVEVNCRNEYAMIGNNMITCINGIWTELPMTVCVATHQ LKRCKIAGVNIKTLLKLSGKEFNHNSRIRYRCSDFIRYRHSVCINGKWNVEIDCTE KREQFCPPPPQIPNAQNMTT TVNYQDGEKVAVLCKENYLLPEAKEIVCKDGRWQ SLPRCVSTAYCGPPPSINNGDTTSFPLSVYPPGSTVTYRCQSFYKLQGSVTVT CRNKQWSEPPRCCLDPCVVSEENMNKNNIQLKWRNDGKLYAKTGDAVEFQCKF PHKAMISSPPFRAICQEGKFEYPICE
11	Complement Factor I (FI)	MKLLHVFLFLCFHLRFCKVTYTSQEDLVEKKCLAKKYTHLSCDKVFCQPWQRCI EGTCVCKLPYQCPKNGTAVCATNRRSFPTYCQKSLECLHPGTKFLNNGTCTA

	Uniprot: P05156 Entry version 147 (11 Dec 2019) Sequence version 1 (01 Jun 2001)	EGKFSVSLKHGNTDSEGIVEVKLVDDQDKTMFICKSSWSMREANVACLDLGFQQG ADTQRRFKLSDSLINSITECLHVHCRGLETSLAECTFTKRRTMGYQDFADVVCYT QKADSPMDDFQCVNGKYISQMKACDGINDCGDQSDCLCKACQKGFHCKS GVCIPSQYQCNGEVDICITGEDEVGCAGFASVTQEETEILTADMDAERRRIKSLLP KLSCGVKNRMHIRRKRVGGKRAQLGDLWPQVAIKDASGITCGGIYIGGCWILTA AHCLRASKTHRYQIWTTVVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQNDIALIEM KKDGNKKDCELPSPACVPWSPYLFQPNDCIVSGWGREKDNERVFSLQWGE VKLISNCSKFYGNRFYEKEMECAGTYDGSIDACKGDSGGPLVCMANNVITYVW GVVSWGENCCKPEFPGVYTKVANYFDWISYHVGRPFISQYNV
12	Complement factor C3 (Uniprot: P01024) Entry version 242 (11 Dec 2019) Sequence version 2 (12 Dec 2006)	MGPTSGPSLLLLLTHLPLALGSPMYSIITPNILRLESEETMVLEAHDAAQGDVPVT VTVHDFPGKKLVLSSEKTVLTPATNHMGNTFTIPANREFKSEKGRNKFVTVQAT FGTQVVEKVVLSLQSGYLFIQTDKTIYTPGSTVLRYFTVNHKLLPVGRTVMVNIE NPEGIPVKQDSLSSQNQLGVLPLSWDIPELVNMGWKIRAYYENSPQQVFSTEF EVKEYVLPSEFVIEPTKFFYYIYNEKGLEVTITARFLYGKKVEGTAFFVFIQDGE QRISLPESLKRIPIEDGSGEVVLSRKVLLDGVQNPRAEDLVGKSLYVSATVILHSG SDMVQAERSGIPVITSPYQIHFTKTPKYFKPGMPFDLMVFVTNPDGSPAYRVPVA VQGEDTVQSLTQGDGVAKLSINTHPSQKPLSITVTRTKKQELSEAEQATRTMQALP YSTVGNSNNYLHLSVLRTELRLPGETLNVNFLLRMDRAHEAKIRYYTYLIMNKGRL LKAGRQVREPGQDLVVLPLSITTDIFPSFRLVAYYTLIGASGQREVADSVWVDV KDSCVGSLLVKSQSEDRQVPVPGQMTLKIEGDHARVVLVAVDKGVFVNLKK NKLTSKIWDVVEKADIGCTPGSGKDYAGVFSAGLTFTSSSGQQAQRAELQCPQAA PQPAARRRRSVQLTEKRMKVGKYPKELRKCCEDGMRENPMRFSCQRRTRFIS LGEACKKVLDCCNITELRRQHARASHLGLARSLNDEIIAENIVSRSEFPESW LWNVEDLKEPPKNGISTKLMNIFLKDSITTWEILAVSMSDKKGCIVADPFVTVMQ DFIDLRPLPYSVVRNEQVEIRAVLYNYRQNLKVRVELLHNPFCSLATTKRRH QQTVTIPPKSSLSVPYVIVPLKTGLQEVEVKAAYVHHFISDGVRSKLVVPEGIRM NKTAVRTLDPERLREGVQKEDIPPADLSDQVPDTESETRILLQGTTPVAQMTE AVDAERLKHILVTPSGCGEQNMIGMTPTVIAVHYLDETEQWEKFGLEKRGAL IKKGYTQQLAFRQPSAFAAFVKRAPSTWLTA YVVKVFLAVNLIAIDSQVLCGAV KWLILEKQKPDGVFQEDAPVIHQEMIGGLRNNNEKDMALTAFLVLSLQEAKDICEE QVNSLPGSITKAGDFLEANYMNLQRSYTVIAAGYALAQMGRLKGPLLNKFLTTAK DKNRWEDPGKQLYNVEATSYALLALLQLKDFDFVPPVVRWLNEQRYGGGYGS TQATFMVFQALAQYQKDAPDHQELNLDVSLQLPSRSSKITHRIHWESASLLRSEE TKENEGFTVTAEGKGQGTLSVVTMYHAKAKDQLTCNKFDLKVTIKPAPETEKRP QDAKNTMILEICTRYRGDQDATMSILDISMGTGFAPDTDDLKQLANGVDRYISKY ELDKAFSDRNTLIYLDKVSHEDDCLAFKVHQYFNVELIQPGAVKVYAYYNLEES CTRFYHPEKEDGKLNKLCRDELRCRAEENCIFQKSDDKVTLERLDKACEPGVD YVYKTRLVKVQLSNDFDEYIMAIEQTIKSGSDEVQVGQRTFISPIKCREALKLEE KKHYLMWGLSSDFWGEKPNLSYIIGKDTWVEHWPEEDECCQDEENQKQCQDLG AFTESMVVFGCPN
13	Human C3 β chain UniProt: P01024; Entry version 242 (11 Dec 2019) Sequence version 2 (12 Dec 2006); residues 23-667	SPMYSIITPNILRLESEETMVLEAHDAAQGDVPVTVTVHDFPGKKLVLSSEKTVLTP ATNHMGNTFTIPANREFKSEKGRNKFVTVQATFGTQVVEKVVLSLQSGYLFIQ TDKTIYTPGSTVLRYFTVNHKLLPVGRTVMVNIEENPEGIPVKQDSLSSQNQLGVL PLSWDIPELVNMGWKIRAYYENSPQQVFSTEFVEKEYVLPSEFVIEPTKFFYYI YNEKGLEVTITARFLYGKKVEGTAFFVFIQDGEQRISLPESLKRIPIEDGSGEVV SRKVLLDGVQNPRAEDLVGKSLYVSATVILHSGSDMVQAERSGIPVITSPYQIHFT KTPKYFKPGMPFDLMVFVTNPDGSPAYRVPVAVQGEDTVQSLTQGDGVAKLSIN THPSQKPLSITVTRTKKQELSEAEQATRTMQALPYSTVGNSNNYLHLSVLRTELRLP GETLNVNFLLRMDRAHEAKIRYYTYLIMNKGRLKAGRQVREPGQDLVVLPLSITT DFPSFRLVAYYTLIGASGQREVADSVWVDKDCVGSLLVKSQSEDRQVPV GQQMTLKIEGDHARVVLVAVDKGVFVNLKKNKLTQSKIWDVVEKADIGCTPGS GKDYAGVFSAGLTFTSSSGQQAQRAELQCPQAA
14	Human C3 α' chain UniProt: P01024; Entry version 242 (11 Dec 2019) Sequence version 2 (12 Dec 2006); residues 749-1663	SNLDEIIAENIVSRSEFPESWLVNVEDLKEPPKNGISTKLMNIFLKDSITTWEIL AVSMSDKKGCIVADPFVTVMQDFIDLRPLPYSVVRNEQVEIRAVLYNYRQNL KVRVELLHNPFCSLATTKRRHQQTVTIPPKSSLSVPYVIVPLKTGLQEVEVKAAY YHHFISDGVRSKLVVPEGIRMNKTAVRTLDPERLREGVQKEDIPPADLSDQV PDTESETRILLQGTTPVAQMTEAVDAERLKHILVTPSGCGEQNMIGMTPTVIAVH YLDTEQWEKFGLEKRGALIKKGYTQQLAFRQPSAFAAFVKRAPSTWLTA YVVKVFLAVNLIAIDSQVLCGAVKWLILEKQKPDGVFQEDAPVIHQEMIGGLRNN NEKDMALTAFLVLSLQEAKDICEEQVNSLPGSITKAGDFLEANYMNLQRSYTVIA GYALAQMGRLKGPLLNKFLTTAKDKNRWEDPGKQLYNVEATSYALLALLQLKDF DFVPPVVRWLNEQRYGGGYGSTQATFMVFQALAQYQKDAPDHQELNLDVSL QLPSRSSKITHRIHWESASLLRSEETKENEGFTVTAEGKGQGTLSVVTMYHAKAK DQLTCNKFDLKVTIKPAPETEKRPQDAKNTMILEICTRYRGDQDATMSILDISMGT GFAPDTDDLKQLANGVDRYISKYELDKAFSDRNTLIYLDKVSHEDDCLAFKVHQ YFNVELIQPGAVKVYAYYNLEESCTRFYHPEKEDGKLNKLCRDELRCRAEENCIF QKSDDKVTLERLDKACEPGVDYVYKTRLVKVQLSNDFDEYIMAIEQTIKSGSDE

		VQVGQQRTEFISPIKCREALKLEEKHHYLMWGLSSDFWGEKPNLSYIIGKDTWVEH WPEEDECQDEENQKQCQDLGAFTESMVVFGCPN
15	Human C3a UniProt: P01024; Entry version 242 (11 Dec 2019) Sequence version 2 (12 Dec 2006); residues 672-748	SVQLTEKRMCKVGVKYPKELRKCCEDGMRENPMRFSCQRRTRFISLGEACKKV LDCCNYITELRRQHARASHLGLAR
16	Human C3b α' chain fragment 1 UniProt: P01024; Entry version 242 (11 Dec 2019) Sequence version 2 (12 Dec 2006); residues 749-1663	SNLDEDIAEENIVSRSEFPESWLWNVEDLKEPPKNGISTKLMNIFLKDSITTWEIL AVSMDDKKGICVADPFVETVMQDFFIDLRPLYSVVRNEQVEIRAVLYNYRQNKEL KVRVELLHNPFCSLATTKRRHQQTVTIPPKSSLSVPYVIVPLKTGLQEVEVKA YHHFISDGVKSLKVPEGIRMNKTVAVRTLDPERLGRGREGVQKEDIPPADLSQV PDTESETRILLQGTPVAQMTEDAVDAERLKHILVTPSGCGEQNMIGMTPTVIAVH YLDETEQWEKFGLEKRGGALELIKGGYTQQLAFRQPSAFAAFVKRAPSTWLTA YVVKVFLAVNLIAIDSQVLCGAVKWLILEKQKPDGVFQEDAPVIHQEMIGGLRNN NEKDMALTAFLVLSLQEAKDICEEQVNSLPGSITKAGDFLEANYMNLQRSYTVIA GYALAQMGRLKGPLLNKFLTTAKDKNRWEDPGKQLYNVEATSYALLALLQLKDF DFVPPVVRWLNEQRYGGGGYGSTQATFMVFQALAQYQKDAPDHQELNLDVSL QLPSRSSKITHRIHWESASLLRSEETKENEGFTVTAEGKGQGTLSVVTMYHAKAK DQLTCNKFDLKVTIKPAPEKTEKRPQDAKNTMILEICTRYRGDQDATMSILDISMT GFAPDTPDLKQLANGVDYRISKYELDKAFSDRNTLIYLDKVSHSEDDCLAFKVHQ YFNVELIQPGAVKVYAYYNLEESCTRFYHPEKEDGKLNKLCRDELRCRCAEENC FIQKSDDKVTLEERLDKACEPGVDYVYKTRLVKVQLSNDFDEYIMAIEQTIKSGSDE VQVGQQRTEFISPIKCREALKLEEKHHYLMWGLSSDFWGEKPNLSYIIGKDTWVEH WPEEDECQDEENQKQCQDLGAFTESMVVFGCPN
17	Human C3 α' chain fragment 2 UniProt: P01024; Entry version 242 (11 Dec 2019) Sequence version 2 (12 Dec 2006); residues 1321-1663	SEETKENEGFTVTAEGKGQGTLSVVTMYHAKAKDQLTCNKFDLKVTIKPAPEK RPQDAKNTMILEICTRYRGDQDATMSILDISMTGFAPDTPDLKQLANGVDYRIS KYELDKAFSDRNTLIYLDKVSHSEDDCLAFKVHQYFNVELIQPGAVKVYAYYNLE ESCTRFYHPEKEDGKLNKLCRDELRCRCAEENCFIQKSDDKVTLEERLDKACEPG VDYVYKTRLVKVQLSNDFDEYIMAIEQTIKSGSDEVQVGQQRTEFISPIKCREALK LEEKHHYLMWGLSSDFWGEKPNLSYIIGKDTWVEHWPEEDECQDEENQKQCQD LGAFTESMVVFGCPN
18	C3f UniProt: P01024; Entry version 242 (11 Dec 2019) Sequence version 2 (12 Dec 2006) residues 1304-1320	SSKITHRIHWESASLLR
19	Human C3c α' chain fragment 1 UniProt: P01024; Entry version 242 (11 Dec 2019) Sequence version 2 (12 Dec 2006); residues 749-954	SNLDEDIAEENIVSRSEFPESWLWNVEDLKEPPKNGISTKLMNIFLKDSITTWEIL AVSMDDKKGICVADPFVETVMQDFFIDLRPLYSVVRNEQVEIRAVLYNYRQNKEL KVRVELLHNPFCSLATTKRRHQQTVTIPPKSSLSVPYVIVPLKTGLQEVEVKA YHHFISDGVKSLKVPEGIRMNKTVAVRTLDPERLGR
20	FH MS peptide	VTYKCFE
21	FHL-1 MS peptide	NGWSPTPRCIRVSFTL
22	FHR-1 MS peptide	ATFCDFPKINHGIYDEE
23	FHR-2 MS peptide 1	RGWSTPPKCRSTISAE
24	FHR-2 MS peptide 2	AMFCDFPKINHGIYDEE
25	FHR-3 MS peptide	VACHPGYGLPKAQTTVTCTE
26	FHR-4 MS peptide	YQCQSYE
27	FHR-5 MS peptide	RGWSTPPICSFTKGE
28	C3.1 peptide	GTAFVIFGIQDGE
29	C3.2 peptide	LRRQHARASHLGLARSNLDE
30	C3.3 peptide	LRRQHARASHLGLAR
31	C3.4 peptide	LNLDVSLQLPSRSSKITHRIHWE
32	C3.5 peptide	LNLDVSLQLPSR
33	C3.6 peptide	RLGRE

34	C3.7 peptide	SSKITHRIHWE
35	C3.8 peptide	SASLLR
36	C3.9 peptide	RLGR
37	C3.10 peptide	HLIVTPSGCGE
38	Fl peptide 1	CAGTYDGSIDACKGDSGGPLVCMDANNVTYVWGVVSWGE
39	Fl peptide 2	GTCVCKLPYQCPKNGTAVCATNRRSFPTYCQKSLE
40	Fl peptide 3	FPGVYTKVANYFDWISYHVGRPFISQYNV
41	Fl peptide 4	ANVACLDLGFQGGADTQRRFKLSDSLINSTE
42	Fl peptide 5	LPRSIPACVPWSPYLFQPNDCIVSGWGRE
43	Fl peptide 6	KKCLAKKYTHLSCDKVFCQPWQRCIE
44	Fl peptide 7	LCCKACQGKGFHCKSGVCIPSQYQCNGE
45	Fl peptide 8	VKLVDQDKTMFICKSSWSMRE
46	Fl peptide 9	VKLISNCSKFYGNRFYE
47	Fl peptide 10	CLHPGTKFLNNGTCTAE
48	Fl peptide 11	NYNAGTYQNDIALIE
49	Fl peptide 12	GKFSVSLKHGNTDSE
50	Fl peptide 13	VGCAGFASVTQEE
51	Fl peptide 14	MKKDGNKKDCE
52	Fl peptide 15	YVDRIIFHE
53	Fl peptide 16	CLHVHCRGLE
54	Fl peptide 17	RVFSLQWGE
55	Fl peptide 18	ILTADMDAE
56	Fl peptide 19	KVTYTSQE
57	Fl peptide 20	VDCITGE
58	Fl peptide 21	NCGKPE
59	Fl peptide 22	TSLAE
60	Fl peptide 23	KDNE
61	C3.1.1	AKIRYYTYLIMNKGRLKAGRQVREPGQDLVVLPLSITDFIPSFRLVAYYTLIGAS GQRE
62	C3.1.2	RSGIPIVTSPIYQIHFTKPKYFKPGMPFDLMVFVTNPDS PAYRVPVAVQGE
63	C3.1.3	KVVLVSLQSGYLFIQTDKTIYTPGSTVLYRIFTVNHKLLPVGRTVMVNIE
64	C3.1.4	DTVQSLTQGDGVAKLSINTHPSQKPLSITVRTKKQE
65	C3.1.5	GDHGARVVLVAVDKGVFVLNKKNKLTQSKIWDVVE
66	C3.1.6	KADIGCTPGSGKDYAGVFSDAGLTFTSSSGQQTAAQRAE
67	C3.1.7	QATRTMQALPYSTVGNSNNYLHLSVLRTE
68	C3.1.8	AHDAQGDVPVTVTVHDFPGKKLVLSSE
69	C3.1.9	GIPVKQDSLSSQNQLGVLPLSWDIPE
70	C3.1.10	VVADSVWVDVKDSCVGSLLVKSQGE
71	C3.1.11	DLVGKSLYVSATVILHSGSDMVQAE
72	C3.1.12	KTVLTPATNHMGNVFTIPANRE
73	C3.1.13	KGRNKFVTVQATFGTQVVE
74	C3.1.14	VVLSRKVLLDGVQNPRAE
75	C3.1.15	TLNVNFLLRMDRAHE
76	C3.1.16	LVNMGQWKIRAYYE
77	C3.1.17	SPMYSIITPNILRE
78	C3.1.18	DRQPVPGQMQMTLKIE
79	C3.1.19	VTITARFLYGKKVE
80	C3.1.20	GTAFVIFGIQDGE
81	C3.1.21	KFYIYNE
82	C3.1.22	NSPQQVFSTE
83	C3.1.23	SLKRIPIE
84	C3.1.24	YVLPSFE
85	C3.1.25	QRISLPE
86	C3.1.26	LQCPQPAA
87	C3.1.27	VIVEPTE
88	C3.1.28	TMVLE
89	C3.1.29	LRPGE
90	C3.1.30	FKSE

91	FHR-1 peptide 2	NYNIALRWTAQKQKLYLRTGE
92	FHR-2 peptide 3	YNFVSPSKSFWTRITCAEE
93	FHR-3 peptide 2	KGWSPTPRCIRVRTCSKSDIE
94	FHR-3 peptide 3	NGYNQNYGRKFVQGNSTE
95	FHR-3 peptide 4	QVKPCDFPDIKHGGLFHE
96	FHR-3 peptide 5	FMCKLGYNANTSILSFQAVCRE
97	FHR-3 peptide 6	YQCQPYEE
98	FHR-4 peptide 2	NSRAKSNGMRFKLHDTLDYE
99	FHR-4 peptide 3	DGWSHFPTCYNSE
100	FHR-4 peptide 4	ISYGNTTGSIVCGE
101	FHR-4 peptide 5	FMCKLGYNANTSIVLSFQAVCRE
102	FHR-5 peptide 2	GTLCDFPKIHGFLYDEE
103	FHR-5 peptide 3	YAMIGNNMITCINGIWTE
104	FHR-5 peptide 4	YGYVQPSVPPYQHGVSV
105	FHR-5 peptide 5	GDTVQIICNTGYSLQNE
106	FHR-5 peptide 6	IVCKDGRWQSLPRCVE
107	FHR-5 peptide 7	DYNPFSQVPTGE
108	FHR-5 peptide 8	QVKTCGYIPE
109	FHR-5 peptide 9	ANVDAQPKKE
110	FHR-5 peptide 10	WTTLPCTVE
111	FHR-5 peptide 11	KVAVLCKE
112	FH peptide 2	SNTGSTTGSIVCGYNGWSDLPICYE
113	FH peptide 3	NGWSPTPRCIRVKTCSSSIDIE
114	FH peptide 4	LPKIDVHLVPDRKKDQYKVE
115	FH peptide 5	YYCNPRLMKGPNIQCVDE
116	FH peptide 6	NYNIALRWTAQKQKLYSRTGE
117	FH peptide 7	KWSHPPSCIKTDCSLPSFE
118	FH peptide 8	HGWAQLSSPPYYGDSVE
119	FH peptide 9	ISHGVVAHMSDSYQYGE
120	FH peptide 10	FDHNSNIRYRCRGKE
121	FH peptide 11	ITCKDGRWQSIPLCVE
122	FH peptide 12	GWHTVCINGRWDPE
123	FH peptide 13	KAKYQCKLGYVTADGE
124	FH peptide 14	TTCYMGKWSSPPQCE
125	FH peptide 15	SYAHGTKLSYTCE
126	FH peptide 16	RVRYQCRSPYE
127	FH peptide 17	GFGIDGPAIAKCLGE
128	FH peptide 18	HGTINSSRSSQE
129	FH peptide 19	YQCQNLYQLE
130	FH peptide 20	WTTLPVCIVVE
131	FH peptide 21	KIPCSQPPQIE
132	FH peptide 22	SQYTYALKE
133	FH peptide 23	QVQSCGPPPE
134	FH peptide 24	KKDVYKAGE
135	FH peptide 25	GLPCKSPPE
136	FH peptide 26	KVSVLCQE
137	FH peptide 27	HLKNKKE
138	FH peptide 28	GGFRISSE
139	FH peptide 29	LLNGNVKE
140	FH peptide 30	YPTCAKR
141	FH peptide 31	STCGDIPE
142	C3dg UniProt: P01024; Entry version 242 (11 Dec 2019) Sequence version 2 (12 Dec 2006) residues 955-1303	EGVQKEDIPPADLSDQVPDTESETRILLQGTPVAQMTEDAVDAERLKHIVTPSG CGEQNMIGMTPTVIAVHYLDETEQWEKFGLEKRRQGALELIKKGYTQQLAFRQPS SAFAAFVKRAPSTWLTAAYVVKVFLAVNLIAIDSQVLCGAVKWLILEKQKPDGVFQ EDAPVIHQEMIGGLRNNEKDMALTAFVLISLQEAKDICEEQVNSLPGSITKAGDF LEANYMNLQRSYTVIAGYALAAQMGRLKGPLLNKFLTAKDKNRWEDPGKQLYN VEATSYALLALLQLKDFDFVPPVVRWLNEQRYGGGYGSTQATFMVFQALAQY QKDAPDHELNLDVSLQLPSR

