



US 20100324114A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2010/0324114 A1**
Dewald (43) **Pub. Date: Dec. 23, 2010**

(54) **METHODS AND KITS TO DETECT HEREDITARY ANGIOEDEMA TYPE III**

(57) **ABSTRACT**

(76) Inventor: **Georg Dewald, Bonn (DE)**

Correspondence Address:
**BIRCH STEWART KOLASCH & BIRCH
PO BOX 747
FALLS CHURCH, VA 22040-0747 (US)**

(21) Appl. No.: **12/245,645**

(22) Filed: **Oct. 3, 2008**

Related U.S. Application Data

(63) Continuation of application No. 11/600,739, filed on Nov. 17, 2006, now abandoned, which is a continuation-in-part of application No. PCT/EP2005/005404, filed on May 18, 2005.

(30) **Foreign Application Priority Data**

May 18, 2004 (EP) 04011790.5

Publication Classification

(51) **Int. Cl.**

- A61K 31/7105* (2006.01)
- C12Q 1/68* (2006.01)
- G01N 33/50* (2006.01)
- G01N 33/53* (2006.01)
- C07K 2/00* (2006.01)
- C07K 14/00* (2006.01)
- C07H 21/04* (2006.01)
- A01K 67/033* (2006.01)
- C07K 16/18* (2006.01)
- C12N 5/12* (2006.01)
- A61P 9/00* (2006.01)

(52) **U.S. Cl.** **514/44 A; 435/6; 436/94; 435/7.1; 435/7.21; 530/300; 536/23.1; 530/350; 514/44 R; 800/13; 800/9; 800/3; 536/23.5; 530/381; 530/389.1; 530/389.3; 435/331**

The present invention relates to a method of diagnosing hereditary angioedema type III (HAE III) or a predisposition thereto in a subject being suspected of having developed or of having a predisposition to develop a hereditary angioedema type III or in a subject being suspected of being a carrier for hereditary angioedema type III, the method comprising determining in vitro from a biological sample of said subject the presence or absence of a disease-associated mutation in a nucleic acid molecule regulating the expression of or encoding coagulation factor XII; wherein the presence of such a mutation is indicative of a hereditary angioedema type III or a predisposition thereto. The present invention also relates to a method of diagnosing hereditary angioedema type III (HAE III) or a predisposition thereto in a subject being suspected of having developed or of having a predisposition to develop a hereditary angioedema type III or in a subject being suspected of being a carrier for hereditary angioedema type III, the method comprising assessing the presence, amount and/or activity of coagulation factor XII in said subject and including the steps of: (a) determining from a biological sample of said subject in vitro, the presence, amount and for activity of: (i) a (poly)peptide encoded by the coagulation factor XII gene; (ii) a substrate of the (poly)peptide of (i); or (iii) a (poly)peptide processed by the substrate mentioned in (ii); (b) comparing said presence, amount and/or activity with that determined from a reference sample; and (c) diagnosing, based on the difference between the samples compared in step (b), the pathological condition of a hereditary angioedema type III or a predisposition thereto. The present invention also relates to a method of identifying a compound modulating coagulation factor XII activity which is suitable as a medicament or a lead compound for a medicament for the treatment and/or prevention of hereditary angioedema type III, the method comprising the steps of: (a) in vitro contacting a coagulation factor XII (poly)peptide or a functionally related (poly)peptide with the potential modulator; and (b) testing for modulation of coagulation factor XII activity, wherein modulation of coagulation factor XII activity is indicative of a compound's suitability as a medicament for the treatment and/or prevention of hereditary angioedema type III. Furthermore, the present invention relates to gene therapy methods and to a kit for diagnosing hereditary angioedema type III.

```

1 ctattgatct ggaactcttg ataggaactt ggaacaagg aaggaagpcca tgaaggctct
61 gctgctctctt gggttctctc tggtagcttt tgaagcaaca ctltgatttc cacttggaga
121 agcccccaag gggcataagt acaaaagctga agagaacaca gtgtgttcca ctgtcaacgg
181 gggpccctgc cacttccctt tccagtacca cgggagctgy taccacaagt jkaccacaca
241 gggagggcca ggcctcagtc cctgtgtgtc taccaccccc aadtgtatgc agagcaagp
301 atgggatac tgtttggagc ccaagaagct gaaagaccac tgcagaaac aagacccttg
361 ccagaagaga ggaactcttg tgaacatgc aagggcccc cactgtctct gtccacaaca
421 cctcactgga aaccactgcc agaagaaga gtgctttgag octagcttcc tccggttttt
481 ccacaagaat ggaataggt atgaacatga ccaagagctt gtagccagat gccagtgaca
541 ggttctgagc ggcacctgac agcgggtgca caagagagct tggcaacaca accagctctt
601 caatggaggt cgtctctcag agtggagggp ccaacgctgtg tgcactctgc cgtgtgctc
661 caaccgaccr tctctgagc tgaacacaaa ggaacagctc tatgtgtccc ggggtctag
721 ctaccgagcc ctggcagaga caacgctctc ggtgtgcccc tatgtgtccc ggggtctag
781 ggcacactac cggaaactga ctgcagagca agcggcaaac tgggactgag gggccacagc
841 ctctctgagc aaccgggacc agcaactctc ccggtgtctc tctgtgtgca accggagag
901 gctgtgtgct ggttactgag accgtgagcc ctgcagagcc acccaacagc agagctctc
961 gaccctcagt tccctcagtc ttcagtctcc actatgccc gggcagccgg caaccgagaa
          a
          a
1021 gecttaagcc aggaaccgga cccgcgctca gtcccagacc cgggagpct tggccggaga
          Thr
          Lys
          Arg
1081 cgggagacag cagctctctc tgaacagaa cggccactgt agctcgggc agagctctcg
1141 caagagctgt tctctgtga cctggctgct tggagagctc gtagagctcc cggggagca
1201 cccctaccct gcccgctctg actggggcca cagttctctc gctggagccc tcaatggccc
1261 ctgctgggtg ctgagcagc ctraactgct cggagaccgg cccgcaaccg agactgtgac
1321 ggtgtgtctc ggcaggaac cgcataacca cagctgtgag cagtgcaaga cgttggpctt
1381 ggcctctcac cgtctctcag agcctctctc gccctctcag taccagcagc accctgctct
1441 gttgagctct cagggagag tggagagcag ctgagctctc ctgctgctct agctctcagc
1501 gttgtgtctc caagagagc cagctgagcc ctgcagagcc accctctctc agtggagag
1561 ctgggcaacc cagttctgag ggcacagaga atatgcaagc tctctcagag aggcctgagt
1621 accgtctctc tccctgagc gctgtgagc cccgagctgt cagcagctct caactctccc
1681 cggcatgctc tgcagaggt tctctgagag cggcaaccgt ggttctcag gtagctctgg
1741 aggcctgctc gttgtgagc accacagctc agagcagcag ctaccctctc aagcatctac
1801 cagctgggga tggcctgta gtagccacca caagcagagc gttcaaacgc atgtgagcta
1861 ctactctgac tgaatcagp agcaacactt tctctgtgtg ctggagagat cactctctcc
1921 tctctgtgta tctccagta agagctgctc tggagctatp aagcagatc tgtatctcat
1981 tccccagtg cggcagctc cgcgcagga tggcagaga ctcaataag tctttgaaa
2041 atgctgag

```

1 ctattgatct ggactcctgg ataggcagct ggaccaacgg acggacgcca tgagggctct
 61 gctgctcctg gggttcctgc tggtagctt ggagtcaaca ctttcgattc caccttgga
 121 agccccaag gagcataagt acaaagctga agagcacaca gtcgttctca ctgtcaccgg
 181 ggagccctgc cacttcccct tccagtacca ccggcagctg taccacaaat gtaccacaa
 241 gggccggcca ggccctcagc cctggtgtgc taccaccccc aactttgatc aggaccagcg
 301 atggggatac tgtttggagc ccaagaaagt gaaagaccac tgcagcaaac acagcccctg
 361 ccagaaagga gggacctgtg tgaacatgcc aagcggcccc cactgtctct gtccacaaca
 421 cctcactgga aaccactgcc agaaagagaa gtgctttgag cctcagcttc tccggtttt
 481 ccacaagaat gagatatggt atagaactga gcaagcagct gtggccagat gccagtgcaa
 541 gggtcctgat gccactgcc agcggctggc cagccaggcc tgccgcacca acccgtgcct
 601 ccatgggggt cgctgcctag aggtggaggg ccaccgcctg tgccactgcc cgggtgggta
 661 caccggacc tctgcgacg tggacaccaa ggcaagctgc tatgatggcc gcgggctcag
 721 ctaccgcggc ctggccagga ccacgctctc gggtgcgccc tgtcagccgt gggcctcgga
 781 ggccacctac cggaacgtga ctgccagca agcgcggaac tggggactgg gcggccacgc
 841 cttctgccg aaccgggaca acgacatccg cccgtggtgc ttcgtgctga accgcgaccg
 901 gctgagctgg gagtactgcg acctggcaca gtgccagacc ccaaccagc cggcgcctcc
 961 gaccccggtg tcccctaggc ttcatgtccc actcatgccc gcgcagccgg caccgccgaa

g

a

1021 gcctcagccc acgacccgga cccgcctca gtcccagacc ccgggagcct tgccggcgaa
 Thr
 Lys
 Arg

1081 gcgggagcag ccgccttccc tgaccaggaa cggcccactg agctgcgggc agcggctccg
 1141 caagagtctg tcttcgatga cccgcgtcgt tggcgggctg gtggcgctac gcggggcgca
 1201 cccctacatc gccgcgctgt actggggcca cagtttctgc gccggcagcc tcatcgcccc
 1261 ctgctgggtg ctgacggccg ctcactgcct gcaggaccgg cccgcaccg aggatctgac
 1321 ggtggtgctc ggccaggaac gccgtaacca cagctgtgag ccgtgccaga cgttgccgt
 1381 gcgctcctac cgcttgacg aggccttctc gcccgtcagc taccagcacg acctggctct
 1441 gttgcgcctt caggaggatg cggacggcag ctgcgcgctc ctgtcgcctt acgttcagcc
 1501 ggtgtgcctg ccaagcggcg ccgcgcgacc ctccgagacc acgctctgcc aggtggccgg
 1561 ctggggccac cagttcgagg gggcggagga atatgccagc ttcctgcagg aggcgcaggt
 1621 accgttcctc tccctggagc gctgctcagc cccggacgtg cacggatcct ccatcctccc
 1681 cggcatgctc tgcgcagggt tcttcgaggg cggcaccgat gcgtgccagg gtgattccgg
 1741 aggcccgctg gtgtgtgagg accaagctgc agagcgcgg ctcacctgc aaggcatcat
 1801 cagctgggga tcgggctgtg gtgaccgcaa caagccagc gtctacaccg atgtggccta
 1861 ctacctggcc tggatccggg agcacaccgt ttcttgattg ctcagggaact catcttccc
 1921 tccttggtga ttccgcagtg agagagtggc tggggcatgg aaggcaagat tgtgtcccat
 1981 tccccagtg cgccagctc cgcgccagga tggcgaggaa ctcaataaag tgctttgaaa
 2041 atgctgag

FIG. 1

```

1  acatgctctg tgcttagtaa ccccagtgca acttttttgc ttccccaaaa gttctggcaa
61  aagtcccaag cttagcacttt aattggccta aattgtgtat atgcttatct ctgaaccaat
121 cactgtggat tagagatgtc atgctctgat tgaccagacc taggccacat ctctagccct
181 agctctgagg gtagagttgg cagcactaga gcccatggaa gaagtaagag aggagtcggt
241 gctaaaggaa aaatcaaagt gtcattaccg aaccaggaca gatgctgggc agcacatgtg
301 caccctgtct tcttctcatg ttccagctgc acatcttagt gcccttgggt ttagcacttt
361 tctcattaaa tcatttgctt tcttgctca cttcctgtgg ttggtagaat gctaagatgg
421 cccaagatc tctaccctg gtgttgcac acctcccagt tattctgtca aacatgaatg
481 tagatgcttc tgtgaaagaa ttttgacat gtaatttaag tcccaaattg tttgacctta
541 aaataaggag aatggcaggg ccaggcatgg tggctcatac ctgtaatccc agcactttgg

601 gaggccaagg cgggcagatc acgaggtcag gagatcgaga ccatcctggc taacacagtg
661 aaaccccatc tctactaaaa atacaaaaaa ttagctgggc gtggtggcgg gtgctgtat

721 tcccagctac ccaggaggct gaggcaggag aatggcgtga acccgggagg cgtagcttgc
781 agtgagccaa gatcgtgcca ctgcactcca gcctgggtga cagagccaga ctctgtctca
841 aaaaaaaaaa aaaaaggag aatggctttg gtgggcctga cctagtcagg ttagttctta
901 aaaggcgaca catggcccgg tgcagtggct caggcctgta atcccagcac tttgggaggc
961 cgaggcgggt ggalcacgag gtcaggagat cgagaccatc ctggctaaca tggtgaaacc
1021 ccgtctctac taaaagaca aaaaattagc tgggcgtgggt ggtgggctcc tgtagtccca
1081 gctactcggg aggtgaggc aggagaatgg cgtgaaccgg ggaggcggag cttgcagtga
1141 gcggagatg cgccactgca ctccagcctg ggcgacagag cgagactccg tctcaaaaaa
1201 aaaaaaaaaa aaaaagaaaa ttaaagtgg gtattgtgtg aagatgctga gtttatggta
1261 gtttgttaca tgacaataga aaatgaacac acttcacagt ggactccaag atccccatga
1321 tctttgatct ccttaacctc ctgatctcca caggaccag agcataagaa tgtcccttct
1381 tctgtctcca gtcccactat ctagaaaaga gaggaggagc ccagctcttc atttaccctc
1441 caccacaaa ctccaactt tccggccctc aaggggtgac caaggaagt gctccacttg
1501 gctttccaca aacagcctgt gccccaccag gctcaggagg gcagcttgac caatctctat
1561 ttccaagacc tttggccagt cCTATTGATC TGGACTCCTG GATAGGCAGC TGGACCAACG

1621 GACGGACGCC ATGAGGGCTC TGCTGCTCCT GGGGTTCCCTG CTGGTGAGCT TGGAGTCAAC

1681 ACTTTCGgtg agtgcgtggt gaaccaggat tgtcccagga ttgttctggg gggctcgtat
1741 cacagccatg agccatggcc tctgctcatg acctgtgggt ccagggtgact aggaggccta
1801 tgtggaagg tgaggccagc ccggaaggcc caggcagagg agacagacaa ccagactggg
1861 tggatacaag ggcacagcct gcatttctgg gggagatggg ccttaagaag acaacggggg
1921 gaggtagaaa gggtttgggt cttgggaaga aatctctgca tttctgggct gtgagaggaa
1981 gctgcagact agcaacagat cgggtggcagg ctatgactta tagtcagttc cctgccttct
2041 tctctccctt gtagattcca ccttgggaag ccccaagga gcataagtac aaagctgaag
2101 agcacacagt cggtaatggt cctggctcct cctcccggga acccttgggt ggggatgtgt
2161 atgggtgcat gtgtgcagc tcagggcagt ctagtctagt gcctacctgg tcttaggtct
2221 tatgcccata ggactagag tgatcgtgag ctgtgtgata cttgagggca gggatggggc
2281 tgtgtctaag tgcccacgag cctggctcgg agcagggtgct tgagatatgt gctgctggcg
2341 ccatacacacc tgggctcctg ccagccttcc tcagtttccc cagcttctcc ccttcttttc
2401 ctttccccag tacgtctcat gggcatcatt catgccacac agaggccagg gccttcaatg
2461 ggcaagggaag gatcaagagc ttgtctctgg catctgaatg cctctgaagc ccagctttat
2521 cacttatgag ctgggtgact ctgggcgagg gatttgagtt ctccaagctt caatttcccc
2581 ttctgtgaaa ccaggttgat aacagtaaac ctcttagggt tgttgagaag ggaacccat
2641 gtgaggatatt cagcccatca cctggtgcat ggaatgctt tacaaatatt agcttttatt
2701 atgaaactac cttttagatg aagggtacct gccatttccc ccttctcaa gctctgccat
2761 agctccccat tgctttcatt cttccagaca ctaaattacc tacatgccag gcatgggtggc
2821 tcatgcctgt aatcccagca ctttgggagg ccaaggctcg tggatcatga ggtcaggagt
2881 tcgagaccag cctggccaac atggtgaaat gctgtctcta ctaaagtac aaaaattagc
2941 caggcatggt ggcatgcgcc agtagtccca gctactcggg aggctgaggc agaagaattg
3001 ctgaaactg ggaggtgaag gttgcagtga acgaagatca caccattgca ctccagcttg
3061 ggcaacacag caagactccg tctcaaaaaa aaaaaaaaaa ttacctaga gtgtggcaca
3121 tagcagggcc tgtgaaccag atggacctta ccctggtggg cctgacttgg tggggttag
3181 tctctaagca tggcgttag gccagcaca ttccaacctt ggactccctc agcctcctct
3241 cttcacccca caccaaaag tttctcctct ctcttgctt acccaactt ggtgcctat
3301 ccttgccctaa tcccctgect aaggtcccc tctctctgt ccgtccatcc catctgcatc

```

FIG.2A

```

3361 tttttttttt tttgagatgg agtctcgctc tgtcccctag gctagagtgc aatggcgcga
3421 tcttggtcca ttgcaacctc cgctcctggt gtcaagcga ttctctgcct cagcctcccg
3481 agttgctggg attacaggca cacaacttca tgctcagcta atttttgtat ttttagtag
3541 agacagggtt tcaccatggt ggcaggctg gtctcgaact cctgccctca ggtggtccgc
3601 ccaccttagc ctcccaaagt gctgggatta caggcgtgag ccaccgccc tggcccccac
3661 ttgcatctta aaggtccatc tcagatccat ttccatttac tgccttagtt ctggtttggt
3721 ccttggaag tgcactttgc cttgaacaaa atagtggcaa aagcttatg agcaggtact
3781 ttgtgccaga cactgctcag cattcatggt cattaatctca tgaagcccca cgacaattcc
3841 tctgaagaag acacaggcaa ttctcattat tcgctgaggt tatgttctat aaaaacacag
3901 tgaacattga actagcaaac agtattaggt tcctgtgagc cctcggtcac aacattttca
3961 tcaaccaaca gcatataatc tggttttatg tatgattctg tttaaagaca ttttatttag
4021 tatatgtgtt gctgattcat caatgctaag ctgatggcac tatagcacac acctgaatca
4081 agtgtctaac acacgctttc tccctaaggt agccttcttg tgcttaggaa ctacacagct
4141 cttcagcagg aggtcagag gccatttcca aaagccaaat cccagcaaa agcacaagt
4201 gtgaaaaacg ttgcactaag tagactgaga aggacactca tcaatagga gagctgaaac
4261 aagcagcagc agcgtgacgc ctgtttgaaac cttaactggg aatgtgcaa ttttctactg
4321 ctctgtgcat gccacaaaat ggccatgaaa acatttcaag tatgacttg ggagttacaa
4381 ataaaattca gcaagtaggc acattctcaa tgtagaacca gagaagaatg aggatcaact
4441 gtactattat tactgccgtt ttacagataa ggaaccaag gctcagatca gagtggtaa
4501 cagtgacttc aacattcaac aagtattatt aagtgcctac tttgtggcaa gtgctcttcc
4561 tggccttggg actgaagact tacccaaggt cacacagcta gcaggtttg gagtcaggag
4621 tctactccag ctatctgact cctgaacca agttttttt ttttctita agatggagtc
4681 tcactctgtc acccaggctg gagtgcagtg gcgcgatctc ggctcactgc aagctccgcc
4741 tcccgggttc acaccattct cctgcctcgg cctcccagat agctgggact acaggcacct
4801 gccaccacc ccagctaatt ttttgtatf ttagtagag agggggttc actgtattag
4861 ccaggatggt ctgatctcc tgacctcgtg atctgcccgc cttggcctcc caaagtgtg
4921 ggattacagg cttagccac cgcgccggc cctgaacca acttttagag cagaagtgtg
4981 tttcaatgca cagcgacctt tttgagggc tgtccttttc ctgaccagac cctgagggac
5041 agtgcctgag cagttagta caggggaagt cctcagagag tgtgtgtcc ctgcagttct
5101 cactgtcacc ggggagcct gccacttccc cttccagtac caccggcagc tgtaccacaa
5161 atgtaccac aagggccggc caggccctca gccctggtaa gactacgcag aggagttgga
5221 gcaggggcct gggagacatg tacctgcct gtcttctgt ccaaggaact ctgcttgag
5281 agaggggact gtgataggc agggggggc agggccctgg gttaggcagg gaagccttgt
5341 ctctttctac aggtgtgcta ccaccccaa cttgatcag gaccagcgt gaccagactg
5401 tttggagccc aagaaagtga aaggtgctac acacagcctc tgggtggcc tgggctctc
5461 tctcccgcc tcattactct cctggtatca ccagaccca cacacctggg attctggacc
5521 cagcccctc tctcctcca caataccct tggagtcca gaggagagt tctgggaagg
5581 agtgggtcca tttgacaggt ggtaaacca agcttggaaa ctggagtag caaggtcaca
5641 aggcaagtag gttcaagaag gccttggcc ccagctgtg tgaactcagc cctgtctct
5701 cctccacca tgtccatctc tcagaccact gcagcaaaaca cagcccctgc cagaaggag
5761 ggacctgtgt gaacatgcca agcggccccc actgtctctg tccacaacac ctactggaa
5821 accactgcca gaaaggtgag gagatgtgga ggacctggg ggggtgctgg gggacagggg
5881 caaccctgg cctacagaat aggttgctgg atactcggag acttggcatg gtcttagact
5941 ctctgagac cactatcctt cttgtcccc agagaagtgc tttgagcctc agcttctccg
6001 gtttttccac aagaatgaga tatggtatag aactgagcaa gcagctgtgg ccagatgcca
6061 gtgcaagggt cctgatgccc actcccagc gctggcccagc cagggtgagc agatggttg
6121 gaacgggcca gggaggagc tcaggaagac aggtggcag gaggccgggt ggtgtgccgg
6181 gaaggagagc tctctggggg ggtctttagg cccaggggtg gctcactgcg ttccctccc
6241 aagcctgccc caccaaccgc tgcctccatg ggggtcgtg cctagaggtg gaggccacc
6301 gctgtgcca ctgcccgtg ggtacaccg gacccttctg cgactgggt gagtgaggt
6361 ctggggcaag cagaaggcca gcccccaggt gggacgggct tgccaggaag gaggagggag
6421 agtgcggaaa gcagatgaga gggaggcagg agagcccagc cttggctgcc caggagccc
6481 ctttctctct cagacaccaa ggcaagctgc tatgatggc gcgggctcag ctaccgcggc

6541 ctggccagga ccacgtctc ggtgcgccc tgtcagccgt gggcctcgga gggcacctac

6601 cggaacgtga ctgccgagca agcgcggaac tggggactgg gggccacgc cttctgccgg
6661 tgcgcgcgct ggggtgggt gacccctccg ccccagggt cgggctccc ggcctctaa
6721 cggcgcctccg tctgtggct acaggaacc ggacaacgc atccgctctg tctgtctctg
6781 gctgaaccgc gaccggctga gctgggagta ctgcgacctg gcacagtgc agaccccaac
6841 ccaggcggcg cctccgacc cggtgtccc taggcttcat gtcccactca tgcccgcga
6901 gccggcaccg ccgaagcctc agcccagcag ccggacccc cctcagctcc agaccccg

6961 aggttaggaa gtggggggg gaaggaggag ccgagaggg gccggcgag ctagattccc

```

FIG.2B

```

7021 gccagccggc cgcgggctcc ccgctctcag ccctgtctcc tccacagcct tgccggcgaa
7081 gcgggagcag ccgccttccc tgaccaggaa cggcccactg agctgcgggc agcggctccg
7141 caagagtctg tcttcgatga cccgcgtcgt tggcgggtg gtggcgctac gcgggcgca
7201 cccctacatc gccgcgctgt actggggcca cagtttctgc gccggcagcc tcatcgcgcc
7261 ctgctgggtg ctgacggccg ctactgcct gcaggaccgg cgagtaccgg cccgcccaga
7321 gccgccccag gggccgcggc tcctcgtct cccagcgcag ctccacgct gcaccggaac
7381 ccgtgcccta ctttctccc ccccaccctt cttccacgc ccctccggag ctcccgggga
7441 ggaagctgga acacgggatt ggggttcggg agcagggggc ttcccagaa cgcttgggc
7501 caggctctgag agcgtgcct ctcccctacc ctcccgcag gcccgaccc gaggatctga
7561 cgttggtgct cggccaggaa cgccgtaacc aCagctgtga gccgtgccag acgttggccg
7621 tgcgtccta ccgctgcac gaggccttct cgcccgctag ctaccagcac gacctgggtg
7681 cgtggggggc ccccggggg acgggaagag agcttggggc cccggcgctc ccgcctcacg
7741 ctctctccc cccgggttag ctctgttgcg ccttcaggag gatgcggacg gcagctgcgc
7801 gctcctgtcg ccttacgttc agccggtgtg cctgccaagc ggcgcgcgc gacctccga
7861 gaccacgctc tgccaggtgg ccggctgggg ccaccagttc gagggtaggc acaactgcta
7921 ggggcagggg taggggagga gacctttgat cactgggtta ggcggaagaa gcccgcgact
7981 ttggtatcgt tccgggtgcc tacagaatgg gtggcgctga cctgatgggt tgtgagaatg
8041 tgtaggtgaa tcccaggtag aatcccaggg cctgggattc actgctggga tcccctaatc
8101 tcctggggat acagggagaa tcgaacttc tcttggttcc ctctggggcg cgggtgcaa
8161 agccaacta ggacgtggc cccgcgtccc gggctagtgt gggagccagg ttctgcgact
8221 ctggatgggt ggtgggggag ggtttctgt ttccgctccg cccattcaaa tcttgctttt
8281 tcctgggacc tcagcctcct tgcctatgaa attgaattaa tggcacctcc tcccctcgg
8341 gcttgctgag agagaggaag ggcattgagtg ggtttacaag cgctggagc agctttgtcc
8401 atcgtccggg cggcaagcgt tgtcagatgg ggtgtgaaga aggcgctctg tgttcgagg
8461 ggcggaggaa tatgccagct tcctgcagga ggcgcaggtta ccgttctct ccctggagcg
8521 ctgctcagcc ccggacgtgc acggatcctc catcctcccc ggcattgctc ggcaggggtt
8581 cctcgagggc ggcaccgatg cgtgccaggt gactcttag cccggttggc gccctcccc
8641 gagcccgta ggcacaaatc tcaggctcac agcgtgagc tgcgtgtttc cgaccagggg
8701 tgattccgga ggcccgtgg tgtgtgagga ccaagctgca gagcgcggc tcaccctgca
8761 aggcattcag agctgggat cgggctgtgg tgaccgcaac aagccaggcg tctacaccga
8821 tgtggcctac tacctggcct ggatccggga gcacaccgt tcctgattgc tcagggactc
8881 atctttccct ccttgggtgat tccgcagtg gagagtggct ggggcatgga aggcaagatt
8941 gtgtcccatt ccccagtgcc ggccagctcc gcgccaggat ggcgcaggaa ctcaataaag
9001 tgctttgaaa atgctgagaa ggaagctct ttcttcctg ggtccgccc ggaatagcca
9061 agacagaaaa gcgattcaca gcttctccac agctctcaga gaacaaggtc tatgagatct
9121 taacgtgcaa aatctagatg ccagcccagc taatgtttac tgagcctagg atactgtata
9181 ccaagccctg tgcaaggaga agctgcatgt tattccttat gagaactaa cttttgttt
9241 acagagcagt agttctcaga ccatacatta agatcacttg gggagcgttt tgagccaatc
9301 tatgcccaag ttccacctca gaccaattaa atcagtatgt ctagggatgg ggcattgggtg
9361 gtggtatatt tgtaaaactc ccagataat tccatgtaca gccaaagtg agaactcgtg
9421 ttagaataat ttagcattgg cccggcgcgg tggctcagc ctgtaatcct agcactttaa
9481 gaggccaagg caggtggatt gctcaggagt tcgaaaccag cctgggcaac acgatgaaac
9541 cccgtctcta ctaaaataca agaaattagc cgggcacggc ggcgtgcgcc tgtagtcca
9601 gctactcagg aggtgagggc aggagaatca ctgaaccgg caggaaggaa ggaaggagg
9661 aacagaggga gggaaagaga gagacagaaa gaaaagaaa aagaaaatag aaaaaagag
9721 cattgactgt ggcgtggacc ctaagggtc ggtgacatat cgttgtcccc accccaacac
9781 gcactagtgt agtgggtctg agagtccctt ggctagcagt accatcacca ggaacttgt
9841 tacacataac aaattctcgg gctacacttt atactgctga acagaaagtc tggggtgggg
9901 cccagcaatc tgtttaacag ccttgcgggg gattctgatg ttctctcatg cttagaacc
9961 acaatctggg ggttgaatgg ttggttccct tacaagtga ggtctggctg tccagacaca
10021 acatcctttt ttcaaaaac cagcttttta aaattaaata tagattggcc agatgcggtg
10081 gttcacgcct gtaatctcgt cactttgaga gctgaggcg ggaggattgt ttgagctcaa
10141 gacttcttga cccgcctggg caacatagtg agacctcctc tcaaaaaaat ttttttaat
10201 taaaatttgt ttttgccttt tttagacggg ggcctcgctc tgtggctcag ctggcgctgc
10261 agcgatacga tcctataata gtttactata atctcgctac tgagttcaag gcacccgcc
10321 gctcggcct cccaaagcgc tgggattaca ggagtgagcc gctgcgctct gccaaacca
10381 tcctacagga taaccttaga actgcgacag cactaaacgc ccacgcccc cgtgccccag
10441 cctgggtggt cgctccggga cggcgcttg tgtgacgta cagccccgc cagctgcct
10501 cacagcggc caggccttcc ccgctggcg cctctatatt tcccggagag gtgcgaggcg
10561 gctgggcgca ctcgagcgc gatgggcgac tggaggctc acatcagtc agtgc

```

FIG.2C

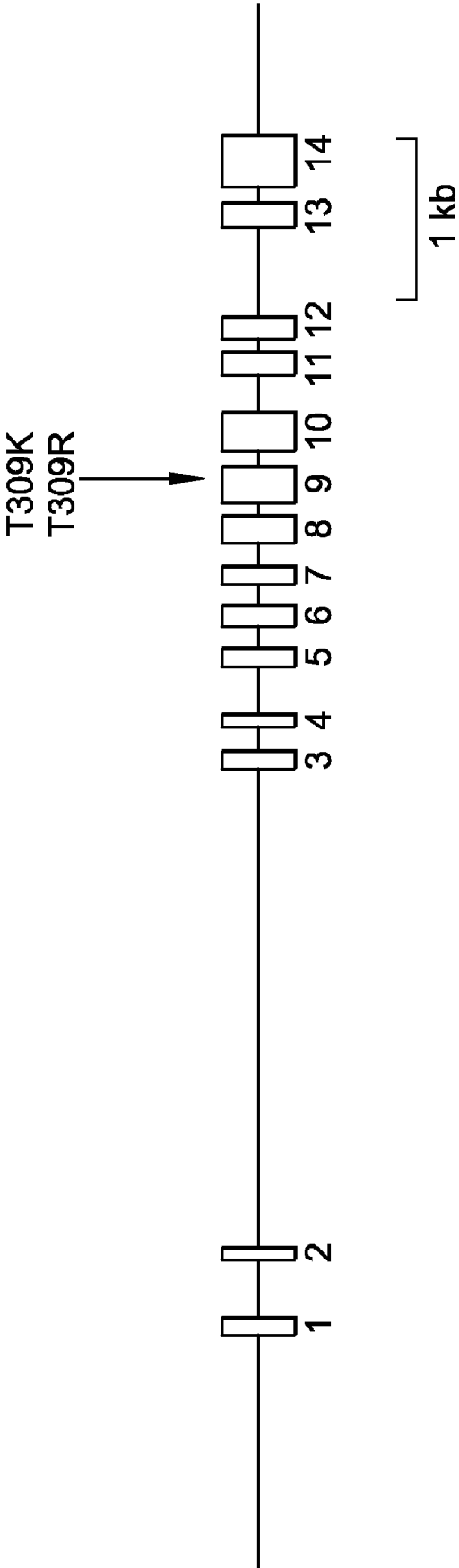


FIG.3

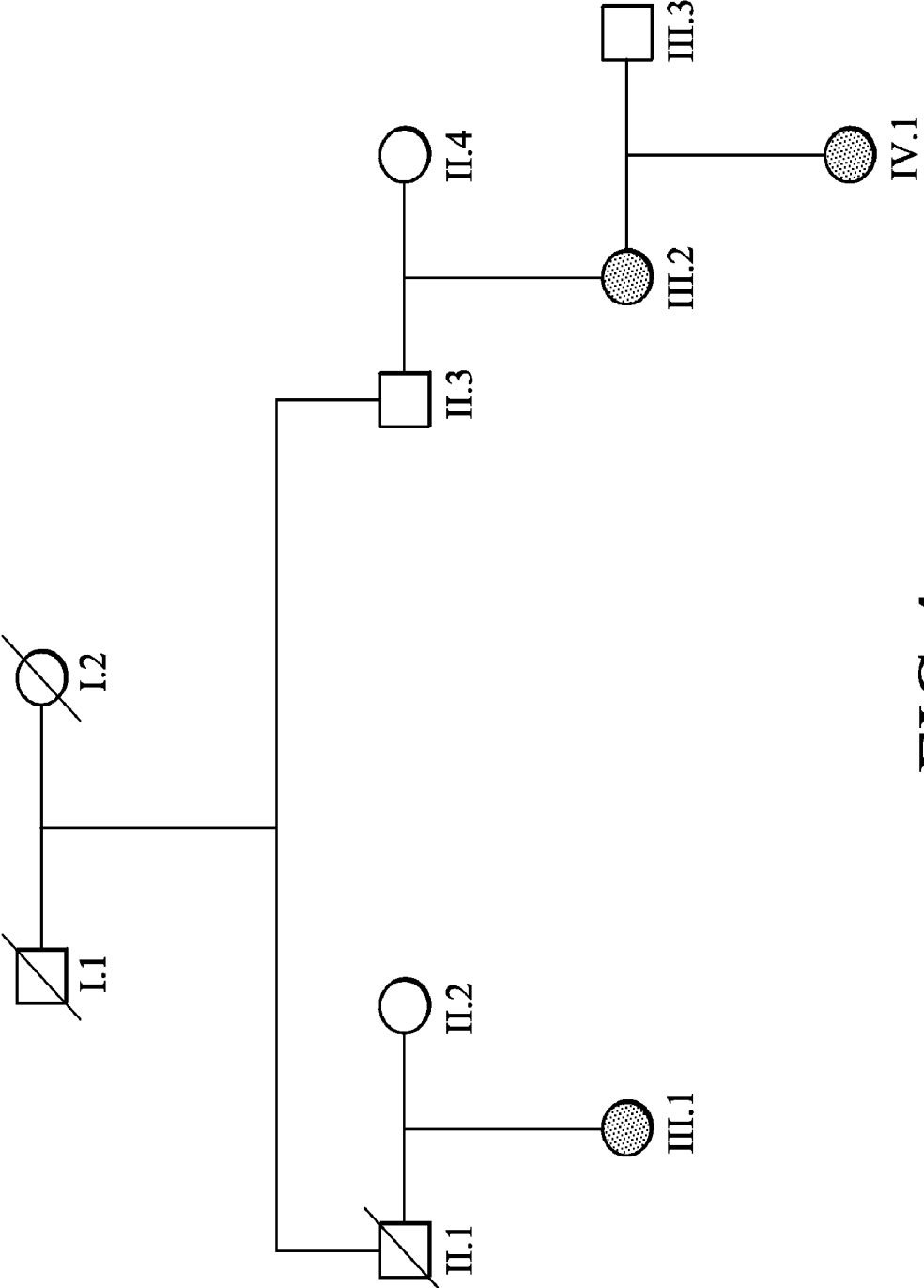


FIG.4

METHODS AND KITS TO DETECT HEREDITARY ANGIOEDEMA TYPE III

[0001] This application is a Continuation of co-pending application Ser. No. 11/600,739 filed on Nov. 17, 2006, which is a Continuation-In-Part of International Application No. PCT/EP2005/005404 filed on May 18, 2005, which designated the United States and on which priority is claimed under 35 U.S.C. §120, and under 35 U.S.C. 119(a) on Patent Application No. EP 04011790.5 filed in the European Patent Office on May 18, 2004; the entire contents of all are hereby incorporated by reference.

[0002] The present invention relates to a method of diagnosing hereditary angioedema type III (HAE III) or a predisposition thereto in a subject being suspected of having developed or of having a predisposition to develop a hereditary angioedema type III or in a subject being suspected of being a carrier for hereditary angioedema type III, the method comprising determining in vitro from a biological sample of said subject the presence or absence of a disease-associated mutation in a nucleic acid molecule regulating the expression of or encoding coagulation factor XII; wherein the presence of such a mutation is indicative of a hereditary angioedema type III or a predisposition thereto. The present invention also relates to a method of diagnosing hereditary angioedema type III (HAE III) or a predisposition thereto in a subject being suspected of having developed or of having a predisposition to develop a hereditary angioedema type III or in a subject being suspected of being a carrier for hereditary angioedema type III, the method comprising assessing the presence, amount and/or activity of coagulation factor XII in said subject and including the steps of: (a) determining from a biological sample of said subject in vitro, the presence, amount and/or activity of: (i) a (poly)peptide encoded by the coagulation factor XII gene; (ii) a substrate of the (poly)peptide of (i); or (iii) a (poly)peptide processed by the substrate mentioned in (ii); (b) comparing said presence, amount and/or activity with that determined from a reference sample; and (c) diagnosing, based on the difference between the samples compared in step (b), the pathological condition of a hereditary angioedema type III or a predisposition thereto. The present invention also relates to a method of identifying a compound modulating coagulation factor XII activity which is suitable as a medicament or a lead compound for a medicament for the treatment and/or prevention of hereditary angioedema type III, the method comprising the steps of: (a) in vitro contacting a coagulation factor XII (poly)peptide or a functionally related (poly)peptide with the potential modulator; and (b) testing for modulation of coagulation factor XII activity, wherein modulation of coagulation factor XII activity is indicative of a compound's suitability as a medicament or a lead compound for a medicament for the treatment and/or prevention of hereditary angioedema type III. Furthermore, the present invention relates to gene therapy methods and to a kit for diagnosing hereditary angioedema type III.

[0003] Several documents are cited throughout the text of this specification. The disclosure content of the documents cited herein (including any manufacturer's specifications, instructions, etc.) is herewith incorporated by reference.

[0004] All or any combination of steps (including single steps only) carried out in the method of the present invention and cited throughout this specification can be carried out in any combination of in vivo, ex vivo or in vitro.

[0005] The conventional or classic forms of hereditary angioedema (HAE) are known to be autosomal dominant disorders. Two types are recognized (Rosen et al. 1965, *Science* 148: 957-958), both related to a C1 inhibitor deficiency caused by mutations in the C1 inhibitor gene (Bissler et al. 1997, *Proc. Assoc. Am. Physicians* 109: 164-173; Zuraw & Herschbach 2000, *J. Allergy Clin. Immunol.* 105: 541-546; Bowen et al. 2001, *Clin. Immunol.* 98: 157-163). The defective gene produces either no C1 inhibitor (HAE type I) or a dysfunctional C1 inhibitor (HAE type II). Recently, a further type of hereditary angioedema has been described (Bork et al. 2000, *Lancet* 356: 213-217; Binkley & Davis 2000, *J. Allergy Clin. Immunol.* 106: 546-550; Martin et al. 2001, *J. Allergy Clin. Immunol.* 107: 747), and it appears that this new type is closely related, possibly identical, to a disease entity described already in 1986 by Warin et al. (*Br. J. Dermatol.* 115:731-734) in two sisters. In patients with this new type of inherited/familial angioedema, C1 inhibitor protein levels and C1 inhibitor function (as determined by antigenic and functional assays) are normal. This disease has been termed HAE type III by Bork et al. 2000 (*Lancet* 356: 213-217). The genetic defect underlying hereditary angioedema type III is still unknown. Until now, this disease has been reported exclusively in women, but from pedigree analysis one must postulate the existence of male carriers (Binkley & Davis 2000; Martin et al. 2001). Inheritance is assumed to be autosomally dominant (Binkley and Davis, 2000; Martin et al., 2001; Binkley and Davis 2001, *J. Allergy Clin. Immunol.* 107: 747-748), male-to-male transmission has been observed in one family (Martin et al., 2001). Nevertheless, the possibility of genetic heterogeneity, including the possibility of X-chromosomal inheritance eventually in some families, has been discussed (Bork et al. 2000; Martin et al. 2001; Binkley & Davis 2001). The clinical manifestation of HAE type III, in particular regarding frequency and intensity of symptoms, appears to be quite variable, and penetrance of the disease can be reduced (Bork et al. 2000, *Lancet* 356: 213-217; Bork et al. 2003, *Am. J. Med.* 114: 294-298). One therefore might speculate that some patients diagnosed as 'idiopathic angioedema' eventually are affected by hereditary angioedema type III, and that the disease has not (yet) manifested in any of their relatives (Bork et al. 2003, *Am. J. Med.* 114: 294-298). In about two thirds of women affected with HAE type III angioedema symptoms are precipitated or exacerbated by oral contraceptives or hormone replacement therapy (Bork et al. 2003), pregnancy might also be an important precipitating factor, at least in some families (Binkley & Davis 2000). It is assumed that exogenous, respectively endogenous estrogens are responsible for these precipitating or exacerbating effects (Binkley & Davis 2000, 2001; Bork et al. 2000, 2003). Based on presently available information, the clinical presentation of HAE type III appears to be highly similar to HAE type I or II, except for the unique occurrence in women.

[0006] The deficiency of functional C1 esterase inhibitor provides a useful means of detecting hereditary angioedema types I and II, as the substitution with a pharmaceutical preparation of human C1 inhibitor does provide a useful means of treating as well as of preventing these types of hereditary angioedema. In contrast, an effective method for the detection, treatment, and prevention of hereditary angioedema type III still remains to be identified.

[0007] Thus, the technical problem underlying the present invention was to provide means and methods for diagnosis of hereditary angioedema type III or a predisposition thereto, as

well as for prevention and treatment of hereditary angioedema type III. The solution to this technical problem is achieved by providing the embodiments characterized in the claims.

[0008] Accordingly, the present invention relates to a method of diagnosing hereditary angioedema type III (HAE III) or a predisposition thereto in a subject being suspected of having developed or of having a predisposition to develop a hereditary angioedema type III or in a subject being suspected of being a carrier for hereditary angioedema type III, the method comprising determining in vitro from a biological sample of said subject the presence or absence of a disease-associated mutation in a nucleic acid molecule regulating the expression of or encoding coagulation factor XII; wherein the presence of such a mutation is indicative of a hereditary angioedema type III or a predisposition thereto.

[0009] The term “nucleic acid” or “nucleic acid molecule” refers to DNA or RNA, including genomic DNA, cDNA, mRNA, hnRNA etc as well as chimeras thereof. Included are artificially modified nucleic acid molecules carrying chemically modified bases. All nucleic acid molecules may be either single or double stranded.

[0010] In principle, the detection of at least one disease-associated mutation such as 2, 3, 4, 5, 6, 7, 8, 9, 10 or more mutations or combinations of various different mutations in at least one allele is an indication that the subject to be diagnosed either with respect to a potentially existing disease predisposition or susceptibility or because of being affected by the disease is a carrier. In general, if a disease-associated mutation is dominant, it may be causative for the onset or progress of the disease and a diagnosis of heterozygosity as only of its presence in the genome at all, will be indicative of the subject being prone to developing the disease if it does not already suffer from it. A recessive character of a mutation will more likely indicate that only its homozygous occurrence will have a direct impact on the onset or progress of the disease, whereas its occurrence in heterozygous form will rather qualify the subject as a carrier only, unless other concomitantly occurring mutations contribute to the onset or progress of the disease.

[0011] The term “diagnosing” means assessing whether or not an individual or a subject has a specific mutation linked with hereditary angioedema type III and concluding from the presence of said mutation that the individual or subject has a hereditary angioedema type III (HAE III), or a predisposition thereto, a predisposition to develop a hereditary angioedema type III or is a carrier for hereditary angioedema type III.

[0012] The term “hereditary angioedema” refers to an inherited abnormality to cause skin swellings, gastrointestinal symptoms (abdominal pain attacks) due to edema of the intestinal wall, or edema of the tongue, or laryngeal edema, which may ultimately result in death by asphyxiation. Until recently two forms of hereditary angioedema (HAE), type I and type II, were recognized (Rosen et al. 1965, *Science* 148: 957-958); both are inherited in an autosomal dominant fashion and are related to a C1 inhibitor deficiency caused by mutations in the C1 inhibitor gene (Bissler et al. 1997, *Proc. Assoc. Am. Physicians* 109: 164-173; Zuraw & Herschbach 2000, *J. Allergy Clin. Immunol.* 105: 541-546; Bowen et al. 2001, *Clin. Immunol.* 98: 157-163). The defective gene produces either no C1 inhibitor (HAE type I) or a dysfunctional C1 inhibitor (HAE type II). With respect to hereditary angioedema due to C1 inhibitor deficiency, it is generally assumed that bradykinin (and eventually related kinins) is an

important mediator of angioedema development (Nussberger et al. 1998, *Lancet* 351: 1693-1697; Kaplan et al. 2002, *J. Allergy Clin. Immunol.* 109: 195-209; Han et al. 2002, *J. Clin. Invest.* 109: 1057-1063; Cugno et al. 2003, *Int. Immunopharmacol.* 3: 311-317). However, also a kinin derived from complement component C2—following an eventually increased or uncontrolled activation of the classical complement pathway—has been considered to be of pathophysiological significance (Donaldson et al. 1977, *Trans. Assoc. Am. Physicians* 90: 174-183; Strang et al. 1988, *J. Exp. Med.* 168: 1685-1698).

[0013] The term “hereditary angioedema type III”, as used throughout the description of the present invention, relates to the disease described by Bork et al. 2000 (*Lancet* 356: 213-217) and Binkley and Davis 2000 (*J. Allergy Clin. Immunol.* 106: 546-550), the disease described by Warin et al. 1986 (*Br. J. Dermatol.* 115: 731-734) probably being closely related or even identical. Type III of hereditary angioedema is characterized by similar symptoms as observed in HAE I and II, however, the blood plasma level and activity of C1 esterase inhibitor are normal and until now the disease has exclusively been reported in women. According to the present invention, hereditary angioedema type III may include several potential subtypes: Binkley & Davis 2001 (*J. Allergy Clin. Immunol.* 107: 747-748) suggested to differentiate between (1) an estrogen-sensitive type (in which symptoms worsen but are not strictly dependent on high estrogen levels) and (2) an estrogen-dependent type (in which there is an absolute dependence of symptoms). In addition, according to data presented by Bork et al. (2003, 2000), it appears that in almost one third of patients symptoms are not influenced by estrogen exposure; these patients may represent a third subtype (3) of HAE type III regarding estrogen-sensitivity. A fourth subtype (4) of HAE type III appears to be the disease described by Warin et al. 1986: a familial type of recurrent angioedema with normal C1 inhibitor levels, oestrogen-induced, but with occasional symptoms of urticaria. A fifth subtype (5) may be ‘HAE type III with negative family history’, a term that is understood here as being equivalent to ‘idiopathic angioedema’, i.e. a recurrent angioedema that cannot be attributed to any of the C1 inhibitor-related forms of hereditary or acquired angioedema or to any of the known drug-induced and physical causes: certain patients classified as cases of “idiopathic angioedema” may, in fact, represent patients with HAE type III, namely HAE type III patients where the disease has not yet manifested in any relative (e.g. because of reduced penetrance), or HAE type III patients with a negative family history because of a de novo mutation. Finally, a sixth subtype (6) of HAE type III may be manifested in men, eventually only in the presence of certain (genetic or environmental) precipitating factors.

[0014] The term “predisposition”, in accordance with the present invention, refers to a genetic condition that (a) increases the risk for the development of a disease or promotes or facilitates the development of a disease and/or that (b) facilitates to pass on to the offspring specific alleles of a gene increasing the risk for or promoting the development of such condition or disease.

[0015] The term “biological sample”, in accordance with the present invention, relates to the specimen taken from a mammal. Preferably, said specimen is taken from hair, skin, mucosal surfaces, body fluids, including blood, plasma, serum, urine, saliva, sputum, tears, liquor cerebrospinalis,

semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum, bronchial secretion, or stool.

[0016] The term “mutation” comprises, *inter alia*, substitutions, additions, insertions, inversions, duplications or deletions within nucleic acid molecules, wherein one or more nucleotide positions can be affected by a mutation. These mutations occur with respect to the wild-type nucleic acid sequence. As the “wild-type” or “normal” nucleic acid sequence of the coagulation factor XII gene is considered herein the sequence (bases 1 to 10616) given under GenBank acc. no. AF 538691 and, with respect to extended flanking sequences, the sequence given in the July 2003 human reference sequence of the UCSC Genome Browser, v.53 (*vide infra*). A mutation may affect preferably up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or even of up to 20, 30, 40, 50, or up to 1000 nucleotides. However, it is also conceivable that even larger sequences are affected. Therefore, the term “mutation” also relates to, e.g., a nucleotide deletion, substitution or insertion of up to 10000 or up to 20000 nucleotides, also comprising the situation when the entire coding, non-coding and/or regulating sequence of a gene is affected. Mutations can involve coding or non-coding gene regions. The term “non-coding” preferably relates to introns, to the non-coding parts of exons, to 5'- and 3'-flanking regulatory sequences, thus also to expression control sequences including control elements such as promoter, enhancer, silencer, transcription terminator, polyadenylation site. It is well known to the person skilled in the art that mutations in these regions of a gene can have a substantial impact on gene expression, eventually also with respect to specific tissues. For example, mutations in these sites can result in a nearly complete shut-down of gene expression or in a drastic overexpression. However, mutations in non-coding regions can also exert important effects by altering the splicing process; such mutations, for example, can affect the intron consensus sequences at the splice and branch sites, sometimes they activate cryptic sites, or create ectopic splice sites.

[0017] On the other hand, a mutation can also reside in the coding region of a gene and severely affect the protein's structural and/or functional characteristics, for example by causing amino acid substitutions. However, even so-called silent or synonymous mutations must not necessarily be silent. For example, mutations within exonic splicing enhancers or silencers may affect mRNA splicing, which may for example alter protein structure or cause phenotypic variability and variable penetrance of mutations elsewhere in the gene (Liu H.-X. et al. 2001, *Nature Genet.* 27: 55-58; Blencowe 2000, *TIBS* 25: 106-110; Verlaan et al. 2002, *Am. J. Hum. Genet.* 70; Pagani et al. 2003, *Hum. Mol. Genet.* 12: 1111-1120).

[0018] However, it is well known in the art that not any deviation from a given reference sequence must necessarily result in a disease condition or a predisposition thereto. For example the gene encoding human coagulation factor XII is known to occur in a number of variations comprising polymorphisms or polymorphic variants such as those deposited in the databank of Seattle (<http://pga.gs.washington.edu>, University of Washington, ‘Seattle SNPs’).

[0019] The term “polymorphism” or “polymorphic variant” means a common variation in the sequence of DNA among individuals (NHGR1 glossary). “Common” means that there are two or more alleles that are each present at a frequency of at least 1% in a population. Usually it is understood, that polymorphisms, or at least the majority of poly-

morphisms, represent variations that are benign, functionally neutral, not having an adverse effect on gene function. However, it is also clear that polymorphic variants exist which can have an impact with respect to the development of a disease. This impact can be not only a disease-predisposing one, but, in certain cases, it can also be a protective effect reducing the risk of disease manifestation.

[0020] Taking into account the existence of polymorphic variants, it is reasonable to consider the existence of numerous alternative wild-type sequences. For various purposes of the present invention, for example for the design of nucleotide probes and primers and also for the design of oligonucleotides to be used therapeutically, it will be important to carefully take into account the existence of such variant sequences.

[0021] Although the term “mutation” basically describes any alteration or change in a gene from its natural state, it is often understood as a disease-causing change, as a change that causes a disorder or the inherited susceptibility to a disorder.

[0022] For the skilled artisan and under certain circumstances, the terms “polymorphic variant” (“polymorphism”) and “mutation” have the same connotation and refer to the same molecular phenomenon, namely alteration in or deviation from a paradigmatic wild-type sequence.

[0023] For the purpose of the present invention, the term “disease-associated mutation” refers to a mutation in a nucleic acid molecule regulating the expression of or encoding coagulation factor XII and which is linked with hereditary angioedema type III or a predisposition thereto. In accordance with the present invention, a “disease-associated mutation” is preferably a rare mutation, preferably with a frequency <1%, and more preferably a mutation with an important disease-causing effect, a dominant mutation. Nevertheless, in accordance with the present invention, it is also envisaged that polymorphic variants exist that can have an influence on disease predisposition and/or the onset or progress of a disease (*vide infra*), and which, thus, also represent a “disease-associated mutation”. It is important to note that an affected individual may carry more than one disease-associated mutation. In order to determine whether or not a mutation is disease-associated, the person skilled in the art may for example compare the frequency of a specific sequence change in patients affected by HAE type III with the frequency of this sequence change in appropriately chosen control individuals, preferably individuals who never showed any angioedema symptoms, and conclude from a statistically significantly deviating frequency in the patient group that said mutation is a disease-associated mutation. The person skilled in the art knows how to design such a comparison of patients and controls. For example, patients and controls should be matched for age and sex. Controls could be individuals assumed to be healthy, like blood donors, but also a population-based control sample appears to be possible, although it is appreciated that among such samples there might be a small percentage of individuals included who have a predisposition for the disease. Thus, preferably, controls should be individuals who never experienced any angioedema symptoms. According to the present invention, the term “statistically significant” describes a mathematical measure of difference between groups. The difference is said to be statistically significant if it is greater than what might be expected to happen by chance alone. Preferably, a P-value < 0.10, more preferred a P-value < 0.05, even more preferred, a P-value < 0.

01, calculated without using any corrections like those for multiple testing, is considered to be indicative of a significant difference.

[0024] In cases where more than one mutation is present in a nucleic acid molecule, wherein said mutation is linked with HAE type III, it may suffice to detect the presence of one mutation only or of a lower number of mutations than are actually present in the nucleic acid molecule and associated with HAE type III. Normally, it is not relevant for the purpose of diagnosis, whether such associated mutations are solely indicative, thus having for example a bystander effect, and not causative or whether they are causative for the onset or progress of the disease.

[0025] GenBank accession number AF538691 lists a consensus sequence of the human coagulation factor XII gene and a number of polymorphic variants observed in Caucasian and Negroid individuals. For a large part, these and potentially existing other polymorphic variants may be functionally neutral. Nevertheless, it is possible that at least some polymorphic variants are not neutral, i.e. that they can exhibit functional, quantitative or qualitative consequences like, for example, influencing directly the susceptibility or predisposition for the development of HAE type III or modulating the pathogenic effect of another mutation associated with hereditary angioedema type III.

[0026] For example, it is envisaged that a common polymorphism (46C/T) in the 5'-UTR (in exon 1) of the human coagulation factor XII gene can be of importance for the present invention. It is known that this polymorphism is significantly associated with the plasma concentration of coagulation factor XII (Kanaji et al. 1998, Blood 91: 2010-2014), the T allele being associated with a decreased translation efficiency; in functional and antigenic assays, individuals with the genotype C/C show 170% of the concentration seen in pooled normal plasma, whereas in individuals with the genotype T/T the factor XII plasma concentration is 80% of that seen in pooled normal plasma. In accordance with the present invention, one, therefore, may consider the C allele being a risk factor whose presence can increase the risk for the development of angioedema, for example in case that it is present in one haplotype with a dominant disease-associated mutation.

[0027] Thus, in a less preferred alternative, it is conceivable that, in fact, some of said polymorphic variants represent a disease-associated mutation. It is also envisaged that such a situation might arise from linkage disequilibrium phenomena. With these limitations in mind, the deposited consensus sequence mentioned above, is considered herein to represent the "wild-type" sequence.

[0028] It is important to note that the term "nucleic acid molecule regulating the expression of or encoding coagulation factor XII" preferably comprises the complete genomic sequence of the coagulation factor XII gene including extended flanking regulatory sequences (vide infra) as well as sequences or nucleic acid molecules which are physically unrelated to the coagulation factor XII gene but which exert regulatory effects on the expression of coagulation factor XII. The term "nucleic acid molecule regulating the expression of or encoding coagulation factor XII" also refers to portions of the above sequences, for example the promoter of said gene.

[0029] The term "regulating the expression" means influencing, including increasing or decreasing transcription or translation. Accordingly, increasing or decreasing means producing more or less, respectively, RNA or (poly)peptides.

The term "regulating the expression" also refers to influencing splicing processes, as well as the tissue-specific expression of a gene. The skilled person knows that expression may be regulated, for example, by enhancer or silencer sequences, splicing signals as well as other sequences which affect splicing processes, binding of transcription factors, polyadenylation sequences, transport signals, transcription terminator and the like. It is also envisaged that nucleic acid sequences physically unrelated to the coagulation factor XII gene locus can participate in the regulation of the expression of coagulation factor XII, and thus may have an impact on the development of angioedema symptoms. For example, a gene locus on the short arm of chromosome 10, around marker D10S1653, envisaged to be located within the nucleotide sequence comprising nucleotides chr10:10,554,416 to chr10:18,725,506 (UCSC Genome Browser/July 2003) has been demonstrated to affect coagulation factor XII plasma level (Soria et al. 2002, Am. J. Hum. Genet. 70:567-574) and may, thus, also affect disease susceptibility or disease development.

[0030] Sequences "encoding coagulation factor XII" refer to the coding sequence of the coagulation factor XII gene. Said term relates to the genomic coding sequence as well as the coding sequence in a RNA or cDNA molecule.

[0031] The term "coagulation factor XII" preferably relates to coagulation factor XII, which is a serine protease circulating in plasma as a single-chain inactive zymogen of approximately 80 kDa. Particularly preferred in accordance with the present invention is the coagulation factor XII corresponding to the mRNA sequence given under GenBank accession no. NM_000505.2 and encoded by the nucleic acid molecule deposited under GenBank accession number AF538691 which is considered by the present invention as the wild-type coagulation factor XII gene sequence and which includes 5' promoter sequences (up to 1581 by upstream from exon 1), coding and non-coding exon sequences, intronic sequences, and 3' flanking regulatory sequences, including 1598 by downstream from the end of exon 14 which corresponds to the end of the coagulation factor XII mRNA as given under GenBank accession number NM_000505.2. With respect to genomic sequences further extending into upstream and downstream direction, the sequence considered here to represent the wild-type sequence may be taken from the July 2003 human reference sequence of the UCSC Genome Browser, v.53, namely from the reverse complement sequence of chr5:176,807,093-176,821,530 (representing 4000 by upstream of exon 1 and 3000 by downstream of exon 14). The GenBank entry AF538691 relates to the gene of *Homo sapiens* coagulation factor XII (Hageman factor) (F12) of which several variants are known in the art (vide supra). The term "coagulation factor XII" also relates to sequences with an identity of at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% when compared with the sequence of GenBank accession number AF538691. In addition, the present invention also relates to various protein isoforms corresponding to different transcripts produced by alternative splicing (for example, those shown in "http://www.ncbi.nih.gov/IEB/Research/Acembly/ay.cgi?db=human&I=F12"). Further, the present invention also relates to species homologues in other animals, preferably mammals including rat, mouse, guinea pig, pig, cattle or rabbit. Polymorphic variants of coagulation factor XII may also comprise variants with large deletions in, for example, intron regions. Said variants may nevertheless encode a coagulation factor XII (poly)peptide of wild-type sequence. It is important to note that when

aligned to the sequence of AF538691, the calculated sequence identity may be considerably lower than expected for normal polymorphic variation. Thus, preferred in accordance with the present invention are biologically active variants and also fragments of coagulation factor XII encoded by a nucleic acid molecule with a sequence identity of at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% when compared with the sequence of databank accession number AF538691. Sequence identity may be determined by using the Bestfit® program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). Bestfit® uses the local homology algorithm of Smith and Waterman to find the best segment of homology between two sequences (*Advances in Applied Mathematics* 2:482-489 (1981)). When using Bestfit® or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed. The identity between a first sequence and a second sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag and colleagues (*Comp. App. Biosci.* 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

[0032] The present invention is related to the observation that patients affected by hereditary angioedema type III show no deficiency of C1 esterase inhibitor. According to the present invention, the symptoms observed in patients affected by hereditary angioedema type III can be associated with a mutation in a nucleic acid molecule regulating the expression of or encoding coagulation factor XII.