143	C3g UniProt: P01024; Entry version 242 (11 Dec 2019) Sequence version 2 (12 Dec 2006) residues 955-1001	EGVQKEDIPPADLSDQVPDTESETRILLQGTPVAQMTEDAVDAERLK
144	C3d UniProt: P01024; Entry version 242 (11 Dec 2019) Sequence version 2 (12 Dec 2006) residues 1002-1303	HLIVTPSGCGEQNMIGMTPTVIAVHYLDETEQWEKFGLEKRQGALELIKGYTQQ LAFRQPSSAFAAFVKRAPSTWLTAYVVKVFLAVNLIADSQVLCGAVKWLILEKQ KPDGVFQEDAPVIHQEMIGGLRNNNEKDMALTAFVLISLQEAKDICEEQVNSLPG SITKAGDFLEANYMNLQRSYTVIAIAGYALAQMGRLKGPLLNKFLTAKDKNRWE DPGKQLYNVEATSYALLALLQLKDFDFPPVVRWLNEQRYGGGYGSTQATFM VFQALAQYQKDAPDHQELNLDVSLQLPSR
145	Human Complement Receptor 1; consensus sequence for CCPs 8-10, 15-17 (UniProt: P17927 residues 491 to 684; residues 941 to 1134) Without leader sequence	GHCQAPDHFLFAKLKTQTNASDFPIGTSLKYECRPEYYGRPFSITCLDNLVWSSP KDVCKRKSKCTPPDPVNGMVHVITDIQVGSRINYSCTTGHRHIGHSSAECILSGN X ₁ AHWSTKPPICQRI PCGLPPTIANGDFISTNREN FHYGSVVTYRCN X ₂ GS X ₃ GRK VFELVGEPsiYCTSNDDQVGiWsgPAPQCII
146	Human Complement Receptor 1 CCPs 8- 10 (UniProt: P17927 residues 491 to 684) Without leader sequence	GHCQAPDHFLFAKLKTQTNASDFPIGTSLKYECRPEYYGRPFSITCLDNLVWSSP KDVCKRKSKCTPPDPVNGMVHVITDIQVGSRINYSCTTGHRHIGHSSAECILSGN AA HWSTKPPICQRI PCGLPPTIANGDFISTNREN FHYGSVVTYRCN PGSG GRKVF ELVGEPsiYCTSNDDQVGiWsgPAPQCII
147	Human Complement Receptor 1 CCPs 15-17 (UniProt: P17927 residues 941 to 1134) Without leader sequence	GHCQAPDHFLFAKLKTQTNASDFPIGTSLKYECRPEYYGRPFSITCLDNLVWSSP KDVCKRKSKCTPPDPVNGMVHVITDIQVGSRINYSCTTGHRHIGHSSAECILSGN TA HWSTKPPICQRI PCGLPPTIANGDFISTNREN FHYGSVVTYRCN LGSR GRKVF ELVGEPsiYCTSNDDQVGiWsgPAPQCII
148	Human Complement Receptor 1 CCPs 8-10 and 15- 17 (contiguous; without leader sequence) [amino acid differences between CCPs 8-10 and 15- 17 indicated with wavy underline]	GHCQAPDHFLFAKLKTQTNASDFPIGTSLKYECRPEYYGRPFSITCLDNLVWSSP KDVCKRKSKCTPPDPVNGMVHVITDIQVGSRINYSCTTGHRHIGHSSAECILSGN AA HWSTKPPICQRI PCGLPPTIANGDFISTNREN FHYGSVVTYRCN PGSG GRKVF ELVGEPsiYCTSNDDQVGiWsgPAPQCIIIGHCQAPDHFLFAKLKTQTNASDFPIGT SLKYECRPEYYGRPFSITCLDNLVWSSPKDVCKRKSKCTPPDPVNGMVHVITDIQ VGSRINYSCTTGHRHIGHSSAECILSGN TA HWSTKPPICQRI PCGLPPTIANGDFIS TNREN FHYGSVVTYRCN LGSR GRKVFELVGEPsiYCTSNDDQVGiWsgPAPQCI I
149	Human Complement Factor I proteolytic domain (UniProt: P05156 residues 340-574)	IVGGKRAQLGDLPWQVAIKDASGITCGGIYIGGCWILTAHCLRASKTHRYQIWTT VVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQNDIALIEMKKDGNKKDCELP RSIPA CVPWSPYLFQPNDCIVSGWGREKDNERVFLQWGEVKLISNCSKFYGNRFYE KEMECAGTYDGSIDACKGDSGGPLVCM DANNVTVVWGVVSWGENCGKPEFPG VYTKVANYFDWISYHVG
150	Human Complement Factor H co-factor region (UniProt: P08603 residues 19-264)	EDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPGYRSLGNVIMVCRKGEWW ALNPLRKCKRCPGHPGDTFPGTFTLTGGNVFEYGVKAVYTCNEGYQLLGEINY RECDTDGWTNDIPICEVVKCLPVTAPENGKIVSSAMEPDREYHFGQAVRFVCNS GYKIEGDEEMHCSDDGFWSEKPKCIVEISCKSPDVINGSPISQKIYKENERFQY KCNMGYEYSERGDAVCTESGWRPLPSC EE

151	Human Complement Receptor 1 CCPs 8-10 (UniProt: P17927 residues 491 to 684)	GHCQAPDHFLFAKLKTQTNASDFPIGTS�KYECRPEYYGRPFISITCLDNLVWSSPKDVCKRKSCKTPPDVNGMVHVITDIQVGSRINYSCTTGHRIGHSSAECILSGNAAHWSTKPPICQRIPCGLPTIANGDFISTNRENPHYGSVVTYRCNP GSGGRKV FELVGESYICTSNDDQVGIW SG PAPQCII
152	Human Complement Receptor 1 CCPs 15-17 (UniProt: P17927 residues 941 to 1134)	GHCQAPDHFLFAKLKTQTNASDFPIGTS�KYECRPEYYGRPFSITCLDNLVWSSPKDVCKRKSCKTPPDVNGMVHVITDIQVGSRINYSCTTGHRIGHSSAECILSGNTAHWSTKPPICQRIPCGLPTIANGDFISTNRENPHYGSVVTYRCNLGSRGRKV FELVGESYICTSNDDQVGIW SG PAPQCII
153	GluC (Uniprot Q7A6A6 including N-terminal signal peptide residues 1-29 and propeptide residues 30-68)	MKGKFLKVSSLFVATLTTATLVSSPAANALSSKAMDNH PQQTQSSKQQTPKIKKGGNLKPLEQREHANVILPNNDRHQITDTTNGHYAPVTYIQVEAPTGTFIASGVVVGKDTLLTNKHVV DATHGDPHALKA FPSAINQDNYPNGGFTAEQITKYSGEGLAI VKFSPNEQNKHIGEVVKPATMSNNAETQVNQNI TVTGYPGD KPVATMWESKGKITYLKGEAMQYDLSTTGGNSGSPVFNEKNEVIGIHWGGVPNEFN GAVFINENVRNFLKQNI EDIH FANDDQPNNPDNPDNPNPNPDNPNNPDEPNNP DNPNP DNP DNPGDNNNSDNPDA A
154	GluC (Uniprot Q7A6A6; without signal peptide or propeptide)	VILPNNDRHQITDTTNGHYAPVTYIQVEAPTGTFIASGVVVGKDTLLTNKHVV DATHGDPHALKA FPSAINQDNYPNGGFTAEQITKYSGEGLAI VKFSPNEQNKHIGEVVKPATMSNNAETQVNQNI TVTGYPGD KPVATMWESKGKITYLKGEAMQYDLSTTGGNSGSPVFNEKNEVIGIHWGGVPNEFN GAVFINENVRNFLKQNI EDIH FANDDQPNNPDNPDNPNPNPDNPNNPDEPNNP DNPNP DNP DNPGDNNNSDNPDA A
155	Fl peptide 24	VGCAGFASVTQE
156	C3a-desArg	LRRQHARASHLG LA
157	C3f-desArg	SASLL

Brief Description of the Figures

Figure 1. Schematic showing the C3 proteolytic cascade and the proteolytic events leading to the generation, breakdown and inactivation of C3b (modified from Maillard et al, J Am Soc Nephrol. 2015 Jul;26(7):1503-12). Proteoform-specific peptides for mass spectrometry are underlined.

Figure 2. LC-SRM Trace showing detection of the heavy-labelled synthetic standard peptides of each individual RCA locus protein from a plasma sample.

Figure 3. Linearity data for peptides derived from FH, FHL-1, and FHR1-5.

Figures 4A to 4D. Data confirming that C3 and C3 breakdown products in human plasma can be detected by MS with sufficient specificity and sensitivity. **4A:** Total ion chromatograph from SRM-MS analysis showing specific and simultaneous detection of C3b fragment-specific peptides. **4B:** Linearity data for seven of the ten peptides spiked into a plasma background. **4C:** Coomassie-stained electrophoresis gel of C3 breakdown products obtained *in vitro*. **4D:** MS quantification of key C3 fragments from the *in vitro* assay products shown in 4C.

EXAMPLES**Example 1: Generation of peptides from complement proteins for mass spectrometry**

GluC digestion was performed on FH, FHL-1, FHR1-5, FI, C3, C3b and C3b breakdown products to achieve distinct peptides for mass spectrometry. GluC digestion is described in Example 2.2.

5

Peptides that can be used to detect each protein or protein fragment are set out in Tables 1-4 below.

Table 1. Distinct FH family peptides after GluC digestion.

Protein	Peptide Sequence	Mass	SEQ ID No:
Factor H	VTYKCFE	888.4051	20
FHL1	NGWSPTPRCIRVSFTL	1832.9355	21
FHR1	ATFCDFPKINHGILYDEE	2110.9669	22
FHR2	RGWSTPPKCRSTISAE and	1774.8784	23
	AMFCDFPKINHGILYDEE	2140.9598	24
FHR3	VACHPGYGLPKAQTTVTCTE	2074.9816	25
FHR4	YQCQSYYE	1082.4015	26
FHR5	RGWSTPPICSFTKGE	1664.7980	27

- 10 The series of proteolytic events leading to the generation, breakdown and inactivation of C3 are shown in Figure 1. Proteoform-specific peptides produced by GluC digestion are underlined in Figure 1 and are shown in Table 2. Table 3 shows how each protein can be detected individually using the peptides in Table 2.

- 15 Table 2. Peptide sequences for MS resulting from GluC digestion of C3, C3b and breakdown products.

Peptide	Peptide sequence	Contained in C3b products	SEQ ID No:
C3.1	GTA FVIFGIQDGE	C3 + C3b + iC3b + C3c	28
C3.2	LRRQHARASHLGLARSNLDE	C3 only	29
C3.3	LRRQHARASHLGLAR	C3a only	30
C3.4	LNLDVSLQLPSRSSKITHRIHWE	C3 + C3b	31
C3.5	LNLDVSLQLPSR	iC3b + C3dg + C3d	32
C3.6	RLGRE	C3 + C3b + iC3b	33
C3.7	SSKITHRIHWE	C3f	34
C3.8	SASLLR	C3f	35
C3.9	RLGR	C3c	36
C3.10	HLIVTPSGCGE	C3d	37

Table 3. Methodology for determining concentration of all C3/C3b breakdown products using GluC digestion peptides of Table 2.

Protein or fragment	Peptide(s)
C3 (total)	C3.1
C3 only	C3.2
C3a	C3.3
C3b	C3.4 – C3.2
iC3b	C3.6 – C3.4
C3f	C3.7 or C3.8
C3c	C3.9
C3dg	C3.5 – C3.10 – iC3b [C3.6 - C3.4]
C3d	C3.10

Table 4. Alternative peptides for C3.1 resulting from GluC digestion, to measure total C3 content.

Peptide	Mass	Position	Peptide sequence	SED ID No.
C3.1.1	6957.8210	463-523	AKIRYYTYLIMNKGRLKAGRQVREPGQDLV VLPLSITTDIFIPSFRLVAYYTLIGASGQRE	61
C3.1.2	5778.9734	321-372	RSGIPIVTSPIYQIHFTKTPKYFKPGMPFDLMV FVTNPDGSPAYRVPVAVQGE	62
C3.1.3	5650.1364	97-146	KVVLVSLQSGYLFQTDKTIYTPGSTVLYRIFT VNHKLLPVGRTVMVNIE	63
C3.1.4	3904.1017	373-408	DTVQSLTQGDGVAKLSINTHPSQKPLSITVRT KKQE	64
C3.1.5	3861.1628	565-599	GDHGARVVLVAVDKGVFVLNKKNKLTQSKI WDVVE	65
C3.1.6	3807.7645	600-637	KADIGCTPGSGKDYAGVFSDAGLTFTSSSG QQTAAQRAE	66
C3.1.7	3263.6357	414-442	QATRTMQALPYSTVGNSNNYLHLSVLRTE	67
C3.1.8	2845.4609	24-50	AHDAQGDVPVTVTVHDFPGKKLVLSSE	68
C3.1.9	2819.4705	150-175	GIPVKQDSLSSQNQLGVLPLSWDIPE	69
C3.1.10	2691.3425	524-549	VVADSVWVDVKDSCVGSVLVKSGQSE	70
C3.1.11	2618.3261	296-320	DLVGKSLYVSATVILHSGSDMVQAE	71
C3.1.12	2511.2904	51-73	KTVLTPATNHMGNTFTIPANRE	72
C3.1.13	2108.1378	78-96	KGRNKFVTVQATFGTQVVE	73
C3.1.14	1992.1480	278-295	VVLSRKVLLDGVQNPRAE	74
C3.1.15	1827.9414	448-462	TLNVNFLLRMDRAHE	75
C3.1.16	1769.8923	176-189	LVNMGQWKIRAYYE	76
C3.1.17	1745.9386	1-15	SPMYSIITPNILRLE	77
C3.1.18	1738.9036	550-564	DRQPVPGQQMTLKIE	78
C3.1.19	1623.9348	231-244	VTITARFLYGKKVE	79
C3.1.20	1352.6612	245-257	GTAFVIFGIQDGE	80
C3.1.21	1138.5335	219-226	KFYIYNE	81
C3.1.22	1135.5146	190-199	NSPQQVFSTE	82
C3.1.23	954.5862	265-272	SLKRIPIE	83
C3.1.24	853.4221	205-211	YVLPSE	84
C3.1.25	841.4657	258-264	QRISLPE	85
C3.1.26	826.4007	638-645	LQCPQPAA	86
C3.1.27	785.4171	212-218	VIVEPTE	87
C3.1.28	591.2938	19-23	TMVLE	88
C3.1.29	570.3125	443-447	LRPGE	89
C3.1.30	509.2485	74-77	FKSE	90

GluC digestion of Factor I (FI) produced the candidate peptides in Table 5 for MS analysis. SEQ ID NO:45 to 56 and 155 contain 8-21 amino acids and are a good length for MS analysis.

Table 5. Peptide sequences resulting from GluC digestion of FI.

Peptide	Mass	Position	Peptide sequence	SEQ ID NO:
1	3996.7183	510-548	CAGTYDGSIDACKGDSGGPLVCMDANNVTYVWGVVS WGE	38
2	3994.8853	57-92	GTCVCKLPYQCPKNGTAVCATNRRSFPTYCQQKSLE	39
3	3467.7211	555-583	FPGVYTKVANYFDWISYHVGRPFISQYNV	40
4	3397.6804	150-180	ANVACLDLGFQQGADTQRRFKLSDSL SINSTE	41
5	3388.6605	446-475	LPRSIPACVPWSPYLFQPNDCIVSGWGRE	42
6	3155.5773	31-56	KKCLAKKYTHLSCDKVFCQPWQRCIE	43
7	2991.2861	254-281	LCCKACQKGKGFHCKSGVCIPSQYQCNGE	44
8	2531.2455	129-149	VKLVDQDKTMFICKSSWSMRE	45
9	2068.0320	489-505	VKLISNCSKFYGNRFYE	46
10	1805.8309	93-109	CLHPGKFLNNGTCTAE	47
11	1698.7969	420-434	NYNAGTYQNDIALIE	48
12	1605.7867	110-124	GKFSVSLKHGNTDSE	49
13	1297.5729	291-303	VGCAGFASVTQEE	50
14	1168.272	291-302	VGCAGFASVTQE	155
15	1295.6082	435-445	MKKDGNKKDCE	51
16	1191.6156	411-419	YVDRIIFHE	52
17	1166.5557	181-190	CLHVHCRGLE	53
18	1121.5738	480-488	RVFSLQWGE	54
19	978.4448	306-314	ILTADMDAE	55
20	955.4731	19-26	KVTYTSQE	56
21	736.3182	282-288	VDCITGE	57
22	647.2817	549-554	NCGKPE	58
23	520.2613	191-195	TSLAE	59
24	505.2252	476-479	KDNE	60

5

Example 2: Mass spectrometry

2.1 Preparation of stable isotopic standards (SIS) spiking solution

High purity heavy-labelled synthetic standards, with S-carboxymethylated (CAM) cysteine residues, were obtained (Cambridge Research Biochemicals, Cambridge, UK) and diluted to 1 µg/µL with 50:50 acetonitrile:water + 0.1 % formic acid (Table 6).

10

A mixed SIS solution was prepared by firstly diluting stock solution of FHL-1, FHR1, FHR2, FHR3, FHR4 and FHR5 by tenfold (no dilution of CFH stock was required), then adding the appropriate amounts of each individual diluted solution to a final volume of 200 µL in 0.1 % TFA. This was then stored at -80 °C in 5 µL aliquots for further dilution immediately prior to spiking.

15

Spiking solution was prepared immediately prior to sample addition by adding 195 μL 50:50 acetonitrile:water to a 5 μL aliquot of the mixed SIS solution. 2 μL of this was carefully added to each digested sample prior to drying down.

- 5 Table 6. Stock solutions of stable isotopic standards (SIS) at 1 $\mu\text{g}/\text{mL}$. The residues in bold were chosen to carry a stable-heavy isotope to enable quantitation. Lower case 'c' denotes a S-carboxymethylated (CAM) cysteine residue. Residue in bold type contained an isotopically heavy amino acid, where K(+8), R(+10), F(+10) and Y(+10).

Protein	Peptide Sequence	MW, g	Purity, %	Net Content, %	Solvent Volume, μL	Conc, pmol/ μL
CFH	VTYKcFE	953.4	98.6	72.6	716	1050
FHL-1	NGWSPTPRcIRVSFTL	1900	97.4	78.9	768	526.3
FHR1	ATFcDFPKINHGILYDEE	2178	100.0	80.7	807	459.1
FHR2	RGWSTPPKcRSTISAE	1845.9	100.0	55.5	555	541.7
FHR3	VAcHPGYGLPKAQTTVTcTE	2207.1	98.4	75.0	738	453.1
FHR4	YQcQSYYE	1149.7	97.6	84.1	821	869.8
FHR5	RGWSTPPKcSFTKGE	1739.8	95.6	74.0	707	574.8

10 2.2 Preparation of samples for analysis by LC-MS/MS

Frozen plasma samples were allowed to thaw to room temperature before being vortexed hard for 5 minutes to dissolve any soluble material, then centrifuged at 13,300g for 30 min to settle any insoluble material.

- 15 To a 5 μL plasma aliquot (equivalent to approximately 350 μL protein), 90 μL of 50 mM ammonium bicarbonate (pH 7.8), 2 μL of ProteaseMAX™ (Promega, Southampton, UK) solution (1 % w/v in 50 mM ammonium bicarbonate) and 1 μL of 500 mM dithiothreitol prepared in 50 mM ammonium bicarbonate was added. This was vortexed briefly to mix, then given a pulse spin before incubating at 56 °C for 25 min.

- 20 After cooling to room temperature, 3 μL 500 mM iodoacetamide (prepared in 50 mM ammonium bicarbonate) was added. This was vortexed briefly to mix, then given a pulse spin before incubating at room temperature and in the dark for 15 min.

- 25 A further 1 μL of ProteaseMAX solution (1 % w/v in 50 mM ammonium bicarbonate) and 5 μL of 1 $\mu\text{g}/\mu\text{L}$ endoproteinase GluC (Roche, Mannheim, Germany) were added. The mixture was vortexed briefly, then given a pulse spin before incubating for 16 hours at 25 °C with slight shaking (400 rpm).

- 30 To the digested peptide mix obtained, 6 μL 10 % v/v trifluoroacetic acid (TFA) and 2 μL of SIS spiking solution were added, vortexed briefly to mix, then pulse spin. The solution was placed into an

evaporator and dried. Finally the peptides were reconstituted in 50 μ L 0.1 % TFA and vortexed to dissolve any residue before centrifuging at 13,300g for 30 min to settle any insoluble/particulate material. Approximately 48 μ L (taking care to leave behind any precipitated material) was transferred to a LC autosampler vial for subsequent analysis by LC-MS/MS.

5 2.3 LC-SRM/MS analysis of plasma digests

SRM analyses of plasma digests were performed on a 6495 triple quadrupole mass spectrometer with iFunnel-equipped electrospray ion source (Agilent, Santa Clara, CA, USA) coupled to an Infinity 1200 Series liquid chromatography system consisting of 1290 autosampler, 1260 Quat Pump VL pump and TCC column oven modules (Agilent, Santa Clara, CA, USA). Samples were injected directly (4 μ L) onto a C18 column (250 mm x 2.1 mm i.d., Thermo Scientific Acclaim 120, 3 μ m particle size) that was maintained at a column temperature of 50 °C. Compounds were developed using a gradient elution of increasing acetonitrile concentration with Buffer A consisting of Water + 0.1 % formic acid and Buffer B being Acetonitrile + 0.1 % formic acid. The flow rate was maintained at 250 μ L/min with an initial composition of 5 % Buffer B.

The following gradient elution profile was used to separate the peptides (time: %B): 0 min: 5 % B; 2 min: 5 % B; 3 min: 12 % B; 12 min: 15 % B; 15 min: 20 % B; 30 min: 25 % B; 31 min: 90 % B; 39 min: 90 % B; 40 min: 5 % B; 49 min: 5 % B.

Optimized SRM settings were determined using SIS solutions and are provided in Table 7.

Table 7. SRM transitions and optimal collision energies for FH family peptides (Quantitation ions in bold).

Protein	Peptide Sequence	Precursor ion <i>m/z</i>	Product ions <i>m/z</i>	Collision energy, eV
CFH	VTYKcFE (Light)	473.7	583.3, 847.4, 746.3	16, 16, 16
CFH	VTYKcFE (Heavy)	477.7	591.3, 855.4, 754.3	16, 16, 16
FHL-1	NGWSPTPRcIRVSFTL (Light)	631.2	723.9, 860.5, 767.4	19, 19, 19
FHL-1	NGWSPTPRcIRVSFTL (Heavy)	634.3	728.9, 865.5, 772.4	19, 19, 19
FHR1	ATFcDFPKINHGILYDEE (Light)	724.2	925.6, 1011.9, 947.1	20, 16, 20
FHR1	ATFcDFPKINHGILYDEE (Heavy)	727.2	930.6, 1016.9, 952.1	20, 16, 20
FHR2	RGWSTPPKcRSTISAE (Light)	459.1 , 611.8	539.2 , 798.9	8 , 26
FHR2	RGWSTPPKcRSTISAE	462.6 , 616.3	543.9 , 806.0	8 , 26

	(Heavy)			
FHR3	VAcHPGYGLPKAQTTVTcTE (Light)	730.7	1022.4, 971.7	16, 18
FHR3	VAcHPGYGLPKAQTTVTcTE (Heavy)	736.7	1031.4, 980.7	16, 18
FHR4	YQcQSYYE (Light)	570.7	830.3, 993.1, 311.1	11, 10, 14
FHR4	YQcQSYYE (Heavy)	575.7	840.3, 1003.1, 311.1	11, 10, 14
FHR5	RGWSTPPIcSFTKGE (Light)	575.2	828.4, 895.5, 588.3	16, 15, 20
FHR5	RGWSTPPIcSFTKGE (Heavy)	581.2	836.4, 905.5, 598.3	16, 15, 20

Table 8. Peptides and transitions for quantitation of C3/C3b breakdown products.

Peptide	Sequence	Contained in...	Transitions
C3.1	GTAfVIFGIQDGE	C3 + C3b + iC3b + C3c	569.0/483.2 569.0/654.3 569.0/801.4
C3.2	LRRQHARASHLGLARSNLDE	C3 only	384.2/431.6 384.2/510.4 461.0/503.3
C3.3	LRRQHARASHLGLAR	C3a only	291.2/304.3 291.2/395.8 436.3/598.7
C3.4	LNLDVSLQLPSRSSKITHRIHWE	C3 + C3b	546.8/578.7 683.2/797.1 683.2/834.9
C3.5	LNLDVSLQLPSR	iC3b + C3dg + C3d	677.9/1014.4 677.9/359.1 677.9/800.2
C3.6	RLGRE	C3 + C3b + iC3b	251.3/175.1 251.3/232.1 251.3/345.2
C3.7	SSKITHRIHWE	C3f	349.3/354.2 349.3/436.4 349.3/489.8
C3.8	SASLLR	C3f	323.7/159.1

			323.7/288.2 323.7/488.3
C3.9	RLGR	C3c	251.3/175.1 251.3/232.1 251.3/345.2
C3.10	HLIVTPSGCGE	C3d	585.3/251.1 585.3/394.5 585.3/404

In order to protect the source region from unwanted contaminants, a switching valve located between the column and source was diverted to the waste position at points in the chromatogram when the analyte peptides were not eluting. This allowed for six windows (two of the peptides, FHR-2 and FHL-1, eluted within the same window) of acquisition, of approximately one minute each, to be acquired with the column on-line to the mass spectrometer.

2.4 Results

FH family proteins

Figure 2 shows a LC-SRM Trace showing detection of the heavy-labelled synthetic standards of each individual RCA locus protein from a plasma sample. This demonstrates that the method is feasible, specific and has the required sensitivity to distinguish between peptides from these seven proteins, in particular between FH and FHL-1.

Figure 3 shows linearity data for FH, FHL-1, and FHR1-5. This demonstrates that the GluC digestion produces peptides that can be detected individually and specifically in native serum at endogenous levels. It also shows that the assay is capable of quantifying the level of each protein in the sample. Increasing amounts of protein increase the signal in a predictable manner, allowing determination of the levels, as well as the presence, of each of the proteins. Also demonstrated is that the assay is free from interference.

Lower limits of quantitation were defined as plasma concentrations of FH = 25nM, FHL-1 = 0.25nM, FHR-1 = 2nM, FHR-2 = 1 nM, FHR-3 = 1nM, FHR-4 = 4nM and FHR-5 = 3nM.

C3 and C3 breakdown products

Synthetic versions of the peptides in Table 2 were synthesised to confirm and optimise their detection by MS to confirm that they could be quantified in a linear manner, and to demonstrate that they could be detected at endogenous levels in a serum or plasma sample. This is shown by Figures 4A to 4D.

Figure 4A shows that all peptides in Table 2 can be detected individually in a plasma sample by SRM-MS using at least three transitions. The specificity of the assay for the peptides of interest is confirmed by the relative intensities of the transitions matching the relative intensity of the relevant product ions in an MS/MS scan. Figure 4B confirms the specificity of the peptides, showing

experiments in which the plasma sample was spiked with crude synthetic peptide which demonstrated the appropriate increase in signal.