[0033] Such mutations may comprise, but are not limited to, for example (1) a mutation that favours, directly or indirectly, the production of a vasoactive kinin, (2) a mutation that alters the interaction of coagulation factor XII with activating surfaces or with a cell surface receptor or a cell surface receptor complex or with another physiologically interacting molecule, (3) a mutation that results in an increased stability of coagulation factor XII and/or an increased stability of its mRNA, (4) a mutation that results in an increased activity of coagulation factor XII, (5) a mutation that results in an alteration of substrate specificity of coagulation factor XII, (6) a mutation that results in an aberrant proteolytic processing of coagulation factor XII or (7) a mutation that results in an irregular interaction with C1 esterase inhibitor.

[0034] Further, without being bound by any theory, it is believed in accordance with the invention, that certain mutations or variations within certain regions of the coagulation factor XII gene may be mutations that affect the splicing, the expression, the structure and/or function of the GPRK6 (G protein-coupled receptor kinase 6) gene or a GPRK6 protein,

respectively. GPRK6 has a direct functional relationship for example with the β 2-adrenergic receptor, the vasoactive intestinal polypeptide type-1 (VPAC1) receptor, and the calcitonin gene-related peptide (CGRP) receptor (Shetzline et al. 2002, *J. Biol. Chem.* 277: 25519-25526; Aiyar et al. 2000, *Eur. J. Pharmacol.* 403: 1-7), thus possibly also being involved in the regulation of mechanisms underlying angioedema pathogenesis. The GPRK6 gene is located ~15 kb telomeric from the coagulation factor XII gene, being encoded on the opposite strand. There appear to exist certain splice variants/isoforms of GPRK6 (c.f. AceView and UCSC Genome Browser; GenBank acc nos. BX355118, BX463737, B1604127 [isoform h]) that arise from or are related to genomic sequences within the coagulation factor XII gene or its extended promoter region.

[0035] As stated above, factor XII (i.e. coagulation factor XII) is preferably a serine protease produced by the liver, circulating in human plasma as a single-chain inactive zymogen at a concentration of approximately 30 μ g/ml. From expression data one has to assume a coagulation factor XII production also by other tissues, possibly as isoforms. Coagulation factor XII has a molecular weight of about 80 kDa on SDS gel electrophoresis and was originally cloned and sequenced by Cool et al. 1985 (*J. Biol. Chem.* 260: 13666-13676) and by Que & Davie 1986 (*Biochemistry* 25: 1525-1528). The human coagulation factor XII gene is located on chromosome 5, at 5q35.3 (Royle et al. 1988, *Somat. Cell Mol. Genet.* 14: 217-221), it is approximately 12 kb in size and consists of 14 exons and 13 introns (Cool & MacGillivray 1987, *J. Biol. Chem.* 262: 13662-13673). The mature plasma protein consists of 596 amino acids (following a leader peptide of 19 residues) and is organized in several domains. From N-terminus to C-terminus, these domains are: a fibronectin type-II domain, an epidermal growth factor-like domain, a fibronectin type-I domain, another epidermal growth factor-like domain, a kringle domain, a proline-rich region, and a serine-protease catalytic region.

[0036] In vitro activation of factor XII occurs on negatively charged surfaces (including glass, kaolin, Celite, dextran sulfate, and ellagic acid), by autoactivation, by proteolytic cleavage, by conformational change, or by some combination of these mechanisms (Pixley & Colman 1993, *Methods Enzymol.* 222: 51-65). Further activating substances include sulfatides, chondroitin sulfate, endotoxin, some mast cell proteoglycans, and also aggregated A β protein of Alzheimer's disease. In vivo, the subendothelial vascular basement membrane and/or the stimulated endothelial cell surface might be important for factor XII activation (Pixley & Colman 1993). On endothelial cell membranes, urokinase plasminogen activator receptor, gC1qR (the receptor that binds to the globular heads of complement C1q), and cytokeratin 1 might be involved in the interaction with factor XII (Joseph K. et al. 1996, *Proc. Natl. Acad. Sci. USA* 93: 8552-8557; Joseph K. et al. 2001, *Thromb. Haemost.* 85: 119-124; Mahdi et al. 2002, *Blood* 99: 3585-3596).

[0037] Primary activation of factor XII is due to cleavage of the molecule at a critical Arg₃₅₃-Val₃₅₄ bond contained within a disulfide bridge, mediated for example by kallikrein or plasmin (or factor XIIa itself). The resultant factor XIIa (α -coagulation factor XIIa) is thus a two-chain, disulfide-linked 80-kDa enzyme consisting of a heavy chain (353 residues; 50 kDa) and a light chain (243 residues; 28 kDa). The heavy chain binds to negatively charged surfaces, the light chain represents the serine protease part of the molecule

containing the canonical Asp₄₄₂, His₃₉₃, Ser₅₄₄ triad. Two subsequent cleavages are responsible for the formation of the two forms of factor XIIIf (Kaplan et al. 2002, *J. Allergy Clin. Immunol.* 109: 195-209): these cleavages occur at Arg334-Asn335 and Arg343-Leu344 and result in the formation of "factor XII fragment", FXIIIf, also called β -FXIIa. FXIIIf consists of the light chain of factor XIIa, corresponding to the serine protease domain, and a very small piece, either 19 or 9 amino acids in length, of the original heavy chain. Factor XIIIf lacks the binding site for the activating surface as well as the ability of factor XIIa to convert factor XI to factor XIa. However, FXIIIf is still a potent activator of prekallikrein. In summary, activation of the factor XII zymogen results in an enzyme with decreasing size, a decrease in surface-binding properties, and a decrease in coagulant activity, but retained, eventually increased kinin-forming capacity (Colman & Schmaier 1997, *Blood* 90: 3819-3843).

[0038] The present invention's disclosure allows to specifically identify individuals with (a) mutation(s) in a nucleic acid molecule encoding coagulation factor XII or regulating the expression of coagulation factor XII and link this/these mutation(s) with the individual's hereditary angioedema type III or its predisposition to develop HAE type III or to pass on to their offspring (a) specific mutation(s) which is/are associated with an increased risk for the development of HAE type III. Said nucleic acid molecule may be DNA or RNA.

[0039] Any method including those known to the person skilled in the art may be used to determine the presence or absence of such a mutation.

[0040] In a preferred embodiment of the present invention's method of diagnosing, said determination comprises hybridizing under stringent conditions to said nucleic acid molecule at least one pair of nucleic acid probes, the first probe of said pair being complementary to the wild-type sequence of said nucleic acid molecule and the second probe of said pair being complementary to the mutant sequence of said nucleic acid molecule, wherein a perfect match, the presence of stable hybridization, between (i) the first hybridization probe and the target nucleic acid molecule indicates the presence of a wild-type sequence, and (ii) the second hybridization probe and the target nucleic acid molecule, indicates the presence of a mutant sequence, wherein the first hybridization probe and the second hybridization probe allow a differential detection. Preferably, said mutant sequence is a disease-associated mutant sequence.

[0041] The term "hybridizing under stringent conditions", as used in the description of the present invention, is well known to the skilled artisan and corresponds to conditions of high stringency or selectivity. Appropriate stringent hybridization conditions for each sequence may be established by a person skilled in the art on well-known parameters such as temperature, composition of the nucleic acid molecules, salt conditions etc.; see, for example, Sambrook et al., "Molecular Cloning, A Laboratory Manual"; ISBN: 0879695765, CSH Press, Cold Spring Harbor, 2001, or Higgins and Hames (eds.), "Nucleic acid hybridization, a practical approach", IRL Press, Oxford 1985, see in particular the chapter "Hybridization Strategy" by Britten & Davidson, 3 to 15. Stringent hybridization conditions are, for example, conditions comprising overnight incubation at 42° C. in a solution comprising: 50% formamide, 5×SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5×Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml denatured, sheared salmon sperm DNA, followed

by washing the filters in 0.1×SSC at about 65°. Other stringent hybridization conditions are for example 0.2×SSC (0.03 M NaCl, 0.003 M sodium citrate, pH 7) at 65° C.

[0042] Depending on the particular conditions, for example the base composition of the probe, the person skilled in the art may have to vary, for example the salt concentration and temperature in order to find conditions which (a) prevent the hybridization of probes differing from the target nucleic acid molecule in only one position and (b) still allow hybridization of probes which completely match the same region of the target nucleic acid molecule. However, said conditions can be established by standard procedures known to the person skilled in the art and by routine experimentation.

[0043] The probe of hybridization is usually a nucleic acid molecule containing one or more labels. The label can be located at the 5' and/or 3' end of the nucleic acid molecule or be located at an internal position. Preferred labels include, but are not limited to, fluorochromes, e.g. carboxyfluorescein (FAM) and 6-carboxy-X-rhodamine (ROX), fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin, allophycocyanin, 6-carboxyfluorescein (6-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), 6-carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), radioactive labels, e.g. ³²P, ³⁵S, ³H; etc. The label may also be a two stage system, where the probe is conjugated to biotin, haptens, etc. having a high affinity binding partner, e.g. avidin, specific antibodies, etc., where the binding partner is conjugated to a detectable label.

[0044] As stated above, two probes used as a pair must allow a differential detection. This can be accomplished, for example, by labeling the probes with two different labels that can be differentiated in a detection process.

[0045] The hybridization probe is usually a nucleic acid molecule of about 20 to about 2000 bases in length. When used for hybridization reactions such as southern or northern blot reactions, the probe can be an oligonucleotide or primer which are typically in the range of about 15 to 50 bases in length or can be considerably longer and may range from about 50 bases to about 2000 bases. The term "oligonucleotide", when used in an amplification reaction, refers to a nucleic acid molecule of typically 15 to 50 bases in length with sufficient complementarity to allow specific hybridization to a nucleic acid sequence encoding or regulating the expression of coagulation factor XII. Preferably, an oligonucleotide used for hybridization or amplification is about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 bases in length. However, probes of about 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 bases are also contemplated by the present invention. Moreover, according to the particular conditions chosen for hybridization, the nucleotide probe may even be several hundred or thousand bases longer. Said probe or oligonucleotide may be composed of DNA or RNA. When used as a hybridization probe, it may be, e.g., desirable to use nucleic acid analogs, in order to improve the stability and binding affinity. The term "nucleic acid" shall be understood to encompass such analogs. A number of modifications have been described that alter the chemistry of the phosphodiester backbone, sugars or heterocyclic bases. Among useful changes in the backbone chemistry are phosphorothioates; phosphorodithioates, where both of the non-bridging oxygens are substituted with sulfur; phosphoroam-

idites; alkyl phosphotriesters and boranophosphates. Achiral phosphate derivatives include, but are not limited to, 3'-O'-5'-S-phosphorothioate, 3'-S-5'-O-phosphorothioate, 3'-CH₂-5'-O-phosphonate and 3'-NH-5'-O-phosphoroamidate. Peptide nucleic acids replace the entire phosphodiester backbone with a peptide linkage. Sugar modifications are also used to enhance stability and affinity. The α-anomer of deoxyribose may be used, where the base is inverted with respect to the natural β-anomer. The 2'-OH of the ribose sugar may be altered to form 2'-O-methyl or 2'-O-allyl sugars, which provides resistance to degradation without comprising affinity. Modification of the heterocyclic bases must maintain proper base pairing. Some useful substitutions include deoxyuridine for deoxythymidine; 5-methyl-2'-deoxycytidine and 5-bromo-2'-deoxycytidine for deoxycytidine; 5-propynyl-2'-deoxyuridine and 5-propynyl-2'-deoxycytidine for deoxythymidine and deoxycytidine, respectively.

[0046] In another preferred embodiment of the present invention's method of diagnosing, said method comprises hybridizing under stringent conditions to said nucleic acid molecule a hybridization probe specific for a mutant sequence. Preferably, said mutant sequence is a disease-associated mutant sequence.

[0047] In another preferred embodiment of the present invention, the method of diagnosing comprises a step of nucleic acid amplification and/or nucleic acid sequencing. Preferably, nucleic acid sequencing is DNA sequencing. A widely used method of diagnosing is for example direct DNA sequencing of PCR products containing a mutation to be diagnosed. The term "amplification" or "amplify" means increase in copy number. The person skilled in the art knows various methods to amplify nucleic acid molecules, these methods may also be used in the present invention's method of diagnosing. Amplification methods include, but are not limited to, "polymerase chain reaction" (PCR), "ligase chain reaction" (LCR, EPA320308), "cyclic probe reaction" (CPR), "strand displacement amplification" (SDA, Walker et al. 1992, *Nucleic Acid Res.* 7: 1691-1696), "transcription based amplification systems" (TAS, Kwok et al. 1989, *Proc. Nat. Acad. Sci. USA* 86: 1173; Gingeras et al., PCT Application WO 88/10315). Preferably, amplification of DNA is accomplished by using polymerase chain reaction (PCR) [Methods in Molecular Biology, Vol. 226 (Bartlett J. M. S. & Stirling D., eds.): PCR protocols, 2nd edition; PCR Technology: Principles and Applications for DNA Amplification (Erich H. A., ed.), New York 1992; PCR Protocols: A guide to methods and applications (Innis M. A. et al., eds.), Academic Press, San Diego 1990]. Nucleic acid amplification methods may be particularly useful in cases when the sample contains only minute amounts of nucleic acid. If said nucleic acid is RNA, an RT-PCR might be performed. Subsequently, another amplification step involving PCR may be performed. Alternatively, if said nucleic acid contained in the sample is DNA, PCR may be performed.

[0048] The PCR, generally, consists of many repetitions of a cycle which consists of: (a) a denaturing step, which melts both strands of a DNA molecule; (b) an annealing step, which is aimed at allowing the primers to anneal specifically to the melted strands of the DNA molecule; and (c) an extension step, which elongates the annealed primers by using the information provided by the template strand. Generally, PCR can be performed for example in a 50 μl reaction mixture containing 5 μl of 10×PCR buffer with 1.5 mM MgCl₂, 200 μM of each deoxynucleoside triphosphate, 0.5 μl of each primer

(10 μM), about 10 to 100 ng of template DNA and 1 to 2.5 units of Taq Polymerase. The primers for the amplification may be labeled or be unlabeled. DNA amplification can be performed, e.g., with a model 2400 thermal cycler (Applied Biosystems, Foster City, Calif.): 2 min at 94° C., followed by 35 cycles consisting of annealing (30 s at 50° C.), extension (1 min at 72° C.), denaturing (10 s at 94° C.) and a final annealing step at 55° C. for 1 min as well as a final extension step at 72° C. for 5 min. However, the person skilled in the art knows how to optimize these conditions for the amplification of specific nucleic acid molecules or to scale down or increase the volume of the reaction mix.

[0049] A further method of nucleic acid amplification is the "reverse transcriptase polymerase chain reaction" (RT-PCR). This method is used when the nucleic acid to be amplified consists of RNA. The term "reverse transcriptase" refers to an enzyme that catalyzes the polymerization of deoxyribonucleoside triphosphates to form primer extension products that are complementary to a ribonucleic acid template.

[0050] The enzyme initiates synthesis at the 3'-end of the primer and proceeds toward the 5'-end of the template until synthesis terminates. Examples of suitable polymerizing agents that convert the RNA target sequence into a complementary, copy-DNA (cDNA) sequence are avian myeloblastosis virus reverse transcriptase and *Thermus thermophilus* DNA polymerase, a thermostable DNA polymerase with reverse transcriptase activity marketed by Perkin Elmer. Typically, the genomic RNA/cDNA duplex template is heat denatured during the first denaturation step after the initial reverse transcription step leaving the DNA strand available as an amplification template. Suitable polymerases for use with a DNA template include, for example, *E. coli* DNA polymerase I or its Klenow fragment, T.sub.4 DNA polymerase, Tth polymerase, and Taq polymerase, a heat-stable DNA polymerase isolated from *Thermus aquaticus* and developed and manufactured by Hoffmann-La Roche and commercially available from Perkin Elmer. The latter enzyme is widely used in the amplification and sequencing of nucleic acids. The reaction conditions for using Taq polymerase are known in the art and are described, e.g., in: PCR Technology, Erlich, H. A. 1989, Stockton Press, New York; or in: Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White. 1990, PCR Protocols: A guide to methods and applications. Academic Press, New York. High-temperature RT provides greater primer specificity and improved efficiency. Copending U.S. patent application Ser. No. 07/746,121, filed Aug. 15, 1991, describes a "homogeneous RT-PCR" in which the same primers and polymerase suffice for both the reverse transcription and the PCR amplification steps, and the reaction conditions are optimized so that both reactions occur without a change of reagents. *Thermus thermophilus* DNA polymerase, a thermostable DNA polymerase that can function as a reverse transcriptase, can be used for all primer extension steps, regardless of template. Both processes can be done without having to open the tube to change or add reagents; only the temperature profile is adjusted between the first cycle (RNA template) and the rest of the amplification cycles (DNA template). The RT Reaction can be performed, for example, in a 20 μl reaction mix containing: 4 μl of 5×ANV-RT buffer, 2 μl of Oligo dT (100 μg/ml), 2 μl of 10 mM dNTPs, 1 μl total RNA, 10 Units of AMV reverse transcriptase, and H₂O to 20 μl final volume. The reaction may be, for example, performed by using the following conditions: The reaction is held at 70° C. for 15 minutes to allow for reverse transcription. The reaction

temperature is then raised to 95° C. for 1 minute to denature the RNA-cDNA duplex. Next, the reaction temperature undergoes two cycles of 95° C. for 15 seconds and 60° C. for 20 seconds followed by 38 cycles of 90° C. for 15 seconds and 60° C. for 20 seconds. Finally, the reaction temperature is held at 60° C. for 4 minutes for the final extension step, cooled to 15° C., and held at that temperature until further processing of the amplified sample.

[0051] The term “primer” or “oligonucleotide” refers to a short nucleic acid molecule from about 8 to about 30, eventually to about 50 nucleotides in length, whether natural or synthetic, capable of acting as a point of initiation of nucleic acid synthesis under conditions in which synthesis of a primer extension product complementary to a template nucleic acid strand is induced, i.e., in the presence of four different nucleoside triphosphates or analogues thereof and an agent for polymerisation (i.e., DNA polymerase or reverse transcriptase) in an appropriate buffer and at a suitable temperature. Preferably, a primer is a single-stranded oligodeoxyribonucleotide. The appropriate length of a primer depends on the intended use of the primer but typically ranges for PCR primers and primers used in sequencing reactions from 10 to 25 nucleotides. Short primer molecules generally require cooler temperatures to form sufficiently stable hybrid complexes with the template. A primer need not reflect the exact sequence of the template but must be sufficiently complementary to hybridize specifically with a template, provided its ability to mediate amplification is not compromised. “Hybridize” refers to the binding of two single stranded nucleic acids via complementary base pairing, i.e. A to T (in RNA: U), G to C. The term “primer pair” refers to two primers that hybridize with the + and – strand, respectively, of a double stranded nucleic acid molecule, and allow the amplification of e.g. DNA fragments, as for example in a PCR reaction. A primer can be labeled, if desired, by incorporating a compound detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include, but are not limited to, fluorescent dyes, electron-dense reagents, biotin, or small peptides for which antisera or monoclonal antibodies are available. A label can also be used to “capture” the primer, so as to facilitate a selection of amplified nucleic acid or fragments thereof. Carboxyfluorescein (FAM) and 6-carboxy-X-rhodamine (ROX) are preferred labels. However, other preferred labels include fluorochromes, e.g. fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin, allophycocyanin, 6-carboxyfluorescein (6-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), 6-carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), radioactive labels, e.g. ³²P, ³⁵S, ³H; etc. The label may also be a two stage system, where the primer is conjugated to biotin, haptens, etc. having a high affinity binding partner, e.g. avidin, specific antibodies, etc., where the binding partner is conjugated to a detectable label. The label may be conjugated to one or both of the primers.

[0052] During said method for diagnosing, a step of nucleic acid sequencing may be performed. Any methods known in the art may be used for sequencing. Preferably, the nucleic acid sequence is determined by a method based on the sequencing techniques of Sanger or Maxam/Gilbert (see for example: *Methods in Molecular Biology*, Vol. 167 (Graham C. A. & Hill A. J. M., eds.): DNA sequencing protocols. 2nd

edition, 2001; Galas D. J. & McCormack S. J., *Genomic Technologies: Present and Future*. Caister Academic Press, Wymondham, UK, 2002).

[0053] In another preferred embodiment of the present invention's method of diagnosing, said method is or comprises an allele discrimination method selected from the group consisting of allele-specific hybridization, allele-specific primer extension including allele-specific PCR, allele-specific oligonucleotide ligation, allele-specific cleavage of a flap probe and/or allele-specific cleavage using a restriction endonuclease. These methods are known to the skilled person and described and further referenced for example by Kwok P-Y & Chen X 2003, *Curr. Issues Mol. Biol.* 5:43-60; Kwok P-Y 2001, *Annu. Rev. Genomics Hum. Genet.* 2:235-258; Syvanen, A.-Ch. 2001, *Nature Rev. Genet.* 2: 930-942.

[0054] In yet a further preferred embodiment, the present invention's method of diagnosing may comprise a detection method selected from the group consisting of fluorescence, time-resolved fluorescence, fluorescence resonance energy transfer (FRET), fluorescence polarization, colorimetric methods, mass spectrometry, (chemi)luminescence, electrophoretic detection and electrical detection methods. These methods for the detection of an allele discrimination reaction are known to the skilled person and described and further referenced for example by Kwok P-Y & Chen X 2003, *Curr. Issues Mol. Biol.* 5:43-60; Kwok P-Y 2001, *Annu. Rev. Genomics Hum. Genet.* 2:235-258; Syvanen, A.-Ch. 2001, *Nature Rev. Genet.* 2: 930-942.

[0055] In certain cases it might be necessary to detect large deletions, insertions, or duplications. Preferably, this may be done by using methods well known in the art and comprising, for example, Southern blotting methods; quantitative or semi-quantitative gene dosage methods including competitive PCR, differential PCR, real-time PCR, multiplex amplifiable probe hybridization; or long-range PCR (Armour et al. 2002, *Human Mutation* 20: 325-337).

[0056] It may often be desirable to obtain, from a single individual, an allelic diagnosis at several regions or positions of the nucleic acid molecule(s) encoding coagulation factor XII or regulating its expression. For this purpose, nucleic acid arrays may be useful, such as those described in: WO 95/11995.

[0057] Further, for some purposes it may be desirable to determine the presence of two or more mutations/variations as a haplotype, i.e. to determine which alleles from several mutant/variant positions occur together on one haplotype. This can be achieved by methods known in the art, for example by a segregation analysis within families, and also and preferably by methods allowing molecular haplotyping. For example, a double digest of a single PCR product, containing two mutant/variant positions, with two restriction endonucleases, each one of these two enzymes being able to differentiate the allelic situation at one of the two investigated positions, can yield such haplotype information from the fragment sizes obtained. However, numerous other methods are known to the person skilled in the art (see, for example: Tost et al. 2002, *Nucleic Acids Res.* 30: e96; Eitan & Kashi 2002, *Nucleic Acids Res.* 30: e62; Pettersson et al. 2003, *Genomics* 82: 390-396; Ding et al. 2003, *Proc. Natl. Acad. Sci. U.S.A.* 100: 7449-7453; Odeberg et al. 2002, *Biotechniques* 33: 1104, 1106, 1108; McDonald et al. 2002, *Pharmacogenetics* 12: 93-99; Woolley et al. 2000, *Nature Biotechnol.* 18: 760-763), and are envisaged to be applicable for the purposes of the present invention.

[0058] In yet another preferred embodiment of the present invention's method of diagnosing, the probe or the subject's nucleic acid molecule is attached to a solid support. Solid supports that may be employed in accordance with the invention include filter material, chips, wafers, microtiter plates, to name a few.

[0059] The present invention also relates to a method of diagnosing hereditary angioedema type III (HAE III) or a predisposition thereto in a subject being suspected of having developed or of having a predisposition to develop a hereditary angioedema type III or in a subject being suspected of being a carrier for hereditary angioedema type III, the method comprising assessing the presence, amount and/or activity of coagulation factor XII in said subject and including the steps of: (a) determining from a biological sample of said subject in vitro, the presence, amount and/or activity of: (i) a (poly)peptide encoded by the coagulation factor XII gene; (ii) a substrate of the (poly)peptide of (i); or (iii) a (poly)peptide processed by the substrate mentioned in (ii); (b) comparing said presence, amount and/or activity with that determined from a reference sample; and (c) diagnosing, based on the difference between the samples compared in step (b), the pathological condition of a hereditary angioedema type III or a predisposition thereto. The term "(poly)peptide" refers alternatively to peptide or to (poly)peptides. Peptides conventionally are covalently linked amino acids of up to 30 residues, whereas polypeptides (also referred to herein as "proteins") comprise 31 and more amino acid residues. The term "assessing the amount" or "determining the amount" means assessing or determining the amount of a (poly)peptide encoded by the coagulation factor XII gene, comprising for example the coagulation factor XII precursor or any of its maturation products generated for example by activating processes including autoactivation and proteolytic processing of coagulation factor XII. Therefore, assessing or determining the amount of coagulation factor XII also may refer to determining the amount of (1) mature FXII, (2) FXIIa (80 kDa, arising from the cleavage at Arg353-Val354); (3) FXIIf (2 subforms: 30 kDa/28.5 kDa; 19-peptide or nonapeptide linked via S—S to the catalytic chain; arising from the cleavage of Arg334-Asn335 and the additional cleavage of Arg343-Leu344); (4) a third form of activated factor XII, a 40 kDa molecule (mainly produced by autoactivation), in which the serine protease domain is linked to a 12,000-MW fragment of the heavy chain (Kaplan & Silverberg 1987); (5) potential protein isoforms (AceView, <http://www.ncbi.nlm.nih.gov/IEB/Research/Aceembly/ay.cgi?db=33&c=Gene&I=F12>); (6) coagulation factor XII forms or fragments that arise from an irregular proteolytic processing, eventually caused by a mutation of the present invention; or (7) a mutant of any one of the forms (1) to (5), including any of the mutants of the present invention. However, "assessing the amount" or "determining the amount" also refers to determining the amount of substrates and/or their activation products of any of the above-mentioned coagulation factor XII forms. Preferably, the ratio of activated and native (non-activated) forms of these substrates is determined. Also included are (poly)peptides processed by these (activated) substrates. These substrates and processed (poly)peptides include, for example, (8) coagulation factor XIa/coagulation factor XI; (9) coagulation factor Vila/coagulation factor VII; (10) kallikrein/prekallikrein; (11) plasmin/plasminogen; (12) activated complement C1r/C1r; (13) activated complement C1s/C1s; (14) activated hepatocyte growth fac-

tor (HGF)/hepatocyte growth factor; (15) activated macrophage stimulating protein (MSP)/macrophage stimulating protein. Also included is (16) the determination of "cleavage products of high-molecular weight kininogen" or the ratio of the "cleavage products of high-molecular weight kininogen" with "high-molecular weight kininogen". Said cleavage products comprise cleaved kininogen, bradykinin and/or other kinins. Furthermore included are (17) cleavage products of complement component C2/complement component C2; (18) cleavage products of complement component C4/complement component C4; and (19) activated bradykinin type 2 receptor/bradykinin type 2 receptor.

[0060] The term "assessing the activity" or "determining the activity" means determining a biological activity, wherein biological activity refers to (a) the known activities, preferably those of wild-type (poly)peptides, and (b) aberrant activities, including those of mutant coagulation factor XII (poly)peptides which are apparent from comparing the activity of a mutant with that of a wild-type (poly)peptide. The known and aberrant activities may comprise the activity of any of the proteins (1) to (19) mentioned above. The term "assessing the presence" or "determining the presence" means determining which of the aforementioned (poly)peptides or proteins is present in the sample. Said term also refers to determining whether wild-type or a mutant (poly)peptide is present in the sample. Preferably, said (poly)peptide is any of the (poly)peptides (1) to (7) as mentioned above. In some cases, it may also be useful to analyze any of the (poly)peptides (8) to (19) as mentioned above, their native and/or activated forms.

[0061] Step (i) of the method, which reads "a (poly)peptide encoded by the coagulation factor XII gene", may comprise the determination of at least one of the (poly)peptides listed above under (1), (2), (3), (4), (5), (6) and (7).

[0062] Step (ii) of the method, which reads "a substrate of the (poly)peptide of (i)", may comprise the determination of at least one of the polypeptides listed above under (8), (9), (10), (11), (12), (13), (14), (15) and (16).

[0063] Step (iii) of the method, which reads "a (poly)peptide processed by the substrate mentioned in (ii)", may comprise the determination of at least one of the polypeptides listed above under (16), (17), (18), and (19).

[0064] This method of diagnosing is based on determining from a sample of an individual to be diagnosed and a reference sample the quantity and/or quality of, for example, any of the proteins listed under (1) to (19) and determining, based on the difference between said samples, a pathological condition in said individual's sample. Said pathological condition is hereditary angioedema type III or a predisposition thereto. The reference sample is a standard sample obtained from a healthy subject or healthy subjects, preferably from a subject or subjects particularly not affected by angioedema symptoms.

[0065] Generally, any of the known protein detection methods may be used. These include, for example, immunochemical, antibody-based methods such as ELISA, RIA, Western Blotting, preferably following any kind of electrophoretic separation step, and the like. Such methods are, for example, described by Clark & Hales: *Immunoassays*. In: *Clinical Aspects of Immunology* (P. J. Lachmann et al., eds.), vol. 2, 5th ed., Boston 1993; or in Weir's *Handbook of Experimental Immunology*, 5th ed., 1996 (Herzenberg L. et al., eds.); see also e.g. Lammle et al. 1987 (*Semin. Thromb. Hemost.* 13: 106-114). Methods for the determination of biological activi-

ties of the polypeptides listed above are known in the art. Biological activity can be measured for example by providing substrates for the (poly)peptides and measuring substrate conversion by the methods known in the art. For example, measuring the activity of (pre)kallikrein on a chromogenic substrate, which may be monitored by detecting cleavage of said substrate, has been described by Klufft 1978 (*J. Lab. Clin. Med.*, 91:83-95), Klufft 1988 (*Meth. Enzymol.* 163: 170-179). Functional assays for measuring prekallikrein have also been described by de la Cadena et al. 1987 (*J. Lab. Clin. Med.* 109: 601-607) and Silverberg & Kaplan 1988 (*Meth. Enzymol.* 163: 85-95). A functional assay for high molecular weight kininogen using a chromogenic substrate has been described by Scott et al. 1987 (*Thromb. Res.* 48: 685-700) and also by Gallimore et al. 2002 (*Blood Coagul. Fibrinolysis* 13: 561-568).

[0066] The present invention also employs methods for determining the amino acid sequence of a (poly)peptide. Such methods are known in the art (see for example: *Methods in Molecular Biology*, Vol. 211 (Smith B. J., ed.): *Protein Sequencing Protocols*. 2nd edition, 2002). Preferably, protein sequence analysis is performed by Edman degradation (P. Edman, *Acta Chem. Scand.* 4: 283 (1950)) or by Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS). Hence, by using amino acid sequence analysis, the skilled person may determine whether a wild-type or mutant coagulation factor XII (poly)peptide is present in a sample.

[0067] The proteins listed above, include on the one hand coagulation factor XII and its various forms. These are part of a cascade known as, for example, the intrinsic coagulation pathway or contact system or kinin-forming pathway (see e.g. Kaplan et al. 1997, *Adv. Immunol.* 66: 225-272; Kaplan et al. 2002, *J. Allergy Clin. Immunol.* 109: 195-209). On the other hand, proteins listed above are proteins which follow coagulation factor XII downstream in said cascade, and, in addition, proteins which are not directly related to the kinin-forming pathway but for which it has been shown that they can be activated by coagulation factor XII, eventually indirectly. It is important to note that mutations of coagulation factor XII may have an impact on these downstream steps in the cascade and, for example, can result in a quantitatively or qualitatively abnormal activation of (poly)peptides located downstream in the cascade. This effect may be measured and may allow for deductions on the nature of the specific coagulation factor XII expressed in the individual under study.

[0068] The methods of the present invention are not limited to measuring individual (poly)peptides as listed above, but also refer to the measuring or determination of complexes of said (poly)peptides. Such complexes are for example complexes consisting of activated factor XII and complement C1 inhibitor; or complexes consisting of kallikrein and complement C1 inhibitor; or complexes consisting of kallikrein and alpha2-macroglobulin. Such complexes can be detected, for example, by using ELISA or RIA based techniques (Nuijens et al., 1987 *Thromb. Hemost.* 58: 778-785; Kaplan et al., 1985, *Blood* 66: 636-641; Kaplan et al., 1989, *Clin. Immunol. Immunopathol.* 50: S41-S51; Dors et al. 1992, *Thromb. Haemost.* 67: 644-648).

[0069] In a preferred embodiment of the present invention's method, the biological sample consists of or is taken from hair, skin, mucosal surfaces, body fluids, including blood, plasma, serum, urine, saliva, sputum, tears, liquor cerebrospi-

nalis, semen, synovial fluid, amniotic fluid, milk, lymph, pulmonary sputum, bronchial secretion, or stool.

[0070] The term "biological sample" relates to the specimen taken from a mammal. Preferably, said specimen is taken from hair, skin, mucosal surfaces, body fluids, including blood, plasma, serum, urine, saliva, sputum, tears, liquor cerebrosplanis, semen, synovial fluid, amniotic fluid, milk, lymph, pulmonary sputum, bronchial secretion, or stool. However, it is important to note that many other samples might be useful for this purpose, for example a sample taken for histological or cytological purposes.

[0071] A variety of techniques for extracting nucleic acids from biological samples are known in the art. For example, see those described in Rotbart et al., 1989, in *PCR Technology* (Erlich ed., Stockton Press, New York) and Han et al. 1987, *Biochemistry* 26:1617-1625. If the sample is fairly readily disruptable, the nucleic acid need not be purified prior to amplification by the PCR technique, i.e., if the sample is comprised of cells, e.g. peripheral blood lymphocytes or monocytes, lysis and dispersion of the intracellular components may be accomplished merely by suspending the cells in hypotonic buffer. Suitable methods will vary depending on the type of specimen and are well known to the person skilled in the art (see e.g. Sambrook et al., "Molecular Cloning, A Laboratory Manual"; ISBN: 0879695765, CSH Press, Cold Spring Harbor, 2001).

[0072] It is apparent that, for analysis of mRNA, cDNA, or protein, the sample must be obtained from a tissue in which coagulation factor XII/the coagulation factor XII gene is expressed, or, respectively, from a tissue or body fluid, in which coagulation factor XII is expressed or in which it is secreted.

[0073] In another preferred embodiment, said presence, amount and/or activity is determined by using an antibody or an aptamer, wherein the antibody or aptamer is specific for (a) a (poly)peptide encoded by the coagulation factor XII gene, (b) a substrate of the (poly)peptide of (a), or (c) a (poly)peptide processed by the substrate mentioned in (b). The term "antibody" refers to monoclonal antibodies, polyclonal antibodies, chimeric antibodies, single chain antibodies, or a fragment thereof. Preferably the antibody is specific for a polypeptide listed under (1) to (19). The antibodies may be bispecific antibodies, humanized antibodies, synthetic antibodies, antibody fragments, such as Fab, F(ab₂), Fv or scFv fragments etc., or a chemically modified derivative of any of these, all comprised by the term "antibody". Monoclonal antibodies can be prepared, for example, by the techniques as originally described in Köhler and Milstein, *Nature* 256 (1975), 495, and Galfré, *Meth. Enzymol.* 73 (1981), 3, which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized mammals with modifications developed by the art. Furthermore, antibodies or fragments thereof to the aforementioned (poly)peptides can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1998. When derivatives of said antibodies are obtained by the phage display technique, surface plasmon resonance as employed in the BIAcore system can be used to increase the efficiency of phage antibodies S which bind to an epitope of the peptide or polypeptide to be analyzed (Schier, *Human Antibodies Hybridomas* 7 (1996), 97-105; Malmberg, *J. Immunol. Methods* 183 (1995), 7-13). The production of chimeric antibodies is described, for example, in WO89/09622.

[0074] Antibodies may be labelled. Preferably said label is selected from the group consisting of fluorochromes, e.g. fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin, allophycocyanin, 6-carboxyfluorescein (6-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), 6-carboxy-X-rhodamine (ROX), 6-carboxy-2',4',7',4',7-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), radioactive labels, e.g. ³²P, ³⁵S, ³H; etc. The label may also be a two stage system, where the antibody is conjugated to biotin, haptens, etc. having a high affinity binding partner, e.g. avidin, specific antibodies, etc., where the binding partner is conjugated to a detectable label. In another preferred embodiment of the present invention the label is a toxin, radioisotope, or fluorescent label.

[0075] The term "aptamers" refers to RNA and also DNA molecules capable of binding target proteins with high specificity, comparable with the specificity of antibodies. Methods for obtaining or identifying aptamers specific for a desired target are known in the art. Preferably, these methods may be based on the "systematic evolution of ligands by exponential enrichment" (SELEX) process (Ellington and Szostak, *Nature*, 1990, 346: 818-822; Tuerk and Gold, 1990, *Science* 249: 505-510; Fitzwater & Polisky, 1996, *Methods Enzymol.* 267: 275-301). Preferably, said aptamers may be specific for any of the (poly)peptides listed under (1) to (19). The use of aptamers for detection and quantification of polypeptide targets is described in, for example, McCauley et al., 2003, *Anal. Biochem.*, 319:244-250; Jayasena, 1999, *Clin. Chem.* 45:1628-1650.

[0076] In a more preferred embodiment, said antibody or aptamer is specific for a (poly)peptide encoded by the coagulation factor XII gene. Said reagents will allow for assessing the quantity and/or quality of (a) coagulation factor XII (poly)peptide(s), and eventually also for the differentiation between wild-type and mutant, preferably disease-associated mutant coagulation factor XII (poly)peptides. For example, the identification of coagulation factor XII (poly)peptides by an immunoblotting procedure following an electrophoretic separation step, might well allow for the recognition of a mutant coagulation factor XII (poly)peptide. However, regarding the preferred differentiation between wild-type and disease-associated mutant coagulation factor XII (poly)peptides, preferably, said antibody or aptamer is specific for a disease-associated mutant of the present invention. Such an antibody or aptamer would fail to bind to wild-type coagulation factor XII (poly)peptide(s) but bind to a disease-associated mutant with high specificity. This antibody or aptamer would therefore be most useful to discriminate between wild-type and mutant coagulation factor XII (poly)peptides. More preferably, the epitope or target region recognized by the antibody or aptamer comprises the mutant position/region in coagulation factor XII.

[0077] Various antibody-based methods for the determination of coagulation factor XII (poly)peptide(s), like radial immunodiffusion, electroimmunoassay according to Laurell, dot immunobinding assay, radioimmunoassay, enzyme immunoassay, enzyme-linked immunosorbent assay, immunoblotting, or alike, have been described or employed for example by Mannhalter et al. 1987 (*Fibrinolysis* 1: 259-263), Gevers Leuven et al. 1987 (*J. Lab. Clin. Med.*), Wuillemin et al. 1990 (*J. Immunol. Methods* 130: 133-140), Saito et al. 1976 (*J. Lab. Clin. Med.* 88: 506-514), Ford et al. 1996 (*J.*

Immunoassay 17: 119-131), Lammler et al. 1987 (*Semin. Thromb. Hemost.* 13: 106-114).

[0078] In a preferred embodiment of the present invention, the presence, amount and/or activity of the (poly)peptide(s) encoded by the coagulation factor XII gene is determined in (a) a coagulation assay; or in (b) a functional amidolytic assay; or in (c) a mitogenic assay; or in (d) a binding assay measuring binding of a (poly)peptide encoded by the coagulation factor XII gene to a binding partner.

[0079] Coagulant activity of coagulation factor XII may be quantified using methods in which correction of the abnormal clotting time, the prolonged activated partial thromboplastin time, of plasma of a person with a severe hereditary deficiency of coagulation factor XII is measured (see for example: Pixley R. A. & Colman R. W. 1993; *Methods in Enzymology* 222: 51-65). Functional amidolytic assays for coagulation factor XII using various synthetic chromogenic substrates (for example S2302, S2337, S2222) have been described for example by Vinazzer 1979 (*Thrombosis Research* 14: 155-166), Tans et al. 1987 (*Eur. J. Biochem.* 164: 637-642), Gallimore et al. 1987 (*Fibrinolysis* 1: 123-127), Walshe et al. 1987 (*Thrombosis Research* 47: 365-371), Klufft 1988 (*Methods Enzymol.* 163: 170-179), Stürzebecher et al. 1989 (*Thrombosis Research* 55: 709-715). Another example for assessing a coagulation factor XII functional activity may be a measurement of the hepatocyte growth factor activating activity of coagulation factor XII (Shimomura et al. 1995, *Eur. J. Biochem.* 229: 257-261).

[0080] Schmeidler-Sapiro et al. 1991 (*Proc. Natl. Acad. Sci. U.S.A.* 88: 4382-4385) described assay systems allowing to assess a mitogenic activity of coagulation factor XII on HepG2 cells; coagulation factor XII as well as coagulation factor XIIa (kaolin-activated coagulation factor XII) enhanced cell proliferation and thymidine and leucine incorporation in HepG2 cells. Gordon et al. 1996 (*Proc. Natl. Acad. Sci. U.S.A.* 93: 2174-2179) assessed a growth factor activity of factor XII on several other target cells. Any of the aforementioned methods may be modified and used for determining the activity of (poly)peptides encoded by the coagulation factor XII gene. Various activators can be used in these assays, for example dextran sulfate, kaolin, a cephalin ellagic acid based reagent (Walshe et al. 1987, *Thromb. Res.* 47: 365-371), or others, and it is conceivable that the extent and/or the nature of activation achieved could be different for disease-associated mutant forms of coagulation factor XII when compared to wild-type coagulation factor XII (poly)peptide(s).

[0081] The term "binding partner" refers to a molecule capable of interacting with a (poly)peptide encoded by the coagulation factor XII gene. The binding activity of coagulation factor XII (poly)peptides may be determined by using a binding assay. The skilled person knows from *in vitro* studies that coagulation factor XII may bind for example to activating surfaces or substances, proteins or protein complexes. The prior art reported for example about the binding of coagulation factor XII to complexes of gC1q-R, cytokeratin 1 and urokinase plasminogen activator receptor present on the surface of endothelial cells (Joseph et al. 1996, *Proc. Natl. Acad. Sci. USA* 93: 8552-8557; Joseph et al. 2001, *Thromb. Haemost.* 85: 119-124; Mandi et al. 2002, *Blood* 99: 3585-3596). The binding partner can also be an antibody. Binding assays are described in detail in the prior art and may be used by the skilled person in order to determine whether a sample contains coagulation factor XII (poly)peptide(s) with normal or

aberrant binding characteristics. This will allow deductions on the nature of the coagulation factor XII (poly)peptide(s) present in the sample under study.

[0082] The present invention also relates to a method of identifying a compound modulating coagulation factor XII activity which is suitable as a medicament or a lead compound for a medicament for the treatment and/or prevention of hereditary angioedema type III, the method comprising the steps of: (a) in vitro contacting a coagulation factor XII (poly)peptide or a functionally related (poly)peptide with the potential modulator; and (b) testing for modulation of coagulation factor XII activity, wherein modulation of coagulation factor XII activity is indicative of a compound's suitability as a medicament or a lead compound for a medicament for the treatment and/or prevention of hereditary angioedema type III.

[0083] The term "modulator" or "modulating compound" refers to a compound which alters the activity and/or the expression and/or the secretion of coagulation factor XII. This includes also the modulation of a "functionally related (poly)peptide", thus of (a) (poly)peptide(s) or the expression thereof being related to the function and/or expression and/or secretion of coagulation factor XII, preferably functionally related to coagulation factor XII upstream or downstream within the contact system/kinin pathway. In principle, a modulator can have an activating or an inhibiting effect. It is also envisaged that the modulator can differentially modulate only one or more of the various functions of coagulation factor XII. The modulator can be, for example, a 'small molecule', an aptamer, or an antibody (see below). In accordance with the present invention, the modulator is preferably a compound interacting with a coagulation factor XII (poly)peptide, and, more preferably, an inhibiting compound.

[0084] The term "contacting" means bringing in contact the targeted (poly)peptide, preferably a coagulation factor XII (poly)peptide with a potential modulator. Said coagulation factor XII (poly)peptide is preferably a polypeptide selected from any of the aforementioned (poly)peptides (1) to (7). By bringing in contact the (poly)peptide with a potential modulator of activity, the skilled person can test the impact of the modulator on the (poly)peptide's activity. Examples for assays for measuring various activities of coagulation factor XII (poly)peptides, including the binding to activating substances or other binding partners, have been described above and can be used for testing of potential modulators.

[0085] Coagulation factor XII (poly)peptide(s) used for contacting with a potential modulator may generate from various sources. For example, coagulation factor XII (poly)peptide(s) may be isolated from human plasma, either from healthy individuals or from patients affected by HAE type III; to this end, various methods known in the art may be used, for example those described by Pixley & Colman 1993 (Methods Enzymol. 222: 51-65). Alternatively, coagulation factor XII (poly)peptide(s) may also be produced synthetically. Further, coagulation factor XII (poly)peptide(s) may be recombinantly expressed. To this end, nucleic acid molecules encoding coagulation factor XII (poly)peptides may be introduced into a host cell. The term "introducing" refers to the process of transfecting or transforming a host cell with such a nucleic acid molecule. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard labo-

ratory manuals, such as Davis et al., *Basic Methods In Molecular Biology* (1986). Said nucleic acid molecule introduced into the host cell comprises an open reading frame encoding a coagulation factor XII (poly)peptide in expressible form. A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements might include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from retroviruses, e.g., RSV, HTLV1, HIV1, and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells. Alternatively, the recombinant (poly)peptide can be expressed in stable cell lines that contain the gene construct integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells. The transfected nucleic acid can also be amplified to express large amounts of the encoded (poly)peptide. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al. 1991, *Biochem J.* 227:277-279; Bebbington et al. 1992, *Bio/Technology* 10:169-175). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins. The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al. 1985, *Molecular and Cellular Biology* 5: 438-447) plus a fragment of the CMV-enhancer (Boshart et al. 1985, *Cell* 41:521-530). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites Barn HI, Xba I and Asp 718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene. As indicated above, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, 293 and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

[0086] The recombinantly expressed polypeptide may contain additional amino acid residues in order to increase the stability or to modify the targeting of the protein. For

instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to stabilize and purify proteins. For example, EP-A-0 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0 232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when the Fc portion proves to be a hindrance for example for the catalytic activity of a coagulation factor XII (poly)peptide. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., *J. Molecular Recognition* 8:52-58 (1995) and K. Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995). Coagulation factor XII (poly)peptide(s) can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography and/or hydroxylapatite chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

[0087] The step of contacting the recovered coagulation factor XII (poly)peptide with a potential modulator is essentially a step by which the efficacy of a potential modulator is tested. Generally, the coagulation factor XII (poly)peptide is present at conditions assumed to be physiological conditions or in a test solution representing such conditions. When examining, for example, enzymatic activity, the following may be of importance: after optimum substrate and enzyme concentrations are determined, a candidate modulator is added to the reaction mixture at a range of concentrations. The assay conditions ideally should resemble the conditions under which the modulator is to be active, i.e., under physiologic pH, temperature, ionic strength, etc. For example, when the modulator is an inhibitor of protease activity, suitable inhibitors will exhibit strong protease inhibition at concentrations which do not raise toxic side effects in the subject. Inhibitors which compete for binding to the protease's active site may require concentrations equal to or greater than the substrate concentration, while inhibitors capable of binding irreversibly to the protease's active site may be added in concentrations in the order of the enzyme concentration. Substrate conversion, i.e. proteolytic cleavage is conveniently measured by using labelled substrates such as labelled peptides representing the cleavage site of a natural substrate of coagulation factor XII.

[0088] One of the more popular protease detection methods is the use of fluorescence resonance energy transfer between a donor fluorophore at one end of the peptide chain, and a quencher at the other end of the peptide chain. These methods were reviewed by Knight "Fluorimetric assays of proteolytic enzymes," *Methods in Enzymol.* (1995) 248:18-34, the contents of which are incorporated herein by reference. Here, proteolytic cleavage of the peptide link connecting the fluorophore and quencher liberates the quencher to diffuse away from the fluorophore. This results in an increase in fluorescence. A variation on this quencher method is taught by U.S. Pat. Nos. 5,605,809 and 6,037,137. This variation brings a first fluorophore in close proximity to a second fluorophore via a folded peptide backbone. This technique has the advantage that the protease cleavage site need not be immediately adjacent to either of the fluorophores. However it has the disadvantage that to avoid disrupting the folded structure, the length of the protease cleavage site should ideally fall between 2-15 amino acid residues in length. Another very popular method is the use of peptide-quenched fluorescent moieties, such as the 7-amino-4-methylcoumarin (AMC) fluorophore, the 7-amino-4-carbamoylmethylcoumarin fluorophore (Harris, et. al. *PNAS* 97: 7754-7759 (2000)), or the peptide quenched Rhodamine 110 fluorophore (Mange) et. al., U.S. Pat. No. 4,557,862). Here the intrinsic fluorescence of a fluorophore is quenched by one or more covalently linked peptides, and the fluorescence is restored upon cleavage of the peptide. Although the Rhodamine 110 molecule operates with high efficiency, uses visible light for excitation and emission, and is otherwise an excellent label for fluorescence based protease assays, it has a few drawbacks that limit its use. The Rhodamine 110 molecule is divalent and normally incorporates two peptides of identical sequence, with both "N" terminal peptide groups exposed. This has the drawback that peptides with this polarity can not be incorporated into the interior of a larger peptide chain. Thus this label has primarily been used for protease substrate assays where the Rhodamine 110 molecule effectively represents the final "C" terminal group on the substrate. Variations on Rhodamine 110 molecule methods, suitable for caspase assays, are taught by U.S. Pat. No. 6,248,904.

[0089] The test for protease activity of coagulation factor XII (poly)peptides may be performed in solution or with the coagulation factor XII (poly)peptide or the substrate or the modulator arrayed on a solid support, e.g. a microtiter plate. Microarray methods have become widely used for pharmaceutical and biochemical research, and a large number of microarrays are commercially available. Use of peptide microarrays, constructed by photochemical methods, for antibody recognition of peptide patterns was taught by Fodor et. al. 1991, *Science* 251: 767-773. Use of peptide microarrays for protein kinase or protein-protein binding was taught by MacBeath and Schreiber 2000, *Science* 289: 1760-1763. Here glass slides were chemically activated to covalently bind peptides, and various peptides were spotted onto the slides using conventional spotting equipment. The peptides formed a covalent bond with the derivatized glass. Alternative methods to attach peptides to solid supports are taught by U.S. Pat. No. 6,150,153, which teaches the use of polyethyleneimine layers to facilitate peptide linkages. U.S. Pat. No. 4,762,881 teaches the use of incorporating an artificial benzoylphenylalanine into a peptide and allowing the peptide to attach to a solid substrate having an active hydrogen (such as polystyrene) using ultraviolet light. U.S. Pat. No. 4,681,870 teaches

methods for derivatizing silica surfaces to introduce amino or carboxyl groups, and then coupling proteins to these groups. U.S. Pat. Nos. 5,527,681 and 5,679,773 teach methods for immobilized polymer synthesis and display suitable for microarrays, and various fluorescent-labeling methods to detect proteolytic cleavage.

[0090] For protease substrate microarrays, the peptides on the microarray will further contain detection moieties (fluorescent tags, fluorescent quenchers, etc.) to generate a detectable signal corresponding to the level of proteolytic cleavage of the particular peptide zone in question. The peptides are bound to the surface of the solid support (either covalently or non-covalently) to the extent sufficient to prevent diffusion of the bound peptides upon application of liquid sample, and subsequent digestion and processing steps. In use, the completed microarray is exposed to a liquid sample, which contains a coagulation factor XII (poly)peptide under study. The sample will typically be covered with an optional cover to help distribute the sample evenly over the array, and to prevent evaporation. Typically the cover will be of a transparent flat material, such as a glass or plastic cover slip, to enable observation of the peptide zones during the course of the digestion reaction. During the protease digestion reaction, peptides with differential sequences or different modifications will typically be digested to a differential amount. The detectable signal generated by the detection moieties attached to each peptide region will be interrogated, typically at multiple time points during the digestion reaction. This conveys information as to the relative proteolytic activity of the studied coagulation factor XII (poly)peptide in the presence of a potential protease modulator or inhibitor, thus providing information on the suitability of the modulator for modulating, eventually inhibiting coagulation factor XII activity. Optionally, at the end of the reaction, a non-specific protease or a non-specific labeled moiety reacting agent may be added to the microarray to serve as a positive or negative control.

[0091] In a preferred embodiment of the present invention's method of identifying a modulator compound, the coagulation factor XII (poly)peptide of step (a) is present in cell culture or cell culture supernatant or in a subject's sample or purified from any of these sources. The cell culture could be for example a cell culture in which a coagulation factor XII (poly)peptide is recombinantly expressed or a culture of cells, for example hepatocytes, and preferably of human origin, that naturally express coagulation factor XII. The subject's sample could be for example blood plasma, and the subject could be either affected or non-affected by hereditary angioedema type III.

[0092] In another preferred embodiment of the present invention's method of identifying a modulator compound, said testing is performed by assessing the physical interaction between a coagulation factor XII (poly)peptide and the modulator and/or the effect of the modulator on the function of said coagulation factor XII (poly)peptide.

[0093] The person skilled in the art knows of various methods for detecting the interaction between a protein and a potential binding partner or modulator. One such method, for example, may be based on the testing of potential binding partners which are spotted onto a solid support. If bound to a solid support, incubation of said potential binding partners with a solution containing, for example, coagulation factor XII (poly)peptide might identify positions on the solid support, occupied with candidate binding partners. Binding of, for example coagulation factor XII (poly)peptide(s) to said

binding partner may be detected by various methods known in the art. For example, binding of coagulation factor XII to a binding partner could be visualized by incubating the solid support with a labeled antibody specific for coagulation factor XII. Preferred methods comprise biacore based detection methods, ELISA based methods.

[0094] It is also envisaged here, that the (poly)peptide targeted by the potential modulator can be—instead of a coagulation factor XII (poly)peptide—a (poly)peptide functionally related, upstream or downstream within the contact system, with coagulation factor XII, i.e. interacting with coagulation factor XII. Nevertheless, as further envisaged here, this may cause a modulation of coagulation factor XII activity.

[0095] A modulator may be based on known compounds which may also be modified in order to adapt the compound to the requirements of the specific (poly)peptide to be targeted. The modulator can be, for example, a small molecule, an aptamer, or an antibody (*vide infra*).

[0096] Preferably, the modulator is a small molecule or small molecular compound and may be selected by screening a library of small molecules ("small molecule library"). The term "small molecule" or "small molecular compound" refers to a compound having a relative molecular weight of not more than 1000 D and preferably of not more than 500 D. It can be of organic or inorganic nature. A large number of small molecule libraries, which are commercially available, are known in the art. Thus, for example, a modulator may be any of the compounds contained in such a library or a modified compound derived from a compound contained in such a library. Preferably, such a modulator binds to the targeted (poly)peptide encoded by the coagulation factor XII gene with sufficient specificity, wherein sufficient specificity means preferably a dissociation constant (K_d) of less than 500 nM, more preferable less than 200 nM, still more preferable less than 50 nM, even more preferable less than 10 nM and most preferable less than 1 nM.

[0097] It is also envisaged to design small molecular compounds using so called molecular modeling methods. Small molecular compounds can be for example peptide derived. Preferred are compounds which mimic the transition state of substrates of coagulation factor XII. Suitable compounds may be, for example, peptide-derived substrates which do not contain a cleavable peptide bond. Preferably, such compounds contain a cleavage site of a natural substrate of coagulation factor XII, wherein the peptide bond between P1 and P1' is replaced by a non-cleavable bond.

[0098] The peptide-based compounds and others, like compounds based on heterocyclic structures, may be for example known inhibitors of serine proteases or new compounds or compounds derived from preexisting inhibitors by derivatization. Preferably, such compounds are designed by computer modeling, wherein computer modeling means using virtual-screening tools for the search of compounds that bind, for example, to the substrate binding site of coagulation factor XII by using homology-modeling tools. Generally, these methods rely on the three-dimensional structure of proteins, preferably of proteins crystallized together with a substrate. More preferably, the substrate is replaced with a candidate modulator or inhibitor.

[0099] The design of molecules with particular structural relationships to part of a protein molecule like coagulation factor XII is well established and described in the literature (see for example Cochran 2000, *Chem. Biol.* 7, 85-94; Grzybowski et al. 2002, *Acc. Chem. Res.* 35, 261-269; Velasquez-

Campoy et al. 2001, *Arch. Biochem. Biophys.* 380, 169-175; D'Aquino et al. 2000, *Proteins: Struc. Func. Genet. Suppl.* 4, 93-107.). Any of these so-called "molecular modeling" methods for rational drug design can be used to find a modulator of coagulation factor XII. Most of these molecular modeling methods take into consideration the shape, charge distribution and the distribution of hydrophobic groups, ionic groups and hydrogen bonds in the site of interest of the protein molecule. Using this information, that can be derived e.g. from the crystal structure of proteins and protein-substrate complexes, these methods either suggest improvements to existing proposed molecules, construct new molecules on their own that are expected to have good binding affinity, screen through virtual compound libraries for such molecules, or otherwise support the interactive design of new drug compounds in silico. Programs such as GOLD (G. Jones, et al., *Development and J. Mol. Biol.*, 267, 727-748 (1997)); FLEXX (B. Kramer et al., *Structure, Functions, and Genetics*, Vol. 37, pp. 228-241, 1999); FLEXE (M. Rarey et al., *JMB*, 261, 470-489 (1996)) DOCK (Kuntz, I.D. *Science* 257: 1078-1082, 1992); AUTODOCK (Morris et al., (1998), *J. Computational Chemistry*, 19: 1639-1662) are virtual screening programs designed to calculate the binding position and conformation as well as the corresponding binding energy of an organic compound to a protein. These programs are specially trimmed to allow a great number of "dockings", that is calculations of the conformation with the highest binding energy of a compound to a binding site, per time unit. Their binding energy is not always a real value, but can be statistically related to a real binding energy through a validation procedure. These methods lead to molecules, termed here "hits" that have to be evaluated by experimental biochemical, structural-biological, molecular-biological or physiological methods for their expected biological activity. The term "molecular modeling" or "molecular modeling techniques" refers to techniques that generate one or more 3D models of a ligand binding site or other structural feature of a macromolecule. Molecular modeling techniques can be performed manually, with the aid of a computer, or with a combination of these. Molecular modeling techniques can be applied for example to the atomic co-ordinates to derive a range of 3D models and to investigate the structure of ligand binding sites. A variety of molecular modeling methods are available to the skilled person for use according to the invention (G. Klebe and H. Gohlke, *Angew. Chem. Int. Ed.* 2002, 41, 2644-2676; Jun Zeng: *Combinatorial Chemistry & High Throughput Screening*, 2000, 3, 355-362; Andrea G Cochran, *Current Opinion in Chemical Biology* 2001, 5:654-659).