C3b breakdown was further analysed in an *in vitro* assay. C3b was incubated along with FI and a fragment of cofactor CR1, selected over FH as CR1 drives the reaction to cleavage of iC3b to C3c + C3dg, whereas FH will only support cleavage of C3b to iC3b. Sequential samples were taken from the reaction and stopped by boiling.

Figure 4C shows the time course of the C3b breakdown via gel electrophoresis. Analysis using MS and the peptides of Table 2 demonstrates that the formation of C3b fragments iC3b, C3f and C3c, and loss of intact C3b can be clearly detected over time (Figure 4D). Not all peptides are shown since some (e.g. C3a) will not be present in the *in vitro* set-up, and others represent multiple products.

These data demonstrate that C3/C3b breakdown can be measured in a quantitative manner using GluC-derived peptides and MS. This enables the presence and levels of complement proteins to be detected in complement-related diseases such as AMD, as well as providing information as to successful treatment outcomes.

A single assay which can measure all FH family, C3 fragments and FI proteins allows for the simultaneous analysis of all key proteins in the complement amplification loop from just one sample and with efficient throughput.

Claims

1. A method for detecting at least one complement protein in a sample, the method comprising:
digesting the protein(s) with endoproteinase GluC to obtain one or more peptides; and
5 detecting the one or more peptides by mass spectrometry.
2. A method for determining the level of at least one complement protein in a sample, the method comprising:
digesting the protein(s) with endoproteinase GluC to obtain one or more peptides; and
10 determining the level of the one or more peptides by mass spectrometry.
3. A method according to claim 1 or claim 2, wherein the step of detecting the one or more peptides or the step of determining the level of the one or more peptides consists of measuring the one or more peptides by mass spectrometry.
15
4. A method according to any one of claims 1 to 3, wherein the method comprises determining the concentration of the complement protein(s) in the sample.
5. A method for preparing at least one complement protein for analysis, the method comprising
20 digesting the protein(s) with endoproteinase GluC to obtain one or more peptides.
6. A method according to any one of claims 1 to 5, wherein the complement protein(s) is one or more of FH, FHL-1, FHR1, FHR2, FHR3, FHR4 and/or FHR5.
7. A method according to any one of claims 1 to 6, wherein the complement protein is FH and/or FHL-1.
25
8. A method according to any one of claims 1 to 7, wherein the complement protein(s) is involved in the complement amplification loop and/or C3 convertase activity.
30
9. A method according to any one of claims 1 to 8, wherein the complement protein(s) is a breakdown product of C3b.
10. A method according to any one of claims 1 to 9, wherein the complement protein(s) is one or
35 more of C3, C3b, C3a, iC3b, C3f, C3c, C3dg, and/or C3d.
11. A method according to any one of claims 1 to 10, wherein the complement protein(s) is C3b and/or iC3b.

12. A method according to any one of claims 1 to 11, wherein the complement protein is FI.

13. A method according to any one of claims 1 to 12, wherein the sample has been obtained from a subject.

5

14. A method according to any one of claims 1 to 12, wherein the method comprises a step of obtaining the sample from a subject.

15. A method according to any one of claims 1 to 14, wherein the sample comprises, or is derived from, blood, lymph, plasma, serum, tissue or cells.

10

16. A method according to any one of claims 1 to 15, wherein the one or more peptides are selected from the group consisting of:

(a) VTYKCFE (SEQ ID NO:20);

15

(b) NGWSPTPRCIRVSFTL (SEQ ID NO:21);

(c) ATFCDFPKINHGILYDEE (SEQ ID NO:22);

(d) RGWSTPPKCRSTISAE (SEQ ID NO:23) or AMFCDFPKINHGILYDEE (SEQ ID NO:24);

(e) VACHPGYGLPKAQTTVTCTE (SEQ ID NO:25);

(f) YQCQSYYE (SEQ ID NO:26);

20

(g) RGWSTPPICSFTKGE (SEQ ID NO:27);

(h) GTAFVIFGIQDGE (SEQ ID NO:28);

(i) LRRQHARASHLGLARSNLDE (SEQ ID NO:29);

(j) LRRQHARASHLGLAR (SEQ ID NO:30) and/or LRRQHARASHLGLA (SEQ ID NO:156);

(k) LNLDVSLQLPSRSSKITHRIHWE (SEQ ID NO:31);

25

(l) LNLDVSLQLPSR (SEQ ID NO:32);

(m) RLGRE (SEQ ID NO:33);

(n) SSKITHRIHWE (SEQ ID NO:34);

(o) SASLLR (SEQ ID NO:35) and/or SASLL (SEQ ID NO:157);

(p) RLGR (SEQ ID NO:36);

30

(q) HLIVTPSGCGE (SEQ ID NO:37); and/or

(r) any one or more of SEQ ID NO:38 to 60, or 155.

17. Use of endoproteinase GluC for preparing at least one complement protein for detection by mass spectrometry, optionally for preparing at least two complement proteins for detection by mass spectrometry.

35

18. A method of determining the presence and/or level of a complement protein in a subject, the method comprising performing a method according to any one of claims 1 to 17.

19. A method of identifying a subject having a complement-related disorder or at risk of developing a complement-related disorder, the method comprising:

- a) digesting at least one complement protein in a sample obtained from the subject with endoproteinase GluC to obtain one or more peptides;
- b) determining the presence and/or level of the one or more peptides by mass spectrometry; and
- c) using the results of (b) to determine whether the subject has or is likely to develop a complement-related disorder.

20. A method according to claim 19, comprising (d) treating a subject who has been determined to be at risk of developing, or to have, a complement-related disorder, optionally wherein treating a subject comprises administering a therapeutically effective amount of a complement-targeted therapeutic to the subject.

21. A method of selecting a subject for treatment of a complement-related disorder with a complement-targeted therapeutic, the method comprising:

- a) digesting at least one complement protein in a sample obtained from the subject with endoproteinase GluC to obtain one or more peptides;
- b) determining the presence and/or level of the one or more peptides by mass spectrometry; and
- c) using the results of (b) to determine whether the subject is in need of a complement-targeted therapeutic.

22. A complement-targeted therapeutic for use in a method of treating a complement-related disorder in a subject, the method comprising:

- a) digesting at least one complement protein in a sample obtained from the subject with endoproteinase GluC to obtain one or more peptides;
- b) determining the presence and/or level of the one or more peptides by mass spectrometry; and
- c) based on the results of (b), administering an effective amount of the complement-targeted therapeutic.

23. A method according to any one of claims 18 to 22, wherein the method comprises obtaining a sample from a subject comprising at least one complement protein, optionally wherein the sample comprises or is derived from blood, lymph, plasma, serum, tissue or cells.

24. A method according to any one of claims 19 to 23, wherein the complement-related disorder is selected from macular degeneration, age related macular degeneration (AMD), geographic atrophy ('dry' (i.e. non-exudative) AMD), early AMD, early onset macular degeneration (EOMD), intermediate AMD, late/advanced AMD, 'wet' (neovascular or exudative) AMD, choroidal neovascularisation (CNV), retinal dystrophy, Haemolytic Uremic Syndrome (HUS), atypical Haemolytic Uremic Syndrome (aHUS), DEAP HUS (Deficiency of FHR plasma proteins and Autoantibody Positive form of Hemolytic Uremic Syndrome), autoimmune uveitis, Membranoproliferative Glomerulonephritis Type II (MPGN II),

sepsis, Henoch-Schönlein purpura (HSP), IgA nephropathy, chronic kidney disease, paroxysmal nocturnal hemoglobinuria (PNH), autoimmune hemolytic anemia (AIHA), systemic lupus erythematosus (SLE), Sjogren's syndrome (SS), rheumatoid arthritis (RA), C3 glomerulopathy (C3G), dense deposit disease (DDD), C3 nephritic factor glomerulonephritis (C3 NF GN), FHR5 nephropathy, hereditary angioedema (HAE), acquired angioedema (AAE), encephalomyelitis, atherosclerosis, neurodegeneration/neurodegenerative disease, dementia, multiple sclerosis (MS), cancer, stroke, Parkinson's disease, and/or Alzheimer's disease.

25. A method according to any one of claims 18 to 24, wherein an increase in the level of one or more of C3, C3b, C3a, iC3b, FHR1, FHR2, FHR3, FHR4 and/or FHR5; and/or a decrease in the level of one or more of iC3b, C3f, C3c, C3dg, C3d, C3g, FI, FH, FHL-1, FHR1, FHR2, FHR3, FHR4 and/or FHR5 as compared to reference value(s), indicates that the subject is at risk of developing or has a complement-related disorder.

26. A kit for use in a method of detecting and/or determining the level of one or more complement protein(s) in a sample, the kit comprising endoproteinase GluC.

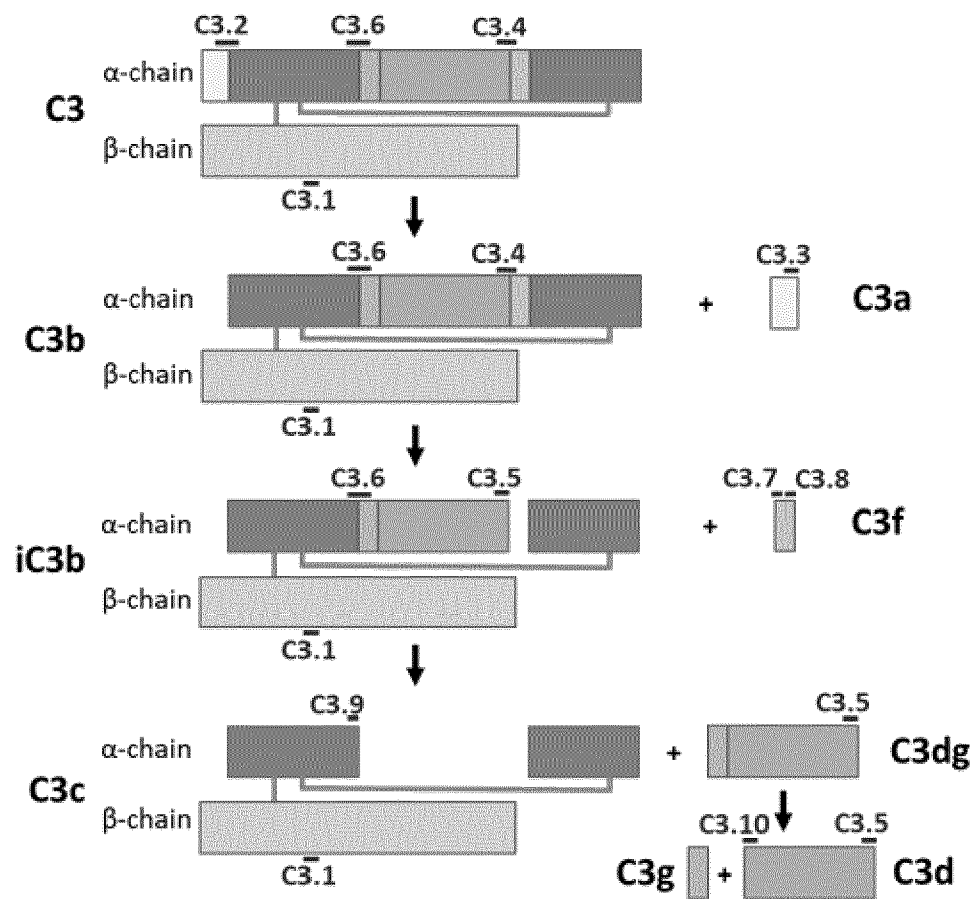


Figure 1

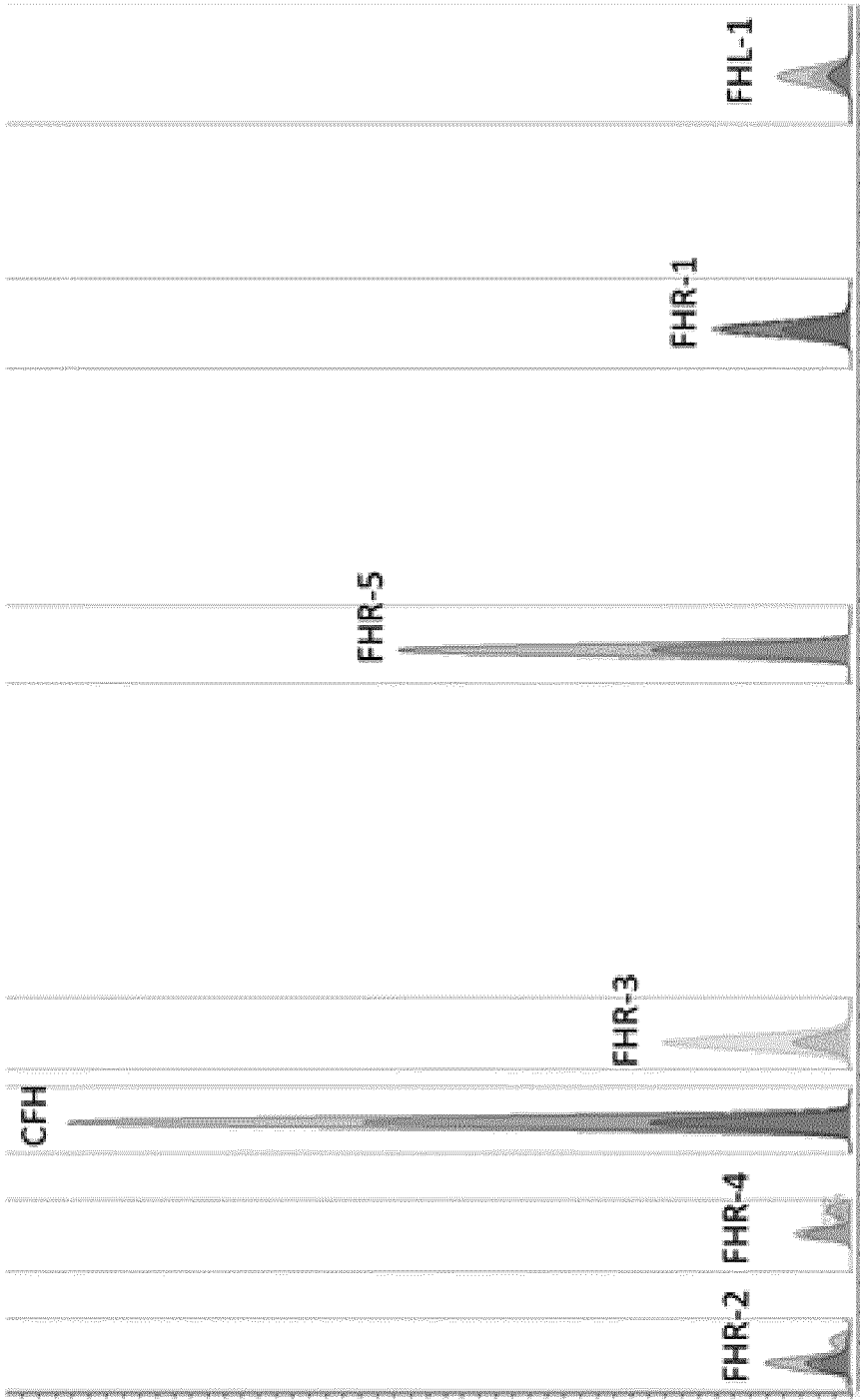


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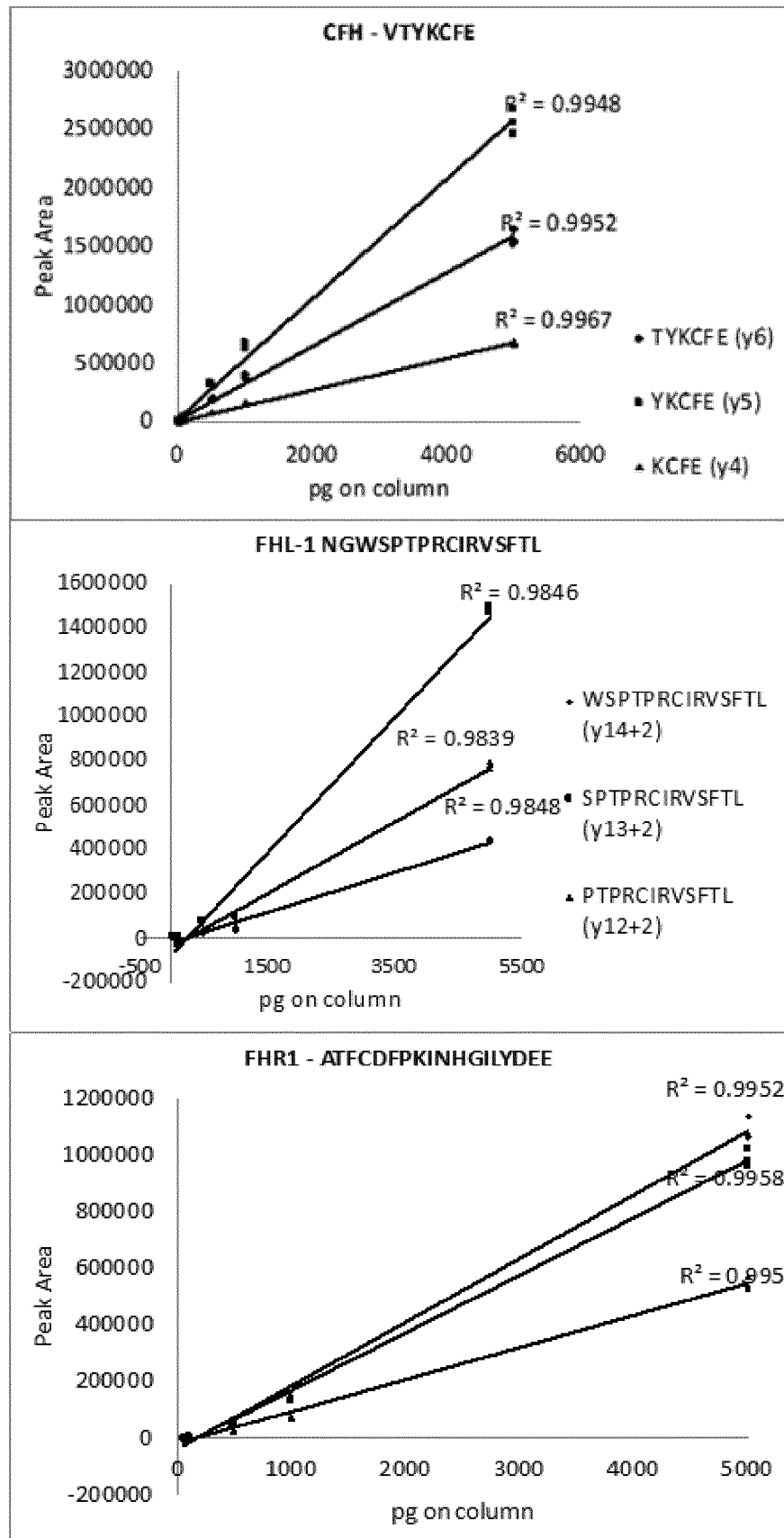


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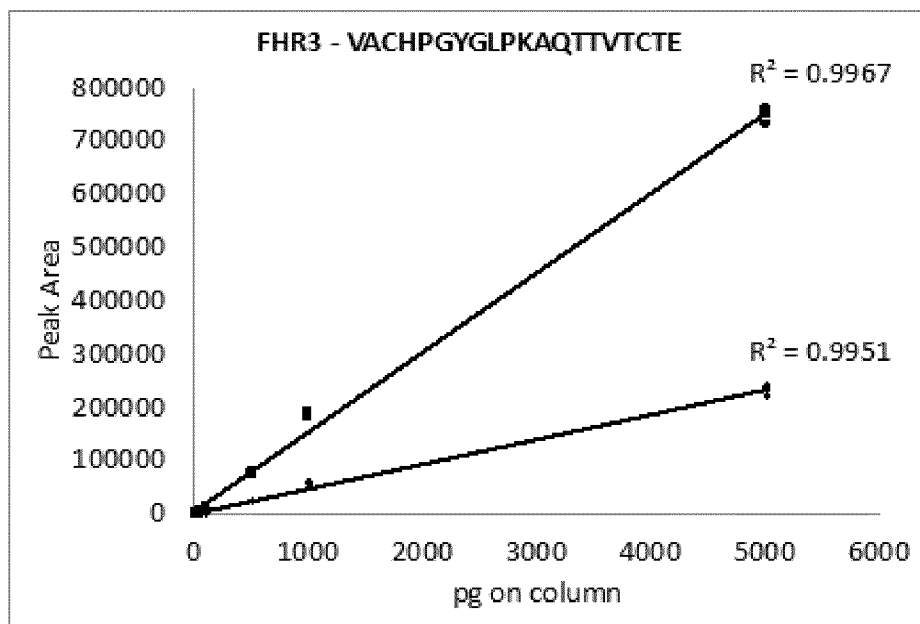
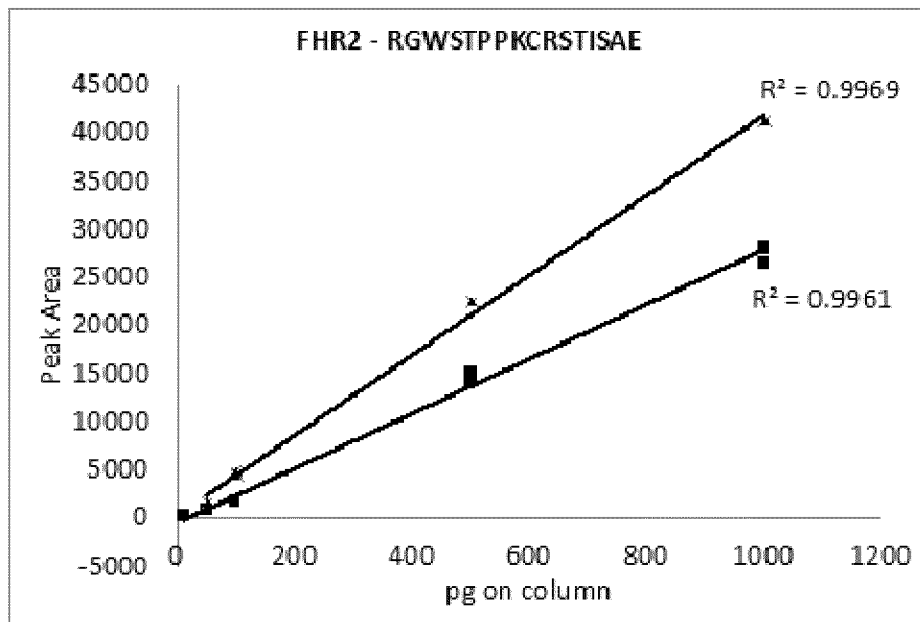


Figure 3 (continued)