[0100] In a preferred embodiment, the modulator is an inhibitor of coagulation factor XII activity, selected from the group consisting of: (a) an aptamer or inhibitory antibody or fragment or derivative thereof, specifically binding to a coagulation factor XII (poly)peptide and/or specifically inhibiting a coagulation factor XII activity; (b) a small molecule inhibitor of coagulation factor XII and/or coagulation factor XII activity; and (c) a serine protease inhibitor selected from group (I) consisting of wild-type and modified or engineered proteinaceous inhibitors of serine proteases including C1 esterase inhibitor, antithrombin III, α 2-antiplasmin, α 1-antitrypsin, ovalbumin serpins, and α 2-macroglobulin, or selected from group (II) of Kunitz-type inhibitors including bovine pancreatic trypsin inhibitor.

[0101] The inhibitor can be an aptamer, preferably an aptamer specifically binding to coagulation factor XII. The

term "aptamer" refers to RNA and also DNA molecules capable of binding target proteins with high affinity and specificity, comparable with the affinity and specificity of monoclonal antibodies. Methods for obtaining or identifying aptamers specific for a desired target are known in the art. Preferably, these methods may be based on the "systematic evolution of ligands by exponential enrichment" (SELEX) process (Ellington and Szostak, *Nature*, 1990, 346: 818-822; Tuerk and Gold, 1990, *Science* 249: 505-510; Fitzwater & Polisky, 1996, *Methods Enzymol.* 267: 275-301). Various chemical modifications, for example the use of 2'-fluoropyrimidines in the starting library and the attachment of a polyethylene glycol to the 5' end of an aptamer can be used to ensure stability and to enhance bioavailability of aptamers (see e.g. Toulme 2000, *Current Opinion in Molecular Therapeutics* 2: 318-324).

[0102] The inhibitor can also be an antibody or fragment or derivative thereof. As used herein, the term "antibody or fragment or derivative thereof" relates to a polyclonal antibody, monoclonal antibody, chimeric antibody, single chain antibody, single chain Fv antibody, human antibody, humanized antibody or Fab fragment specifically binding to coagulation factor XII and/or to a mutant of coagulation factor XII.

[0103] The antibodies described herein may be prepared by any of a variety of methods known in the art. For example, polyclonal antibodies may be induced by administration of purified protein, a coagulation factor XII (poly)peptide or an antigenic fragment thereof, to a host animal.

[0104] As pointed out above, the antibody may also be a monoclonal antibody. Such monoclonal antibodies can be prepared using hybridoma technology (Köhler et al., *Nature* 256:495 (1975); Köhler et al., *Eur. J. Immunol.* 6:511 (1976); Köhler et al., *Eur. J. Immunol.* 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., 1981, pp. 563-681). In general, such procedures involve immunizing an animal (preferably a mouse) with a coagulation factor XII protein antigen. The splenocytes of such immunized mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2/0), available from the American Type Culture Collection, Rockville, Md. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. 1981 (*Gastroenterology* 80:225-232). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the coagulation factor XII protein antigen.

[0105] It will be appreciated that Fab and F(ab')₂ and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments).

[0106] For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Cabilly et al., U.S. Pat. No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al.,

WO 8601533; Robinson et al., WO 8702671; Boulianne et al., *Nature* 312:643 (1984); Neuberger et al., *Nature* 314:268 (1985).

[0107] Preferably, the antibodies specifically bind a coagulation factor XII (poly)peptide and include IgG (including IgG1, IgG2, IgG3, and IgG4), IgA (including IgA1 and IgA2), IgD, IgE, or IgM, and IgY. As used herein, the term “antibody” is meant to include whole antibodies, including single-chain whole antibodies, and antigen-binding fragments thereof. Most preferably the antibodies are human antigen binding antibody fragments and include, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. The antibodies may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, rabbit, goat, guinea pig, camel, horse, or chicken.

[0108] “Specific binding” of antibodies may be described, for example, in terms of their cross-reactivity. Preferably, specific antibodies are antibodies that do not bind polypeptides with less than 98%, less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70% and less than 65% identity (as calculated using methods known in the art) to a (poly)peptide encoded by the coagulation factor XII gene. Antibodies may, however, also be described or specified in terms of their binding affinity. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10⁻⁶M, 10⁻⁶M, 5×10⁻⁷M, 10⁻⁷M, 5×10⁻⁸M, 10⁻⁸M, 5×10⁻⁹M, 10⁻⁹M, 5×10⁻¹⁰M, 10⁻¹⁰M, 5×10⁻¹¹M, 10⁻¹¹M, 5×10⁻¹²M, 10⁻¹²M, 5×10⁻¹³M, 10⁻¹³M, 5×10⁻¹⁴M, 10⁻¹⁴M, 5×10⁻¹⁵M, and 10⁻¹⁵M.

[0109] Further, the inhibitor can be a “small molecule” or “small molecular compound”. As pointed out above, the term “small molecule” refers to a compound having a relative molecular weight of not more than 1000 D and preferably of not more than 500 D. Said compound may be of differing chemical nature, for example, it may be peptide-based or based on heterocyclic structures. Small molecule inhibitors of serine proteases have been extensively reviewed for example by Leung et al. 2000 (*J. Med. Chem.* 43: 305-341) and Walker & Lynas 2001 (*Cell. Mol. Life. Sci.* 58: 596-624). Substances discussed by these authors include, for example, (i) peptide-based inhibitors, like phosphorus-based inhibitors (including α-aminoalkyl diphenylphosphonate esters and mixed phosphonate esters), fluorine-containing inhibitors (including for example trifluoromethyl ketones [as well as analogues containing the trifluoromethyl ketone moiety with lower peptidic characteristics], difluoromethyl ketone-based and pentafluoroethyl ketone-based inhibitors), inhibitors based on peptidyl boronic acids (including, for example, boroArg- or boroLys- or boro-methoxy-propylglycine- or boroPro-containing substances), inhibitors based on so-called ‘inverse substrates’ (including, for example, compounds containing a p-methoxybenzoic acid function), and peptide-based inhibitors with novel functional groups (including, for example, compounds with O-terminal electron-withdrawing groups based on α-keto heterocycles, like α-keto benzoxazoles or α-keto thiazoles); (ii) natural product-derived inhibitors, like cyclotheonamides (macrocyclic pentapeptides analogues), aeruginosins, and radiosumin; (iii) inhibitors based on heterocyclic and other nonpeptide scaffolds, like N-hydroxysuccinimide heterocycles and related compounds, compounds based on the isocoumarin scaffold, and β-lactam-based inhibitors (including, for example, cephalosporin-derived compounds and

analogues of monocyclic and bicyclic β-lactams); and (iv) metal-potentiating compounds, like compounds based on bis (5-amidino-2-benzimidazolyl)methane (BABIM). All these (types of) substances, as well as derivatives thereof, are considered to be applicable for the purposes of the present invention.

[0110] Any of the known protease inhibitors may be useful for developing modulators or inhibitory modulators of coagulation factor XII activity, although inhibitors of serine proteases may be particularly useful. Any of the known compounds may be modified, for example in order to change their binding characteristics or their specificity.

[0111] With respect to natural or engineered proteinaceous inhibitors of serine proteases, selective changes or modifications of the natural inhibitory characteristics, of the natural specificity have been achieved, for example, with P2 mutants of C1 inhibitor (Zahedi et al. 2001, *J. Immunol.* 167: 1500-1506), a P1 mutant of α1-antitrypsin (Schapira et al. 1985, *J. Clin. Invest.* 76: 645-647), various P1-P2-P3 mutants of α1-antitrypsin (Sulikowski et al. 2002, *Protein Science* 11: 2230-2236), a P1-P2 mutant of α1-antitrypsin (Schapira et al. 1987, *J. Clin. Invest.* 80: 582-585), various P3-P4 mutants of bovine pancreatic trypsin inhibitor (Grzesiak et al. 2000, *J. Biol. Chem.* 275: 33346-33352), among them one P3 mutant with high specificity for factor XIIa.

[0112] Particularly with respect to (a) and (b), it is also envisaged that the “inhibitor of coagulation factor XII activity” could be a compound that does not primarily target a coagulation factor XII (poly)peptide, but still inhibits coagulation factor XII activity, for example by inhibiting the activation of coagulation factor XII due to interference with an activating protein. The present invention also relates to a method of identifying a compound modulating coagulation factor XII expression and/or secretion which is suitable as a medicament or lead compound for a medicament for the treatment and/or prevention of hereditary angioedema type III, the method comprising the steps of: (a) in vitro contacting a cell that expresses or is capable of expressing coagulation factor XII with a potential modulator of expression and/or secretion; and (b) testing for altered expression and/or secretion, wherein the modulator is (i) a small molecule compound, an aptamer or an antibody or fragment or derivative thereof, specifically modulating expression and/or secretion of coagulation factor XII; or (ii) a siRNA or shRNA, a ribozyme, or an antisense nucleic acid molecule specifically hybridizing to a nucleic acid molecule encoding coagulation factor XII or regulating the expression of coagulation factor XII. “Specific hybridization” means that the siRNA, shRNA, ribozyme or antisense nucleic acid molecule hybridizes to the targeted nucleic acid molecule, encoding coagulation factor XII or regulating its expression. Preferably, “specific hybridization” also means that no other genes or transcripts are affected.

[0113] A modulating compound will affect expression and/or secretion of coagulation factor XII. The skilled person knows a number of techniques for monitoring an effect on protein expression or secretion. For example, protein expression may be monitored by using techniques such as western blotting, immunofluorescence or immunoprecipitation. Alternatively, expression may also, for example, be monitored by analyzing the amount of RNA transcribed from a coagulation factor XII gene.

[0114] The term “contacting a cell” refers to the introduction of a potential modulator compound into a cell. As far as

the compound is a nucleic acid molecule, the contacting may be performed by any of the known transfection techniques such as electroporation, calcium phosphate transfection, lipofection and the like. However, the nucleic acid may also be entered into the cell by virus based vector systems.

[0115] As used herein, the term “siRNA” means “short interfering RNA”, the term “shRNA” refers to “short hairpin RNA”. In RNA interference, small interfering RNAs (siRNA) bind the targeted mRNA in a sequence-specific manner, facilitating its degradation and thus preventing translation of the encoded protein. Transfection of cells with siRNAs can be achieved, for example, by using lipophilic agents (among them Oligofectamine™ and Transit-TKO™) and also by electroporation.

[0116] Methods for the stable expression of small interfering RNA or short hairpin RNA in mammalian, also in human cells are known to the person skilled in the art and are described, for example, by Paul et al. 2002 (Nature Biotechnology 20: 505-508), Brummelkamp et al. 2002 (Science 296: 550-553), Sui et al. 2002 (Proc. Natl. Acad. Sci. U.S.A. 99: 5515-5520), Yu et al. 2002 (Proc. Natl. Acad. Sci. U.S.A. 99: 6047-6052), Lee et al. 2002 (Nature Biotechnology 20: 500-505), Xia et al. 2002 (Nature Biotechnology 20: 1006-1010). It has been shown by several studies that an RNAi approach is suitable for the development of a potential treatment of dominantly inherited diseases by designing a siRNA that specifically targets the disease-associated mutant allele, thereby selectively silencing expression from the mutant gene (Miller et al. 2003, Proc. Natl. Acad. Sci. U.S.A. 100: 7195-7200; Gonzalez-Alegre et al. 2003, Ann. Neurol. 53: 781-787).

[0117] The siRNA molecules are essentially double-stranded but may comprise 3' or 5' overhangs. They may also comprise sequences that are not identical or essentially identical with the target gene but these sequences must be located outside of the sequence of identity. The sequence of identity or substantial identity is at least 14 and more preferably at least 19 nucleotides long. It preferably does not exceed 23 nucleotides. Optionally, the siRNA comprises two regions of identity or substantial identity that are interspersed by a region of non-identity. The term “substantial identity” refers to a region that has one or two mismatches of the sense strand of the siRNA to the targeted mRNA or 10 to 15% over the total length of siRNA to the targeted mRNA mismatches within the region of identity. Said mismatches may be the result of a nucleotide substitution, addition, deletion or duplication etc. dsRNA longer than 23 but no longer than 40 by may also contain three or four mismatches.

[0118] The interference of the siRNA with the targeted mRNA has the effect that transcription/translation is reduced by at least 50%, preferably at least 75%, more preferred at least 90%, still more preferred at least 95%, such as at least 98% and most preferred at least 99%.

[0119] Further, the modulator can be an antisense nucleic acid molecule specifically hybridizing to a nucleic acid molecule encoding coagulation factor XII or regulating the expression of coagulation factor XII. The term “antisense nucleic acid molecule” refers to a nucleic acid molecule which can be used for controlling gene expression. The underlying technique, antisense technology, can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); “Oligodeoxynucleotides as Antisense Inhibitors of

Gene Expression.” CRC Press, Boca Raton, Fla. (1988), or in: Phillips MI (ed.), Antisense Technology, Methods in Enzymology, Vol. 313, Academic Press, San Diego (2000). Triple helix formation is discussed in, for instance, Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). The methods are based on binding of a target polynucleotide to a complementary DNA or RNA. For example, the 5' coding portion of a polynucleotide that encodes a coagulation factor XII (poly)peptide may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a gene region involved in transcription thereby preventing transcription and the production of coagulation factor XII. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into coagulation factor XII polypeptide.

[0120] The term “ribozyme” refers to RNA molecules with catalytic activity (see, e.g., Sarver et al, Science 247:1222-1225 (1990)); however, DNA catalysts (deoxyribozymes) are also known. Ribozymes and their potential for the development of new therapeutic tools are discussed, for example, by Steele et al. 2003 (Am. J. Pharmacogenomics 3: 131-144) and by Puerta-Fernandez et al. 2003 (FEMS Microbiology Reviews 27: 75-97). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy coagulation factor XII mRNAs, the use of trans-acting hairpin or hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of the coagulation factor XII mRNA which will be apparent to the person skilled in the art. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the coagulation factor XII mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts. RNase P is another ribozyme approach used for the selective inhibition of pathogenic RNAs. Ribozymes may be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express coagulation factor XII. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of other nucleic acid molecules. A preferred method of delivery involves using a DNA construct “encoding” the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous coagulation factor XII messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is generally required for efficiency. Ribozyme-mediated RNA repair is another therapeutic option applying ribozyme technologies (Watanabe & Sullenger 2000, Adv. Drug Deliv. Rev. 44: 109-118) and may also be useful for the purpose of the present invention. To this end, catalytic group I introns can be employed in a trans-splicing reaction to replace a defective segment of target mRNA in order to alleviate a mutant phenotype.

[0121] In a preferred embodiment of the method of the present invention, coagulation factor XII is a disease-associated mutant of coagulation factor XII. As pointed out above, in order to determine whether or not a mutation is disease-associated, the person skilled in the art may, for example, compare the frequency of a specific sequence change, for example in the coagulation factor XII gene, in patients affected by HAE type III with the frequency in appropriately chosen control individuals and conclude from a statistically significantly deviating frequency in the patient group that said mutation is a disease-associated mutation.

[0122] In another preferred embodiment of the present invention, said modulator is selective for a disease-associated mutant of coagulation factor XII, the method comprising (a) comparing the effect of the modulator on wild-type and disease-associated coagulation factor XII activity or their expression and/or secretion; and (b) selecting a compound which (i) modulates disease-associated coagulation factor XII activity or its expression and/or secretion and which (ii) does not affect wild-type coagulation factor XII activity or its expression and/or secretion. By using this method, the skilled person can determine whether a modulating compound is a general modulator of coagulation factor XII or selective for disease-associated coagulation factor XII. It is also possible and envisaged that a modulator affects preferably disease-associated coagulation factor XII, and partially, but to a lesser extent, also wild-type coagulation factor XII.

[0123] In yet another preferred embodiment of the present invention's methods, the disease-associated mutant or mutation is: (a) a mutant located in the fibronectin type II domain, within the region of amino acid position 1 to 76, and/or a mutation located in the nucleic acid sequence encoding the fibronectin type II domain, within mRNA position 107 to 334; (b) a mutant located in the EGF-like domain 1, within the region of amino acid position 77 to 113, and/or a mutation located in the nucleic acid sequence encoding the EGF-like domain 1, within mRNA position 335 to 445; (c) a mutant located in the fibronectin type I domain, within the region of amino acid position 114 to 157, and/or a mutation located in the nucleic acid sequence encoding the fibronectin type I domain, within mRNA position 446 to 577; (d) a mutant located in the EGF-like domain 2, within the region of amino acid position 158 to 192, and/or a mutation located in the nucleic acid sequence encoding the EGF-like domain 2, within mRNA position 578 to 682; (e) a mutant located in the kringle domain, within the region of amino acid position 193 to 276, and/or a mutation located in the nucleic acid sequence encoding the kringle domain, within mRNA position 683 to 934; (f) a mutant located in the proline-rich region, within the region of amino acid position 277 to 331, and/or a mutation located in the nucleic acid sequence encoding the proline-rich region, within mRNA position 935 to 1099; (g) a mutant located in the region of proteolytic cleavage sites, within the region of amino acid position 332 to 353, and/or a mutation located in the nucleic acid sequence encoding the region of proteolytic cleavage sites, within mRNA position 1100 to 1165; (h) a mutant located in the serine protease domain, within the region of amino acid position 354 to 596, and/or a mutation located in the nucleic acid sequence encoding the serine protease domain, within mRNA position 1166 to 1894; (i) a mutant located in the signal peptide, within the region of amino acid position -19 to -1, and/or a mutation located in the nucleic acid sequence encoding the signal peptide, within mRNA position 50 to 106; (j) a mutation located in the

untranslated regions (UTRs) of coagulation factor XII mRNA, within mRNA position 1 to 49 and/or 1895 to 2048; (k) a mutation located in an intron of the coagulation factor XII gene; and/or (l) a mutation located in a flanking regulatory genomic sequence of the coagulation factor XII gene, within the region encompassing 4000 bp upstream of the transcription initiation site of the coagulation factor XII gene and/or within the region encompassing 3000 bp downstream of the nucleotide sequence representing the 3'-UTR of the coagulation factor XII mRNA.

[0124] The above numbering of amino acid residues of human coagulation factor XII refers to the numbering as given for example in Cool & MacGillivray 1987 (J. Biol. Chem. 262: 13662-13673). The numbering of mRNA positions refers to GenBank acc. no. NM_000505.2. Introns of the coagulation factor XII gene are preferably introns one to thirteen as given for example in the Seattle data (<http://pga.gs.washington.edu/data/f12/f12.ColorFasta.html>) or in the UCSC Genome Browser/July 2003 human reference sequence/chr5:176,810,093-176,817,530. Also according to the July 2003 human reference sequence of the UCSC Genome Browser, flanking regulatory sequences of the coagulation factor XII gene, as given above, encompass nucleotide positions chr5:176,817,531 to 176,821,030 and nucleotide positions chr5:176,807,093 to 176,810,092.

[0125] In a more preferred embodiment of the present invention said mutant located in the proline-rich region is a mutant affecting amino acid residues 309 or 310 of human coagulation factor XII. This preferred embodiment refer to the observation, as described in detail in the Examples of the present invention, that in a significant number of patients studied a mutation in the region encoding the proline-rich region of the human coagulation factor XII gene could be detected. This mutation is one example of the mutations expected according to the teaching of the present invention and useful for diagnosis of HAE III as well as for diagnostic evaluation of any patient presenting with angioedema or angioedema-related symptoms. The skilled person can now use the knowledge disclosed herein and adapt conventional methods or use the methods of the present invention in order to e.g. diagnose a patient suspected of having developed or of having a predisposition to develop a hereditary angioedema type III or in a subject being suspected of being a carrier for hereditary angioedema type III. Moreover, the skilled person can search for the additional specific mutations within the coagulation factor XII gene, as expected according to the teaching of the present invention.

[0126] As used herein, position 309 and 310 refer to the numbering of the mature coagulation factor XII protein, wherein the first amino acid at the N-terminal end of the mature factor XII protein is amino acid residue number 1.

[0127] In another more preferred embodiment of the present invention, said amino acid residue at position 309 is substituted by a basic or positively charged amino acid residue. The term "basic or positively charged amino acid residue" refers to arginine, lysine and histidine.

[0128] In another more preferred embodiment of the present invention, said basic or positively charged amino acid residue is a lysine or arginine.

[0129] In a preferred embodiment, the present invention's method comprises the additional step of producing the modulator identified in said methods.

[0130] In another preferred embodiment, the present invention's method comprises in vitro testing of a sample of a blood

donor for determining whether the blood of said donor or components thereof may be used for transfusion to a patient in need thereof, wherein a positive testing indicates a predisposition for hereditary angioedema type III, excluding the transfusion of blood or components thereof from said donor.

[0131] The present invention also relates to the use of (a) a (poly)peptide encoded by the coagulation factor XII gene or a fragment thereof, (b) a modulator of coagulation factor XII identified by any of the methods of claims 13 to 21; (c) a nucleic acid molecule capable of expressing coagulation factor XII or a fragment thereof; and/or (d) a nucleic acid molecule capable of expressing a modulator of coagulation factor XII activity or its expression and/or secretion, for the preparation of a pharmaceutical composition for the treatment and/or prevention of hereditary angioedema type III. Said modulator of coagulation factor XII may be any of the modulating compounds identified by the methods of the present invention or any of the modulating compounds disclosed in the present invention. As such, the modulator may be affecting the expression from the coagulation factor XII gene or may modulate the secretion or function of coagulation factor XII. Preferably, the modulating compound is an inhibitor of coagulation factor XII activity or of its expression or secretion. The use of (a) and (c) may be envisaged, for example, with the purpose of a vaccination, either protein-based or DNA-based, to stimulate an immune response against coagulation factor XII (*vide infra*).

[0132] The active components of a pharmaceutical composition such as, e.g. a small molecular compound or an antibody, will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient, the site of delivery of pharmaceutical composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of the components of the pharmaceutical composition for purposes herein is thus determined by such considerations.

[0133] As a general proposition, the total pharmaceutically effective amount of for example a proteinaceous compound administered parenterally per dose will be in the range of about 1 µg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect. Pharmaceutical compositions may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers for example to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

[0134] The pharmaceutical composition is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers* 22:547-556

(1983)), poly (2-hydroxyethyl methacrylate (R. Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and R. Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., *Id.*) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include for example liposomally entrapped components. Liposomes containing the active components of the pharmaceutical composition are prepared by methods known per se: DE 3,218, 121; Epstein et al., *Proc. Natl. Acad. Sci. (USA)* 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci. (USA)* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal therapy.

[0135] Components to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0136] It is further envisaged in a preferred embodiment of the present invention's use, that said coagulation factor XII or said (poly)peptide is a mutant coagulation factor XII or mutant (poly)peptide or a fragment thereof. In one embodiment, the mutant is a disease-associated mutant of coagulation factor XII or a fragment thereof, which may be used, for example, for preparation of a vaccine to stimulate an immune response. In such a case, a fragment of coagulation factor XII would comprise at least 5, 6, 7, 8 or 9 consecutive amino acid residues of coagulation factor XII to provide an effective immunogen. Preferably, for this purpose the fragment would be a fragment comprising the mutant position of the disease-associated coagulation factor XII (poly)peptide. The use of modified, chimeric peptide constructs and other methods for creating a sufficient immunogenicity are known in the art (see e.g. Rittershaus et al. 2000, *Arterioscler. Thromb. Vasc. Biol.* 20:2106-2112). Alternatively, it is conceivable to engineer coagulation factor XII in such a way that the resulting mutant can for example displace a disease-associated mutant coagulation factor XII (poly)peptide from one of its interaction partners. Administering such a recombinant, i.e. mutant coagulation factor XII construct to a host may therefore be useful in treating, eventually also in preventing HAE type III. With respect to a modulator used for the preparation of a pharmaceutical composition and/or a nucleic acid molecule expressing a modulator it is envisaged here that the targeted coagulation factor XII (poly)peptide, or gene or mRNA species, is or contains a disease-associated mutant or mutation.

[0137] In a more preferred embodiment of the present invention's use, it is envisaged that the mutant is or is based on: (a) a mutant located in the fibronectin type II domain, within the region of amino acid position 1 to 76, and/or a mutation located in the nucleic acid sequence encoding the fibronectin type II domain, within mRNA position 107 to 334; (b) a mutant located in the EGF-like domain 1, within the region of amino acid position 77 to 113, and/or a mutation located in the nucleic acid sequence encoding the EGF-like domain 1, within mRNA position 335 to 445; (c) a mutant located in the fibronectin type I domain, within the region of amino acid position 114 to 157, and/or a mutation located in

the nucleic acid sequence encoding the fibronectin type I domain, within mRNA position 446 to 577; (d) a mutant located in the EGF-like domain 2, within the region of amino acid position 158 to 192, and/or a mutation located in the nucleic acid sequence encoding the EGF-like domain 2, within mRNA position 578 to 682; (e) a mutant located in the kringle domain, within the region of amino acid position 193 to 276, and/or a mutation located in the nucleic acid sequence encoding the kringle domain, within mRNA position 683 to 934; (f) a mutant located in the proline-rich region, within the region of amino acid position 277 to 331, and/or a mutation located in the nucleic acid sequence encoding the proline-rich region, within mRNA position 935 to 1099; (g) a mutant located in the region of proteolytic cleavage sites, within the region of amino acid position 332 to 353, and/or a mutation located in the nucleic acid sequence encoding the region of proteolytic cleavage sites, within mRNA position 1100 to 1165; (h) a mutant located in the serine protease domain, within the region of amino acid position 354 to 596, and/or a mutation located in the nucleic acid sequence encoding the serine protease domain, within mRNA position 1166 to 1894; (i) a mutant located in the signal peptide, within the region of amino acid position -19 to -1, and/or a mutation located in the nucleic acid sequence encoding the signal peptide, within mRNA position 50 to 106; (j) a mutation located in the untranslated regions (UTRs) of coagulation factor XII mRNA, within mRNA position 1 to 49 and/or 1895 to 2048; (k) a mutation located in an intron of the coagulation factor XII gene; and/or (l) a mutation located in a flanking regulatory genomic sequence of the coagulation factor XII gene, within the region encompassing 4000 bp upstream of the transcription initiation site of the coagulation factor XII gene and/or within the region encompassing 3000 bp downstream of the nucleotide sequence representing the 3'-UTR of the coagulation factor XII mRNA. Numbering of sequences etc. is as outlined earlier (vide supra).

[0138] In a more preferred embodiment of the present invention's use, said mutant located in the proline-rich region is a mutant affecting amino acid residue 309 or 310 of human coagulation factor XII.

[0139] In another more preferred embodiment of the present invention's use, said amino acid residue at position 309 is substituted by a basic or positively charged amino acid residue. The term "basic or positively charged amino acid residue" refers to arginine, lysine and histidine.

[0140] In another more preferred embodiment of the present invention's use, said basic or positively charged amino acid residue is a lysine or arginine.

[0141] In a more preferred embodiment of the present invention's use, it is envisaged that the modulator is an inhibitor of coagulation factor XII, its activity, its expression and/or its secretion, comprising: (a) an aptamer or an inhibitory antibody or fragment or derivative thereof, specifically binding to and/or specifically inhibiting the activity of (i) disease-associated coagulation factor XII or (ii) wild-type and disease-associated coagulation factor XII; (b) a small molecule inhibitor of (i) disease-associated coagulation factor XII and/or disease-associated coagulation factor XII activity; or (ii) wild-type and disease-associated coagulation factor XII and/or wild-type and disease-associated coagulation factor XII activity; (c) a serine protease inhibitor of (i) disease-associated coagulation factor XII or of (ii) wild-type and disease-associated coagulation factor XII selected from a first group consisting of wild-type and modified or engineered proteina-

ceous inhibitors of serine proteases including C1 esterase inhibitor, antithrombin III, α 2-antiplasmin, α 1-antitrypsin, ovalbumin serpins, and α 2-macroglobulin, or selected from a second group consisting of Kunitz-type inhibitors including bovine pancreatic trypsin inhibitor; or (d) a siRNA or shRNA, a ribozyme or an antisense nucleic acid molecule specifically hybridizing to a nucleic acid molecule encoding coagulation factor XII or regulating the expression of coagulation factor XII, either affecting (i) disease-associated coagulation factor XII or (ii) wild-type and disease-associated coagulation factor XII. In general, it may be a preferable type of treatment to target specifically the disease-associated mutant coagulation factor XII, its activity, expression and/or secretion. However, it may also be possible to use an inhibitor that targets wild-type as well as disease-associated mutant coagulation factor XII, their activity, expression or secretion; such an option appears particularly reasonable whenever the treatment is not a long-term or ultralong-term treatment.

[0142] The present invention also relates to a method of gene therapy in a mammal, characterized by administering an effective amount of a nucleic acid molecule capable of expressing in the mammal: (a) siRNA or shRNA, a ribozyme or an antisense nucleic acid molecule specifically hybridizing to a nucleic acid molecule encoding coagulation factor XII or regulating its expression; (b) an aptamer or an inhibitory antibody or fragment or derivative thereof, specifically binding coagulation factor XII (poly)peptide; (c) coagulation factor XII or a fragment thereof; or (d) a serine protease inhibitor selected from group (i) consisting of wild-type and modified or engineered proteinaceous inhibitors of serine proteases including C1 esterase inhibitor, antithrombin III, α 2-antiplasmin, α 1-antitrypsin, ovalbumin serpins, and α 2-macroglobulin, or selected from group (ii) of Kunitz-type inhibitors including bovine pancreatic trypsin inhibitor.

[0143] The gene therapy method relates to the introduction of nucleic acid sequences, DNA, RNA and/or antisense DNA or RNA sequences, into a mammal. This method requires a nucleic acid construct capable of expressing in the mammal (a) siRNA or shRNA, a ribozyme, or an antisense nucleic acid molecule specifically hybridizing to a nucleic acid molecule encoding or regulating the expression of coagulation factor XII; (b) an aptamer or an inhibitory antibody or fragment or derivative thereof, specifically binding coagulation factor XII (poly)peptide; (c) coagulation factor XII or a fragment thereof; or (d) a proteinaceous serine protease inhibitor, for example C1 esterase inhibitor, antithrombin III, α 2-antiplasmin, α 2-macroglobulin, α 1-antitrypsin, an ovalbumin serpin, or a Kunitz-type inhibitor, modified or engineered in such a way to specifically inhibit coagulation factor XII, preferably disease-associated mutant coagulation factor XII, and any other genetic elements necessary for the expression of the desired (poly)peptide or nucleic acid molecule by the target tissue. Such gene therapy and delivery techniques are known in the art; see, for example, WO90/11092, which is herein incorporated by reference, or: M. I. Phillips (Ed.): Gene Therapy Methods. Methods in Enzymology, Vol. 346, Academic Press, San Diego 2002. Thus, for example, cells from a patient may be engineered ex vivo with a nucleic acid construct comprising a promoter operably linked to the nucleic acid molecule corresponding to the molecule to be introduced, with the engineered cells then being provided to a patient to be treated. Such methods are well-known in the art. For example, see Beldegrun, A., et al., J. Natl. Cancer Inst. 85: 207-216 (1993); Ferrantini, M. et al., Cancer

Research 53: 1107-1112 (1993); Ferrantini, M. et al., *J. Immunology* 153: 4604-4615 (1994); Kaido, T., et al., *Int. J. Cancer* 60: 221-229 (1995); Ogura, H., et al., *Cancer Research* 50: 5102-5106 (1990); Santodonato, L., et al., *Human Gene Therapy* 7:1-10 (1996); Santodonato, L., et al., *Gene Therapy* 4:1246-1255 (1997); and Zhang, J.-F. et al., *Cancer Gene Therapy* 3: 31-38 (1996)), which are herein incorporated by reference. The cells which are engineered may be, for example, blood or liver cells. The nucleic acid construct used in gene therapy can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The nucleic acid molecule used in gene therapy may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

[0144] The nucleic acid molecules may be delivered as a naked nucleic acid molecule. The term "naked" nucleic acid molecule, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the nucleic acid molecules used in gene therapy can also be delivered in liposome formulations and lipofectin formulations and the like that can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Pat. Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

[0145] The vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan. Any strong promoter known to those skilled in the art can be used for driving the expression from the nucleic acid molecule used in gene therapy. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter of coagulation factor XII or of any of the polypeptides expressed in gene therapy. Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the nucleic acid molecule synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

[0146] The nucleic acid molecules used in gene therapy can be delivered to the interstitial space of tissues within an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercel-

lular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

[0147] For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.0005 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid molecules can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose.

[0148] The naked nucleic acid molecules are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art. The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc.

[0149] Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Feigner et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7416, which is herein incorporated by reference); mRNA (Malone et al., *Proc. Natl. Acad. Sci. USA* (1989) 86:6077-6081, which is herein incorporated by reference); and purified transcription factors (Debs et al., *J. Biol. Chem.* (1990) 265: 10189-10192, which is herein incorporated by reference), in functional form. Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxypropyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Feigner et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7416). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication No. WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g.,

Feigner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials. Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art. For example, commercially available dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

[0150] Generally, the ratio of nucleic acid to liposomes will be from about 10:1 to about 1:10. Preferably, the ratio will be from about 5:1 to about 1:5. More preferably, the ratio will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

[0151] In certain embodiments, cells are engineered, ex vivo or in vivo, using a retroviral particle containing RNA which comprises a sequence encoding any of the nucleic acid molecules or (poly)peptides used in the method of gene therapy. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus. The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO₄ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host. The producer cell line generates infectious retroviral vector particles which include the nucleic acid molecule encoding the (poly)peptide or the therapeutically active nucleic acid, such as siRNA, intended to be used for gene therapy. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo.

[0152] In certain other embodiments, cells are engineered, ex vivo or in vivo, with a nucleic acid molecule to be used in gene therapy, contained in an adenovirus vector. Adenovirus can be manipulated such that it expresses a construct of interest, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz, A. R. et al. (1974) Am. Rev. Respir. Dis. 109:233-238). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld, M. A. et al. (1991) Science 252:431-434; Rosenfeld et al., (1992) Cell 68:143-155). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) Proc. Natl. Acad. Sci. USA 76:6606). Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel. 3:499-503 (1993); Rosenfeld et al., Cell 68:143-155 (1992); Engelhardt et al., Human Genet. Ther. 4:759-769 (1993); Yang et al., Nature Genet. 7:362-369 (1994); Wilson et al., Nature 365:691-692 (1993); and U.S. Pat. No. 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express E1a and E1b, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention. Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a gene of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

[0153] The present invention also relates to a non-human transgenic animal, comprising as a transgene: (a) a gene encoding human disease-associated coagulation factor XII; (b) (i) a gene encoding human disease-associated coagulation factor XII and (ii) a gene encoding human wild-type coagulation factor XII; (c) a nucleic acid molecule causing an altered expression of human coagulation factor XII and a gene encoding human wild-type coagulation factor XII; and/or (d) a species-specific coagulation factor XII gene which is specifically altered to contain a human disease-associated mutation.

[0154] Said transgenic animal of (a) to (d) will be very important, for example, for studying the pathophysiological consequences of certain coagulation factor XII alterations, and for the screening of new medicaments effective in the treatment and/or prevention of hereditary angioedema type III. Preferably, said animal is a mammalian animal, including, but not limited to, rat, mouse, cat, hamster, dog, rabbit, pig, or monkey, but can also be, for example, *C. elegans* or a fish, such as Torpedo fish.

[0155] The non-human transgenic animal of (b) will be valuable for example for studying a heterozygous situation,

including possible dominant negative effects of a disease-associated mutation. Further it may allow to investigate potential differential effects of a medicament, including any of the modulators discussed above, on wild-type and disease-associated human coagulation factor XII. The non-human transgenic animal of (c) may allow for example to study the consequences and potential treatment of a mutated nucleic acid that leads to an altered expression of human coagulation factor XII. As envisaged here, such a mutation could relate for example to a nucleic acid molecule which in the human genome is physically unrelated to the coagulation factor XII gene. It is also envisaged that, for example in case of a mutation at a highly conserved position or within a functionally conserved motif, the human disease or disease predisposition can be imitated in the animal by altering the animal's species-specific coagulation factor XII gene to contain a human disease-associated mutation.

[0156] A method for the production of a transgenic non-human animal, for example transgenic mouse, comprises introduction of the desired polynucleotide, for example a nucleic acid encoding human wild-type or disease-associated mutant coagulation factor XII, or targeting vector into a germ cell, an embryonic cell, stem cell or an egg or a cell derived therefrom. Production of transgenic embryos and screening of those can be performed, e.g., as described by A. L. Joyner Ed., *Gene Targeting, A Practical Approach* (1993), Oxford University Press. The DNA of the embryonal membranes of embryos can be analyzed using, e.g., Southern blots with an appropriate probe. A general method for making transgenic non-human animals is described in the art, see for example WO 94/24274. For making transgenic non-human organisms (which include homologously targeted non-human animals), embryonal stem cells (ES cells) are preferred. Murine ES cells, such as AB-1 line grown on mitotically inactive SNL76/7 cell feeder layers (McMahon and Bradley, *Cell* 62: 1073-1085 (1990)), essentially as described in: *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*. E. J. Robertson, ed. (Oxford: IRL Press), 1987, pp. 71-112, may be used for homologous gene targeting. Other suitable ES lines include, but are not limited to, the E14 line (Hooper et al., *Nature* 326: 292-295 (1987)), the D3 line (Doetschman et al., *J. Embryol. Exp. Morph.* 87: 27-45 (1985)), the CCE line (Robertson et al., *Nature* 323: 445-448 (1986)), the AK-7 line (Zhuang et al., *Cell* 77: 875-884 (1994) which is incorporated by reference herein). The success of generating a mouse line from ES cells bearing a specific targeted mutation depends on the pluripotency of the ES cells (i.e., their ability, once injected into a host developing embryo, such as a blastocyst or morula, to participate in embryogenesis and contribute to the germ cells of the resulting animal). The blastocysts containing the injected ES cells are allowed to develop in the uteri of pseudopregnant nonhuman females and are born as chimeric animals. The resultant transgenic animals are chimeric for cells having either the recombinase or reporter loci and are backcrossed and screened for the presence of the correctly targeted transgene (s) by PCR or Southern blot analysis on tail biopsy DNA of offspring so as to identify transgenic animals heterozygous for either the recombinase or reporter locus/loci.

[0157] Methods for producing transgenic flies, such as *Drosophila melanogaster* are also described in the art, see for example U.S. Pat. No. 4,670,388, Brand & Perrimon, *Development* (1993) 118: 401-415; and Phelps & Brand, *Methods* (April 1998) 14: 367-379. Transgenic worms such as *C.*

elegans can be generated as described in Mello, et al., (1991) Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *Embo J* 10, 3959-70, Plasterk, (1995) Reverse genetics: from gene sequence to mutant worm. *Methods Cell Biol* 48, 59-80. **[0158]** In a preferred embodiment of the present invention, the non-human transgenic animal additionally expresses siRNA or shRNA, a ribozyme or an antisense nucleic acid molecule specifically hybridizing to the transgene(s) or to the altered species-specific gene contained in the transgenic animal. Preferably, said transgene(s) is/are of human origin. Such an approach can be useful, for example, for studying options for treatment and/or prevention for example by using RNA interference.

[0159] It may also be desirable to inactivate coagulation factor XII protein expression or function at a certain stage of development and/or life of the transgenic animal. This can be achieved by using, for example, tissue specific, developmental and/or cell regulated and/or inducible promoters which drive the expression of, e.g., an antisense or ribozyme directed against a mRNA encoding a coagulation factor XII (poly)peptide. A suitable inducible system is for example tetracycline-regulated gene expression as described, e.g., by Gossen and Bujard (*Proc. Natl. Acad. Sci.* 89 USA (1992), 5547-5551) and Gossen et al. (*Trends Biotech.* 12 (1994), 58-62). Similar, the expression of a mutant coagulation factor XII protein may be controlled by such regulatory elements.

[0160] In another preferred embodiment, the non-human transgenic animal's native species-specific genes encoding coagulation factor XII are inactivated. The term "inactivation" means reversible or irreversible inactivation. Appropriate methods to obtain such an inactivation are well known in the art. Such an approach may be useful in order to eliminate any effects of the animal's species-specific coagulation factor XII genes when studying for example the pathophysiological effects and/or the possible therapeutic targeting of the human transgene(s).

[0161] The present invention also relates to the use of any of the transgenic animals of the present invention, for screening for compounds for use in the diagnosis, prevention and/or treatment of hereditary angioedema type III.

[0162] The present invention also relates to a nucleic acid molecule comprising the human coagulation factor XII nucleotide sequence or a fragment thereof, having a mutation at a position corresponding to position 6927 of GenBank accession no. AF 538691, wherein the wild-type C is substituted by an A or by a G. This sequence may have, e.g. 5' and 3' of the human coagulation factor XII nucleotide sequence, foreign sequences. Moreover, e.g. intronic regions may be e.g. mutated or replaced with foreign nucleic acid sequence.

[0163] Said molecule may be of any length, however, preferably the nucleic acid molecule comprising the human coagulation factor XII nucleotide sequence or fragment thereof has a length of at least 10, 30, 50, 100, 1000, 2000, 3000, 4000, 5000, 6000. Preferably the maximal length of said nucleic acid molecule is up to 30, 50, 100, 1000, 2000, 3000, 4000, 5000, 6000 or up to 20000 nucleotides or bases. Moreover, the nucleic acid molecule may be a single or double stranded nucleic acid molecule.

[0164] The term "fragment" as used herein refers to a portion of the human coagulation factor XII gene. Said fragment comprised in the nucleic acid molecule of the present invention may have a length of at least 10, 30, 50, 100, 1000, 2000, 3000, 4000, 5000, 6000. Preferably the maximal length of

said fragment is up to 30, 50, 100, 1000, 2000, 3000, 4000, 5000, 6000 nucleotides or bases. Moreover, the nucleic acid molecule may be a single or double stranded nucleic acid molecule.

[0165] The term “nucleic acid molecule” as used herein refers to DNA or RNA, including cDNA, hnRNA, mRNA, unspliced, spliced or partially spliced RNA and genomic DNA.

[0166] Moreover, the present invention also relates to an oligonucleotide containing at least 8 nucleotides of (a) the mutant nucleotide sequence of the present invention, comprising position 6927, wherein the oligonucleotide contains a nucleotide corresponding to mutant position 6927, or the corresponding wild-type sequence of said oligonucleotide or (b) the complementary sequence of (a). The term “oligonucleotide” as used herein refers to a nucleic acid molecule useful e.g. as primer for PCR reactions or as probe for specific detection of the mutation of the present invention. Said oligonucleotide may have a length of at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 nucleotides. Preferably the maximal length of said oligonucleotide is up to 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50 or 100 nucleotides in length. However, also larger oligonucleotides are in accordance with the present invention. The oligonucleotide may be composed of ribonucleic acid bases or desoxyribonucleic acid bases. These may be mixed and/or modified.

[0167] The present invention also relates to a (poly)peptide or a fragment thereof, encoded by the nucleic acid molecule of the present invention. This (poly)peptide contains a basic or positively charged amino acid residue in the position corresponding to position 309 of the human coagulation factor XII amino acid sequence. Preferably, said amino acid residue is a lysine, an arginine or a histidine.

[0168] The present invention also relates to an antibody or antibody fragment specific for the (poly)peptide of the present invention. This antibody is an antibody specifically binding to an epitope containing the mutant position 309. However, it is also conceivable that the mutation may induce a conformational change in the coagulation factor XII (poly)peptide, thereby generating new epitopes outside of this region which may allow specific binding to the mutant (poly)peptide but not to the wild-type coagulation factor (poly)peptide. Preferably, this antibody is capable of discrimination between coagulation factor XII with wild-type in respect of position 309 and the mutant having a basic or positively charged amino acid residue in the same position. The antibody may be any antibody as defined herein, including polyclonal or monoclonal antibody. The term antibody also includes antibody fragments as described herein.

[0169] In a preferred embodiment of the present invention, the antibody is a monoclonal or polyclonal antibody.

[0170] The present invention also relates to a hybridoma producing the monoclonal antibody of the present invention.

[0171] Finally, the present invention also relates to a kit for use in diagnosis of hereditary angioedema type III or a susceptibility or predisposition thereto, said kit comprising: (a) at least one nucleic acid molecule capable of hybridizing under stringent conditions to a nucleic acid molecule encoding or regulating the expression of coagulation factor XII; (b) an antibody or an aptamer specific for coagulation factor XII or a fragment thereof and/or a disease-associated mutant of these; (c) a restriction enzyme capable of discriminating between wild-type and disease-associated mutant nucleic acid encoding or regulating the expression of coagulation

factor XII; and/or (d) a pair of primers complementary to nucleic acid regulating the expression of coagulation factor XII or encoding wild-type and/or disease-associated coagulation factor XII; (e) the nucleic acid molecule of the present invention; and/or (f) the polypeptide of the present invention; (g) the antibody of the present invention; (h) the hybridoma of the present invention; and/or the oligonucleotide of the present invention; and optionally instructions for use.

[0172] In a preferred embodiment of the present invention, the disease-associated mutant is any of the mutants of the present invention or a mutant as defined in any one of claims 21 to 23 or a mutant as defined in claim 38.

[0173] In another preferred embodiment (a) of the present invention's kit is a primer pair capable of amplifying exon 9 of human coagulation factor XII gene or a part thereof comprising the mutant position as defined in the present invention or as defined in claim 38 or a probe or pair of probes and optionally instructions for use.

[0174] The nucleic acid molecule(s) of (a) may be suitable for example for use as probes or primers. Preferably, the kit will also provide means for detection of a reaction, e.g. nucleotide label detection means, labeled secondary antibodies or size detection means. The various compounds of the kit may be packed in one or more containers, optionally dissolved in suitable buffer for storage.

FIGURE LEGENDS

[0175] FIG. 1: mRNA reference sequence (SEQ ID NO: 66) of human coagulation factor XII as given under Genbank accession number NM_000505, together with the amino acid sequence at the mutant position. The nucleotide affected by the two newly identified mutations is highlighted.

[0176] FIG. 2: Genomic reference sequence (SEQ ID NO: 3) of the coagulation factor XII gene as given in Genbank accession number AF538691. Variable positions observed in the patients studied and known from the literature already are underlined, one additional variation newly observed in two patients of the present study, is highlighted by bold/italic printing.

[0177] FIG. 3: Structure of the human coagulation factor XII gene, arrow indicating the position of the missense mutations in exon 9.

[0178] FIG. 4: Pedigree situation illustrating the transmission of the disease through a clinically unaffected male.

[0179] The Examples illustrate the invention:

EXAMPLE 1

Oligonucleotide Primer Design for Coagulation Factor XII Gene Amplification and Sequencing

[0180] Pairs of oligonucleotide primers were designed to amplify the complete human coagulation factor XII gene including flanking sequences. Table 1 lists the corresponding sequences of these primers.

TABLE 1

Oligonucleotide primer sequences (F = forward, R = reverse) (SEQ ID NOs: 6-65)	
Primer ID	Primer Sequence
F12-Ex1-F	5'-aggaagtgtgctccactggcttt-3'
F12-Ex1-R	5'-tgcagagatttcttcccaagacc-3'

TABLE 1-continued

Oligonucleotide primer sequences (F = forward, R = reverse) (SEQ ID NOS: 6-65)	
Primer ID	Primer Sequence
F12-Ex2-F	5'-ctatgtggaaggtgaggccag-3'
F12-Ex2-R	5'-ctcaaggatcacacagctcacg-3'
F12-Ex3-4-F	5'-tgagggtctgtccttttctga-3'
F12-Ex3-4-R	5'-ggtgtgtgggtctggtgatac-3'
F12-Ex5-6-F	5'-gtaggttcaagaaggccttg-3'
F12-Ex5-6-R	5'-gagctctccttccggcac-3'
F12-Ex7-F	5'-gagcagatggttgggaacg-3'
F12-Ex7-R	5'-tgaggagaaaggggctc-3'
F12-Ex8-F	5'-ggtctgggcaagcagaag-3'
F12-Ex8-R	5'-tgtagccacacgacggg-3'
F12-Ex9-F	5'-GAACGTGACTGCCGAGCAAG-3'
F12-Ex9-R	5'-aggagcaggggctgaggac-3'
F12-Ex10-F	5'-gaaggaggagccgagagg-3'
F12-Ex10-R	5'-ggtaggggagagcagcg-3'
F12-Ex11-12-F	5'-aggaagctggaacacgggatt-3'
F12-Ex11-12-R	5'-ataccaaagtgcggcttct-3'
F12-Ex13-F	5'-cccattcaaatcctgcttttc-3'
F12-Ex13-R	5'-AATCACCTGGGTCGGAAC-3'
F12-Ex14-F	5'-GTGCCAGGTGAGCTCTTAGCC-3'
F12-Ex14-R	5'-ccttgctctctgagagctgtgga-3'
F12-Intr2-pt1-F	5'-tgtatggtgcagtggtgcagt-3'
F12-Intr2-pt1-R	5'-ggcatgtaggaatttagtctgga-3'
F12-Intr2-pt2-F	5'-cctttagatgaagggtacctgcc-3'
F12-Intr2-pt2-R	5'-gagaaactttgggtgtgggt-3'
F12-Intr2-pt3-F	5'-ctgacttggtggggtgagtct-3'
F12-Intr2-pt3-R	5'-tgccatctattttgtcaaggca-3'
F12-Intr2-pt4-F	5'-ccatttgcatcttaaggtccatc-3'
F12-Intr2-pt4-R	5'-tcacactttgtgcttttctgg-3'
F12-Intr2-pt5-F	5'-acacacgctttctccctaaagg-3'
F12-Intr2-pt5-R	5'-ggagtagactcctgactcccaa-3'
F12-Intr2-pt6-F	5'-agtattattaagtgcactttgtggc-3'
F12-Intr2-pt6-R	5'-CAGTGAGAActgcaggacaac-3'
F12-Intr4-F	5'-gaggggactgtgataggcag-3'
F12-Intr4-R	5'-ACACAGGTCCCTCCTTCTGG-3'

TABLE 1-continued

Oligonucleotide primer sequences (F = forward, R = reverse) (SEQ ID NOS: 6-65)	
Primer ID	Primer Sequence
F12-Intr12-F	5'-AGACCACGCTCTGCCAGGT-3'
F12-Intr12-R	5'-gtaaacccactcatgcccttcc-3'
F12-P(-1)-F	5'-cgtcttcttctcatgttccagc-3'
F12-P(-1)-R	5'-actggcceaaggctctggaaat-3'
F12-P(-2)-F	5'-cacagcatctttccatccttcc-3'
F12-P(-2)-R	5'-atcttggggccatcttagcatt-3'
F12-P(-3)-F	5'-gtgtcctcacaacacagtggt-3'
F12-P(-3)-R	5'-cacattgatgatcacctttgtcac-3'
F12-P(-4)-F	5'-tgtgcctagccataactgacca-3'
F12-P(-4)-R	5'-tggacttccaagcccaggt-3'
F12-P(-5)-F	5'-gtcacgtcaatgactttgaaacc-3'
F12-P(-5)-R	5'-cgacatttgagaactagtactgagg-3'
F12-3'UTR-pt1-F	5'-TCAATAAAGTGCTTTGAAAATGCTGA-3'
F12-3'UTR-pt1-R	5'-tagagacggggttctcatcgtgt-3'
F12-3'UTR-pt2-F	5'-gaaatacttagcattggccggg-3'
F12-3'UTR-pt2-R	5'-aacattcaaccccagattgt-3'
F12-Ex9-seqint1-R	5'-cccccaacttctaactcccc-3'
F12-P(-1)-S2-R	5'-tttgagacggagtctcgtc-3'
F12-Ex9-ARMS-Mt1-F	5'-cgccgaagcctcagcccaa-3'
F12-Ex9-ARMS-Mt1-R	5'-gcggtgcatcgaagacagact-3'
F12-Ex9-RFLP-Mt2-F	5'-cccgggtgtcccctaggcttc-3'
F12-Ex9-RFLP-Mt2-R	5'-ctgccggcgcagaaactgt-3'
F12-Intr10-RFLP-Mt3-F	5'-aagcgcggaactggggact-3'
F12-Intr10-RFLP-Mt3-R	5'-gctgaacgtaaggcgacaggag-3'

EXAMPLE 2

Coagulation Factor XII Gene Amplification and Direct Sequencing of PCR Products

[0181] 50-100 ng of genomic DNA was amplified by PCR in a total reaction volume of 50 µl containing 2.5 mM MgCl₂, 200 µM each dATP, dCTP, dGTP, dTTP, 5 µl of a 10xPCR buffer (of Invitrogen or Applied Biosystems), 50 µmol of each oligonucleotide primer and 1.25 units Taq DNA polymerase. Occasionally, the buffer had to be optimized by adding denaturing reagents such as DMSO and glycerol or other compounds or compositions known to improve amplification efficiency and specificity.

[0182] In general, reactions were thermocycled with an initial denaturation step of 95° C./5 mins [10 min when AmpliTaq Gold DNA polymerase (Applied Biosystems) was used] followed by 35 cycles of 94° C./40 secs; $T_{annealing}/40$ secs; 72° C./45 secs. For amplimers 20 and 29 subperiods of each cycle of 60 sec/60 sec/120 sec were chosen. A final elongation step of 72° C./10 mins completed the amplification. Annealing temperatures for specific primer pairs and amplimer sizes are presented in Table 2.

[0183] Direct sequencing of PCR products was done according to standard procedures (using BigDye™ terminator cycling conditions; purification of reacted products using ethanol precipitation; ABI Automatic Sequencer 3730) known to the skilled artisan (Sambrook et al., “Molecular Cloning, A Laboratory Manual”; ISBN: 0879695765, CSH Press, Cold Spring Harbor, 2001).

TABLE 2

Amplimer sizes and annealing temperatures			
Amplimer	Primer Pair	Size (bp)	T_{ann} (° C.)
1	F12-Ex1-F and F12-Ex1-R	478	62° C.
2	F12-Ex2-F and F12-Ex2-R	469	62° C.
3	F12-Ex3-4-F and F12-Ex3-4-R	504	62° C.
4	F12-Ex5-6-F and F12-Ex5-6-R	546	62° C.
5	F12-Ex7-F and F12-Ex7-R	386	60° C.
6	F12-Ex8-F and F12-Ex8-R	386	59° C.
7	F12-Ex9-F and F12-Ex9-R	459	59° C.
8	F12-Ex10-F and F12-Ex10-R	550	59° C.
9	F12-Ex11-12-F and F12-Ex11-12-R	548	60° C.
10	F12-Ex13-F and F12-Ex13-R	445	60° C.
11	F12-Ex14-F and F12-Ex14-R	507	60° C.
12	F12-Intr2-pt1-F and F12-Intr2-pt1-R	651	63° C.
13	F12-Intr2-pt2-F and F12-Intr2-pt2-R	557	63° C.
14	F12-Intr2-pt3-F and F12-Intr2-pt3-R	598	60° C.
15	F12-Intr2-pt4-F and F12-Intr2-pt4-R	548	57° C.
16	F12-Intr2-pt5-F and F12-Intr2-pt5-R	540	63° C.
17	F12-Intr2-pt6-F and F12-Intr2-pt6-R	584	57° C.
18	F12-Intr4-F and F12-Intr4-R	489	59° C.
19	F12-Intr12-F and F12-Intr12-R	518	59° C.
20	F12-P(-1)-F and F12-P(-1)-R	1275	64° C.
21	F12-P(-2)-F and F12-P(-2)-R	547	62° C.
22	F12-P(-3)-F and F12-P(-3)-R	642	60° C.
23	F12-P(-4)-F and F12-P(-4)-R	442	60° C.
24	F12-P(-5)-F and F12-P(-5)-R	655	60° C.
25	F12-3'UTR-pt1-F and F12-3'UTR-pt1-R	559	58° C.
26	F12-3'UTR-pt2-F and F12-3'UTR-pt2-R	559	59° C.

TABLE 2-continued

Amplimer sizes and annealing temperatures			
Amplimer	Primer Pair	Size (bp)	T_{ann} (° C.)
27	F12-Ex9-ARMS-Mt1-F and F12-Ex9-ARMS-Mt1-R	257	63° C.
28	F12-Ex9-RFLP-Mt2-F and F12-Ex9-RFLP-Mt2-R	390	64° C.
29	F12-Intr10-RFLP-Mt3-F and F12-Intr10-RFLP-Mt3-R	1204	60° C.

EXAMPLE 3

Sequencing of the Coagulation Factor XII Gene in Unrelated Patients with Hereditary Angioedema Type III

[0184] Initially, twenty unrelated patients diagnosed with hereditary angioedema type III were studied. All patients (as well as family members studied subsequently, see below) had given informed consent. All these patients had experienced recurrent angioedema attacks; in all patients immunochemical as well as functional assays of complement C1 inhibitor had shown normal values. All had a positive family history (at least one relative was reported to have experienced angioedema attacks). All were female; all were Caucasian.

[0185] In these 20 patients, all 14 exons (with flanking intron sequences) of the coagulation factor XII gene were amplified and sequenced. Approximately 1 kb of promoter sequence was studied in 18 of these patients.

[0186] Compared to previously reported data, two missense mutations were newly identified (‘mutation 1’ and ‘mutation 2’):

[0187] Both these missense mutations are located in exon 9 of the coagulation factor XII gene, more specifically, both are located in exactly the same position, namely the second position of the codon encoding amino acid residue 309 of the mature protein (corresponding to amino acid residue 328 in the numbering of the primary translation product [e.g. swissprot acc. No. P00748]).

[0188] The wild-type sequence of this codon is ACG (encoding a threonine residue). ‘Mutation 1’ is a C→A substitution (1032C→A; numbering according to GenBank acc. No. NM_000505) resulting in an AAG triplet encoding a lysine residue.

[0189] ‘Mutation 2’ is a C→G substitution (1032C→G) resulting in an AGG triplet encoding an arginine residue.

[0190] Thus, with respect to both mutations, the wild-type threonine residue is substituted by a basic amino acid residue. Both substitutions are transversions.

[0191] Both substitutions may alter the putative O-glycosylation pattern in this region (McMullen & Fujikawa 1985, J. Biol. Chem. 260: 5328-5341; O’Connell et al. 1991, BBRC 180:1024-1030). Glycosylation is known to affect protein folding, localisation and trafficking, protein solubility, antigenicity, biological activity and half-life, as well as cell-cell interactions. Importantly, in the wild-type protein (according to e.g. OglycBase 6.00 (<http://www.cbs.dtu.dk/databases/OGLYCBASE/Oglyc.base.html>) Thr309 as well as Thr310 of the mature protein (corresponding to Thr328 and Thr329 of acc. No. P00748) are predicted to be glycosylated; and the

missense mutation of Thr309 may also affect the glycosylation at Thr310 (O'Connell et al. 1991, BBRC 180:1024-1030).

[0192] In all cases, patients were heterozygous for the mutation, in accordance with a dominant inheritance pattern of the disease.

[0193] The Thr309Lys mutation ('mutation 1'; 1032C→A) was observed in five of the 20 unrelated patients.

[0194] The Thr309Arg mutation ('mutation 2'; 1032C→G) was observed in one of the 20 unrelated patients.

[0195] Subsequently, an additional 6 unrelated patients diagnosed with HAE type III were selectively examined with respect to their exon 9 sequence. Two of these six patients were heterozygous for the Thr309Lys mutation ('mutation 1'; 1032C→A). Thus, altogether 31% (8/26) of the unrelated patients studied were heterozygous for a missense mutation affecting the Thr309 residue. Considering that among 61 healthy controls no single carrier of such a mutation was observed (see below), these data are highly significant (Fisher's exact test for comparison of 26 patients with the 61 sequenced controls: $p=0.000027$).

[0196] Two out of the 20 patients initially studied revealed a nucleotide substitution (g.7418C>T) in intron 10 which has previously not been observed, in the course of studies on a considerable number of (normal) individuals (<http://pga.gs.washington.edu/>; WO 01/79228). In position 7418 of the genomic reference sequence (GenBank acc. No. AF 538691), these two patients were heterozygous Y (C/T), whereas the wild-type sequence is C. Both these patients were at the same time heterozygous for a missense mutation of codon 309 (one patient 1032C→A; the other patient 1032C→G). For the second patient it could be shown by family studies that the allele carrying the missense mutation (1032C→G) is g.7418C; the affected daughter of this patient inherited the missense mutation, but not the g.7418T allele. Although in this situation there is no co-segregation of the disease and the g.7418T allele, a relationship between this rare variation and the disease cannot be excluded at present. The intron 10 sequence, including the variable position g.7418C>T, may eventually be contained in certain transcripts from the coagulation factor XII gene (c.f. e.g. GenBank acc. nos. CR616520, CR601747).