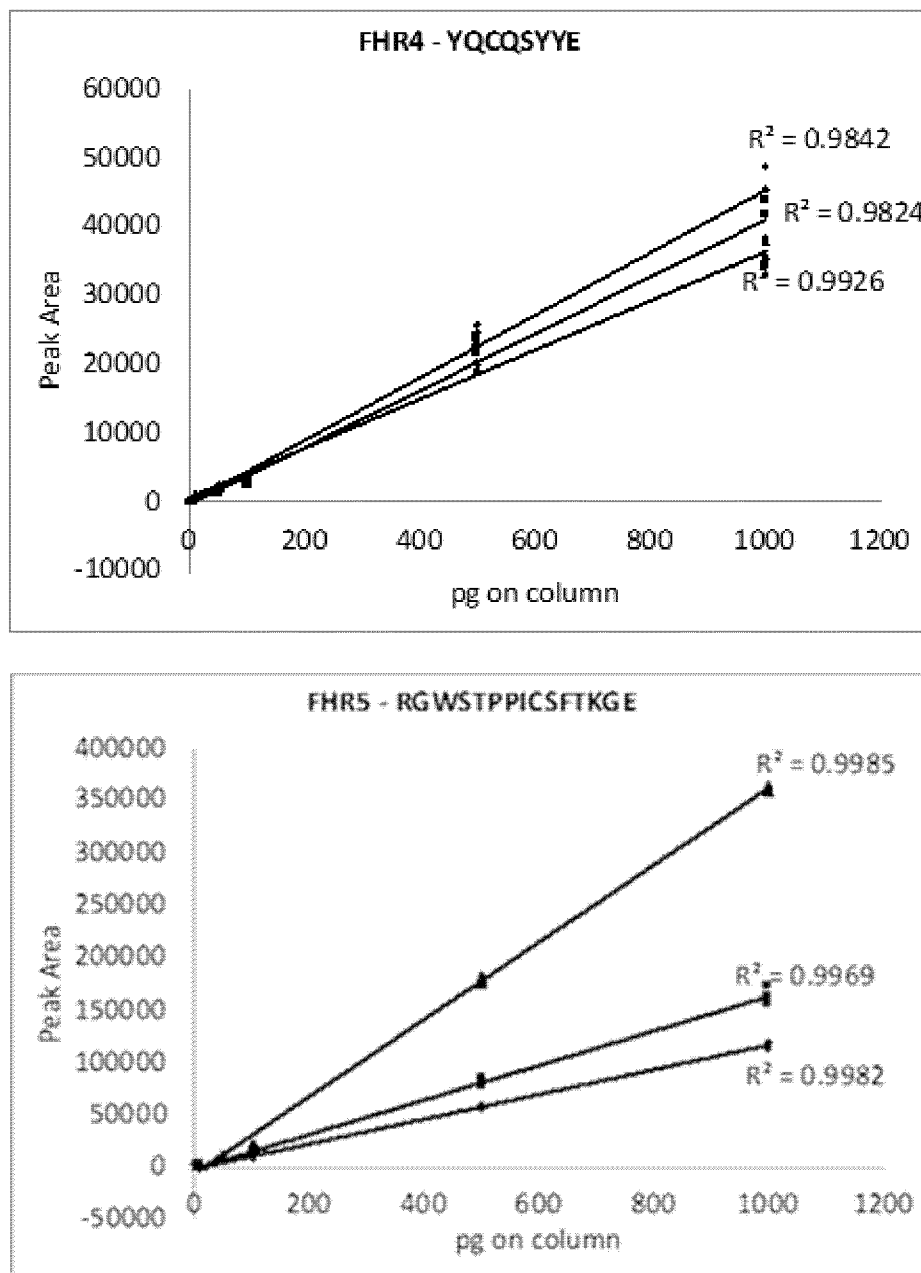


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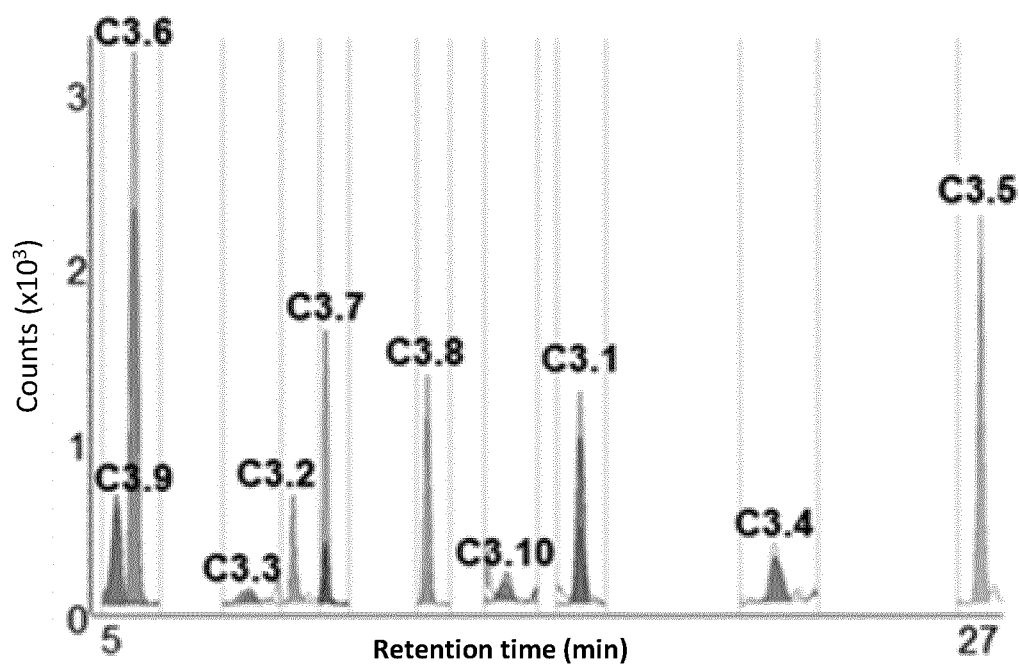


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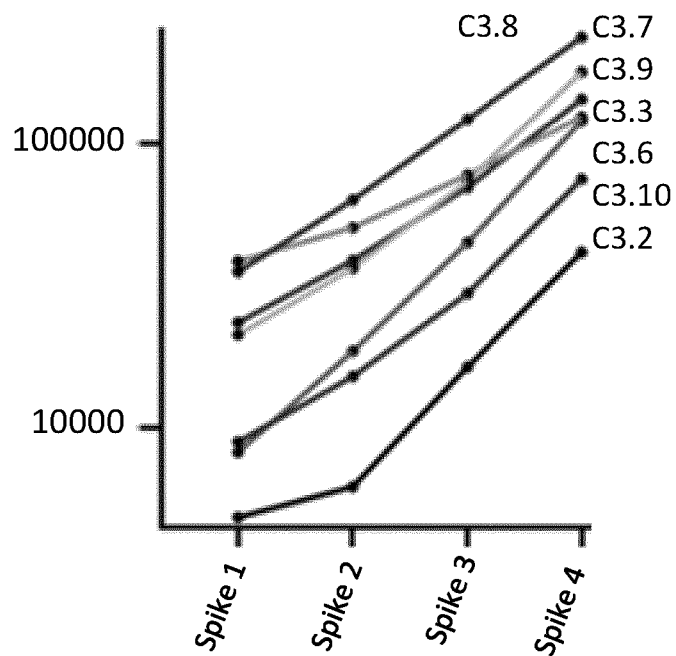


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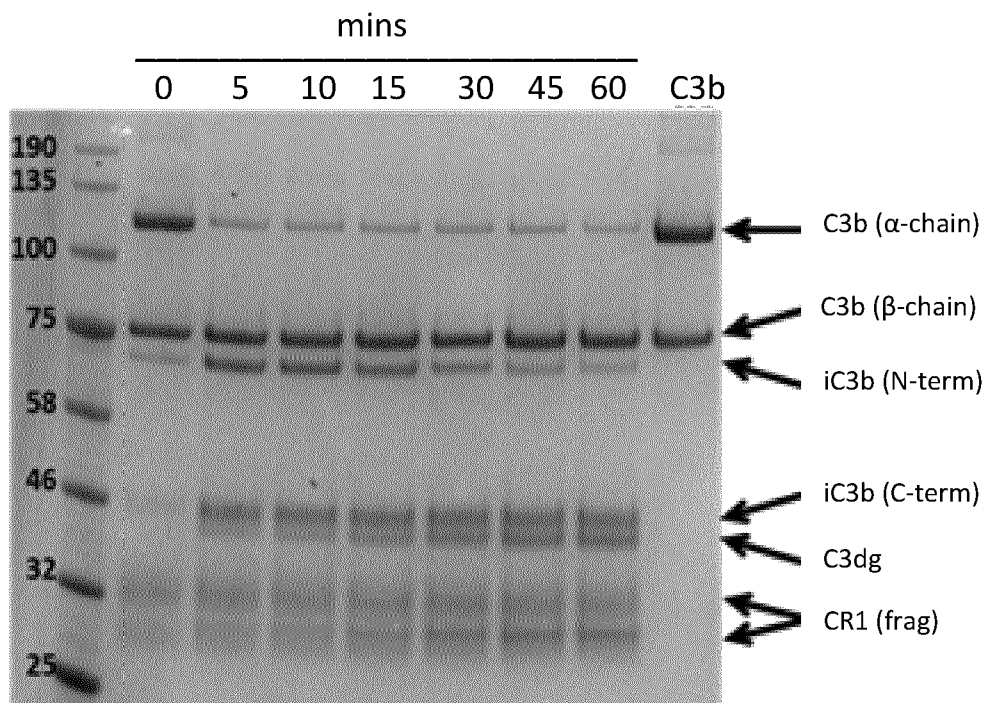


Figure 4C

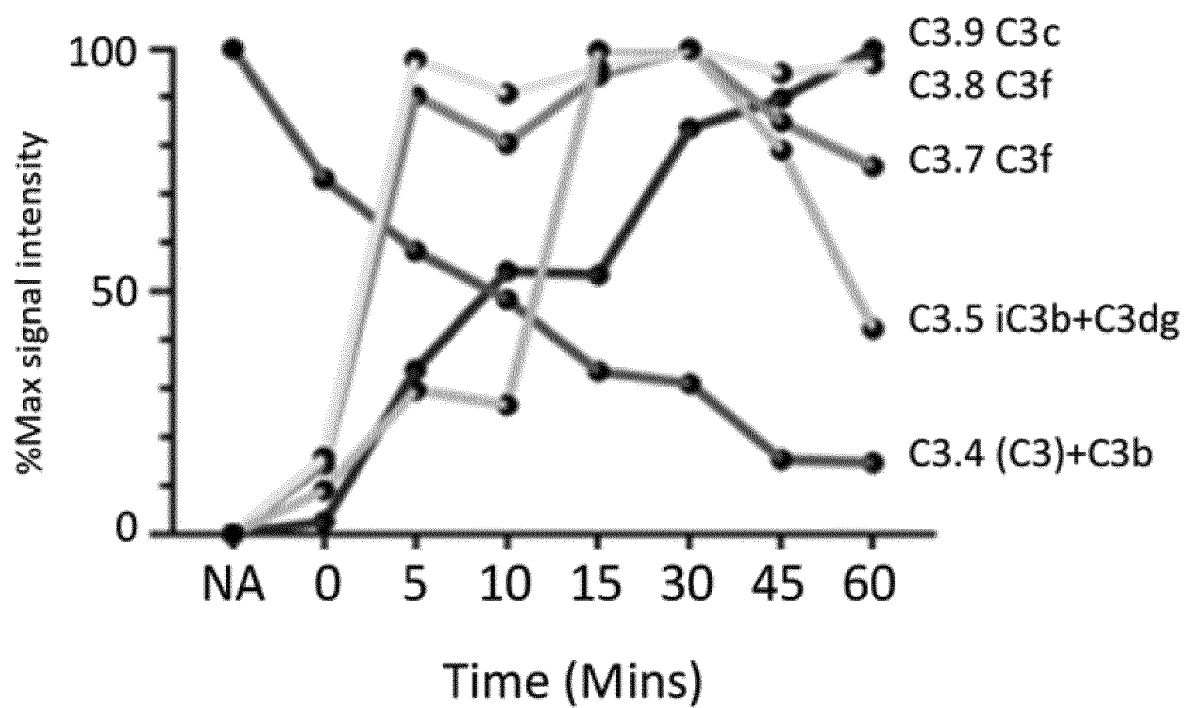


Figure 4D

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<160> 157

<170> PatentIn version 3.5

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Cys Thr Glu Lys Gly Trp Ser Pro Thr Pro Arg Cys Ile Arg Val Arg	130	135	140
Thr Cys Ser Lys Ser Asp Ile Glu Ile Glu Asn Gly Phe Ile Ser Glu	145	150	155
Ser Ser Ser Ile Tyr Ile Leu Asn Lys Glu Ile Gln Tyr Lys Cys Lys	165	170	175
Pro Gly Tyr Ala Thr Ala Asp Gly Asn Ser Ser Gly Ser Ile Thr Cys	180	185	190
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Lys Cys Gly Pro Pro Pro Pro Ile Ser Asn Gly Asp Thr Thr Ser Phe	210	215	220

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Pro Tyr Tyr Glu Leu Gln Gly Ser Asn Tyr Val Thr Cys Ser Asn Gly
245 250 255

Glu Trp Ser Glu Pro Pro Arg Cys Ile His Pro Cys Ile Ile Thr Glu
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Glu Asn Met Asn Lys Asn Asn Ile Lys Leu Lys Gly Arg Ser Asp Arg
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Lys Tyr Tyr Ala Lys Thr Gly Asp Thr Ile Glu Phe Met Cys Lys Leu
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<213> Homo sapiens