[0197] A number of common sequence variations, known from the literature and/or SNP databases (some of them for example observed among the 23 normal individuals within the Seattle sequencing project, see above), were also observed in the present study:

[0198] var(1627) (g.1627C>T with respect to the genomic reference sequence AF 538691; 46C>T with respect to the mRNA reference sequence NM_000505) in exon 1 (amplimer 1);

[0199] var(6570) (g.6570C>T; 760C>T) in exon 8 (amplimer 6);

[0200] a mononucleotide insertion/deletion polymorphism in intron 9 [g.6981delG] with respect to the genomic reference sequence AF 538691], resulting in a variable length (8g/9g) of a mononucleotide (g) repeat (amplimer 7); var (7040) (g.7040C>T) in intron 9 (amplimer 7, as well as in amplimer 8);

[0201] var(7532) (g.7532T>C) in intron 10 (amplimer 9);

[0202] var(640) (g.640A>G) in the promoter region (amplimer 20);

[0203] var(654) (g.654C>T) in the promoter region (amplimer 20);

[0204] var (668) (g.668A>C) in the promoter region (amplimer 20).

EXAMPLE 4

Family Studies

[0205] With respect to four of the patients carrying a missense mutation of the Thr309 residue, the extended family was studied. Three of the four families segregated for the Thr309Lys mutation, the fourth family segregated for the Thr309Arg mutation. Altogether there were ten patients (all women) affected by angioedema symptoms in these four families.

[0206] There was complete co-segregation between the disease and the presence of the (respective) missense mutation: all ten individuals affected by angioedema symptoms were heterozygous for the Thr309Lys or the Thr309Arg mutation, respectively.

[0207] Examination of the exon 9 sequence in altogether 37 members of these four families revealed—in addition to the clinically affected individuals—several further mutation carriers who were apparently asymptomatic until now (mostly men, but in one family also two women); this observation is in complete agreement with the incomplete penetrance of the disease and the preference to affect the female sex. Remarkably, taking a detailed medical history, revealed that at least two men carrying the Thr309Lys mutation had a decade-long history of un-explained abdominal pain attacks, well in agreement with the diagnosis of a monosymptomatic (gastrointestinal) angioedema disease.

[0208] Among the asymptomatic mutation carriers there were also two men for whom it was concluded from the pedigree structure that they must have transmitted the disease (see, e.g. FIG. 4: a pedigree illustrating transmission of the disease through an asymptomatic male carrier).

Haplotypic Data

[0209] In several of the unrelated patients the linkage phase between the (heterozygous) missense mutation of Thr309 residue and further variable positions could be established (in some cases with the help of segregation analysis). For example, it was concluded that the 1032C→G mutation occurs on a g.1627C-g.6981delG-g.7040C-g.7532T-haplotype. Also the 1032C→A mutation appears to be associated with this haplotype.

EXAMPLE 5

Studies in Healthy Control Individuals

[0210] In 61 healthy control individuals (blood donors) the exon 9 fragment (amplicon 7; Table 2) of the coagulation factor XII gene was amplified and sequenced. With respect to the exonic sequence of this fragment, and in particular with respect to codon 309 (of the mature protein), all these control individuals were apparently homozygous for the wild-type sequence. In particular, no control individual showed the 1032C→A mutation or the 1032C→G mutation.

[0211] Thus, regarding the presence of a missense mutation of codon 309, there is a highly significant difference between patients and healthy controls (Fisher's exact test for comparison of 26 patients with the 61 sequenced controls: $p=0.000027$).

[0212] Two (known) intronic polymorphic variabilities were observed among the control individuals: a mononucleotide insertion/deletion polymorphism in intron 9 [g.6981delG with respect to the genomic reference sequence AF 538691], resulting in a variable length of a mononucleotide (g) repeat (8g-allele: 0.54; 9g-allele: 0.46); and a single nucleotide polymorphism (g.7040C>T) at the end of intron 9 (=var(7040) in the 'Seattle SNPs' data) (C, 0.55; T: 0.45, as counted from 57 individuals). 57 individuals could be diagnosed with respect to both these variabilities. There was complete linkage disequilibrium between the 8g-allele and g.7040C, and between the 9g-allele and g.7040T, respectively.

[0213] In addition to the 61 control individuals examined by sequencing of the exon 9 fragment, another 35 control individuals were studied by means of a specific RFLP assay (see below) designed for the detection of the 1032C→G mutation. The results indicated that the 1032C→G mutation was not present in any of these 35 control individuals. Thus, altogether none out of 96 control individuals carried the 1032C→G mutation.

EXAMPLE 6

Specific Detection of Mutant Alleles—Assay Design for Genotyping

[0214] A. Allele Specific Amplification (ARMS) Assay for the Detection of the 1032C→A Mutation (Thr309Lys)

[0215] For the specific amplification of the 1032C→A mutation the following primer pair was designed (see also Table 1):

F12-EX9-ARMS-MT1-F:
5'-cgccgaagcctcagcccaa-3' (SEQ ID NO: 60)

F12-EX9-ARMS-MT1-R:
5'-gcggtcatcgaaacagact-3' (SEQ ID NO: 61)

[0216] The 3' end of the forward primer (underlined) is located on the mutant position, and, in this case, the primer sequence corresponds to the mutant allele (1032C→A); with respect to the wild-type sequence the primer sequence represents a mismatch (so that no successful amplification is possible).

[0217] In the presence of the 1032C→A mutation (a 1032C→A allele), this primer pair will amplify a fragment of size 256 bp. However, if the 1032C→A mutation is not present in the sample under study (in the absence of the mutation) no product will be amplified.

[0218] To provide an internal control for the successful PCR amplification reaction, a second primer pair (F12-Ex11-12-F/F12-Ex11-12-R; see Table 1) is included in the reaction mixture, resulting in a constant fragment of size 548 bp.

[0219] The assay was validated on approximately 90 samples from which the exon 9 fragment had been sequenced previously (these samples included 15 samples heterozygous for the 1032C→A mutation). There was complete concordance between the sequencing results and the result of the ARMS assay.

[0220] It should be noted, that to exclude the remote possibility of homozygous occurrence of the 1032C→A mutation, it may be necessary to sequence those samples positive in this assay, or to perform on such samples in addition a similar procedure specific for the wild-type allele.

[0221] B. RFLP (Restriction Fragment Length Polymorphism) Assay for the Detection of the Thr309Arg (1032C→G) Mutation

[0222] The 1032C→G mutation creates a new restriction site for restriction endonuclease BstN I (recognition sequence: cc↓wgg).

[0223] A primer pair (see also Table 1) was designed so that the amplified product contains a constant BstN I site—in addition to the mutation-dependent variable site:

F12-EX9-RFLP-Mt2-F:
5'-cccgggtgtcccctaggcttc-3' (SEQ ID NO: 62)

F12-EX9-RFLP-Mt2-R:
5'-ctgcgggcgcagaaactgt-3' (SEQ ID NO: 63)

[0224] The PCR conditions were as those for the exon 9 amplicon, except that an annealing temperature of 64°C was used (Table 2).

[0225] The undigested product has a size of 390 bp. The presence of a constant BstN I restriction site in the amplified fragment provides a convenient internal digestion control. Cleavage in the this constant BstN I site produces in all individuals a fragment of size 143 bp. Then, depending on the absence or presence of the 1032C→G mutation, either a fragment of 247 by (wild-type allele) or two fragments of 67 by and 180 by (1032C→G allele) are produced.

[0226] C. RFLP (Restriction Fragment Length Polymorphism) Assay for the Detection of the g.7418C>T Mutation in Intron 10

[0227] The g.7418C>T mutation in intron 10 of the coagulation factor XII gene creates a new Nla III restriction site (recognition sequence: CATG1).

[0228] A primer pair (F12-Intr10-RFLP-Mt3-F, F12-Intr10-RFLP-Mt3-R; see Table 1) was designed so that the amplified product contains a constant Nla III site—in addition to the mutation-dependent variable site.

[0229] The undigested product has a size of 1203 bp. The presence of two constant Nla III restriction sites in the amplified fragment provides a convenient internal digestion control. Cleavage in the these constant Nla III sites will produce in all individuals a fragment of size 262 by (beside a second constant fragment of size 11 bp). Then, depending on the absence or presence of the g.7418C>T mutation, either a fragment of 930 by (wild-type allele) or two fragments of 526 by and 404 by (g.7418C>T allele) will be produced.

EXAMPLE 7

Demonstration of the Presence of an Abnormal Coagulation Factor XII Protein in Individuals Carrying the Thr309Lys Missense Mutation

[0230] Isoelectric focusing in polyacrylamide gels followed by an immunoblotting procedure for the specific detection of factor XII revealed the presence of an abnormal factor XII protein, located in a more cathodal position, in the plasma of all individuals carrying the Thr309Lys mutation.

[0231] Plasma samples from n=15 individuals were studied by isoelectric focusing in thin-layer polyacrylamide gels followed by an immunoblotting procedure (Dewald, Ann. Inst. Pasteur/Immunol. 139: 507-515, 1988). Isoelectric focusing was performed in a pH 5-8 gradient using Ampholine carrier ampholytes.

[0232] The focused proteins were transferred from the polyacrylamide gel onto a nitrocellulose membrane filter by using a passive 'press blotting' procedure.

[0233] For the subsequent specific detection of coagulation factor XII on the nitrocellulose membrane an enzyme immunoassay was performed, using goat anti-human coagulation factor XII antiserum (IgG) as a primary antibody and a peroxidase-conjugated rabbit anti-goat-immunoglobulin antiserum as a secondary antibody. Finally, to visualize the factor XII banding pattern, peroxidase activity was developed using o-dianisidine.

[0234] Two different protein banding patterns were observed:

[0235] Pattern I consisted of a set of 3 to 4 bands located in a more anodal position of the gel; pattern II showed the banding set of pattern I and in addition a set of 3 to 4 bands located in a more cathodal position; the bands of the cathodal set showing a considerably stronger intensity than the bands of the anodal set.

[0236] Among the 15 individuals studied, pattern I was observed in 5 individuals, whereas pattern II was seen in the remaining 10 individuals. All individuals with pattern I were homozygous for the wild-type 1032C (Thr309); in contrast, all individuals with protein pattern II were heterozygous for the 1032C→A transversion (Thr309Lys) ('mutation 1'). In conclusion, there was complete concordance between genotype and protein phenotype. These observations indicate that the 1032A allele encodes an abnormal coagulation factor XII protein characterized by an isoelectric point higher than the one of the wild-type protein.

[0237] Considering that 'mutation 2' is a nucleotide substitution (1032C→G) that also—like 'mutation 1'—predicts the substitution of the neutral wild-type Thr309 residue by a basic (positively charged) residue (arginine in the case of 'mutation 2'), it is envisaged that individuals heterozygous for the 1032C→G transversion will show a protein pattern corresponding to pattern II.

SEQUENCE LISTING

```

<160> NUMBER OF SEQ ID NOS: 66

<210> SEQ ID NO 1
<211> LENGTH: 2048
<212> TYPE: RNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: variation
<222> LOCATION: (1032)..(1032)
<223> OTHER INFORMATION: /replace="a"
                        /replace="g"

<400> SEQUENCE: 1

cuaaugaucc ggacuccugg auaggcagcu ggaccaacgg acggacgcca ugagggcucu      60
gcugcuccgg ggguuccugc uggugagcuu ggagucaaca cuuucgauuc caccuuggga      120
agccccaag gagcauagu acaaagcuga agagcacaca gucguucua cugucaccgg      180
ggagccugc cacuuccccc uccaguacca cggcagcug uaccacaaau guaccacaa      240
gggccggcca gggccucagc ccuggugugc uaccacccc aacuuugauc aggaccagcg      300
auggggaucc uguuuggagc ccaagaaagu gaaagaccac ugcagcaaac acagcccccg      360
ccagaaagga gggaccugug ugaacaugcc aagcggcccc cacugucucu guccacaaca      420
ccucacugga aaccacugcc agaaagagaa gugcuuugag ccucagcuuc uccgguuuuu      480
ccacaagaau gagauauggu auagaacuga gcaagcagcu guggccagau gccagugcaa      540
ggguccugau gccacugcc agcggcuggc cagccaggcc ugccgcacca acccgugccu      600
ccaugggggu cgcugccuag agguggaggg ccaccgccug ugccacugcc cggugggcuu      660
caccggaccc uucugcgacg uggacaccaa ggcaagcugc uaugauggcc gcgggcucag      720
cuaccggcgg cuggccagga ccacgcucuc gggugcgccc ugucagccgu gggccucgga      780
ggccaccuac cggaaacuga cugccgagca agcgcggaac uggggacugg gcggccacgc      840
cuucugccgg aaccgggaca acgacaucgg cccgugugc uucgugcuga acccgacccg      900
gcugagcugg gaguacugcg accuggcaca gugccagacc ccaaccagg cggcgccucc      960
gaccccgug ucccuaggc uucaugucc acucaugccc gcgcagccgg caccgccgaa      1020
gccucagccc acgaccggga ccccgccuca gucccagacc cggggagccu ugccggcgaa      1080

```

-continued

```

gcgggagcag cgcuuucc ugaccaggaa cggcccacug agcugcgggc ageggcuccg 1140
caagagucug ucuucgauga cccgcgucgu uggcgggcug guggcgcuac gcggggcgca 1200
cccuacauc gccgcgcugu acuggggcca caguucucg gccggcagcc ucaucgccc 1260
cugcugggug cugacggccg cucacugccu gcaggaccgg cccgcacccg aggaucugac 1320
gguggugcuc ggcagggaac gccguaacca cagcugugag ccgugccaga cguuggccgu 1380
gcgcuccuac cguugcacg aggccuucuc gcccgucagc uaccagcacg accuggcucu 1440
guugcgccuu caggaggaug cggacggcag cugcgcguc cugucgccuu acguucagcc 1500
ggugugccug ccaagcggcg ccgcgcgacc cuccgagacc acgcucugcc agguggccgg 1560
cuggggccac caguucgagg gggcggagga auaugccagc uuccugcagg aggcgcaggu 1620
accguuccuc ucccuggagc gcugcucagc cccggacgug cacggauccu ccauccucc 1680
cggcaugcuc ugcgcagggu uccucgaggg cggcaccgau gcgugccagg gugauccgg 1740
aggcccgug gugugugagg accaagcugc agagcgccgg cucaccucg aaggcaucau 1800
cagcugggga ucgggcugug gugaccgcaa caagccaggc gucuacaccg auguggccua 1860
cuaccuggcc uggauccggg agcacaccgu uuccugauug cucagggacu caucuuccc 1920
uccuugguga ucccgcagug agagaguggc uggggcaugg aaggcaagau uguguccau 1980
ucccccagug cggccagcuc cgcgccagga uggcgaggaa cucaauaaag ugcuuugaaa 2040
augcugag 2048

```

```

<210> SEQ ID NO 2
<211> LENGTH: 615
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

```

```

<400> SEQUENCE: 2

```

```

Met Arg Ala Leu Leu Leu Gly Phe Leu Leu Val Ser Leu Glu Ser
1           5           10           15
Thr Leu Ser Ile Pro Pro Trp Glu Ala Pro Lys Glu His Lys Tyr Lys
          20           25           30
Ala Glu Glu His Thr Val Val Leu Thr Val Thr Gly Glu Pro Cys His
          35           40           45
Phe Pro Phe Gln Tyr His Arg Gln Leu Tyr His Lys Cys Thr His Lys
          50           55           60
Gly Arg Pro Gly Pro Gln Pro Trp Cys Ala Thr Thr Pro Asn Phe Asp
          65           70           75           80
Gln Asp Gln Arg Trp Gly Tyr Cys Leu Glu Pro Lys Lys Val Lys Asp
          85           90           95
His Cys Ser Lys His Ser Pro Cys Gln Lys Gly Gly Thr Cys Val Asn
          100          105          110
Met Pro Ser Gly Pro His Cys Leu Cys Pro Gln His Leu Thr Gly Asn
          115          120          125
His Cys Gln Lys Glu Lys Cys Phe Glu Pro Gln Leu Leu Arg Phe Phe
          130          135          140
His Lys Asn Glu Ile Trp Tyr Arg Thr Glu Gln Ala Ala Val Ala Arg
          145          150          155          160
Cys Gln Cys Lys Gly Pro Asp Ala His Cys Gln Arg Leu Ala Ser Gln
          165          170          175

```

-continued

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

Glu Gly His Arg Leu Cys His Cys Pro Val Gly Tyr Thr Gly Pro Phe
195 200 205

Cys Asp Val Asp Thr Lys Ala Ser Cys Tyr Asp Gly Arg Gly Leu Ser
210 215 220

Tyr Arg Gly Leu Ala Arg Thr Thr Leu Ser Gly Ala Pro Cys Gln Pro
225 230 235 240

Trp Ala Ser Glu Ala Thr Tyr Arg Asn Val Thr Ala Glu Gln Ala Arg
245 250 255

Asn Trp Gly Leu Gly Gly His Ala Phe Cys Arg Asn Pro Asp Asn Asp
260 265 270

Ile Arg Pro Trp Cys Phe Val Leu Asn Arg Asp Arg Leu Ser Trp Glu
275 280 285

Tyr Cys Asp Leu Ala Gln Cys Gln Thr Pro Thr Gln Ala Ala Pro Pro
290 295 300

Thr Pro Val Ser Pro Arg Leu His Val Pro Leu Met Pro Ala Gln Pro
305 310 315 320

Ala Pro Pro Lys Pro Gln Pro Thr Thr Arg Thr Pro Pro Gln Ser Gln
325 330 335

Thr Pro Gly Ala Leu Pro Ala Lys Arg Glu Gln Pro Pro Ser Leu Thr
340 345 350

Arg Asn Gly Pro Leu Ser Cys Gly Gln Arg Leu Arg Lys Ser Leu Ser
355 360 365

Ser Met Thr Arg Val Val Gly Gly Leu Val Ala Leu Arg Gly Ala His
370 375 380

Pro Tyr Ile Ala Ala Leu Tyr Trp Gly His Ser Phe Cys Ala Gly Ser
385 390 395 400

Leu Ile Ala Pro Cys Trp Val Leu Thr Ala Ala His Cys Leu Gln Asp
405 410 415

Arg Pro Ala Pro Glu Asp Leu Thr Val Val Leu Gly Gln Glu Arg Arg
420 425 430

Asn His Ser Cys Glu Pro Cys Gln Thr Leu Ala Val Arg Ser Tyr Arg
435 440 445

Leu His Glu Ala Phe Ser Pro Val Ser Tyr Gln His Asp Leu Ala Leu
450 455 460

Leu Arg Leu Gln Glu Asp Ala Asp Gly Ser Cys Ala Leu Leu Ser Pro
465 470 475 480

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

Thr Thr Leu Cys Gln Val Ala Gly Trp Gly His Gln Phe Glu Gly Ala
500 505 510

Glu Glu Tyr Ala Ser Phe Leu Gln Glu Ala Gln Val Pro Phe Leu Ser
515 520 525

Leu Glu Arg Cys Ser Ala Pro Asp Val His Gly Ser Ser Ile Leu Pro
530 535 540

Gly Met Leu Cys Ala Gly Phe Leu Glu Gly Gly Thr Asp Ala Cys Gln
545 550 555 560

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

Arg Leu Thr Leu Gln Gly Ile Ile Ser Trp Gly Ser Gly Cys Gly Asp

-continued

	580	585	590	
Arg Asn Lys Pro Gly Val Tyr Thr Asp Val Ala Tyr Tyr Leu Ala Trp				
	595	600	605	
Ile Arg Glu His Thr Val Ser				
	610	615		
<210> SEQ ID NO 3 <211> LENGTH: 10616 <212> TYPE: DNA <213> ORGANISM: Homo sapiens <400> SEQUENCE: 3				
acatgctctg	tgcttagtaa	ccccagtgca	acttttttgc	tttcccaaaa gttctgcaaa 60
aagtccaag	ctagcacttt	aattggccta	aattgtgat	atgcttatct ctgaaccaat 120
cactgtggat	tagagatgtc	atgctctgat	tgaccagacc	taggccacat ctctagccct 180
agctctgagg	gtagagtgg	cagcactaga	gccccatgaa	gaagtaagag aggagtcgtt 240
gctaaaggaa	aaatcaaatg	gtcattaccg	aaccaggaca	gatgctgggc agcacatgtg 300
caccccgctc	tcttctcatg	ttccagctgc	acatcttagt	gccccctggg ttagcacttt 360
tctcattaaa	tcatttgctt	tcttgctcca	cttccctgtg	ttggtagaat gctaagatgg 420
ccccaaagtc	tctaccctgt	gtgtttgcac	acctcccagt	tattctgtca aacatgaatg 480
tagatgcttc	tgtgaaagaa	ttttgcacat	gtaatttaag	tcccaaattg tttgacctta 540
aaataaggag	aatggcaggg	ccaggcatgg	tggtctatac	ctgtaatccc agcactttgg 600
gaggccaagg	cgggcagatc	acgaggctag	gagatcgaga	ccatcctggc taacacagtg 660
aaaccccatc	tctactaaaa	atacaaaaaa	ttagctgggc	gtggtggcgg gtgcctgtat 720
tcccagctac	ccaggaggct	gaggcaggag	aatggcgtga	accggggagg cgtagcttgc 780
agtgagccaa	gatcgtgcca	ctgcactcca	gctgggtgta	cagagccaga ctctgtctca 840
aaaaaaaaaa	aaaaaaggag	aatggctttg	gtgggcctga	cctagtcagg tgagttctta 900
aaaggcgaca	catggcccgg	tgcagtggct	caggcctgta	atcccagcac tttgggaggc 960
cgaggcgggt	ggatcacgag	gtcaggagat	cgagaccatc	ctggctaaca tgggaaacc 1020
ccgtctctac	taaaaaagaca	aaaaattagc	tgggcgtggg	ggtgggctcc tgtagtccca 1080
gctactcggg	aggctgaggc	aggagaatgg	cgtaaccctg	ggaggcggag cttgcagtga 1140
gcgagatgtg	cgccactgca	ctccagcctg	ggcgacagag	cgagactccg tctcaaaaaa 1200
aaaaaaaaaa	aaaaagaaaa	ttaaaagtgg	gtattgttgt	aagatgctga gtttatggta 1260
gtttgttaca	tgacaataga	aaatgaacac	acttcacagt	ggactccaag atccccatga 1320
tctttgatct	ccttaacctc	ctgatctcca	caggaccctg	agcataagaa tgtcccttct 1380
tctgcttcca	gtcccactat	ctagaaaaga	gaggaggagc	ccagctcttc atttcacccc 1440
caccacaaaa	ctcccacttt	tccggccctc	aaggggtgac	caaggaagtt gctccacttg 1500
gctttccaca	aacagcctgt	gccccaccag	gctcaggagg	gcagcttgac caatctctat 1560
ttccaagacc	tttgccagct	cctattgata	tggaactcctg	gataggcagc tggaccaacg 1620
gaaggagccc	atgagggtct	tgtctctcct	ggggctcctg	ctggtgagct tggagtcaac 1680
actttcggtg	agtgtctgtg	gaaccaggat	tgtcccagga	ttgttctggg gggctgctat 1740
cacagccatg	agccatggcc	tctgtctcat	acctgtgggt	ccaggctgact aggaggccta 1800

-continued

tgtgaaagg tgagccagc ccggaagcc caggcagagg agacagacaa ccagactggg	1860
tggatacaag ggcacagcct gcatttctgg gggagatggg ccttaagaag acaacggggg	1920
gaggtagaaa gggtttgggt cttgggaaga aatctctgca tttctgggct gtgagaggaa	1980
gctgcagact agcaacagat cgggtggcagg ctatgactta tagtcagtcc cctgccttct	2040
tctctccctt gtagattcca ccttgggaag cccccaagga gcataagtac aaagctgaag	2100
agcacacagt cggtaagtgg cctggctcct cctcccggga acccttgggt ggggatgtgt	2160
atggtgcagt ggtgcagtc tcagggcagt ctagtctagt gcctacctgg tgctaggtct	2220
tatgcccatg ggcactagag tgatcgtgag ctgtgtgac cttgagggca gggatgggc	2280
tgtgtctaag tgcccacag cctggctcgg agcaggtgct tgagatatgt gctgctggcg	2340
ccatcacacc tgggctcctg ccagccttcc tcagtttccc cagcttctcc ccttcttttc	2400
ctttcccag tacgtctcat gggcatcatt catgccacac agaggccagg gccttcaatg	2460
ggcaaggaag gatcaagac ttgtctctgg catctgaatg cctctgaagc ccagctttat	2520
cacttatgag ctgggtgact ctgggcgagg gatttgagtt ctccaagctt caatttccc	2580
ttctgtgaaa ccaggttgat aacagtaaac ctcttagggt tgttgagaag ggaaccat	2640
gtgaggtatt cagcccatca cctgggtgcat ggaaatgctt tacaatatt agcttttatt	2700
atgaaactac cttttagatg aagggtacct gccatttccc ccttctcaa gctctgcat	2760
agctcccat tgctttcatt cttccagaca ctaaattacc tacatgccag gcatggtgga	2820
tcatgcctgt aatcccagca ctttgggagg ccaaggtcgg tggatcatga ggtcaggagt	2880
tcgagaccag cctggccaac atggtgaaat gctgtctcta ctaaaagtac aaaaattagc	2940
caggcatggt ggcactgccc agtagtccc gctactcggg aggctgagga agaagaattg	3000
cttgaacctg gagggtgaag gttgcagtga acgaagatca caccattgca ctccagcttg	3060
ggcaacacag caagactccg tctcaaaaa aaaaaaaaaa tttaactaga gtgtggcaca	3120
tagcagggcc tgtgaaccag atggacctta ccctggtggg cctgacttgg tggggtgag	3180
tctctaagca tggcgttgag gccagcaca ttccaacctt ggaactccctc agcctctct	3240
cttcaacca caccaaaag tttctctct ctcttgcctt acccaactt ggtgacctat	3300
ccttgctaa tcccctgct aaggtecccc tctctctgt ccgtccatcc catctgcatc	3360
ttttttttt tttgagatgg agtctcctc tgtcccctag gctagagtgc aatggcgcga	3420
tcttggctca ttgcaacctc cgcctctgg gttcaagcga ttctctgct cagcctccc	3480
agttgctggg attacaggca cacaacttca tgctcagcta atttttgtat tttttagtag	3540
agacagggtt tcaccatgtt gcccaggctg gtctcgaact cctgccctca ggtggtccgc	3600
ccaccttagc ctcccagaat gctgggatta caggcgtgag ccacgcgcgc tggccccat	3660
ttgcatctta aaggtccatc tcagatccat ttccatttac tgtcctagtt ctggtttgg	3720
ccttggcaag tgcaacttgc cttgaacaaa atagtggcaa aagcttattg agcaggtact	3780
ttgtgccaga cactgctcag catttcatgg cattatctca tgaagcccca cgacaattcc	3840
tctgaagaag acacaggcaa ttctcattat tcgcatggt tatgttctat aaaatcacag	3900
tgaacattga actagcaaac agtattaggt tctgtgagc ctctggtcac aacattttca	3960
tcaaccaaca gcataaatc tggttttatg tatgattctg tttaaagaca ttttatttag	4020
tatatgtgtt gctgattcat caatgctaag ctgatggcac tatagcacac acctgaatca	4080

-continued

agtgtctaac acacgctttc tccctaaggt agccttcttg tgcttaggaa ctacacagct	4140
cttcagcagg aggctcagag gccatttoca aaagccaaat ccccagcaaa agcacaaggt	4200
gtgaaaaacg ttgcactaag tagactgaga aggacactca ttcaatagga gagctgaaac	4260
aagcagcagc agcgtgacgc cttgttgaac cttaactggg aatgtgcaa tttttcactg	4320
ctctgtgcat gccacaaat ggccatgaaa acatttcaag tattgacttg ggagttacaa	4380
ataaaattca gcaagtaggc acatttctca tgtagaacca gagaagaatg aggatcaact	4440
gtactattat tactgccgtt ttacagataa gaaaccaag gctcagatca gagtggttaa	4500
cagtgacttc aacattcaac aagtattatt aagtgcctac tttgtggcaa gtgctcttc	4560
tggccttggg actgaagact tacccaaggt cacacagcta gcaggttgtg gagtcaggag	4620
tctactccag ctatctgact cctgaaccca agttttttt tttttctta agatggagtc	4680
tcactctgtc acccaggctg gagtgacgtg gcgcatctc ggctcactgc aagctccgcc	4740
tcccgggttc acaccattct cctgcctcgg cctcccaggt agctgggact acaggcacct	4800
gccaccacc ccagctaatt tttttgtatt tttagtagag aggggggttc actgtattag	4860
ccaggatggt cttgatctcc tgacctcgtg atctgcccgc cttggcctcc caaagtgtg	4920
ggattacagg cttgagccac cgcgcccggc cctgaaccca acttttagag cagaaagtgt	4980
tttcaatgca cagcgacctt tttgaggtc tgtcctttc ctgaccagac cctgagggac	5040
agtgcctgag cagttgagta caggggaagt cctcagagag tgtgtgtcc ctgcagtct	5100
cactgtcacc ggggagccct gccacttccc cttccagtac caccggcagc tgtaccacaa	5160
atgtaccac aagggccggc caggccctca gccttgtaa gactacgagc aggagttgga	5220
gcaggggcct gggagacatg tacctgcct gtctctctgt ccaaggaact ctgcttgag	5280
agaggggact gtgatagggc aggggtgggc agcccctgg gtagagcagg gaagcctgt	5340
ctctttctac aggtgtgcta ccacccccaa ctttgatcag gaccagcagc ggggatactg	5400
tttgagccc aagaaagtga aaggtgtac acacagcctc tggggtgcc tggggctctc	5460
tcctcccgc tcattactct cctggtatca ccagaccca cacacctggg attctggacc	5520
cagcccctc tctcctcca caataccctt tggaaagcca gagggagagt tctgggaagg	5580
agtgtgcca ttttgacgtg ggttaaacca agcttgaaa cttggagtag caagtcaca	5640
aggcaagtag gttcaagaag ggccttgcc cccagctgtg tgactcagct cctgtctct	5700
cctccacca tgtccatctc tcagaccact gcagcaaca cagcccctgc cagaaaggag	5760
ggacctgtgt gaacatgcca agcggcccc actgtctctg tcacaacac ctactggaa	5820
accactgcca gaaaggtgag gagatgtgga ggacctgggc ggggtgctgg gggacagggg	5880
caacctggg cctacagaat aggttctgg atactcggag acttggcatg gtctagact	5940
ctctgagac cactatccct cttgtcccc agagaagtgc tttgagcctc agcttctccg	6000
gtttttccac aagaatgaga tatggtatag aactgagcaa gcagctgtgg ccagatgcca	6060
gtgcaagggc cctgatgccc actgccagc gctggccagc cagggtgagc agatggttg	6120
gaacgggcca gggaggagc tcaggaagac aggctggcag gagccgggt ggtgtgccg	6180
gaaggagagc tctctgggg ggtctttag cccaggggtg gctcactgcg ttcctcccc	6240
aagcctgcc caccaaccg tgctccatg ggggtcgtg cctagagggt gagggccacc	6300
gcctgtgcca ctgcccgggt ggctacacc gacctctctg cgacgtgggt gagtgagggt	6360