<400> 7

Met Leu Leu Leu Ile Asn Val Ile Leu Thr Leu Trp Val Ser Cys Ala
1 5 10 15

Asn Gly Gln Val Lys Pro Cys Asp Phe Pro Asp Ile Lys His Gly Gly
20 25 30

Leu Phe His Glu Asn Met Arg Arg Pro Tyr Phe Pro Val Ala Val Gly
35 40 45

Lys Tyr Tyr Ser Tyr Tyr Cys Asp Glu His Phe Glu Thr Pro Ser Gly
50 55 60

Ser Tyr Trp Asp Tyr Ile His Cys Thr Gln Asn Gly Trp Ser Pro Ala
65 70 75 80

Val Pro Cys Leu Arg Lys Cys Tyr Phe Pro Tyr Leu Glu Asn Gly Tyr
85 90 95

Asn Gln Asn Tyr Gly Arg Lys Phe Val Gln Gly Asn Ser Thr Glu Val
100 105 110

Ala Cys His Pro Gly Tyr Gly Leu Pro Lys Ala Gln Thr Thr Val Thr
115 120 125

Cys Thr Glu Lys Gly Trp Ser Pro Thr Pro Arg Cys Ile Arg Val Asn
130 135 140

Ser Ser Glu Lys Cys Gly Pro Pro Pro Pro Ile Ser Asn Gly Asp Thr
145 150 155 160

Thr Ser Phe Leu Leu Lys Val Tyr Val Pro Gln Ser Arg Val Glu Tyr
165 170 175

Gln Cys Gln Pro Tyr Tyr Glu Leu Gln Gly Ser Asn Tyr Val Thr Cys
180 185 190

Ser Asn Gly Glu Trp Ser Glu Pro Pro Arg Cys Ile His Pro Cys Ile
195 200 205

Ile Thr Glu Glu Asn Met Asn Lys Asn Asn Ile Lys Leu Lys Gly Arg
210 215 220

Ser Asp Arg Lys Tyr Tyr Ala Lys Thr Gly Asp Thr Ile Glu Phe Met
225 230 235 240

Cys Lys Leu Gly Tyr Asn Ala Asn Thr Ser Ile Leu Ser Phe Gln Ala
245 250 255

Val Cys Arg Glu Gly Ile Val Glu Tyr Pro Arg Cys Glu
260 265

<210> 8
<211> 578
<212> PRT
<213> Homo sapiens

<400> 8

Met Leu Leu Leu Ile Asn Val Ile Leu Thr Leu Trp Val Ser Cys Ala
1 5 10 15

Asn Gly Gln Glu Val Lys Pro Cys Asp Phe Pro Glu Ile Gln His Gly
20 25 30

Gly Leu Tyr Tyr Lys Ser Leu Arg Arg Leu Tyr Phe Pro Ala Ala Ala
35 40 45

Gly Gln Ser Tyr Ser Tyr Tyr Cys Asp Gln Asn Phe Val Thr Pro Ser
50 55 60

Gly Ser Tyr Trp Asp Tyr Ile His Cys Thr Gln Asp Gly Trp Ser Pro
65 70 75 80

Thr Val Pro Cys Leu Arg Thr Cys Ser Lys Ser Asp Val Glu Ile Glu
85 90 95

Asn Gly Phe Ile Ser Glu Ser Ser Ser Ile Tyr Ile Leu Asn Glu Glu
100 105 110

Thr Gln Tyr Asn Cys Lys Pro Gly Tyr Ala Thr Ala Glu Gly Asn Ser
115 120 125

Ser Gly Ser Ile Thr Cys Leu Gln Asn Gly Trp Ser Thr Gln Pro Ile
130 135 140

Cys Ile Lys Phe Cys Asp Met Pro Val Phe Glu Asn Ser Arg Ala Lys
145 150 155 160

Ser Asn Gly Met Trp Phe Lys Leu His Asp Thr Leu Asp Tyr Glu Cys
165 170 175

Tyr Asp Gly Tyr Glu Ser Ser Tyr Gly Asn Thr Thr Asp Ser Ile Val
180 185 190

Cys Gly Glu Asp Gly Trp Ser His Leu Pro Thr Cys Tyr Asn Ser Ser
195 200 205

Glu Asn Cys Gly Pro Pro Pro Pro Ile Ser Asn Gly Asp Thr Thr Ser
210 215 220

Phe Pro Gln Lys Val Tyr Leu Pro Trp Ser Arg Val Glu Tyr Gln Cys
225 230 235 240

Gln Ser Tyr Tyr Glu Leu Gln Gly Ser Lys Tyr Val Thr Cys Ser Asn
245 250 255

Gly Asp Trp Ser Glu Pro Pro Arg Cys Ile Ser Met Lys Pro Cys Glu
260 265 270

Phe Pro Glu Ile Gln His Gly His Leu Tyr Tyr Glu Asn Thr Arg Arg
275 280 285

Pro Tyr Phe Pro Val Ala Thr Gly Gln Ser Tyr Ser Tyr Tyr Cys Asp
290 295 300

Gln Asn Phe Val Thr Pro Ser Gly Ser Tyr Trp Asp Tyr Ile His Cys
305 310 315 320

Thr Gln Asp Gly Trp Leu Pro Thr Val Pro Cys Leu Arg Thr Cys Ser
325 330 335

Lys Ser Asp Ile Glu Ile Glu Asn Gly Phe Ile Ser Glu Ser Ser Ser
340 345 350

Ile Tyr Ile Leu Asn Lys Glu Ile Gln Tyr Lys Cys Lys Pro Gly Tyr
355 360 365

Ala Thr Ala Asp Gly Asn Ser Ser Gly Ser Ile Thr Cys Leu Gln Asn
370 375 380

Gly Trp Ser Ala Gln Pro Ile Cys Ile Lys Phe Cys Asp Met Pro Val
385 390 395 400

Phe Glu Asn Ser Arg Ala Lys Ser Asn Gly Met Arg Phe Lys Leu His
405 410 415

Asp Thr Leu Asp Tyr Glu Cys Tyr Asp Gly Tyr Glu Ile Ser Tyr Gly

420

425

430

Asn Thr Thr Gly Ser Ile Val Cys Gly Glu Asp Gly Trp Ser His Phe
 435 440 445

Pro Thr Cys Tyr Asn Ser Ser Glu Lys Cys Gly Pro Pro Pro Pro Ile
 450 455 460

Ser Asn Gly Asp Thr Thr Ser Phe Leu Leu Lys Val Tyr Val Pro Gln
 465 470 475 480

Ser Arg Val Glu Tyr Gln Cys Gln Ser Tyr Tyr Glu Leu Gln Gly Ser
 485 490 495

Asn Tyr Val Thr Cys Ser Asn Gly Glu Trp Ser Glu Pro Pro Arg Cys
 500 505 510

Ile His Pro Cys Ile Ile Thr Glu Glu Asn Met Asn Lys Asn Asn Ile
 515 520 525

Gln Leu Lys Gly Lys Ser Asp Ile Lys Tyr Tyr Ala Lys Thr Gly Asp
 530 535 540

Thr Ile Glu Phe Met Cys Lys Leu Gly Tyr Asn Ala Asn Thr Ser Val
 545 550 555 560

Leu Ser Phe Gln Ala Val Cys Arg Glu Gly Ile Val Glu Tyr Pro Arg
 565 570 575

Cys Glu

<210> 9

<211> 331

<212> PRT

<213> Homo sapiens

<400> 9

Met Leu Leu Leu Ile Asn Val Ile Leu Thr Leu Trp Val Ser Cys Ala
 1 5 10 15

Asn Gly Gln Glu Val Lys Pro Cys Asp Phe Pro Glu Ile Gln His Gly
20 25 30

Gly Leu Tyr Tyr Lys Ser Leu Arg Arg Leu Tyr Phe Pro Ala Ala Ala
35 40 45

Gly Gln Ser Tyr Ser Tyr Tyr Cys Asp Gln Asn Phe Val Thr Pro Ser
50 55 60

Gly Ser Tyr Trp Asp Tyr Ile His Cys Thr Gln Asp Gly Trp Ser Pro
65 70 75 80

Thr Val Pro Cys Leu Arg Thr Cys Ser Lys Ser Asp Ile Glu Ile Glu
85 90 95

Asn Gly Phe Ile Ser Glu Ser Ser Ser Ile Tyr Ile Leu Asn Lys Glu
100 105 110

Ile Gln Tyr Lys Cys Lys Pro Gly Tyr Ala Thr Ala Asp Gly Asn Ser
115 120 125

Ser Gly Ser Ile Thr Cys Leu Gln Asn Gly Trp Ser Ala Gln Pro Ile
130 135 140

Cys Ile Lys Phe Cys Asp Met Pro Val Phe Glu Asn Ser Arg Ala Lys
145 150 155 160

Ser Asn Gly Met Arg Phe Lys Leu His Asp Thr Leu Asp Tyr Glu Cys
165 170 175

Tyr Asp Gly Tyr Glu Ile Ser Tyr Gly Asn Thr Thr Gly Ser Ile Val
180 185 190

Cys Gly Glu Asp Gly Trp Ser His Phe Pro Thr Cys Tyr Asn Ser Ser
195 200 205

Glu Lys Cys Gly Pro Pro Pro Pro Ile Ser Asn Gly Asp Thr Thr Ser
210 215 220

Phe Leu Leu Lys Val Tyr Val Pro Gln Ser Arg Val Glu Tyr Gln Cys
225 230 235 240

Gln Ser Tyr Tyr Glu Leu Gln Gly Ser Asn Tyr Val Thr Cys Ser Asn
245 250 255

Gly Glu Trp Ser Glu Pro Pro Arg Cys Ile His Pro Cys Ile Ile Thr
260 265 270

Glu Glu Asn Met Asn Lys Asn Asn Ile Gln Leu Lys Gly Lys Ser Asp
275 280 285

Ile Lys Tyr Tyr Ala Lys Thr Gly Asp Thr Ile Glu Phe Met Cys Lys
290 295 300

Leu Gly Tyr Asn Ala Asn Thr Ser Val Leu Ser Phe Gln Ala Val Cys
305 310 315 320

Arg Glu Gly Ile Val Glu Tyr Pro Arg Cys Glu
325 330

<210> 10
<211> 569
<212> PRT
<213> Homo sapiens

<400> 10

Met Leu Leu Leu Phe Ser Val Ile Leu Ile Ser Trp Val Ser Thr Val
1 5 10 15

Gly Gly Glu Gly Thr Leu Cys Asp Phe Pro Lys Ile His His Gly Phe
20 25 30

Leu Tyr Asp Glu Glu Asp Tyr Asn Pro Phe Ser Gln Val Pro Thr Gly
35 40 45

Glu Val Phe Tyr Tyr Ser Cys Glu Tyr Asn Phe Val Ser Pro Ser Lys
50 55 60

Ser Phe Trp Thr Arg Ile Thr Cys Thr Glu Glu Gly Trp Ser Pro Thr
65 70 75 80

Pro Lys Cys Leu Arg Met Cys Ser Phe Pro Phe Val Lys Asn Gly His

85

90

95

Ser Glu Ser Ser Gly Leu Ile His Leu Glu Gly Asp Thr Val Gln Ile
 100 105 110

Ile Cys Asn Thr Gly Tyr Ser Leu Gln Asn Asn Glu Lys Asn Ile Ser
 115 120 125

Cys Val Glu Arg Gly Trp Ser Thr Pro Pro Ile Cys Ser Phe Thr Lys
 130 135 140

Gly Glu Cys His Val Pro Ile Leu Glu Ala Asn Val Asp Ala Gln Pro
 145 150 155 160

Lys Lys Glu Ser Tyr Lys Val Gly Asp Val Leu Lys Phe Ser Cys Arg
 165 170 175

Lys Asn Leu Ile Arg Val Gly Ser Asp Ser Val Gln Cys Tyr Gln Phe
 180 185 190

Gly Trp Ser Pro Asn Phe Pro Thr Cys Lys Gly Gln Val Arg Ser Cys
 195 200 205

Gly Pro Pro Pro Gln Leu Ser Asn Gly Glu Val Lys Glu Ile Arg Lys
 210 215 220

Glu Glu Tyr Gly His Asn Glu Val Val Glu Tyr Asp Cys Asn Pro Asn
 225 230 235 240

Phe Ile Ile Asn Gly Pro Lys Lys Ile Gln Cys Val Asp Gly Glu Trp
 245 250 255

Thr Thr Leu Pro Thr Cys Val Glu Gln Val Lys Thr Cys Gly Tyr Ile
 260 265 270

Pro Glu Leu Glu Tyr Gly Tyr Val Gln Pro Ser Val Pro Pro Tyr Gln
 275 280 285

His Gly Val Ser Val Glu Val Asn Cys Arg Asn Glu Tyr Ala Met Ile
 290 295 300

Gly Asn Asn Met Ile Thr Cys Ile Asn Gly Ile Trp Thr Glu Leu Pro
305 310 315 320

Met Cys Val Ala Thr His Gln Leu Lys Arg Cys Lys Ile Ala Gly Val
325 330 335

Asn Ile Lys Thr Leu Leu Lys Leu Ser Gly Lys Glu Phe Asn His Asn
340 345 350

Ser Arg Ile Arg Tyr Arg Cys Ser Asp Ile Phe Arg Tyr Arg His Ser
355 360 365

Val Cys Ile Asn Gly Lys Trp Asn Pro Glu Val Asp Cys Thr Glu Lys
370 375 380

Arg Glu Gln Phe Cys Pro Pro Pro Pro Gln Ile Pro Asn Ala Gln Asn
385 390 395 400

Met Thr Thr Thr Val Asn Tyr Gln Asp Gly Glu Lys Val Ala Val Leu
405 410 415

Cys Lys Glu Asn Tyr Leu Leu Pro Glu Ala Lys Glu Ile Val Cys Lys
420 425 430

Asp Gly Arg Trp Gln Ser Leu Pro Arg Cys Val Glu Ser Thr Ala Tyr
435 440 445

Cys Gly Pro Pro Pro Ser Ile Asn Asn Gly Asp Thr Thr Ser Phe Pro
450 455 460

Leu Ser Val Tyr Pro Pro Gly Ser Thr Val Thr Tyr Arg Cys Gln Ser
465 470 475 480

Phe Tyr Lys Leu Gln Gly Ser Val Thr Val Thr Cys Arg Asn Lys Gln
485 490 495

Trp Ser Glu Pro Pro Arg Cys Leu Asp Pro Cys Val Val Ser Glu Glu
500 505 510

Asn Met Asn Lys Asn Asn Ile Gln Leu Lys Trp Arg Asn Asp Gly Lys

515

520

525

Leu Tyr Ala Lys Thr Gly Asp Ala Val Glu Phe Gln Cys Lys Phe Pro
 530 535 540

His Lys Ala Met Ile Ser Ser Pro Pro Phe Arg Ala Ile Cys Gln Glu
 545 550 555 560

Gly Lys Phe Glu Tyr Pro Ile Cys Glu
 565

<210> 11
 <211> 583
 <212> PRT
 <213> Homo sapiens

<400> 11

Met Lys Leu Leu His Val Phe Leu Leu Phe Leu Cys Phe His Leu Arg
 1 5 10 15

Phe Cys Lys Val Thr Tyr Thr Ser Gln Glu Asp Leu Val Glu Lys Lys
 20 25 30

Cys Leu Ala Lys Lys Tyr Thr His Leu Ser Cys Asp Lys Val Phe Cys
 35 40 45

Gln Pro Trp Gln Arg Cys Ile Glu Gly Thr Cys Val Cys Lys Leu Pro
 50 55 60

Tyr Gln Cys Pro Lys Asn Gly Thr Ala Val Cys Ala Thr Asn Arg Arg
 65 70 75 80

Ser Phe Pro Thr Tyr Cys Gln Gln Lys Ser Leu Glu Cys Leu His Pro
 85 90 95

Gly Thr Lys Phe Leu Asn Asn Gly Thr Cys Thr Ala Glu Gly Lys Phe
 100 105 110

Ser Val Ser Leu Lys His Gly Asn Thr Asp Ser Glu Gly Ile Val Glu
 115 120 125

Val Lys Leu Val Asp Gln Asp Lys Thr Met Phe Ile Cys Lys Ser Ser
130 135 140

Trp Ser Met Arg Glu Ala Asn Val Ala Cys Leu Asp Leu Gly Phe Gln
145 150 155 160

Gln Gly Ala Asp Thr Gln Arg Arg Phe Lys Leu Ser Asp Leu Ser Ile
165 170 175

Asn Ser Thr Glu Cys Leu His Val His Cys Arg Gly Leu Glu Thr Ser
180 185 190

Leu Ala Glu Cys Thr Phe Thr Lys Arg Arg Thr Met Gly Tyr Gln Asp
195 200 205

Phe Ala Asp Val Val Cys Tyr Thr Gln Lys Ala Asp Ser Pro Met Asp
210 215 220

Asp Phe Phe Gln Cys Val Asn Gly Lys Tyr Ile Ser Gln Met Lys Ala
225 230 235 240

Cys Asp Gly Ile Asn Asp Cys Gly Asp Gln Ser Asp Glu Leu Cys Cys
245 250 255

Lys Ala Cys Gln Gly Lys Gly Phe His Cys Lys Ser Gly Val Cys Ile
260 265 270

Pro Ser Gln Tyr Gln Cys Asn Gly Glu Val Asp Cys Ile Thr Gly Glu
275 280 285

Asp Glu Val Gly Cys Ala Gly Phe Ala Ser Val Thr Gln Glu Glu Thr
290 295 300

Glu Ile Leu Thr Ala Asp Met Asp Ala Glu Arg Arg Arg Ile Lys Ser
305 310 315 320

Leu Leu Pro Lys Leu Ser Cys Gly Val Lys Asn Arg Met His Ile Arg
325 330 335

Arg Lys Arg Ile Val Gly Gly Lys Arg Ala Gln Leu Gly Asp Leu Pro
340 345 350

Trp Gln Val Ala Ile Lys Asp Ala Ser Gly Ile Thr Cys Gly Gly Ile
355 360 365

Tyr Ile Gly Gly Cys Trp Ile Leu Thr Ala Ala His Cys Leu Arg Ala
370 375 380

Ser Lys Thr His Arg Tyr Gln Ile Trp Thr Thr Val Val Asp Trp Ile
385 390 395 400

His Pro Asp Leu Lys Arg Ile Val Ile Glu Tyr Val Asp Arg Ile Ile
405 410 415

Phe His Glu Asn Tyr Asn Ala Gly Thr Tyr Gln Asn Asp Ile Ala Leu
420 425 430

Ile Glu Met Lys Lys Asp Gly Asn Lys Lys Asp Cys Glu Leu Pro Arg
435 440 445

Ser Ile Pro Ala Cys Val Pro Trp Ser Pro Tyr Leu Phe Gln Pro Asn
450 455 460

Asp Thr Cys Ile Val Ser Gly Trp Gly Arg Glu Lys Asp Asn Glu Arg
465 470 475 480

Val Phe Ser Leu Gln Trp Gly Glu Val Lys Leu Ile Ser Asn Cys Ser
485 490 495

Lys Phe Tyr Gly Asn Arg Phe Tyr Glu Lys Glu Met Glu Cys Ala Gly
500 505 510

Thr Tyr Asp Gly Ser Ile Asp Ala Cys Lys Gly Asp Ser Gly Gly Pro
515 520 525

Leu Val Cys Met Asp Ala Asn Asn Val Thr Tyr Val Trp Gly Val Val
530 535 540

Ser Trp Gly Glu Asn Cys Gly Lys Pro Glu Phe Pro Gly Val Tyr Thr
545 550 555 560

Lys Val Ala Asn Tyr Phe Asp Trp Ile Ser Tyr His Val Gly Arg Pro
565 570 575

Phe Ile Ser Gln Tyr Asn Val
580

<210> 12
<211> 1663
<212> PRT
<213> Homo sapiens

<400> 12

Met Gly Pro Thr Ser Gly Pro Ser Leu Leu Leu Leu Leu Leu Thr His
1 5 10 15

Leu Pro Leu Ala Leu Gly Ser Pro Met Tyr Ser Ile Ile Thr Pro Asn
20 25 30

Ile Leu Arg Leu Glu Ser Glu Glu Thr Met Val Leu Glu Ala His Asp
35 40 45

Ala Gln Gly Asp Val Pro Val Thr Val Thr Val His Asp Phe Pro Gly
50 55 60

Lys Lys Leu Val Leu Ser Ser Glu Lys Thr Val Leu Thr Pro Ala Thr
65 70 75 80

Asn His Met Gly Asn Val Thr Phe Thr Ile Pro Ala Asn Arg Glu Phe
85 90 95

Lys Ser Glu Lys Gly Arg Asn Lys Phe Val Thr Val Gln Ala Thr Phe
100 105 110

Gly Thr Gln Val Val Glu Lys Val Val Leu Val Ser Leu Gln Ser Gly
115 120 125

Tyr Leu Phe Ile Gln Thr Asp Lys Thr Ile Tyr Thr Pro Gly Ser Thr
130 135 140

Val Leu Tyr Arg Ile Phe Thr Val Asn His Lys Leu Leu Pro Val Gly
145 150 155 160

Arg Thr Val Met Val Asn Ile Glu Asn Pro Glu Gly Ile Pro Val Lys
165 170 175

Gln Asp Ser Leu Ser Ser Gln Asn Gln Leu Gly Val Leu Pro Leu Ser
180 185 190

Trp Asp Ile Pro Glu Leu Val Asn Met Gly Gln Trp Lys Ile Arg Ala
195 200 205

Tyr Tyr Glu Asn Ser Pro Gln Gln Val Phe Ser Thr Glu Phe Glu Val
210 215 220

Lys Glu Tyr Val Leu Pro Ser Phe Glu Val Ile Val Glu Pro Thr Glu
225 230 235 240

Lys Phe Tyr Tyr Ile Tyr Asn Glu Lys Gly Leu Glu Val Thr Ile Thr
245 250 255

Ala Arg Phe Leu Tyr Gly Lys Lys Val Glu Gly Thr Ala Phe Val Ile
260 265 270

Phe Gly Ile Gln Asp Gly Glu Gln Arg Ile Ser Leu Pro Glu Ser Leu
275 280 285

Lys Arg Ile Pro Ile Glu Asp Gly Ser Gly Glu Val Val Leu Ser Arg
290 295 300

Lys Val Leu Leu Asp Gly Val Gln Asn Pro Arg Ala Glu Asp Leu Val
305 310 315 320

Gly Lys Ser Leu Tyr Val Ser Ala Thr Val Ile Leu His Ser Gly Ser
325 330 335

Asp Met Val Gln Ala Glu Arg Ser Gly Ile Pro Ile Val Thr Ser Pro
340 345 350

Tyr Gln Ile His Phe Thr Lys Thr Pro Lys Tyr Phe Lys Pro Gly Met
355 360 365

Pro Phe Asp Leu Met Val Phe Val Thr Asn Pro Asp Gly Ser Pro Ala

370

375

380

Tyr Arg Val Pro Val Ala Val Gln Gly Glu Asp Thr Val Gln Ser Leu
385 390 395 400

Thr Gln Gly Asp Gly Val Ala Lys Leu Ser Ile Asn Thr His Pro Ser
405 410 415

Gln Lys Pro Leu Ser Ile Thr Val Arg Thr Lys Lys Gln Glu Leu Ser
420 425 430

Glu Ala Glu Gln Ala Thr Arg Thr Met Gln Ala Leu Pro Tyr Ser Thr
435 440 445

Val Gly Asn Ser Asn Asn Tyr Leu His Leu Ser Val Leu Arg Thr Glu
450 455 460

Leu Arg Pro Gly Glu Thr Leu Asn Val Asn Phe Leu Leu Arg Met Asp
465 470 475 480

Arg Ala His Glu Ala Lys Ile Arg Tyr Tyr Thr Tyr Leu Ile Met Asn
485 490 495

Lys Gly Arg Leu Leu Lys Ala Gly Arg Gln Val Arg Glu Pro Gly Gln
500 505 510

Asp Leu Val Val Leu Pro Leu Ser Ile Thr Thr Asp Phe Ile Pro Ser
515 520 525

Phe Arg Leu Val Ala Tyr Tyr Thr Leu Ile Gly Ala Ser Gly Gln Arg
530 535 540

Glu Val Val Ala Asp Ser Val Trp Val Asp Val Lys Asp Ser Cys Val
545 550 555 560

Gly Ser Leu Val Val Lys Ser Gly Gln Ser Glu Asp Arg Gln Pro Val
565 570 575

Pro Gly Gln Gln Met Thr Leu Lys Ile Glu Gly Asp His Gly Ala Arg
580 585 590

Val Val Leu Val Ala Val Asp Lys Gly Val Phe Val Leu Asn Lys Lys
595 600 605

Asn Lys Leu Thr Gln Ser Lys Ile Trp Asp Val Val Glu Lys Ala Asp
610 615 620

Ile Gly Cys Thr Pro Gly Ser Gly Lys Asp Tyr Ala Gly Val Phe Ser
625 630 635 640

Asp Ala Gly Leu Thr Phe Thr Ser Ser Ser Gly Gln Gln Thr Ala Gln
645 650 655

Arg Ala Glu Leu Gln Cys Pro Gln Pro Ala Ala Arg Arg Arg Arg Ser
660 665 670

Val Gln Leu Thr Glu Lys Arg Met Asp Lys Val Gly Lys Tyr Pro Lys
675 680 685

Glu Leu Arg Lys Cys Cys Glu Asp Gly Met Arg Glu Asn Pro Met Arg
690 695 700

Phe Ser Cys Gln Arg Arg Thr Arg Phe Ile Ser Leu Gly Glu Ala Cys
705 710 715 720

Lys Lys Val Phe Leu Asp Cys Cys Asn Tyr Ile Thr Glu Leu Arg Arg
725 730 735

Gln His Ala Arg Ala Ser His Leu Gly Leu Ala Arg Ser Asn Leu Asp
740 745 750

Glu Asp Ile Ile Ala Glu Glu Asn Ile Val Ser Arg Ser Glu Phe Pro
755 760 765

Glu Ser Trp Leu Trp Asn Val Glu Asp Leu Lys Glu Pro Pro Lys Asn
770 775 780

Gly Ile Ser Thr Lys Leu Met Asn Ile Phe Leu Lys Asp Ser Ile Thr
785 790 795 800

Thr Trp Glu Ile Leu Ala Val Ser Met Ser Asp Lys Lys Gly Ile Cys

Ala Val His Tyr Leu Asp Glu Thr Glu Gln Trp Glu Lys Phe Gly
1025 1030 1035

Leu Glu Lys Arg Gln Gly Ala Leu Glu Leu Ile Lys Lys Gly Tyr
1040 1045 1050

Thr Gln Gln Leu Ala Phe Arg Gln Pro Ser Ser Ala Phe Ala Ala
1055 1060 1065

Phe Val Lys Arg Ala Pro Ser Thr Trp Leu Thr Ala Tyr Val Val
1070 1075 1080

Lys Val Phe Ser Leu Ala Val Asn Leu Ile Ala Ile Asp Ser Gln
1085 1090 1095

Val Leu Cys Gly Ala Val Lys Trp Leu Ile Leu Glu Lys Gln Lys
1100 1105 1110

Pro Asp Gly Val Phe Gln Glu Asp Ala Pro Val Ile His Gln Glu
1115 1120 1125

Met Ile Gly Gly Leu Arg Asn Asn Asn Glu Lys Asp Met Ala Leu
1130 1135 1140

Thr Ala Phe Val Leu Ile Ser Leu Gln Glu Ala Lys Asp Ile Cys
1145 1150 1155

Glu Glu Gln Val Asn Ser Leu Pro Gly Ser Ile Thr Lys Ala Gly
1160 1165 1170

Asp Phe Leu Glu Ala Asn Tyr Met Asn Leu Gln Arg Ser Tyr Thr
1175 1180 1185

Val Ala Ile Ala Gly Tyr Ala Leu Ala Gln Met Gly Arg Leu Lys
1190 1195 1200

Gly Pro Leu Leu Asn Lys Phe Leu Thr Thr Ala Lys Asp Lys Asn
1205 1210 1215

Arg Trp Glu Asp Pro Gly Lys Gln Leu Tyr Asn Val Glu Ala Thr

1220						1225					1230			
Ser	Tyr	Ala	Leu	Leu	Ala	Leu	Leu	Gln	Leu	Lys	Asp	Phe	Asp	Phe
1235						1240					1245			
Val	Pro	Pro	Val	Val	Arg	Trp	Leu	Asn	Glu	Gln	Arg	Tyr	Tyr	Gly
1250						1255					1260			
Gly	Gly	Tyr	Gly	Ser	Thr	Gln	Ala	Thr	Phe	Met	Val	Phe	Gln	Ala
1265						1270					1275			
Leu	Ala	Gln	Tyr	Gln	Lys	Asp	Ala	Pro	Asp	His	Gln	Glu	Leu	Asn
1280						1285					1290			
Leu	Asp	Val	Ser	Leu	Gln	Leu	Pro	Ser	Arg	Ser	Ser	Lys	Ile	Thr
1295						1300					1305			
His	Arg	Ile	His	Trp	Glu	Ser	Ala	Ser	Leu	Leu	Arg	Ser	Glu	Glu
1310						1315					1320			
Thr	Lys	Glu	Asn	Glu	Gly	Phe	Thr	Val	Thr	Ala	Glu	Gly	Lys	Gly
1325						1330					1335			
Gln	Gly	Thr	Leu	Ser	Val	Val	Thr	Met	Tyr	His	Ala	Lys	Ala	Lys
1340						1345					1350			
Asp	Gln	Leu	Thr	Cys	Asn	Lys	Phe	Asp	Leu	Lys	Val	Thr	Ile	Lys
1355						1360					1365			
Pro	Ala	Pro	Glu	Thr	Glu	Lys	Arg	Pro	Gln	Asp	Ala	Lys	Asn	Thr
1370						1375					1380			
Met	Ile	Leu	Glu	Ile	Cys	Thr	Arg	Tyr	Arg	Gly	Asp	Gln	Asp	Ala
1385						1390					1395			
Thr	Met	Ser	Ile	Leu	Asp	Ile	Ser	Met	Met	Thr	Gly	Phe	Ala	Pro
1400						1405					1410			
Asp	Thr	Asp	Asp	Leu	Lys	Gln	Leu	Ala	Asn	Gly	Val	Asp	Arg	Tyr
1415						1420					1425			

Ile Ser Lys Tyr Glu Leu Asp Lys Ala Phe Ser Asp Arg Asn Thr
1430 1435 1440

Leu Ile Ile Tyr Leu Asp Lys Val Ser His Ser Glu Asp Asp Cys
1445 1450 1455

Leu Ala Phe Lys Val His Gln Tyr Phe Asn Val Glu Leu Ile Gln
1460 1465 1470

Pro Gly Ala Val Lys Val Tyr Ala Tyr Tyr Asn Leu Glu Glu Ser
1475 1480 1485

Cys Thr Arg Phe Tyr His Pro Glu Lys Glu Asp Gly Lys Leu Asn
1490 1495 1500

Lys Leu Cys Arg Asp Glu Leu Cys Arg Cys Ala Glu Glu Asn Cys
1505 1510 1515

Phe Ile Gln Lys Ser Asp Asp Lys Val Thr Leu Glu Glu Arg Leu
1520 1525 1530

Asp Lys Ala Cys Glu Pro Gly Val Asp Tyr Val Tyr Lys Thr Arg
1535 1540 1545

Leu Val Lys Val Gln Leu Ser Asn Asp Phe Asp Glu Tyr Ile Met
1550 1555 1560

Ala Ile Glu Gln Thr Ile Lys Ser Gly Ser Asp Glu Val Gln Val
1565 1570 1575

Gly Gln Gln Arg Thr Phe Ile Ser Pro Ile Lys Cys Arg Glu Ala
1580 1585 1590

Leu Lys Leu Glu Glu Lys Lys His Tyr Leu Met Trp Gly Leu Ser
1595 1600 1605

Ser Asp Phe Trp Gly Glu Lys Pro Asn Leu Ser Tyr Ile Ile Gly
1610 1615 1620

Lys Asp Thr Trp Val Glu His Trp Pro Glu Glu Asp Glu Cys Gln

1625

1630

1635

Asp Glu Glu Asn Gln Lys Gln Cys Gln Asp Leu Gly Ala Phe Thr
 1640 1645 1650

Glu Ser Met Val Val Phe Gly Cys Pro Asn
 1655 1660

<210> 13
 <211> 645
 <212> PRT
 <213> Homo sapiens

<400> 13

Ser Pro Met Tyr Ser Ile Ile Thr Pro Asn Ile Leu Arg Leu Glu Ser
 1 5 10 15

Glu Glu Thr Met Val Leu Glu Ala His Asp Ala Gln Gly Asp Val Pro
 20 25 30

Val Thr Val Thr Val His Asp Phe Pro Gly Lys Lys Leu Val Leu Ser
 35 40 45

Ser Glu Lys Thr Val Leu Thr Pro Ala Thr Asn His Met Gly Asn Val
 50 55 60

Thr Phe Thr Ile Pro Ala Asn Arg Glu Phe Lys Ser Glu Lys Gly Arg
 65 70 75 80

Asn Lys Phe Val Thr Val Gln Ala Thr Phe Gly Thr Gln Val Val Glu
 85 90 95

Lys Val Val Leu Val Ser Leu Gln Ser Gly Tyr Leu Phe Ile Gln Thr
 100 105 110

Asp Lys Thr Ile Tyr Thr Pro Gly Ser Thr Val Leu Tyr Arg Ile Phe
 115 120 125

Thr Val Asn His Lys Leu Leu Pro Val Gly Arg Thr Val Met Val Asn
 130 135 140

Ile Glu Asn Pro Glu Gly Ile Pro Val Lys Gln Asp Ser Leu Ser Ser
145 150 155 160

Gln Asn Gln Leu Gly Val Leu Pro Leu Ser Trp Asp Ile Pro Glu Leu
165 170 175

Val Asn Met Gly Gln Trp Lys Ile Arg Ala Tyr Tyr Glu Asn Ser Pro
180 185 190

Gln Gln Val Phe Ser Thr Glu Phe Glu Val Lys Glu Tyr Val Leu Pro
195 200 205

Ser Phe Glu Val Ile Val Glu Pro Thr Glu Lys Phe Tyr Tyr Ile Tyr
210 215 220

Asn Glu Lys Gly Leu Glu Val Thr Ile Thr Ala Arg Phe Leu Tyr Gly
225 230 235 240

Lys Lys Val Glu Gly Thr Ala Phe Val Ile Phe Gly Ile Gln Asp Gly
245 250 255

Glu Gln Arg Ile Ser Leu Pro Glu Ser Leu Lys Arg Ile Pro Ile Glu
260 265 270

Asp Gly Ser Gly Glu Val Val Leu Ser Arg Lys Val Leu Leu Asp Gly
275 280 285

Val Gln Asn Pro Arg Ala Glu Asp Leu Val Gly Lys Ser Leu Tyr Val
290 295 300

Ser Ala Thr Val Ile Leu His Ser Gly Ser Asp Met Val Gln Ala Glu
305 310 315 320

Arg Ser Gly Ile Pro Ile Val Thr Ser Pro Tyr Gln Ile His Phe Thr
325 330 335

Lys Thr Pro Lys Tyr Phe Lys Pro Gly Met Pro Phe Asp Leu Met Val
340 345 350

Phe Val Thr Asn Pro Asp Gly Ser Pro Ala Tyr Arg Val Pro Val Ala
355 360 365

Val Gln Gly Glu Asp Thr Val Gln Ser Leu Thr Gln Gly Asp Gly Val
370 375 380

Ala Lys Leu Ser Ile Asn Thr His Pro Ser Gln Lys Pro Leu Ser Ile
385 390 395 400

Thr Val Arg Thr Lys Lys Gln Glu Leu Ser Glu Ala Glu Gln Ala Thr
405 410 415

Arg Thr Met Gln Ala Leu Pro Tyr Ser Thr Val Gly Asn Ser Asn Asn
420 425 430

Tyr Leu His Leu Ser Val Leu Arg Thr Glu Leu Arg Pro Gly Glu Thr
435 440 445

Leu Asn Val Asn Phe Leu Leu Arg Met Asp Arg Ala His Glu Ala Lys
450 455 460

Ile Arg Tyr Tyr Thr Tyr Leu Ile Met Asn Lys Gly Arg Leu Leu Lys
465 470 475 480

Ala Gly Arg Gln Val Arg Glu Pro Gly Gln Asp Leu Val Val Leu Pro
485 490 495

Leu Ser Ile Thr Thr Asp Phe Ile Pro Ser Phe Arg Leu Val Ala Tyr
500 505 510

Tyr Thr Leu Ile Gly Ala Ser Gly Gln Arg Glu Val Val Ala Asp Ser
515 520 525

Val Trp Val Asp Val Lys Asp Ser Cys Val Gly Ser Leu Val Val Lys
530 535 540

Ser Gly Gln Ser Glu Asp Arg Gln Pro Val Pro Gly Gln Gln Met Thr
545 550 555 560

Leu Lys Ile Glu Gly Asp His Gly Ala Arg Val Val Leu Val Ala Val
565 570 575

Asp Lys Gly Val Phe Val Leu Asn Lys Lys Asn Lys Leu Thr Gln Ser
580 585 590

Lys Ile Trp Asp Val Val Glu Lys Ala Asp Ile Gly Cys Thr Pro Gly
595 600 605

Ser Gly Lys Asp Tyr Ala Gly Val Phe Ser Asp Ala Gly Leu Thr Phe
610 615 620

Thr Ser Ser Ser Gly Gln Gln Thr Ala Gln Arg Ala Glu Leu Gln Cys
625 630 635 640

Pro Gln Pro Ala Ala
645

<210> 14
<211> 915
<212> PRT
<213> Homo sapiens

<400> 14

Ser Asn Leu Asp Glu Asp Ile Ile Ala Glu Glu Asn Ile Val Ser Arg
1 5 10 15

Ser Glu Phe Pro Glu Ser Trp Leu Trp Asn Val Glu Asp Leu Lys Glu
20 25 30

Pro Pro Lys Asn Gly Ile Ser Thr Lys Leu Met Asn Ile Phe Leu Lys
35 40 45

Asp Ser Ile Thr Thr Trp Glu Ile Leu Ala Val Ser Met Ser Asp Lys
50 55 60

Lys Gly Ile Cys Val Ala Asp Pro Phe Glu Val Thr Val Met Gln Asp
65 70 75 80

Phe Phe Ile Asp Leu Arg Leu Pro Tyr Ser Val Val Arg Asn Glu Gln
85 90 95

Val Glu Ile Arg Ala Val Leu Tyr Asn Tyr Arg Gln Asn Gln Glu Leu
100 105 110

Lys Val Arg Val Glu Leu Leu His Asn Pro Ala Phe Cys Ser Leu Ala
115 120 125

Thr Thr Lys Arg Arg His Gln Gln Thr Val Thr Ile Pro Pro Lys Ser
130 135 140

Ser Leu Ser Val Pro Tyr Val Ile Val Pro Leu Lys Thr Gly Leu Gln
145 150 155 160

Glu Val Glu Val Lys Ala Ala Val Tyr His His Phe Ile Ser Asp Gly
165 170 175

Val Arg Lys Ser Leu Lys Val Val Pro Glu Gly Ile Arg Met Asn Lys
180 185 190

Thr Val Ala Val Arg Thr Leu Asp Pro Glu Arg Leu Gly Arg Glu Gly
195 200 205

Val Gln Lys Glu Asp Ile Pro Pro Ala Asp Leu Ser Asp Gln Val Pro
210 215 220

Asp Thr Glu Ser Glu Thr Arg Ile Leu Leu Gln Gly Thr Pro Val Ala
225 230 235 240

Gln Met Thr Glu Asp Ala Val Asp Ala Glu Arg Leu Lys His Leu Ile
245 250 255

Val Thr Pro Ser Gly Cys Gly Glu Gln Asn Met Ile Gly Met Thr Pro
260 265 270

Thr Val Ile Ala Val His Tyr Leu Asp Glu Thr Glu Gln Trp Glu Lys
275 280 285

Phe Gly Leu Glu Lys Arg Gln Gly Ala Leu Glu Leu Ile Lys Lys Gly
290 295 300

Tyr Thr Gln Gln Leu Ala Phe Arg Gln Pro Ser Ser Ala Phe Ala Ala
305 310 315 320

Phe Val Lys Arg Ala Pro Ser Thr Trp Leu Thr Ala Tyr Val Val Lys

325

330

335

Val Phe Ser Leu Ala Val Asn Leu Ile Ala Ile Asp Ser Gln Val Leu
 340 345 350

Cys Gly Ala Val Lys Trp Leu Ile Leu Glu Lys Gln Lys Pro Asp Gly
 355 360 365

Val Phe Gln Glu Asp Ala Pro Val Ile His Gln Glu Met Ile Gly Gly
 370 375 380

Leu Arg Asn Asn Asn Glu Lys Asp Met Ala Leu Thr Ala Phe Val Leu
 385 390 395 400

Ile Ser Leu Gln Glu Ala Lys Asp Ile Cys Glu Glu Gln Val Asn Ser
 405 410 415

Leu Pro Gly Ser Ile Thr Lys Ala Gly Asp Phe Leu Glu Ala Asn Tyr
 420 425 430

Met Asn Leu Gln Arg Ser Tyr Thr Val Ala Ile Ala Gly Tyr Ala Leu
 435 440 445

Ala Gln Met Gly Arg Leu Lys Gly Pro Leu Leu Asn Lys Phe Leu Thr
 450 455 460

Thr Ala Lys Asp Lys Asn Arg Trp Glu Asp Pro Gly Lys Gln Leu Tyr
 465 470 475 480

Asn Val Glu Ala Thr Ser Tyr Ala Leu Leu Ala Leu Leu Gln Leu Lys
 485 490 495

Asp Phe Asp Phe Val Pro Pro Val Val Arg Trp Leu Asn Glu Gln Arg
 500 505 510

Tyr Tyr Gly Gly Gly Tyr Gly Ser Thr Gln Ala Thr Phe Met Val Phe
 515 520 525

Gln Ala Leu Ala Gln Tyr Gln Lys Asp Ala Pro Asp His Gln Glu Leu
 530 535 540

Asn Leu Asp Val Ser Leu Gln Leu Pro Ser Arg Ser Ser Lys Ile Thr
545 550 555 560

His Arg Ile His Trp Glu Ser Ala Ser Leu Leu Arg Ser Glu Glu Thr
565 570 575

Lys Glu Asn Glu Gly Phe Thr Val Thr Ala Glu Gly Lys Gly Gln Gly
580 585 590

Thr Leu Ser Val Val Thr Met Tyr His Ala Lys Ala Lys Asp Gln Leu
595 600 605

Thr Cys Asn Lys Phe Asp Leu Lys Val Thr Ile Lys Pro Ala Pro Glu
610 615 620

Thr Glu Lys Arg Pro Gln Asp Ala Lys Asn Thr Met Ile Leu Glu Ile
625 630 635 640

Cys Thr Arg Tyr Arg Gly Asp Gln Asp Ala Thr Met Ser Ile Leu Asp
645 650 655

Ile Ser Met Met Thr Gly Phe Ala Pro Asp Thr Asp Asp Leu Lys Gln
660 665 670

Leu Ala Asn Gly Val Asp Arg Tyr Ile Ser Lys Tyr Glu Leu Asp Lys
675 680 685

Ala Phe Ser Asp Arg Asn Thr Leu Ile Ile Tyr Leu Asp Lys Val Ser
690 695 700

His Ser Glu Asp Asp Cys Leu Ala Phe Lys Val His Gln Tyr Phe Asn
705 710 715 720

Val Glu Leu Ile Gln Pro Gly Ala Val Lys Val Tyr Ala Tyr Tyr Asn
725 730 735

Leu Glu Glu Ser Cys Thr Arg Phe Tyr His Pro Glu Lys Glu Asp Gly
740 745 750

Lys Leu Asn Lys Leu Cys Arg Asp Glu Leu Cys Arg Cys Ala Glu Glu

755

760

765

Asn Cys Phe Ile Gln Lys Ser Asp Asp Lys Val Thr Leu Glu Glu Arg
 770 775 780

Leu Asp Lys Ala Cys Glu Pro Gly Val Asp Tyr Val Tyr Lys Thr Arg
 785 790 795 800

Leu Val Lys Val Gln Leu Ser Asn Asp Phe Asp Glu Tyr Ile Met Ala
 805 810 815

Ile Glu Gln Thr Ile Lys Ser Gly Ser Asp Glu Val Gln Val Gly Gln
 820 825 830

Gln Arg Thr Phe Ile Ser Pro Ile Lys Cys Arg Glu Ala Leu Lys Leu
 835 840 845

Glu Glu Lys Lys His Tyr Leu Met Trp Gly Leu Ser Ser Asp Phe Trp
 850 855 860

Gly Glu Lys Pro Asn Leu Ser Tyr Ile Ile Gly Lys Asp Thr Trp Val
 865 870 875 880

Glu His Trp Pro Glu Glu Asp Glu Cys Gln Asp Glu Glu Asn Gln Lys
 885 890 895

Gln Cys Gln Asp Leu Gly Ala Phe Thr Glu Ser Met Val Val Phe Gly
 900 905 910

Cys Pro Asn
 915

<210> 15
 <211> 77
 <212> PRT
 <213> Homo sapiens

<400> 15

Ser Val Gln Leu Thr Glu Lys Arg Met Asp Lys Val Gly Lys Tyr Pro
 1 5 10 15

Lys Glu Leu Arg Lys Cys Cys Glu Asp Gly Met Arg Glu Asn Pro Met
20 25 30

Arg Phe Ser Cys Gln Arg Arg Thr Arg Phe Ile Ser Leu Gly Glu Ala
35 40 45

Cys Lys Lys Val Phe Leu Asp Cys Cys Asn Tyr Ile Thr Glu Leu Arg
50 55 60

Arg Gln His Ala Arg Ala Ser His Leu Gly Leu Ala Arg
65 70 75

<210> 16
<211> 915
<212> PRT
<213> Homo sapiens

<400> 16

Ser Asn Leu Asp Glu Asp Ile Ile Ala Glu Glu Asn Ile Val Ser Arg
1 5 10 15

Ser Glu Phe Pro Glu Ser Trp Leu Trp Asn Val Glu Asp Leu Lys Glu
20 25 30

Pro Pro Lys Asn Gly Ile Ser Thr Lys Leu Met Asn Ile Phe Leu Lys
35 40 45

Asp Ser Ile Thr Thr Trp Glu Ile Leu Ala Val Ser Met Ser Asp Lys
50 55 60

Lys Gly Ile Cys Val Ala Asp Pro Phe Glu Val Thr Val Met Gln Asp
65 70 75 80

Phe Phe Ile Asp Leu Arg Leu Pro Tyr Ser Val Val Arg Asn Glu Gln
85 90 95

Val Glu Ile Arg Ala Val Leu Tyr Asn Tyr Arg Gln Asn Gln Glu Leu
100 105 110

Lys Val Arg Val Glu Leu Leu His Asn Pro Ala Phe Cys Ser Leu Ala
115 120 125

Thr Thr Lys Arg Arg His Gln Gln Thr Val Thr Ile Pro Pro Lys Ser
130 135 140

Ser Leu Ser Val Pro Tyr Val Ile Val Pro Leu Lys Thr Gly Leu Gln
145 150 155 160

Glu Val Glu Val Lys Ala Ala Val Tyr His His Phe Ile Ser Asp Gly
165 170 175

Val Arg Lys Ser Leu Lys Val Val Pro Glu Gly Ile Arg Met Asn Lys
180 185 190

Thr Val Ala Val Arg Thr Leu Asp Pro Glu Arg Leu Gly Arg Glu Gly
195 200 205

Val Gln Lys Glu Asp Ile Pro Pro Ala Asp Leu Ser Asp Gln Val Pro
210 215 220

Asp Thr Glu Ser Glu Thr Arg Ile Leu Leu Gln Gly Thr Pro Val Ala
225 230 235 240

Gln Met Thr Glu Asp Ala Val Asp Ala Glu Arg Leu Lys His Leu Ile
245 250 255

Val Thr Pro Ser Gly Cys Gly Glu Gln Asn Met Ile Gly Met Thr Pro
260 265 270

Thr Val Ile Ala Val His Tyr Leu Asp Glu Thr Glu Gln Trp Glu Lys
275 280 285

Phe Gly Leu Glu Lys Arg Gln Gly Ala Leu Glu Leu Ile Lys Lys Gly
290 295 300

Tyr Thr Gln Gln Leu Ala Phe Arg Gln Pro Ser Ser Ala Phe Ala Ala
305 310 315 320

Phe Val Lys Arg Ala Pro Ser Thr Trp Leu Thr Ala Tyr Val Val Lys
325 330 335

Val Phe Ser Leu Ala Val Asn Leu Ile Ala Ile Asp Ser Gln Val Leu

340

345

350

Cys Gly Ala Val Lys Trp Leu Ile Leu Glu Lys Gln Lys Pro Asp Gly
 355 360 365

Val Phe Gln Glu Asp Ala Pro Val Ile His Gln Glu Met Ile Gly Gly
 370 375 380

Leu Arg Asn Asn Asn Glu Lys Asp Met Ala Leu Thr Ala Phe Val Leu
 385 390 395 400

Ile Ser Leu Gln Glu Ala Lys Asp Ile Cys Glu Glu Gln Val Asn Ser
 405 410 415

Leu Pro Gly Ser Ile Thr Lys Ala Gly Asp Phe Leu Glu Ala Asn Tyr
 420 425 430

Met Asn Leu Gln Arg Ser Tyr Thr Val Ala Ile Ala Gly Tyr Ala Leu
 435 440 445

Ala Gln Met Gly Arg Leu Lys Gly Pro Leu Leu Asn Lys Phe Leu Thr
 450 455 460

Thr Ala Lys Asp Lys Asn Arg Trp Glu Asp Pro Gly Lys Gln Leu Tyr
 465 470 475 480

Asn Val Glu Ala Thr Ser Tyr Ala Leu Leu Ala Leu Leu Gln Leu Lys
 485 490 495

Asp Phe Asp Phe Val Pro Pro Val Val Arg Trp Leu Asn Glu Gln Arg
 500 505 510

Tyr Tyr Gly Gly Gly Tyr Gly Ser Thr Gln Ala Thr Phe Met Val Phe
 515 520 525

Gln Ala Leu Ala Gln Tyr Gln Lys Asp Ala Pro Asp His Gln Glu Leu
 530 535 540

Asn Leu Asp Val Ser Leu Gln Leu Pro Ser Arg Ser Ser Lys Ile Thr
 545 550 555 560

His Arg Ile His Trp Glu Ser Ala Ser Leu Leu Arg Ser Glu Glu Thr
565 570 575

Lys Glu Asn Glu Gly Phe Thr Val Thr Ala Glu Gly Lys Gly Gln Gly
580 585 590

Thr Leu Ser Val Val Thr Met Tyr His Ala Lys Ala Lys Asp Gln Leu
595 600 605

Thr Cys Asn Lys Phe Asp Leu Lys Val Thr Ile Lys Pro Ala Pro Glu
610 615 620

Thr Glu Lys Arg Pro Gln Asp Ala Lys Asn Thr Met Ile Leu Glu Ile
625 630 635 640

Cys Thr Arg Tyr Arg Gly Asp Gln Asp Ala Thr Met Ser Ile Leu Asp
645 650 655

Ile Ser Met Met Thr Gly Phe Ala Pro Asp Thr Asp Asp Leu Lys Gln
660 665 670

Leu Ala Asn Gly Val Asp Arg Tyr Ile Ser Lys Tyr Glu Leu Asp Lys
675 680 685

Ala Phe Ser Asp Arg Asn Thr Leu Ile Ile Tyr Leu Asp Lys Val Ser
690 695 700

His Ser Glu Asp Asp Cys Leu Ala Phe Lys Val His Gln Tyr Phe Asn
705 710 715 720

Val Glu Leu Ile Gln Pro Gly Ala Val Lys Val Tyr Ala Tyr Tyr Asn
725 730 735

Leu Glu Glu Ser Cys Thr Arg Phe Tyr His Pro Glu Lys Glu Asp Gly
740 745 750

Lys Leu Asn Lys Leu Cys Arg Asp Glu Leu Cys Arg Cys Ala Glu Glu
755 760 765

Asn Cys Phe Ile Gln Lys Ser Asp Asp Lys Val Thr Leu Glu Glu Arg

770

775

780

Leu Asp Lys Ala Cys Glu Pro Gly Val Asp Tyr Val Tyr Lys Thr Arg
 785 790 795 800

Leu Val Lys Val Gln Leu Ser Asn Asp Phe Asp Glu Tyr Ile Met Ala
 805 810 815

Ile Glu Gln Thr Ile Lys Ser Gly Ser Asp Glu Val Gln Val Gly Gln
 820 825 830

Gln Arg Thr Phe Ile Ser Pro Ile Lys Cys Arg Glu Ala Leu Lys Leu
 835 840 845

Glu Glu Lys Lys His Tyr Leu Met Trp Gly Leu Ser Ser Asp Phe Trp
 850 855 860

Gly Glu Lys Pro Asn Leu Ser Tyr Ile Ile Gly Lys Asp Thr Trp Val
 865 870 875 880

Glu His Trp Pro Glu Glu Asp Glu Cys Gln Asp Glu Glu Asn Gln Lys
 885 890 895

Gln Cys Gln Asp Leu Gly Ala Phe Thr Glu Ser Met Val Val Phe Gly
 900 905 910

Cys Pro Asn
 915

<210> 17
 <211> 343
 <212> PRT
 <213> Homo sapiens

<400> 17

Ser Glu Glu Thr Lys Glu Asn Glu Gly Phe Thr Val Thr Ala Glu Gly
 1 5 10 15

Lys Gly Gln Gly Thr Leu Ser Val Val Thr Met Tyr His Ala Lys Ala
 20 25 30

Lys Asp Gln Leu Thr Cys Asn Lys Phe Asp Leu Lys Val Thr Ile Lys
35 40 45

Pro Ala Pro Glu Thr Glu Lys Arg Pro Gln Asp Ala Lys Asn Thr Met
50 55 60

Ile Leu Glu Ile Cys Thr Arg Tyr Arg Gly Asp Gln Asp Ala Thr Met
65 70 75 80

Ser Ile Leu Asp Ile Ser Met Met Thr Gly Phe Ala Pro Asp Thr Asp
85 90 95

Asp Leu Lys Gln Leu Ala Asn Gly Val Asp Arg Tyr Ile Ser Lys Tyr
100 105 110

Glu Leu Asp Lys Ala Phe Ser Asp Arg Asn Thr Leu Ile Ile Tyr Leu
115 120 125

Asp Lys Val Ser His Ser Glu Asp Asp Cys Leu Ala Phe Lys Val His
130 135 140

Gln Tyr Phe Asn Val Glu Leu Ile Gln Pro Gly Ala Val Lys Val Tyr
145 150 155 160

Ala Tyr Tyr Asn Leu Glu Glu Ser Cys Thr Arg Phe Tyr His Pro Glu
165 170 175

Lys Glu Asp Gly Lys Leu Asn Lys Leu Cys Arg Asp Glu Leu Cys Arg
180 185 190

Cys Ala Glu Glu Asn Cys Phe Ile Gln Lys Ser Asp Asp Lys Val Thr
195 200 205

Leu Glu Glu Arg Leu Asp Lys Ala Cys Glu Pro Gly Val Asp Tyr Val
210 215 220

Tyr Lys Thr Arg Leu Val Lys Val Gln Leu Ser Asn Asp Phe Asp Glu
225 230 235 240

Tyr Ile Met Ala Ile Glu Gln Thr Ile Lys Ser Gly Ser Asp Glu Val
245 250 255

Gln Val Gly Gln Gln Arg Thr Phe Ile Ser Pro Ile Lys Cys Arg Glu
260 265 270

Ala Leu Lys Leu Glu Glu Lys Lys His Tyr Leu Met Trp Gly Leu Ser
275 280 285

Ser Asp Phe Trp Gly Glu Lys Pro Asn Leu Ser Tyr Ile Ile Gly Lys
290 295 300

Asp Thr Trp Val Glu His Trp Pro Glu Glu Asp Glu Cys Gln Asp Glu
305 310 315 320

Glu Asn Gln Lys Gln Cys Gln Asp Leu Gly Ala Phe Thr Glu Ser Met
325 330 335

Val Val Phe Gly Cys Pro Asn
340

<210> 18
<211> 17
<212> PRT
<213> Homo sapiens

<400> 18

Ser Ser Lys Ile Thr His Arg Ile His Trp Glu Ser Ala Ser Leu Leu
1 5 10 15

Arg

<210> 19
<211> 206
<212> PRT
<213> Homo sapiens

<400> 19

Ser Asn Leu Asp Glu Asp Ile Ile Ala Glu Glu Asn Ile Val Ser Arg
1 5 10 15

Ser Glu Phe Pro Glu Ser Trp Leu Trp Asn Val Glu Asp Leu Lys Glu
20 25 30

Pro Pro Lys Asn Gly Ile Ser Thr Lys Leu Met Asn Ile Phe Leu Lys
35 40 45

Asp Ser Ile Thr Thr Trp Glu Ile Leu Ala Val Ser Met Ser Asp Lys
50 55 60

Lys Gly Ile Cys Val Ala Asp Pro Phe Glu Val Thr Val Met Gln Asp
65 70 75 80

Phe Phe Ile Asp Leu Arg Leu Pro Tyr Ser Val Val Arg Asn Glu Gln
85 90 95

Val Glu Ile Arg Ala Val Leu Tyr Asn Tyr Arg Gln Asn Gln Glu Leu
100 105 110

Lys Val Arg Val Glu Leu Leu His Asn Pro Ala Phe Cys Ser Leu Ala
115 120 125

Thr Thr Lys Arg Arg His Gln Gln Thr Val Thr Ile Pro Pro Lys Ser
130 135 140

Ser Leu Ser Val Pro Tyr Val Ile Val Pro Leu Lys Thr Gly Leu Gln
145 150 155 160

Glu Val Glu Val Lys Ala Ala Val Tyr His His Phe Ile Ser Asp Gly
165 170 175

Val Arg Lys Ser Leu Lys Val Val Pro Glu Gly Ile Arg Met Asn Lys
180 185 190

Thr Val Ala Val Arg Thr Leu Asp Pro Glu Arg Leu Gly Arg
195 200 205

<210> 20

<211> 7

<212> PRT

<213> Homo sapiens

<400> 20

Val Thr Tyr Lys Cys Phe Glu

1 5

<210> 21
<211> 16
<212> PRT
<213> Homo sapiens

<400> 21

Asn Gly Trp Ser Pro Thr Pro Arg Cys Ile Arg Val Ser Phe Thr Leu
1 5 10 15

<210> 22
<211> 18
<212> PRT
<213> Homo sapiens

<400> 22

Ala Thr Phe Cys Asp Phe Pro Lys Ile Asn His Gly Ile Leu Tyr Asp
1 5 10 15

Glu Glu

<210> 23
<211> 16
<212> PRT
<213> Homo sapiens

<400> 23

Arg Gly Trp Ser Thr Pro Pro Lys Cys Arg Ser Thr Ile Ser Ala Glu
1 5 10 15

<210> 24
<211> 18
<212> PRT
<213> Homo sapiens

<400> 24

Ala Met Phe Cys Asp Phe Pro Lys Ile Asn His Gly Ile Leu Tyr Asp
1 5 10 15

Glu Glu

<210> 25
<211> 20
<212> PRT
<213> Homo sapiens

<400> 25

Val Ala Cys His Pro Gly Tyr Gly Leu Pro Lys Ala Gln Thr Thr Val
1 5 10 15

Thr Cys Thr Glu
20

<210> 26
<211> 8
<212> PRT
<213> Homo sapiens

<400> 26

Tyr Gln Cys Gln Ser Tyr Tyr Glu
1 5

<210> 27
<211> 15
<212> PRT
<213> Homo sapiens

<400> 27

Arg Gly Trp Ser Thr Pro Pro Ile Cys Ser Phe Thr Lys Gly Glu
1 5 10 15

<210> 28
<211> 13
<212> PRT
<213> Homo sapiens

<400> 28

Gly Thr Ala Phe Val Ile Phe Gly Ile Gln Asp Gly Glu
1 5 10

<210> 29
<211> 20
<212> PRT
<213> Homo sapiens

<400> 29

Leu Arg Arg Gln His Ala Arg Ala Ser His Leu Gly Leu Ala Arg Ser
1 5 10 15

Asn Leu Asp Glu
20

<210> 30

<211> 15

<212> PRT

<213> Homo sapiens

<400> 30

Leu Arg Arg Gln His Ala Arg Ala Ser His Leu Gly Leu Ala Arg
1 5 10 15

<210> 31

<211> 23

<212> PRT

<213> Homo sapiens

<400> 31

Leu Asn Leu Asp Val Ser Leu Gln Leu Pro Ser Arg Ser Ser Lys Ile
1 5 10 15

Thr His Arg Ile His Trp Glu
20

<210> 32

<211> 12

<212> PRT

<213> Homo sapiens

<400> 32

Leu Asn Leu Asp Val Ser Leu Gln Leu Pro Ser Arg
1 5 10

<210> 33

<211> 5

<212> PRT

<213> Homo sapiens

<400> 33

Arg Leu Gly Arg Glu
1 5

<210> 34
<211> 11
<212> PRT
<213> Homo sapiens

<400> 34

Ser Ser Lys Ile Thr His Arg Ile His Trp Glu
1 5 10

<210> 35
<211> 6
<212> PRT
<213> Homo sapiens

<400> 35

Ser Ala Ser Leu Leu Arg
1 5

<210> 36
<211> 4
<212> PRT
<213> Homo sapiens

<400> 36

Arg Leu Gly Arg
1

<210> 37
<211> 11
<212> PRT
<213> Homo sapiens

<400> 37

His Leu Ile Val Thr Pro Ser Gly Cys Gly Glu
1 5 10

<210> 38
<211> 39
<212> PRT
<213> Homo sapiens

<400> 38

Cys Ala Gly Thr Tyr Asp Gly Ser Ile Asp Ala Cys Lys Gly Asp Ser
1 5 10 15

Gly Gly Pro Leu Val Cys Met Asp Ala Asn Asn Val Thr Tyr Val Trp
20 25 30

Gly Val Val Ser Trp Gly Glu
35

<210> 39
<211> 36
<212> PRT
<213> Homo sapiens

<400> 39

Gly Thr Cys Val Cys Lys Leu Pro Tyr Gln Cys Pro Lys Asn Gly Thr
1 5 10 15

Ala Val Cys Ala Thr Asn Arg Arg Ser Phe Pro Thr Tyr Cys Gln Gln
20 25 30

Lys Ser Leu Glu
35

<210> 40
<211> 29
<212> PRT
<213> Homo sapiens

<400> 40

Phe Pro Gly Val Tyr Thr Lys Val Ala Asn Tyr Phe Asp Trp Ile Ser
1 5 10 15

Tyr His Val Gly Arg Pro Phe Ile Ser Gln Tyr Asn Val
20 25

<210> 41
<211> 31
<212> PRT
<213> Homo sapiens

<400> 41

Ala Asn Val Ala Cys Leu Asp Leu Gly Phe Gln Gln Gly Ala Asp Thr
1 5 10 15

Gln Arg Arg Phe Lys Leu Ser Asp Leu Ser Ile Asn Ser Thr Glu
20 25 30

<210> 42
<211> 30
<212> PRT
<213> Homo sapiens

<400> 42

Leu Pro Arg Ser Ile Pro Ala Cys Val Pro Trp Ser Pro Tyr Leu Phe
1 5 10 15

Gln Pro Asn Asp Thr Cys Ile Val Ser Gly Trp Gly Arg Glu
20 25 30

<210> 43
<211> 26
<212> PRT
<213> Homo sapiens

<400> 43

Lys Lys Cys Leu Ala Lys Lys Tyr Thr His Leu Ser Cys Asp Lys Val
1 5 10 15

Phe Cys Gln Pro Trp Gln Arg Cys Ile Glu
20 25

<210> 44
<211> 28
<212> PRT
<213> Homo sapiens

<400> 44

Leu Cys Cys Lys Ala Cys Gln Gly Lys Gly Phe His Cys Lys Ser Gly
1 5 10 15

Val Cys Ile Pro Ser Gln Tyr Gln Cys Asn Gly Glu
20 25

<210> 45

<211> 21
<212> PRT
<213> Homo sapiens

<400> 45

Val	Lys	Leu	Val	Asp	Gln	Asp	Lys	Thr	Met	Phe	Ile	Cys	Lys	Ser	Ser
1				5					10					15	

Trp	Ser	Met	Arg	Glu
				20

<210> 46
<211> 17
<212> PRT
<213> Homo sapiens

<400> 46

Val	Lys	Leu	Ile	Ser	Asn	Cys	Ser	Lys	Phe	Tyr	Gly	Asn	Arg	Phe	Tyr
1				5					10					15	

Glu

<210> 47
<211> 17
<212> PRT
<213> Homo sapiens

<400> 47

Cys	Leu	His	Pro	Gly	Thr	Lys	Phe	Leu	Asn	Asn	Gly	Thr	Cys	Thr	Ala
1				5					10					15	

Glu

<210> 48
<211> 15
<212> PRT
<213> Homo sapiens

<400> 48

Asn	Tyr	Asn	Ala	Gly	Thr	Tyr	Gln	Asn	Asp	Ile	Ala	Leu	Ile	Glu
1				5					10					15

<210> 49
<211> 15
<212> PRT
<213> Homo sapiens

<400> 49

Gly	Lys	Phe	Ser	Val	Ser	Leu	Lys	His	Gly	Asn	Thr	Asp	Ser	Glu
1				5					10					15

<210> 50
<211> 13
<212> PRT
<213> Homo sapiens

<400> 50

Val	Gly	Cys	Ala	Gly	Phe	Ala	Ser	Val	Thr	Gln	Glu	Glu
1				5					10			

<210> 51
<211> 11
<212> PRT
<213> Homo sapiens

<400> 51

Met	Lys	Lys	Asp	Gly	Asn	Lys	Lys	Asp	Cys	Glu
1				5					10	

<210> 52
<211> 9
<212> PRT
<213> Homo sapiens

<400> 52

Tyr	Val	Asp	Arg	Ile	Ile	Phe	His	Glu
1				5				

<210> 53
<211> 10
<212> PRT
<213> Homo sapiens

<400> 53

Cys	Leu	His	Val	His	Cys	Arg	Gly	Leu	Glu
1				5					10

<210> 54
<211> 9
<212> PRT
<213> Homo sapiens

<400> 54

Arg Val Phe Ser Leu Gln Trp Gly Glu
1 5

<210> 55
<211> 9
<212> PRT
<213> Homo sapiens

<400> 55

Ile Leu Thr Ala Asp Met Asp Ala Glu
1 5

<210> 56
<211> 8
<212> PRT
<213> Homo sapiens

<400> 56

Lys Val Thr Tyr Thr Ser Gln Glu
1 5

<210> 57
<211> 7
<212> PRT
<213> Homo sapiens

<400> 57

Val Asp Cys Ile Thr Gly Glu
1 5

<210> 58
<211> 6
<212> PRT
<213> Homo sapiens

<400> 58

Asn Cys Gly Lys Pro Glu

1 5

<210> 59
<211> 5
<212> PRT
<213> Homo sapiens

<400> 59

Thr Ser Leu Ala Glu
1 5

<210> 60
<211> 4
<212> PRT
<213> Homo sapiens

<400> 60

Lys Asp Asn Glu
1

<210> 61
<211> 61
<212> PRT
<213> Homo sapiens

<400> 61

Ala Lys Ile Arg Tyr Tyr Thr Tyr Leu Ile Met Asn Lys Gly Arg Leu
1 5 10 15

Leu Lys Ala Gly Arg Gln Val Arg Glu Pro Gly Gln Asp Leu Val Val
20 25 30

Leu Pro Leu Ser Ile Thr Thr Asp Phe Ile Pro Ser Phe Arg Leu Val
35 40 45

Ala Tyr Tyr Thr Leu Ile Gly Ala Ser Gly Gln Arg Glu
50 55 60

<210> 62
<211> 52
<212> PRT
<213> Homo sapiens

<400> 62

Arg Ser Gly Ile Pro Ile Val Thr Ser Pro Tyr Gln Ile His Phe Thr
1 5 10 15

Lys Thr Pro Lys Tyr Phe Lys Pro Gly Met Pro Phe Asp Leu Met Val
20 25 30

Phe Val Thr Asn Pro Asp Gly Ser Pro Ala Tyr Arg Val Pro Val Ala
35 40 45

Val Gln Gly Glu
50

<210> 63
<211> 50
<212> PRT
<213> Homo sapiens

<400> 63

Lys Val Val Leu Val Ser Leu Gln Ser Gly Tyr Leu Phe Ile Gln Thr
1 5 10 15

Asp Lys Thr Ile Tyr Thr Pro Gly Ser Thr Val Leu Tyr Arg Ile Phe
20 25 30

Thr Val Asn His Lys Leu Leu Pro Val Gly Arg Thr Val Met Val Asn
35 40 45

Ile Glu
50

<210> 64
<211> 36
<212> PRT
<213> Homo sapiens

<400> 64

Asp Thr Val Gln Ser Leu Thr Gln Gly Asp Gly Val Ala Lys Leu Ser
1 5 10 15

Ile Asn Thr His Pro Ser Gln Lys Pro Leu Ser Ile Thr Val Arg Thr
20 25 30

Lys Lys Gln Glu
35

<210> 65
<211> 35
<212> PRT
<213> Homo sapiens

<400> 65

Gly Asp His Gly Ala Arg Val Val Leu Val Ala Val Asp Lys Gly Val
1 5 10 15

Phe Val Leu Asn Lys Lys Asn Lys Leu Thr Gln Ser Lys Ile Trp Asp
20 25 30

Val Val Glu
35

<210> 66
<211> 38
<212> PRT
<213> Homo sapiens

<400> 66

Lys Ala Asp Ile Gly Cys Thr Pro Gly Ser Gly Lys Asp Tyr Ala Gly
1 5 10 15

Val Phe Ser Asp Ala Gly Leu Thr Phe Thr Ser Ser Ser Gly Gln Gln
20 25 30

Thr Ala Gln Arg Ala Glu
35

<210> 67
<211> 29
<212> PRT
<213> Homo sapiens

<400> 67

Gln Ala Thr Arg Thr Met Gln Ala Leu Pro Tyr Ser Thr Val Gly Asn
1 5 10 15

Ser Asn Asn Tyr Leu His Leu Ser Val Leu Arg Thr Glu
20 25

<210> 68
<211> 27
<212> PRT
<213> Homo sapiens

<400> 68

Ala His Asp Ala Gln Gly Asp Val Pro Val Thr Val Thr Val His Asp
1 5 10 15

Phe Pro Gly Lys Lys Leu Val Leu Ser Ser Glu
20 25

<210> 69
<211> 26
<212> PRT
<213> Homo sapiens

<400> 69

Gly Ile Pro Val Lys Gln Asp Ser Leu Ser Ser Gln Asn Gln Leu Gly
1 5 10 15

Val Leu Pro Leu Ser Trp Asp Ile Pro Glu
20 25

<210> 70
<211> 26
<212> PRT
<213> Homo sapiens

<400> 70

Val Val Ala Asp Ser Val Trp Val Asp Val Lys Asp Ser Cys Val Gly
1 5 10 15

Ser Leu Val Val Lys Ser Gly Gln Ser Glu
20 25

<210> 71
<211> 25
<212> PRT
<213> Homo sapiens

<400> 71

Asp Leu Val Gly Lys Ser Leu Tyr Val Ser Ala Thr Val Ile Leu His
1 5 10 15

Ser Gly Ser Asp Met Val Gln Ala Glu
20 25

<210> 72

<211> 23

<212> PRT

<213> Homo sapiens

<400> 72

Lys Thr Val Leu Thr Pro Ala Thr Asn His Met Gly Asn Val Thr Phe
1 5 10 15

Thr Ile Pro Ala Asn Arg Glu
20

<210> 73

<211> 19

<212> PRT

<213> Homo sapiens

<400> 73

Lys Gly Arg Asn Lys Phe Val Thr Val Gln Ala Thr Phe Gly Thr Gln
1 5 10 15

Val Val Glu

<210> 74

<211> 18

<212> PRT

<213> Homo sapiens

<400> 74

Val Val Leu Ser Arg Lys Val Leu Leu Asp Gly Val Gln Asn Pro Arg
1 5 10 15

Ala Glu

<210> 75
<211> 15
<212> PRT
<213> Homo sapiens

<400> 75

Thr	Leu	Asn	Val	Asn	Phe	Leu	Leu	Arg	Met	Asp	Arg	Ala	His	Glu
1				5					10					15

<210> 76
<211> 14
<212> PRT
<213> Homo sapiens

<400> 76

Leu	Val	Asn	Met	Gly	Gln	Trp	Lys	Ile	Arg	Ala	Tyr	Tyr	Glu
1				5					10				

<210> 77
<211> 15
<212> PRT
<213> Homo sapiens

<400> 77

Ser	Pro	Met	Tyr	Ser	Ile	Ile	Thr	Pro	Asn	Ile	Leu	Arg	Leu	Glu
1				5					10					15

<210> 78
<211> 15
<212> PRT
<213> Homo sapiens

<400> 78

Asp	Arg	Gln	Pro	Val	Pro	Gly	Gln	Gln	Met	Thr	Leu	Lys	Ile	Glu
1				5					10					15

<210> 79
<211> 14
<212> PRT
<213> Homo sapiens

<400> 79

Val	Thr	Ile	Thr	Ala	Arg	Phe	Leu	Tyr	Gly	Lys	Lys	Val	Glu
1				5					10				

<210> 80
<211> 13
<212> PRT
<213> Homo sapiens

<400> 80

Gly Thr Ala Phe Val Ile Phe Gly Ile Gln Asp Gly Glu
1 5 10

<210> 81
<211> 8
<212> PRT
<213> Homo sapiens

<400> 81

Lys Phe Tyr Tyr Ile Tyr Asn Glu
1 5

<210> 82
<211> 10
<212> PRT
<213> Homo sapiens

<400> 82

Asn Ser Pro Gln Gln Val Phe Ser Thr Glu
1 5 10

<210> 83
<211> 8
<212> PRT
<213> Homo sapiens

<400> 83

Ser Leu Lys Arg Ile Pro Ile Glu
1 5

<210> 84
<211> 7
<212> PRT
<213> Homo sapiens

<400> 84

Tyr Val Leu Pro Ser Phe Glu

1 5

<210> 85
<211> 7
<212> PRT
<213> Homo sapiens

<400> 85

Gln Arg Ile Ser Leu Pro Glu
1 5

<210> 86
<211> 8
<212> PRT
<213> Homo sapiens

<400> 86

Leu Gln Cys Pro Gln Pro Ala Ala
1 5

<210> 87
<211> 7
<212> PRT
<213> Homo sapiens

<400> 87

Val Ile Val Glu Pro Thr Glu
1 5

<210> 88
<211> 5
<212> PRT
<213> Homo sapiens

<400> 88

Thr Met Val Leu Glu
1 5

<210> 89
<211> 5
<212> PRT
<213> Homo sapiens

<400> 89

Leu Arg Pro Gly Glu
1 5

<210> 90
<211> 4
<212> PRT
<213> Homo sapiens

<400> 90

Phe Lys Ser Glu
1

<210> 91
<211> 20
<212> PRT
<213> Homo sapiens

<400> 91

Asn Tyr Asn Ile Ala Leu Arg Trp Thr Ala Lys Gln Lys Leu Tyr Leu
1 5 10 15

Arg Thr Gly Glu
20

<210> 92
<211> 19
<212> PRT
<213> Homo sapiens

<400> 92

Tyr Asn Phe Val Ser Pro Ser Lys Ser Phe Trp Thr Arg Ile Thr Cys
1 5 10 15

Ala Glu Glu

<210> 93
<211> 21
<212> PRT
<213> Homo sapiens

<400> 93

Lys Gly Trp Ser Pro Thr Pro Arg Cys Ile Arg Val Arg Thr Cys Ser
1 5 10 15

Lys Ser Asp Ile Glu
20

<210> 94
<211> 18
<212> PRT
<213> Homo sapiens

<400> 94

Asn Gly Tyr Asn Gln Asn Tyr Gly Arg Lys Phe Val Gln Gly Asn Ser
1 5 10 15

Thr Glu

<210> 95
<211> 18
<212> PRT
<213> Homo sapiens

<400> 95

Gln Val Lys Pro Cys Asp Phe Pro Asp Ile Lys His Gly Gly Leu Phe
1 5 10 15

His Glu

<210> 96
<211> 22
<212> PRT
<213> Homo sapiens

<400> 96

Phe Met Cys Lys Leu Gly Tyr Asn Ala Asn Thr Ser Ile Leu Ser Phe
1 5 10 15

Gln Ala Val Cys Arg Glu
20

<210> 97
<211> 8
<212> PRT

<213> Homo sapiens

<400> 97

Tyr Gln Cys Gln Pro Tyr Tyr Glu
1 5

<210> 98

<211> 20

<212> PRT

<213> Homo sapiens

<400> 98

Asn Ser Arg Ala Lys Ser Asn Gly Met Arg Phe Lys Leu His Asp Thr
1 5 10 15

Leu Asp Tyr Glu
20

<210> 99

<211> 14

<212> PRT

<213> Homo sapiens

<400> 99

Asp Gly Trp Ser His Phe Pro Thr Cys Tyr Asn Ser Ser Glu
1 5 10

<210> 100

<211> 14

<212> PRT

<213> Homo sapiens

<400> 100

Ile Ser Tyr Gly Asn Thr Thr Gly Ser Ile Val Cys Gly Glu
1 5 10

<210> 101

<211> 22

<212> PRT

<213> Homo sapiens

<400> 101

Phe Met Cys Lys Leu Gly Tyr Asn Ala Asn Thr Ser Val Leu Ser Phe
1 5 10 15

Gln Ala Val Cys Arg Glu
20

<210> 102
<211> 18
<212> PRT
<213> Homo sapiens

<400> 102

Gly Thr Leu Cys Asp Phe Pro Lys Ile His His Gly Phe Leu Tyr Asp
1 5 10 15

Glu Glu

<210> 103
<211> 18
<212> PRT
<213> Homo sapiens

<400> 103

Tyr Ala Met Ile Gly Asn Asn Met Ile Thr Cys Ile Asn Gly Ile Trp
1 5 10 15

Thr Glu

<210> 104
<211> 18
<212> PRT
<213> Homo sapiens

<400> 104

Tyr Gly Tyr Val Gln Pro Ser Val Pro Pro Tyr Gln His Gly Val Ser
1 5 10 15

Val Glu

<210> 105
<211> 18
<212> PRT

<213> Homo sapiens

<400> 105

Gly	Asp	Thr	Val	Gln	Ile	Ile	Cys	Asn	Thr	Gly	Tyr	Ser	Leu	Gln	Asn
1				5					10					15	

Asn Glu

<210> 106

<211> 16

<212> PRT

<213> Homo sapiens

<400> 106

Ile	Val	Cys	Lys	Asp	Gly	Arg	Trp	Gln	Ser	Leu	Pro	Arg	Cys	Val	Glu
1				5					10					15	

<210> 107

<211> 12

<212> PRT

<213> Homo sapiens

<400> 107

Asp	Tyr	Asn	Pro	Phe	Ser	Gln	Val	Pro	Thr	Gly	Glu
1				5					10		

<210> 108

<211> 10

<212> PRT

<213> Homo sapiens

<400> 108

Gln	Val	Lys	Thr	Cys	Gly	Tyr	Ile	Pro	Glu
1				5					10

<210> 109

<211> 10

<212> PRT

<213> Homo sapiens

<400> 109

Ala	Asn	Val	Asp	Ala	Gln	Pro	Lys	Lys	Glu
1				5					10

<210> 110
<211> 9
<212> PRT
<213> Homo sapiens

<400> 110

Trp Thr Thr Leu Pro Thr Cys Val Glu
1 5

<210> 111
<211> 8
<212> PRT
<213> Homo sapiens

<400> 111

Lys Val Ala Val Leu Cys Lys Glu
1 5

<210> 112
<211> 25
<212> PRT
<213> Homo sapiens

<400> 112

Ser Asn Thr Gly Ser Thr Thr Gly Ser Ile Val Cys Gly Tyr Asn Gly
1 5 10 15

Trp Ser Asp Leu Pro Ile Cys Tyr Glu
20 25

<210> 113
<211> 23
<212> PRT
<213> Homo sapiens

<400> 113

Asn Gly Trp Ser Pro Thr Pro Arg Cys Ile Arg Val Lys Thr Cys Ser
1 5 10 15

Lys Ser Ser Ile Asp Ile Glu
20

<210> 114
<211> 21
<212> PRT
<213> Homo sapiens

<400> 114

Leu	Pro	Lys	Ile	Asp	Val	His	Leu	Val	Pro	Asp	Arg	Lys	Lys	Asp	Gln
1				5					10					15	

Tyr	Lys	Val	Gly	Glu
			20	

<210> 115
<211> 21
<212> PRT
<213> Homo sapiens

<400> 115

Tyr	Tyr	Cys	Asn	Pro	Arg	Phe	Leu	Met	Lys	Gly	Pro	Asn	Lys	Ile	Gln
1				5					10					15	

Cys	Val	Asp	Gly	Glu
			20	

<210> 116
<211> 20
<212> PRT
<213> Homo sapiens

<400> 116

Asn	Tyr	Asn	Ile	Ala	Leu	Arg	Trp	Thr	Ala	Lys	Gln	Lys	Leu	Tyr	Ser
1				5					10					15	

Arg	Thr	Gly	Glu
			20

<210> 117
<211> 20
<212> PRT
<213> Homo sapiens

<400> 117

Lys	Trp	Ser	His	Pro	Pro	Ser	Cys	Ile	Lys	Thr	Asp	Cys	Leu	Ser	Leu
1				5					10					15	

Pro Ser Phe Glu
20

<210> 118
<211> 18
<212> PRT
<213> Homo sapiens

<400> 118

His Gly Trp Ala Gln Leu Ser Ser Pro Pro Tyr Tyr Tyr Gly Asp Ser
1 5 10 15

Val Glu

<210> 119
<211> 18
<212> PRT
<213> Homo sapiens

<400> 119

Ile Ser His Gly Val Val Ala His Met Ser Asp Ser Tyr Gln Tyr Gly
1 5 10 15

Glu Glu

<210> 120
<211> 15
<212> PRT
<213> Homo sapiens

<400> 120

Phe Asp His Asn Ser Asn Ile Arg Tyr Arg Cys Arg Gly Lys Glu
1 5 10 15

<210> 121
<211> 16
<212> PRT
<213> Homo sapiens

<400> 121

Ile Thr Cys Lys Asp Gly Arg Trp Gln Ser Ile Pro Leu Cys Val Glu
1 5 10 15

<210> 122
<211> 15
<212> PRT
<213> Homo sapiens

<400> 122

Gly Trp Ile His Thr Val Cys Ile Asn Gly Arg Trp Asp Pro Glu
1 5 10 15

<210> 123
<211> 16
<212> PRT
<213> Homo sapiens

<400> 123

Lys Ala Lys Tyr Gln Cys Lys Leu Gly Tyr Val Thr Ala Asp Gly Glu
1 5 10 15

<210> 124
<211> 15
<212> PRT
<213> Homo sapiens

<400> 124

Thr Thr Cys Tyr Met Gly Lys Trp Ser Ser Pro Pro Gln Cys Glu
1 5 10 15

<210> 125
<211> 13
<212> PRT
<213> Homo sapiens

<400> 125

Ser Tyr Ala His Gly Thr Lys Leu Ser Tyr Thr Cys Glu
1 5 10

<210> 126
<211> 11
<212> PRT
<213> Homo sapiens

<400> 126

Arg Val Arg Tyr Gln Cys Arg Ser Pro Tyr Glu
1 5 10

<210> 127
<211> 15
<212> PRT
<213> Homo sapiens

<400> 127

Gly Phe Gly Ile Asp Gly Pro Ala Ile Ala Lys Cys Leu Gly Glu
1 5 10 15

<210> 128
<211> 12
<212> PRT
<213> Homo sapiens

<400> 128

His Gly Thr Ile Asn Ser Ser Arg Ser Ser Gln Glu
1 5 10

<210> 129
<211> 10
<212> PRT
<213> Homo sapiens

<400> 129

Tyr Gln Cys Gln Asn Leu Tyr Gln Leu Glu
1 5 10

<210> 130
<211> 11
<212> PRT
<213> Homo sapiens

<400> 130

Trp Thr Thr Leu Pro Val Cys Ile Val Glu Glu
1 5 10

<210> 131
<211> 11
<212> PRT
<213> Homo sapiens

<400> 131

Lys Ile Pro Cys Ser Gln Pro Pro Gln Ile Glu
1 5 10

<210> 132

<211> 9

<212> PRT

<213> Homo sapiens

<400> 132

Ser Gln Tyr Thr Tyr Ala Leu Lys Glu
1 5

<210> 133

<211> 10

<212> PRT

<213> Homo sapiens

<400> 133

Gln Val Gln Ser Cys Gly Pro Pro Pro Glu
1 5 10

<210> 134

<211> 9

<212> PRT

<213> Homo sapiens

<400> 134

Lys Lys Asp Val Tyr Lys Ala Gly Glu
1 5

<210> 135

<211> 9

<212> PRT

<213> Homo sapiens

<400> 135

Gly Leu Pro Cys Lys Ser Pro Pro Glu
1 5

<210> 136

<211> 8

<212> PRT

<213> Homo sapiens

<400> 136

Lys Val Ser Val Leu Cys Gln Glu
1 5

<210> 137

<211> 7

<212> PRT

<213> Homo sapiens

<400> 137

His Leu Lys Asn Lys Lys Glu
1 5

<210> 138

<211> 8

<212> PRT

<213> Homo sapiens

<400> 138

Gly Gly Phe Arg Ile Ser Glu Glu
1 5

<210> 139

<211> 8

<212> PRT

<213> Homo sapiens

<400> 139

Leu Leu Asn Gly Asn Val Lys Glu
1 5

<210> 140

<211> 7

<212> PRT

<213> Homo sapiens

<400> 140

Tyr Pro Thr Cys Ala Lys Arg
1 5

<210> 141

<211> 8

<212> PRT

<213> Homo sapiens

<400> 141

Ser Thr Cys Gly Asp Ile Pro Glu
1 5

<210> 142

<211> 349

<212> PRT

<213> Homo sapiens

<400> 142

Glu Gly Val Gln Lys Glu Asp Ile Pro Pro Ala Asp Leu Ser Asp Gln
1 5 10 15

Val Pro Asp Thr Glu Ser Glu Thr Arg Ile Leu Leu Gln Gly Thr Pro
20 25 30

Val Ala Gln Met Thr Glu Asp Ala Val Asp Ala Glu Arg Leu Lys His
35 40 45

Leu Ile Val Thr Pro Ser Gly Cys Gly Glu Gln Asn Met Ile Gly Met
50 55 60

Thr Pro Thr Val Ile Ala Val His Tyr Leu Asp Glu Thr Glu Gln Trp
65 70 75 80

Glu Lys Phe Gly Leu Glu Lys Arg Gln Gly Ala Leu Glu Leu Ile Lys
85 90 95

Lys Gly Tyr Thr Gln Gln Leu Ala Phe Arg Gln Pro Ser Ser Ala Phe
100 105 110

Ala Ala Phe Val Lys Arg Ala Pro Ser Thr Trp Leu Thr Ala Tyr Val
115 120 125

Val Lys Val Phe Ser Leu Ala Val Asn Leu Ile Ala Ile Asp Ser Gln
130 135 140

Val Leu Cys Gly Ala Val Lys Trp Leu Ile Leu Glu Lys Gln Lys Pro
145 150 155 160

Asp Gly Val Phe Gln Glu Asp Ala Pro Val Ile His Gln Glu Met Ile
165 170 175

Gly Gly Leu Arg Asn Asn Asn Glu Lys Asp Met Ala Leu Thr Ala Phe
180 185 190

Val Leu Ile Ser Leu Gln Glu Ala Lys Asp Ile Cys Glu Glu Gln Val
195 200 205

Asn Ser Leu Pro Gly Ser Ile Thr Lys Ala Gly Asp Phe Leu Glu Ala
210 215 220

Asn Tyr Met Asn Leu Gln Arg Ser Tyr Thr Val Ala Ile Ala Gly Tyr
225 230 235 240

Ala Leu Ala Gln Met Gly Arg Leu Lys Gly Pro Leu Leu Asn Lys Phe
245 250 255

Leu Thr Thr Ala Lys Asp Lys Asn Arg Trp Glu Asp Pro Gly Lys Gln
260 265 270

Leu Tyr Asn Val Glu Ala Thr Ser Tyr Ala Leu Leu Ala Leu Leu Gln
275 280 285

Leu Lys Asp Phe Asp Phe Val Pro Pro Val Val Arg Trp Leu Asn Glu
290 295 300

Gln Arg Tyr Tyr Gly Gly Gly Tyr Gly Ser Thr Gln Ala Thr Phe Met
305 310 315 320

Val Phe Gln Ala Leu Ala Gln Tyr Gln Lys Asp Ala Pro Asp His Gln
325 330 335

Glu Leu Asn Leu Asp Val Ser Leu Gln Leu Pro Ser Arg
340 345

<210> 143
<211> 47
<212> PRT
<213> Homo sapiens

<400> 143

Glu Gly Val Gln Lys Glu Asp Ile Pro Pro Ala Asp Leu Ser Asp Gln
1 5 10 15

Val Pro Asp Thr Glu Ser Glu Thr Arg Ile Leu Leu Gln Gly Thr Pro
20 25 30

Val Ala Gln Met Thr Glu Asp Ala Val Asp Ala Glu Arg Leu Lys
35 40 45

<210> 144

<211> 302

<212> PRT

<213> Homo sapiens

<400> 144

His Leu Ile Val Thr Pro Ser Gly Cys Gly Glu Gln Asn Met Ile Gly
1 5 10 15

Met Thr Pro Thr Val Ile Ala Val His Tyr Leu Asp Glu Thr Glu Gln
20 25 30

Trp Glu Lys Phe Gly Leu Glu Lys Arg Gln Gly Ala Leu Glu Leu Ile
35 40 45

Lys Lys Gly Tyr Thr Gln Gln Leu Ala Phe Arg Gln Pro Ser Ser Ala
50 55 60

Phe Ala Ala Phe Val Lys Arg Ala Pro Ser Thr Trp Leu Thr Ala Tyr
65 70 75 80

Val Val Lys Val Phe Ser Leu Ala Val Asn Leu Ile Ala Ile Asp Ser
85 90 95

Gln Val Leu Cys Gly Ala Val Lys Trp Leu Ile Leu Glu Lys Gln Lys
100 105 110

Pro Asp Gly Val Phe Gln Glu Asp Ala Pro Val Ile His Gln Glu Met
115 120 125

Ile Gly Gly Leu Arg Asn Asn Asn Glu Lys Asp Met Ala Leu Thr Ala

130

135

140

Phe Val Leu Ile Ser Leu Gln Glu Ala Lys Asp Ile Cys Glu Glu Gln
 145 150 155 160

Val Asn Ser Leu Pro Gly Ser Ile Thr Lys Ala Gly Asp Phe Leu Glu
 165 170 175

Ala Asn Tyr Met Asn Leu Gln Arg Ser Tyr Thr Val Ala Ile Ala Gly
 180 185 190

Tyr Ala Leu Ala Gln Met Gly Arg Leu Lys Gly Pro Leu Leu Asn Lys
 195 200 205

Phe Leu Thr Thr Ala Lys Asp Lys Asn Arg Trp Glu Asp Pro Gly Lys
 210 215 220

Gln Leu Tyr Asn Val Glu Ala Thr Ser Tyr Ala Leu Leu Ala Leu Leu
 225 230 235 240

Gln Leu Lys Asp Phe Asp Phe Val Pro Pro Val Val Arg Trp Leu Asn
 245 250 255

Glu Gln Arg Tyr Tyr Gly Gly Gly Tyr Gly Ser Thr Gln Ala Thr Phe
 260 265 270

Met Val Phe Gln Ala Leu Ala Gln Tyr Gln Lys Asp Ala Pro Asp His
 275 280 285

Gln Glu Leu Asn Leu Asp Val Ser Leu Gln Leu Pro Ser Arg
 290 295 300

<210> 145
 <211> 194
 <212> PRT
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<223> Xaa can be any naturally occurring amino acid

<400> 145

Gly His Cys Gln Ala Pro Asp His Phe Leu Phe Ala Lys Leu Lys Thr
1 5 10 15

Gln Thr Asn Ala Ser Asp Phe Pro Ile Gly Thr Ser Leu Lys Tyr Glu
20 25 30

Cys Arg Pro Glu Tyr Tyr Gly Arg Pro Phe Ser Ile Thr Cys Leu Asp
35 40 45

Asn Leu Val Trp Ser Ser Pro Lys Asp Val Cys Lys Arg Lys Ser Cys
50 55 60

Lys Thr Pro Pro Asp Pro Val Asn Gly Met Val His Val Ile Thr Asp
65 70 75 80

Ile Gln Val Gly Ser Arg Ile Asn Tyr Ser Cys Thr Thr Gly His Arg
85 90 95

Leu Ile Gly His Ser Ser Ala Glu Cys Ile Leu Ser Gly Asn Xaa Ala
100 105 110

His Trp Ser Thr Lys Pro Pro Ile Cys Gln Arg Ile Pro Cys Gly Leu
115 120 125

Pro Pro Thr Ile Ala Asn Gly Asp Phe Ile Ser Thr Asn Arg Glu Asn
130 135 140

Phe His Tyr Gly Ser Val Val Thr Tyr Arg Cys Asn Xaa Gly Ser Xaa
145 150 155 160

Gly Arg Lys Val Phe Glu Leu Val Gly Glu Pro Ser Ile Tyr Cys Thr
165 170 175

Ser Asn Asp Asp Gln Val Gly Ile Trp Ser Gly Pro Ala Pro Gln Cys
180 185 190

Ile Ile

<210> 146
<211> 194
<212> PRT
<213> Homo sapiens

<400> 146

Gly His Cys Gln Ala Pro Asp His Phe Leu Phe Ala Lys Leu Lys Thr
1 5 10 15

Gln Thr Asn Ala Ser Asp Phe Pro Ile Gly Thr Ser Leu Lys Tyr Glu
20 25 30

Cys Arg Pro Glu Tyr Tyr Gly Arg Pro Phe Ser Ile Thr Cys Leu Asp
35 40 45

Asn Leu Val Trp Ser Ser Pro Lys Asp Val Cys Lys Arg Lys Ser Cys
50 55 60

Lys Thr Pro Pro Asp Pro Val Asn Gly Met Val His Val Ile Thr Asp
65 70 75 80

Ile Gln Val Gly Ser Arg Ile Asn Tyr Ser Cys Thr Thr Gly His Arg
85 90 95

Leu Ile Gly His Ser Ser Ala Glu Cys Ile Leu Ser Gly Asn Ala Ala
100 105 110

His Trp Ser Thr Lys Pro Pro Ile Cys Gln Arg Ile Pro Cys Gly Leu
115 120 125

Pro Pro Thr Ile Ala Asn Gly Asp Phe Ile Ser Thr Asn Arg Glu Asn
130 135 140

Phe His Tyr Gly Ser Val Val Thr Tyr Arg Cys Asn Pro Gly Ser Gly

160

Gly Arg Lys Val Phe Glu Leu Val Gly Glu Pro Ser Ile Tyr Cys Thr
165 170 175

Ser Asn Asp Asp Gln Val Gly Ile Trp Ser Gly Pro Ala Pro Gln Cys
180 185 190

Ile Ile

<210>	147
<211>	194
<212>	PRT
<213>	Homo sapiens

<400> 147

Gly His Cys Gln Ala Pro Asp His Phe Leu Phe Ala Lys Leu Lys Thr
1 5 10 15

Gln Thr Asn Ala Ser Asp Phe Pro Ile Gly Thr Ser Leu Lys Tyr Glu
20 25 30

Cys Arg Pro Glu Tyr Tyr Gly Arg Pro Phe Ser Ile Thr Cys Leu Asp
35 40 45

Asn Leu Val Trp Ser Ser Pro Lys Asp Val Cys Lys Arg Lys Ser Cys
50 55 60

Lys Thr Pro Pro Asp Pro Val Asn Gly Met Val His Val Ile Thr Asp
65 70 75 80

Ile Gln Val Gly Ser Arg Ile Asn Tyr Ser Cys Thr Thr Gly His Arg
85 90 95

Leu Ile Gly His Ser Ser Ala Glu Cys Ile Leu Ser Gly Asn Thr Ala
100 105 110

His Trp Ser Thr Lys Pro Pro Ile Cys Gln Arg Ile Pro Cys Gly Leu
115 120 125

Pro Pro Thr Ile Ala Asn Gly Asp Phe Ile Ser Thr Asn Arg Glu Asn
130 135 140

Phe His Tyr Gly Ser Val Val Thr Tyr Arg Cys Asn Leu Gly Ser Arg
145 150 155 160

Gly Arg Lys Val Phe Glu Leu Val Gly Glu Pro Ser Ile Tyr Cys Thr
165 170 175

Ser Asn Asp Asp Gln Val Gly Ile Trp Ser Gly Pro Ala Pro Gln Cys
180 185 190

Ile Ile

<210> 148
<211> 388
<212> PRT
<213> Homo sapiens

<400> 148

Gly His Cys Gln Ala Pro Asp His Phe Leu Phe Ala Lys Leu Lys Thr
1 5 10 15

Gln Thr Asn Ala Ser Asp Phe Pro Ile Gly Thr Ser Leu Lys Tyr Glu
20 25 30

Cys Arg Pro Glu Tyr Tyr Gly Arg Pro Phe Ser Ile Thr Cys Leu Asp
35 40 45

Asn Leu Val Trp Ser Ser Pro Lys Asp Val Cys Lys Arg Lys Ser Cys
50 55 60

Lys Thr Pro Pro Asp Pro Val Asn Gly Met Val His Val Ile Thr Asp
65 70 75 80

Ile Gln Val Gly Ser Arg Ile Asn Tyr Ser Cys Thr Thr Gly His Arg
85 90 95

Leu Ile Gly His Ser Ser Ala Glu Cys Ile Leu Ser Gly Asn Ala Ala
100 105 110

His Trp Ser Thr Lys Pro Pro Ile Cys Gln Arg Ile Pro Cys Gly Leu
115 120 125

Pro Pro Thr Ile Ala Asn Gly Asp Phe Ile Ser Thr Asn Arg Glu Asn
130 135 140

Phe His Tyr Gly Ser Val Val Thr Tyr Arg Cys Asn Pro Gly Ser Gly
145 150 155 160

Gly Arg Lys Val Phe Glu Leu Val Gly Glu Pro Ser Ile Tyr Cys Thr
165 170 175

Ser Asn Asp Asp Gln Val Gly Ile Trp Ser Gly Pro Ala Pro Gln Cys
180 185 190

Ile Ile Gly His Cys Gln Ala Pro Asp His Phe Leu Phe Ala Lys Leu
195 200 205

Lys Thr Gln Thr Asn Ala Ser Asp Phe Pro Ile Gly Thr Ser Leu Lys
210 215 220

Tyr Glu Cys Arg Pro Glu Tyr Tyr Gly Arg Pro Phe Ser Ile Thr Cys
225 230 235 240

Leu Asp Asn Leu Val Trp Ser Ser Pro Lys Asp Val Cys Lys Arg Lys
245 250 255

Ser Cys Lys Thr Pro Pro Asp Pro Val Asn Gly Met Val His Val Ile
260 265 270

Thr Asp Ile Gln Val Gly Ser Arg Ile Asn Tyr Ser Cys Thr Thr Gly
275 280 285

His Arg Leu Ile Gly His Ser Ser Ala Glu Cys Ile Leu Ser Gly Asn
290 295 300

Thr Ala His Trp Ser Thr Lys Pro Pro Ile Cys Gln Arg Ile Pro Cys
305 310 315 320

Gly Leu Pro Pro Thr Ile Ala Asn Gly Asp Phe Ile Ser Thr Asn Arg

325

330

335

Glu Asn Phe His Tyr Gly Ser Val Val Thr Tyr Arg Cys Asn Leu Gly
 340 345 350

Ser Arg Gly Arg Lys Val Phe Glu Leu Val Gly Glu Pro Ser Ile Tyr
 355 360 365

Cys Thr Ser Asn Asp Asp Gln Val Gly Ile Trp Ser Gly Pro Ala Pro
 370 375 380

Gln Cys Ile Ile
 385

<210> 149
 <211> 235
 <212> PRT
 <213> Homo sapiens

<400> 149

Ile Val Gly Gly Lys Arg Ala Gln Leu Gly Asp Leu Pro Trp Gln Val
 1 5 10 15

Ala Ile Lys Asp Ala Ser Gly Ile Thr Cys Gly Gly Ile Tyr Ile Gly
 20 25 30

Gly Cys Trp Ile Leu Thr Ala Ala His Cys Leu Arg Ala Ser Lys Thr
 35 40 45

His Arg Tyr Gln Ile Trp Thr Thr Val Val Asp Trp Ile His Pro Asp
 50 55 60

Leu Lys Arg Ile Val Ile Glu Tyr Val Asp Arg Ile Ile Phe His Glu
 65 70 75 80

Asn Tyr Asn Ala Gly Thr Tyr Gln Asn Asp Ile Ala Leu Ile Glu Met
 85 90 95

Lys Lys Asp Gly Asn Lys Lys Asp Cys Glu Leu Pro Arg Ser Ile Pro
 100 105 110

Ala Cys Val Pro Trp Ser Pro Tyr Leu Phe Gln Pro Asn Asp Thr Cys
115 120 125

Ile Val Ser Gly Trp Gly Arg Glu Lys Asp Asn Glu Arg Val Phe Ser
130 135 140

Leu Gln Trp Gly Glu Val Lys Leu Ile Ser Asn Cys Ser Lys Phe Tyr
145 150 155 160

Gly Asn Arg Phe Tyr Glu Lys Glu Met Glu Cys Ala Gly Thr Tyr Asp
165 170 175

Gly Ser Ile Asp Ala Cys Lys Gly Asp Ser Gly Gly Pro Leu Val Cys
180 185 190

Met Asp Ala Asn Asn Val Thr Tyr Val Trp Gly Val Val Ser Trp Gly
195 200 205

Glu Asn Cys Gly Lys Pro Glu Phe Pro Gly Val Tyr Thr Lys Val Ala
210 215 220

Asn Tyr Phe Asp Trp Ile Ser Tyr His Val Gly
225 230 235

<210> 150
<211> 246
<212> PRT
<213> Homo sapiens

<400> 150

Glu Asp Cys Asn Glu Leu Pro Pro Arg Arg Asn Thr Glu Ile Leu Thr
1 5 10 15

Gly Ser Trp Ser Asp Gln Thr Tyr Pro Glu Gly Thr Gln Ala Ile Tyr
20 25 30

Lys Cys Arg Pro Gly Tyr Arg Ser Leu Gly Asn Val Ile Met Val Cys
35 40 45

Arg Lys Gly Glu Trp Val Ala Leu Asn Pro Leu Arg Lys Cys Gln Lys
50 55 60

Arg Pro Cys Gly His Pro Gly Asp Thr Pro Phe Gly Thr Phe Thr Leu
65 70 75 80

Thr Gly Gly Asn Val Phe Glu Tyr Gly Val Lys Ala Val Tyr Thr Cys
85 90 95

Asn Glu Gly Tyr Gln Leu Leu Gly Glu Ile Asn Tyr Arg Glu Cys Asp
100 105 110

Thr Asp Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val Val Lys Cys
115 120 125

Leu Pro Val Thr Ala Pro Glu Asn Gly Lys Ile Val Ser Ser Ala Met
130 135 140

Glu Pro Asp Arg Glu Tyr His Phe Gly Gln Ala Val Arg Phe Val Cys
145 150 155 160

Asn Ser Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His Cys Ser Asp
165 170 175

Asp Gly Phe Trp Ser Lys Glu Lys Pro Lys Cys Val Glu Ile Ser Cys
180 185 190

Lys Ser Pro Asp Val Ile Asn Gly Ser Pro Ile Ser Gln Lys Ile Ile
195 200 205

Tyr Lys Glu Asn Glu Arg Phe Gln Tyr Lys Cys Asn Met Gly Tyr Glu
210 215 220

Tyr Ser Glu Arg Gly Asp Ala Val Cys Thr Glu Ser Gly Trp Arg Pro
225 230 235 240

Leu Pro Ser Cys Glu Glu
245

<210> 151

<211> 194

<212> PRT

<213> Homo sapiens

<400> 151

Gly His Cys Gln Ala Pro Asp His Phe Leu Phe Ala Lys Leu Lys Thr
1 5 10 15

Gln Thr Asn Ala Ser Asp Phe Pro Ile Gly Thr Ser Leu Lys Tyr Glu
20 25 30

Cys Arg Pro Glu Tyr Tyr Gly Arg Pro Phe Ser Ile Thr Cys Leu Asp
35 40 45

Asn Leu Val Trp Ser Ser Pro Lys Asp Val Cys Lys Arg Lys Ser Cys
50 55 60

Lys Thr Pro Pro Asp Pro Val Asn Gly Met Val His Val Ile Thr Asp
65 70 75 80

Ile Gln Val Gly Ser Arg Ile Asn Tyr Ser Cys Thr Thr Gly His Arg
85 90 95

Leu Ile Gly His Ser Ser Ala Glu Cys Ile Leu Ser Gly Asn Ala Ala
100 105 110

His Trp Ser Thr Lys Pro Pro Ile Cys Gln Arg Ile Pro Cys Gly Leu
115 120 125

Pro Pro Thr Ile Ala Asn Gly Asp Phe Ile Ser Thr Asn Arg Glu Asn
130 135 140

Phe His Tyr Gly Ser Val Val Thr Tyr Arg Cys Asn Pro Gly Ser Gly
145 150 155 160

Gly Arg Lys Val Phe Glu Leu Val Gly Glu Pro Ser Ile Tyr Cys Thr
165 170 175

Ser Asn Asp Asp Gln Val Gly Ile Trp Ser Gly Pro Ala Pro Gln Cys
180 185 190

Ile Ile

<210> 152
<211> 194
<212> PRT
<213> Homo sapiens

<400> 152

Gly His Cys Gln Ala Pro Asp His Phe Leu Phe Ala Lys Leu Lys Thr
1 5 10 15

Gln Thr Asn Ala Ser Asp Phe Pro Ile Gly Thr Ser Leu Lys Tyr Glu
20 25 30

Cys Arg Pro Glu Tyr Tyr Gly Arg Pro Phe Ser Ile Thr Cys Leu Asp
35 40 45

Asn Leu Val Trp Ser Ser Pro Lys Asp Val Cys Lys Arg Lys Ser Cys
50 55 60

Lys Thr Pro Pro Asp Pro Val Asn Gly Met Val His Val Ile Thr Asp
65 70 75 80

Ile Gln Val Gly Ser Arg Ile Asn Tyr Ser Cys Thr Thr Gly His Arg
85 90 95

Leu Ile Gly His Ser Ser Ala Glu Cys Ile Leu Ser Gly Asn Thr Ala
100 105 110

His Trp Ser Thr Lys Pro Pro Ile Cys Gln Arg Ile Pro Cys Gly Leu
115 120 125

Pro Pro Thr Ile Ala Asn Gly Asp Phe Ile Ser Thr Asn Arg Glu Asn
130 135 140

Phe His Tyr Gly Ser Val Val Thr Tyr Arg Cys Asn Leu Gly Ser Arg
145 150 155 160

Gly Arg Lys Val Phe Glu Leu Val Gly Glu Pro Ser Ile Tyr Cys Thr
165 170 175

Ser Asn Asp Asp Gln Val Gly Ile Trp Ser Gly Pro Ala Pro Gln Cys
180 185 190

Ile Ile

<210> 153
<211> 342
<212> PRT
<213> Homo sapiens

<400> 153

Met Lys Gly Lys Phe Leu Lys Val Ser Ser Leu Phe Val Ala Thr Leu
1 5 10 15

Thr Thr Ala Thr Leu Val Ser Ser Pro Ala Ala Asn Ala Leu Ser Ser
20 25 30

Lys Ala Met Asp Asn His Pro Gln Gln Thr Gln Ser Ser Lys Gln Gln
35 40 45

Thr Pro Lys Ile Lys Lys Gly Gly Asn Leu Lys Pro Leu Glu Gln Arg
50 55 60

Glu His Ala Asn Val Ile Leu Pro Asn Asn Asp Arg His Gln Ile Thr
65 70 75 80

Asp Thr Thr Asn Gly His Tyr Ala Pro Val Thr Tyr Ile Gln Val Glu
85 90 95

Ala Pro Thr Gly Thr Phe Ile Ala Ser Gly Val Val Val Gly Lys Asp
100 105 110

Thr Leu Leu Thr Asn Lys His Val Val Asp Ala Thr His Gly Asp Pro
115 120 125

His Ala Leu Lys Ala Phe Pro Ser Ala Ile Asn Gln Asp Asn Tyr Pro
130 135 140

Asn Gly Gly Phe Thr Ala Glu Gln Ile Thr Lys Tyr Ser Gly Glu Gly
145 150 155 160

Asp Leu Ala Ile Val Lys Phe Ser Pro Asn Glu Gln Asn Lys His Ile
165 170 175

Gly Glu Val Val Lys Pro Ala Thr Met Ser Asn Asn Ala Glu Thr Gln
180 185 190

Val Asn Gln Asn Ile Thr Val Thr Gly Tyr Pro Gly Asp Lys Pro Val
195 200 205

Ala Thr Met Trp Glu Ser Lys Gly Lys Ile Thr Tyr Leu Lys Gly Glu
210 215 220

Ala Met Gln Tyr Asp Leu Ser Thr Thr Gly Gly Asn Ser Gly Ser Pro
225 230 235 240

Val Phe Asn Glu Lys Asn Glu Val Ile Gly Ile His Trp Gly Gly Val
245 250 255

Pro Asn Glu Phe Asn Gly Ala Val Phe Ile Asn Glu Asn Val Arg Asn
260 265 270

Phe Leu Lys Gln Asn Ile Glu Asp Ile His Phe Ala Asn Asp Asp Gln
275 280 285

Pro Asn Asn Pro Asp Asn Pro Asp Asn Pro Asn Asn Pro Asp Asn Pro
290 295 300

Asn Asn Pro Asp Asn Pro Asn Asn Pro Asp Glu Pro Asn Asn Pro Asp
305 310 315 320

Asn Pro Asn Asn Pro Asp Asn Pro Asp Asn Gly Asp Asn Asn Asn Ser
325 330 335

Asp Asn Pro Asp Ala Ala
340

<210> 154
<211> 274
<212> PRT
<213> Homo sapiens

<400> 154

Val Ile Leu Pro Asn Asn Asp Arg His Gln Ile Thr Asp Thr Thr Asn

1	5	10	15
Gly His Tyr	Ala Pro Val	Thr Tyr Ile Gln Val Glu Ala	Pro Thr Gly
	20	25	30
Thr Phe Ile	Ala Ser Gly	Val Val Val Gly Lys Asp Thr	Leu Leu Thr
	35	40	45
Asn Lys His	Val Val Asp	Ala Thr His Gly Asp	Pro His Ala Leu Lys
	50	55	60
Ala Phe Pro	Ser Ala Ile	Asn Gln Asp Asn Tyr Pro Asn Gly Gly	Phe
65	70	75	80
Thr Ala Glu	Gln Ile Thr	Lys Tyr Ser Gly Glu Gly Asp	Leu Ala Ile
	85	90	95
Val Lys Phe	Ser Pro Asn	Glu Gln Asn Lys His Ile Gly Glu	Val Val
	100	105	110
Lys Pro Ala	Thr Met Ser	Asn Asn Ala Glu Thr Gln Val	Asn Gln Asn
	115	120	125
Ile Thr Val	Thr Gly Tyr	Pro Gly Asp Lys Pro Val Ala Thr	Met Trp
	130	135	140
Glu Ser Lys	Gly Lys Ile Thr	Tyr Leu Lys Gly Glu Ala Met	Gln Tyr
145	150	155	160
Asp Leu Ser	Thr Thr Gly	Gly Asn Ser Gly Ser Pro Val Phe	Asn Glu
	165	170	175
Lys Asn Glu	Val Ile Gly	Ile His Trp Gly Gly Val Pro	Asn Glu Phe
	180	185	190
Asn Gly Ala	Val Phe Ile	Asn Glu Asn Val Arg Asn Phe	Leu Lys Gln
	195	200	205
Asn Ile Glu	Asp Ile His	Phe Ala Asn Asp Asp Gln Pro	Asn Asn Pro
	210	215	220

Asp Asn Pro Asp Asn Pro Asn Asn Pro Asp Asn Pro Asn Asn Pro Asp
225 230 235 240

Asn Pro Asn Asn Pro Asp Glu Pro Asn Asn Pro Asp Asn Pro Asn Asn
245 250 255

Pro Asp Asn Pro Asp Asn Gly Asp Asn Asn Asn Ser Asp Asn Pro Asp
260 265 270

Ala Ala

<210> 155
<211> 12
<212> PRT
<213> Homo sapiens

<400> 155

Val Gly Cys Ala Gly Phe Ala Ser Val Thr Gln Glu
1 5 10

<210> 156
<211> 14
<212> PRT
<213> Homo sapiens

<400> 156

Leu Arg Arg Gln His Ala Arg Ala Ser His Leu Gly Leu Ala
1 5 10

<210> 157
<211> 5
<212> PRT
<213> Homo sapiens

<400> 157

Ser Ala Ser Leu Leu
1 5