-continued

ctggggcaag cagaaggcca gccccaggt gggacgggct tgccaggaag gaggagggag	6420
agtgcggaag gcagatgaga gggaggcagg agagcccagc cttggctgcc cagggagccc	6480
cctttctcct cagacaccaa ggcaagctgc tatgatggcc gcgggctcag ctaccgcgcc	6540
ctggccagga ccacgctctc ggggtgcgcc tgtcagccgt gggcctcgga ggcacacctac	6600
cggaaactga ctgccgagca agcgcggaac tggggactgg gcggccacgc cttctgccgg	6660
tgcccccgt ggggctgggt gaccctccg ccccagggt cgggctccc ggcgctctaa	6720
cggcgcctcg tegtgtggct acaggaacct ggacaacgac atccgcccgt ggtgcttctg	6780
gctgaaccgc gaccgctga gctgggagta ctgcgacctg gcacagtgcc agacccaac	6840
ccaggcggcg cctccgacct cgggtgtccc taggttcat gtcccactca tgcccgcga	6900
gcccgcaccg ccgaagctc agcccacgac ccggaacctg cctcagtccc agacccggg	6960
aggttaggaa gtgggggggg gaaggaggag ccgagagggc gccgggcgag ctagattccg	7020
gccagccggc cgcgggctcc ccgtcctcag ccctgctcc tccacagcct tgccgcgaa	7080
gcgggagcag ccgcttccc tgaccaggaa cggcccactg agctgcgggc agcggctccg	7140
caagagtctg tcttcgatg cccgcgtcgt tggcgggctg gtggcgctac gggggcgca	7200
ccctacatc gccgcgctg actggggcca cagtttctgc gccggcagcc tcategcccc	7260
ctgctgggtg ctgacggccg ctcaactgct gcaggaccgg cgagtaccg cccgcccaga	7320
gcccggccag gggccgccc tccctcgtct cccagcgcag cttccacgct gcacccgaa	7380
ccgtgcctca ccttctccc ccccaccctt ctttccacgc cctccggag ctcccgggga	7440
ggaagctgga acacgggatt ggggttcggg agcagggggc tccccagaa cgcttgtggc	7500
caggtctgag agcgtgctc ctcccctacc ctcccgcag gcccgcaacc gaggatctga	7560
cgggtgtgct cggccaggaa gcgcgtaacc acagctgtga gccgtgccag acgttggccg	7620
tgcgctccta ccgcttgca caggccttct cgcctcag ctaccagcac gacctgggtg	7680
cgtggggggc ccccggggg acgggaagag agcttggggc cccggcgtcc ccgctcacc	7740
ctcctctccg cccgggttag ctctgttgcg ccttcaggag gatgcggacg gcagctgcgc	7800
gctcctgtcg ccttacgttc agccgggtg cctgccaagc ggcgcccgc gaccctccga	7860
gaccacgctc tgccaggtg ccggtcggg ccaccagttc gagggtagge acaactgcta	7920
ggggcagggg taggggagga gaccttgat cactgggta ggcggaagaa gcccgcgact	7980
ttggtatcgt tccgggtgcc tacagaatgg gtggcgctga cctgatgggt tgtgagaatg	8040
tgtaggtgaa tcccaggtag aatcccaggg cctgggatc actgctggga tccccaaac	8100
tctggggat acaggagaa tcgaactgc tcttggttcc ctctgggcgc cgggctgcaa	8160
aggccaacta ggacgtggc cccgcgctcc gggctagtgt gggagccagg ttctgcgact	8220
ctggatgggt ggtgggggag gggtttctgt ttccgctccg cccattcaaa tctggcctt	8280
tctctggacc tcagctcct tgcctatgaa attgaattaa tggcacctcc tcccctcgg	8340
gcttctgctg agagaggaag ggcagagtg ggtttacaag cgcctggagc agctttgtcc	8400
atcgtccggg cggcaagcgt tgtcagatgg ggtgtgaaga aggcgctctg tgttcgcagg	8460
ggcggaggaa tatgccagct tccctgcagga ggcgcaggta ccgttctct cctggagcg	8520
ctgctcagcc ccgacgtgc acggatcctc catcctccc ggcagctct gcgcaggggt	8580
cctcaggggc ggcaccgatg cgtgccaggt gagctcttag cccggttggc gcccttccc	8640

-continued

```

gaggccgtca ggcacaaatc tcaggtccac agcgtgagc tgcgtgttc cgaccaggg 8700
tgattccgga ggcccgtgg tgtgtgagga ccaagctgca ggcgcccgc tcaccctgca 8760
aggcatcatc agctggggat cgggctgtgg tgaccgcaac aagccaggcg tctacaccga 8820
tgtggcctac tacctggcct ggatccggga gcacaccgtt tctgtattgc tcagggactc 8880
atctttccct ccttggatgat tccgcagtga gagagtggct ggggcatgga aggcaagatt 8940
gtgtcccatt ccccagctgc gccagctcc gcgccaggat ggcgcaggaa ctcaataaag 9000
tgctttgaaa atgctgagaa gaaagctct tttctcatg ggtcccgcg ggaatgcca 9060
agacagaaaa gcgattcaca gcttctccac agctctcaga gaacaaggtc tatgagatct 9120
taactgtcaa aatctagatg ccagcccagc taatgtttac tgagcctagg atactgtata 9180
ccaagccctg tgcaaggaga agctgcatgt tattccttat gagaaactaa cattttgttt 9240
acagagcagt agttctcaga ccatacatta agatcacttg gggagcgttt tgagccaatc 9300
tatgcccaag ttccacctca gaccaattaa atcagtatgt ctagggatgg ggcattggta 9360
gtggtatatt tgtaaaactc ccagataat tccatgtaca gccaaagtgg agaactgtgg 9420
ttagaaatac ttagcattgg ccggggcgcg tggctcacgc ctgtaatcct agcactttaa 9480
gaggccaagg caggtggatt gctcaggagt tcgaaaccag cctgggcaac acgatgaaac 9540
cccgtctcta ctaaaaatac agaaattagc cgggcacggc ggcgtgcgcc tgtagtcca 9600
gctactcagg aggctgaggc aggagaatca cttgaaccgg caggaaggaa ggaaggagg 9660
aacagaggga gggaaagaga gagacagaaa gaaaagaaaa aagaaatag aaaaaagag 9720
cattgactgt ggcgtggacc ctaagggctg ggtgacatat cgttgtcccc accccaacac 9780
gcactagtgt agtgggtctg agagtccctt ggctagcagt accatcacca gggaaactgt 9840
tacacataac aaattctcgg gctacacttt atactgctga acagaaagtc tggggtgggg 9900
cccagcaatc tgtttaacag ccttgccggg gattctgatg ttctctcatg cttaagaacc 9960
acaatctggg ggttgaatgg ttggttccct tacaagtga ggtctggctg tccagacaca 10020
acatcctttt ttcacaaaac cagcttttta aaattaaaa tagattggcc agatgcggtg 10080
gttcaecct gtaatctcgt cactttgaga ggctgaggcg ggaggattgt ttgagctcaa 10140
gacttctga cccgcctggg caacatagtg agacctcctc tcaaaaaaat ttttttaat 10200
taaaatttgt ttttgctttt ttagagacgg ggctcgctc tgggctcag gctggcgtgc 10260
agcgacacga tcctataata gtttactata atctcgctac tgagtcaag cgatccgccc 10320
gcctcggcct cccaaagcgc tgggattaca ggagtgagcc gctgcgctct gccaaacca 10380
tcctacagga taaccttaga actgcgacag cactaaacgc ccacgcccc cgtgccccag 10440
cctgggtggt cgctcgggga gggcgccttg tgtgacgtea cagccccgc cagcctgcct 10500
cacagcgcg caggccttcc ccgcgtggcg cctctatatt tccccgagag gtgcgaggcg 10560
gctgggcgca ctcggagcgc gatgggcgac tggaaagtct acatcagtgc agtgct 10616

```

```

<210> SEQ ID NO 4
<211> LENGTH: 2420
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

```

```

<400> SEQUENCE: 4

```

```

agccgggtgt ggtgctcac gcctgtaatt ccagcacttc gggaggccga ggtgggcaga 60

```

-continued

tcatgaggtc	aggagttcga	gaccaacctg	gccaacatgg	tgaagccctg	gtcttacta	120
aggagacaaa	aaattagccg	ggcatggtgg	cgtgggcctg	taatggcagc	tacttgggag	180
gctgaggcag	gagaatcact	tgaacctggg	aggtaggagt	tgtagtgagc	cgagatcgca	240
ccatggcagt	ccagtttggg	cgacagcgcg	agactccatc	tcaaataaat	aactaaataa	300
aaataataat	aaagtaccct	ttcaccacca	cagctgggga	ggggtgatca	caaaatgaaa	360
ctgggagagg	aagaggaagg	gagatggatg	ttgaacatcc	aatgaccat	taatgtccac	420
ctgacacctc	atctcacact	ccctacaagg	caccttatgc	tcaccacatt	ccccatttga	480
cacgctgcat	tcaggcttct	gtgcagttgt	attactcact	ctgctgcaa	accctactt	540
gtggcgaaga	cctgttcaaa	aggccccttc	catgtagagg	aacagctgcc	ttctcccaa	600
gaggaaggag	ccaatgtggg	agtggcctta	gggccagggt	ctatactcag	gagcaatcaa	660
gaactgacag	agccaggcat	ggtggcatgc	acctgtaatc	ccagctattc	aggaggatga	720
agcgagagga	tcacttgccg	ccagaagttc	caggctagcc	tgggcaactt	aataagaccg	780
agtctctttg	aaacaacaaa	aaaggtgaca	aaggtagttt	cgctttaatg	actcgtcaat	840
atatctaata	gtttcacttt	catcatggag	ttaacagctg	tcacgtcaat	gactttgaaa	900
ccagttacct	ttgaaccag	tggttgaaa	ggtgcccttc	ctctcctgcc	ctctcttttc	960
tgtgtttctg	ttccaagaag	tcttccacag	cctgcctctc	ttccagcaga	attaataaat	1020
tgctacactc	agtcttcatt	tcccataaat	tactttgtat	aacttaggac	ttgtataac	1080
tgaagtgtac	agaagtccac	tcaaattggt	tttattggct	catataactg	agaagtctat	1140
tttttttttt	ttggagata	cagtctcagt	ctgcttccca	gactggagtg	cagtggcaca	1200
atcacggctc	acagcattht	ggacctctgg	gctcaagcaa	tcctcctgcc	tcagactcac	1260
aagtagccgg	aactacaggc	acgcgccacc	atgcctggct	aatgttaaat	ttttttggag	1320
agatgggatc	tcactatggt	gcctacatca	gtctggaact	cctgggctca	agtgatectc	1380
aagtgatccc	cagcctccca	gagtgttggg	attacaggcg	tgagccactg	tgcttagcca	1440
taactgacca	agtcttaagg	atacttctag	ttttaggatg	taggctctca	aagtcatgag	1500
aatcaatcc	atcagtacta	gttctcaaat	gtcgtgtaac	aatcacctc	aacttgtggc	1560
ttaaagcaac	aacatttaat	gatgatttat	cacagtttct	gatggtcagt	tttgccttga	1620
ggtctttcat	aagattgcaa	gccaaatgtc	agcgagggct	gcagtatcca	cttccaaggc	1680
agcttattca	tgtggtctgc	caattggcac	tggtctcag	caggggtgctt	tcctttctcc	1740
ctacatgggc	ttctctgcag	ggctgcatgt	gtgtcctcac	aacacagtgg	ctggcttcct	1800
ccagagtggg	caacctaa	gaccgaggca	gaaactgcaa	tgtcttttat	gacctgggct	1860
tggaaagcca	acacctcac	ttctgcata	tttttttttt	tttgagacag	tcctgtctgc	1920
ccaggctgga	gtgcaatggc	acaatctctg	ctcaactaca	cctccacttc	ctgggttcaa	1980
gcgattctcc	tgctcagcc	tcacgggtag	ctgggattac	aggcacacgc	cactacgccc	2040
ggctaatttt	tgtatttttc	atagagatgg	ggtttgcca	acacgttggc	caggccggtc	2100
ttgaactcct	gacctagagt	gatctgcctg	ccttggcatc	ccaaagtgct	gggattacag	2160
gtgtgagcca	ccacaccg	ccacttctgt	catattctga	tggacacaca	gaacagccct	2220
aactcaatgt	gggaagacac	catacaaggg	catgaatcct	agggtgctgag	gatcaatggg	2280
ggctgtcttt	gaggctgtct	accacagcat	ctttccatcc	ttctgcctc	gtttgccttg	2340

-continued

```
cttttcctat gtgtaggctt cattotcaaa caggccctcc ctagagagtg acaaaggatga 2400
tcacaaatgt gttcagaccc 2420
```

```
<210> SEQ ID NO 5
<211> LENGTH: 1402
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
```

```
<400> SEQUENCE: 5
```

```
gcgggaccag cgcacgcagc acgtggccat cgtgggcat gcggacaaca gctgcgtgtg 60
ggcttcgcgg cccggggggc tgctggcggc catctcggcg caggagggtg gcgtgctcac 120
ggggccggac aggcacacct tcctgcaggc gggcctgagc gtggggggcc gccgctgctg 180
cgtcacccgc gaccacctgc tggccgaggg tgacggcgtg ctggacgcac gcaccaaggg 240
gctggacgcg cgcgccgtgt gcgtgggccc tgcgccgcgc gcgctcctgg tgctaatggg 300
ccgacgcggc gtacatgggg gcacccctca caagacggtg cacgaactca tacgcgggct 360
gcgcatgcag ggcgcctagc cggccagcca ggccgcccac tggtagcgcg ggccaaataa 420
actgtgacct gggcgcggct ggctcctcct ccacttgcgc ggtgggggga gttgtaataa 480
aggaaaactg tctttgcaag acggttaoct ggtggagccg ggattttgag tetagaggct 540
gccaggcccc tgtgcctac acctgtctct cccatggagc ccttcagag gctcctggcc 600
tgactgtgc tccttggcgc gttcccaggg tcctagggac tccgcagctg aggaagagt 660
caagggtggg ggcttctcaa agtctgttcc agccttagcg tcctttctca gagatattcc 720
cacacattag gcaggacaag taaagggagc cccctcccca tcccgcgaac acctctcccc 780
atcagggtgt caggctggag gcccaattgc tcctccccc tccactcata cctcaagcac 840
tagcaagtgt tgagtgggtg acaggatggg cttggtggct tgtaaagcag tcttggggct 900
cacaggcctc tgcactcttg cccacattcc tccaagggga gcctactgag agggctcatg 960
tccaagacca tcgcaattgg gtttgagacc ttacatcctg ccttcccag gccttcgaaa 1020
aggccccgca ggagtcctct gactagaggg aggaactctg gcacccctac ccgggagtct 1080
cactctgcag gcctcagttt cagggtgacc tatggaggag ggggaattga aaagcttggg 1140
taagtttgag gcctggttta ttgcccagg atagtggaca aaagtgggag ggagggtgtc 1200
tggtcctgc cctccatgtg ctggggccca gggcatggcc tctcttggcc acccccacc 1260
ttcccgctcc ctccccagc ggccctgatg gcagacccca cctgtcactt attctggagc 1320
cctgatctta tcccagcagg aaggagtgat gtgtggctga ggtgggtgaa tttagaggc 1380
agagggagac cagaggaagt tc 1402
```

```
<210> SEQ ID NO 6
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex1-F
```

```
<400> SEQUENCE: 6
```

```
aggaagtgc tccacttggc ttt 23
```

```
<210> SEQ ID NO 7
<211> LENGTH: 23
<212> TYPE: DNA
```

-continued

<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex1-R

<400> SEQUENCE: 7

tgcagagatt tcttccaag acc 23

<210> SEQ ID NO 8
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex2-F

<400> SEQUENCE: 8

ctatgtggaa aggtgaggcc ag 22

<210> SEQ ID NO 9
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex2-R

<400> SEQUENCE: 9

ctcaaggatc acacagctca 22

<210> SEQ ID NO 10
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex3-4-F

<400> SEQUENCE: 10

tgagggtctg tccttttct ga 22

<210> SEQ ID NO 11
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex3-4-R

<400> SEQUENCE: 11

ggtgtgtggg gtctggtgat ac 22

<210> SEQ ID NO 12
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex5-6-F

<400> SEQUENCE: 12

gtaggttcaa gaagggcctt gg 22

<210> SEQ ID NO 13
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex5-6-R

-continued

<400> SEQUENCE: 13
gagctctcct tcccggcac 19

<210> SEQ ID NO 14
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex7-F

<400> SEQUENCE: 14
gagcagatgg ttgggaacg 19

<210> SEQ ID NO 15
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex7-R

<400> SEQUENCE: 15
tgaggagaaa gggggctc 18

<210> SEQ ID NO 16
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex8-F

<400> SEQUENCE: 16
ggtctggggc aagcagaag 19

<210> SEQ ID NO 17
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex8-R

<400> SEQUENCE: 17
tgtagccaca cgacgggg 18

<210> SEQ ID NO 18
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex9-F

<400> SEQUENCE: 18
gaacgtgact gccgagcaag 20

<210> SEQ ID NO 19
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex9-R

<400> SEQUENCE: 19
aggagcaggg gctgaggac 19

-continued

<210> SEQ ID NO 20
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex10-F

<400> SEQUENCE: 20

gaaggaggag ccgagaggg 19

<210> SEQ ID NO 21
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex10-R

<400> SEQUENCE: 21

ggtaggggag aggcagcg 18

<210> SEQ ID NO 22
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex11-12-F

<400> SEQUENCE: 22

aggaagctgg aacacgggat t 21

<210> SEQ ID NO 23
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex11-12-R

<400> SEQUENCE: 23

ataccaaagt cgcgggcttc t 21

<210> SEQ ID NO 24
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex13-F

<400> SEQUENCE: 24

cccattcaaa tectggcttt tc 22

<210> SEQ ID NO 25
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex13-R

<400> SEQUENCE: 25

aatcacctg ggtcggaaac 20

<210> SEQ ID NO 26
<211> LENGTH: 21
<212> TYPE: DNA

-continued

<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex14-F

<400> SEQUENCE: 26
gtgccaggtg agctcttagc c 21

<210> SEQ ID NO 27
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex14-R

<400> SEQUENCE: 27
ccttgttctc tgagagctgt gga 23

<210> SEQ ID NO 28
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr2-pt1-F

<400> SEQUENCE: 28
tgtatggtgc agtgtgtgca gt 22

<210> SEQ ID NO 29
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr2-pt1-R

<400> SEQUENCE: 29
ggcatgtagg taatttagtg tctggaa 27

<210> SEQ ID NO 30
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr2-pt2-F

<400> SEQUENCE: 30
ccttttagat gaagggtacc tgcc 24

<210> SEQ ID NO 31
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr2-pt2-R

<400> SEQUENCE: 31
gagaaacttt tgggtgtggg gt 22

<210> SEQ ID NO 32
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr2-pt3-F

-continued

<400> SEQUENCE: 32
ctgacttggt ggggttgagt ct 22

<210> SEQ ID NO 33
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr2-pt3-R

<400> SEQUENCE: 33
tgccactatt ttgttcaagg ca 22

<210> SEQ ID NO 34
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr2-pt4-F

<400> SEQUENCE: 34
ccatttgcat cttaaaggtc catc 24

<210> SEQ ID NO 35
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr2-pt4-R

<400> SEQUENCE: 35
tcacactttg tgcttttgcg gg 22

<210> SEQ ID NO 36
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr2-pt5-F

<400> SEQUENCE: 36
acacacgctt tctccctaag gt 22

<210> SEQ ID NO 37
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr2-pt5-R

<400> SEQUENCE: 37
ggagtagact cctgactcca caa 23

<210> SEQ ID NO 38
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr2-pt6-F

<400> SEQUENCE: 38
agtattatta agtgcctact ttgtggc 27

-continued

<210> SEQ ID NO 39
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr2-pt6-R

<400> SEQUENCE: 39

cagtgagaac tgcaggaca ac 22

<210> SEQ ID NO 40
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr4-F

<400> SEQUENCE: 40

gaggggactg tgataggca g 21

<210> SEQ ID NO 41
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr4-R

<400> SEQUENCE: 41

acacaggtec ctctttctg g 21

<210> SEQ ID NO 42
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr4-R

<400> SEQUENCE: 42

agaccagct ctgccaggt 19

<210> SEQ ID NO 43
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr12-F

<400> SEQUENCE: 43

gtaaaccac tcatgccctt cc 22

<210> SEQ ID NO 44
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr12-R

<400> SEQUENCE: 44

cgttettctt tcatgttcca gc 22

<210> SEQ ID NO 45
<211> LENGTH: 22
<212> TYPE: DNA

-continued

<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-P(-1)-F

<400> SEQUENCE: 45

actggcctaaa ggtcttgtaa at 22

<210> SEQ ID NO 46
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-P(-1)-R

<400> SEQUENCE: 46

cacagcatct ttccatcctt cc 22

<210> SEQ ID NO 47
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-P(-2)-F

<400> SEQUENCE: 47

atcttggggc catcttagca tt 22

<210> SEQ ID NO 48
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-P(-2)-R

<400> SEQUENCE: 48

gtgtcctcac aacacagtgg ct 22

<210> SEQ ID NO 49
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-P(-3)-F

<400> SEQUENCE: 49

cacattgatg atcacctttg tcac 24

<210> SEQ ID NO 50
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-P(-4)-F

<400> SEQUENCE: 50

tgtgcctagc cataactgac ca 22

<210> SEQ ID NO 51
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-P(-4)-R

-continued

<400> SEQUENCE: 51
tggacttcca agcccaggt 19

<210> SEQ ID NO 52
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-P(-5)-F

<400> SEQUENCE: 52
gtcacgtcaa tgactttgaa acc 23

<210> SEQ ID NO 53
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-P(-5)-R

<400> SEQUENCE: 53
cgacatttga gaactagtagt tgatgg 26

<210> SEQ ID NO 54
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-3'UTR-pt1-F

<400> SEQUENCE: 54
tcaataaagt gctttgaaaa tgctga 26

<210> SEQ ID NO 55
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-3'UTR-pt1-R

<400> SEQUENCE: 55
tagagacggg gtttcacgt gt 22

<210> SEQ ID NO 56
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-3'UTR-pt2-F

<400> SEQUENCE: 56
gaaatactta gcattggccg gg 22

<210> SEQ ID NO 57
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-3'UTR-pt2-R

<400> SEQUENCE: 57
aaccattcaa cccccagatt gt 22

-continued

<210> SEQ ID NO 58
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex9-seqint1-R

<400> SEQUENCE: 58

ccccacttc ctaacctccc 20

<210> SEQ ID NO 59
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-P(-1)-S2-R

<400> SEQUENCE: 59

tttgagacgg agtctcgct 19

<210> SEQ ID NO 60
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex9-ARMS-Mt1-F

<400> SEQUENCE: 60

cgccgaagcc tcagcccaa 19

<210> SEQ ID NO 61
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex9-ARMS-Mt1-R

<400> SEQUENCE: 61

gcgggtcatc gaagacagac t 21

<210> SEQ ID NO 62
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex9-RFLP-Mt2-F

<400> SEQUENCE: 62

cccgggtgcc cctaggcttc 20

<210> SEQ ID NO 63
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Ex9-RFLP-Mt2-R

<400> SEQUENCE: 63

ctgccggcgc agaaactgt 19

<210> SEQ ID NO 64
<211> LENGTH: 19
<212> TYPE: DNA

-continued

```

<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr10-RFLP-Mt3-F

<400> SEQUENCE: 64
aagcgcggaa ctggggact 19

<210> SEQ ID NO 65
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: F12-Intr10-RFLP-Mt3-R

<400> SEQUENCE: 65
gctgaacgta aggcgacagg ag 22

<210> SEQ ID NO 66
<211> LENGTH: 2048
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: variation
<222> LOCATION: (1032)..(1032)
<223> OTHER INFORMATION: /replace="a"
                        /replace="g"

<400> SEQUENCE: 66
ctattgatct ggactcctgg ataggcagct ggaccaacgg acggacgcca tgagggtctt 60
gctgctcctg gggttcctgc tggtagctt ggagtcaaca ctttcgattc caccttggga 120
agccccaag gagcataagt acaaagctga agagcacaca gtcgttctca ctgtcaccgg 180
ggagccctgc cacttcccct tccagtacca ccggcagctg taccacaaat gtaccacaaa 240
gggcccggca gcccctcagc cctgggtgtc taccacccc aactttgatc aggaccagcg 300
atggggatac tgtttggagc ccaagaaagt gaaagaccac tgcagcaaac acagcccctg 360
ccagaaagga gggacctgtg tgaacatgcc aagcggcccc cactgtctct gtccacaaca 420
cctcactgga aacctctgcc agaaagagaa gtgctttgag cctcagcttc tccggttttt 480
ccacaagaat gagatatggt atagaactga gcaagcagct gtggccagat gccagtgcaa 540
gggtcctgat gccactgcc agcggctggc cagccaggcc tgcccaccca acccgtgcct 600
ccatgggggt cgctgcctag aggtggaggg ccaccgcctg tgccactgcc cgggtgggcta 660
caccggaccc ttctgogacg tggacaccaa ggcaagctgc tatgatggcc gcgggctcag 720
ctaccgcggc ctggccagga ccacgctctc ggggtgcgcc tgtcagccgt gggcctcgga 780
ggccacctac cggaactgta ctgccagca agcgcggaac tggggactgg gcggccacgc 840
cttctgccgg aacccggaca acgacatccg cccgtggtgc ttcgtgctga accgcgaccg 900
gctgagctgg gactactgcg acctggcaca gtgccagacc ccaaccagg cggcgcctcc 960
gaccccggtg tcccctaggc ttcattgccc actcatgccc gcgcagccgg caccgcccga 1020
gcctcagccc acgaccggga ccccgcctca gtcccagacc ccgggagcct tgccggcgaa 1080
gcgggagcag ccgccttccc tgaccaggaa cggcccactg agctgcgggc agcggctccg 1140
caagagtctg tcttcgatga cccgcgtcgt tggcgggctg gtggcgctac gcggggcgca 1200
cccctacatc gccgcgctgt actggggcca cagtttctgc gccggcagcc tcatcgcccc 1260
ctgctgggtg ctgacggccg ctcactgcct gcaggaccgg cccgcacccg aggatctgac 1320

```

-continued

ggtggtgctc ggccaggaac gccgtaacca cagctgtgag ccgtgccaga cgttgccct	1380
gcgctcctac cgcttgcaag aggccttctc gcccgtcagc taccagcagc acctggctct	1440
ggtgcgctt caggaggatg cggacggcag ctgcgcgctc ctgtgcctt acgttcagcc	1500
ggtgtgctg ccaagcggcg ccgcgcgacc ctccgagacc acgctctgcc agtgggccgg	1560
ctggggccac cagttcgagg gggcggagga atatgccagc ttctgcagg aggcgcaggt	1620
accgttctc tcctggagc gctgctcagc ccgggacgtg cacggatcct ccatectccc	1680
cggcatgctc tgcgcagggt tcctcgaggg cggcaccgat gcgtgccagg gtgattccgg	1740
aggcccctg gtgtgtgagg accaagctgc agagcggcgg ctaccctgc aaggcatcat	1800
cagctgggga tcgggctgtg gtgaccgcaa caagccagc gtctacaccg atgtggccta	1860
ctacctggcc tggatccggg agcacaccgt ttctgattg ctcagggact catctttccc	1920
tccttggtga ttccgcagtg agagagtggc tggggcatgg aaggcaagat tgtgtcccat	1980
tccccagtg cggccagctc cgcgccagga tggcggaggaa ctcaataaag tgctttgaaa	2040
atgctgag	2048

1. A method of diagnosing hereditary angioedema type III (HAE III) or a predisposition thereto in a subject being suspected of having developed or of having a predisposition to develop a hereditary angioedema type III or in a subject being suspected of being a carrier for hereditary angioedema type III, the method comprising determining in vitro from a biological sample of said subject the presence or absence of a disease-associated mutation in a nucleic acid molecule regulating the expression of or encoding coagulation factor XII; wherein the presence of such a mutation is indicative of a hereditary angioedema type III or a predisposition thereto.

2. The method of claim 1, wherein said determination comprises hybridizing under stringent conditions to said nucleic acid molecule at least one pair of nucleic acid probes, the first probe of said pair being complementary to the wild-type sequence of said nucleic acid molecule and the second probe of said pair being complementary to the mutant sequence of said nucleic acid molecule, wherein a perfect match, the presence of stable hybridization, between (i) the first hybridization probe and the target nucleic acid molecule indicates the presence of a wild-type sequence, and (ii) the second hybridization probe and the target nucleic acid molecule, indicates the presence of a mutant sequence, wherein the first hybridization probe and the second hybridization probe allow a differential detection.

3. The method of claim 1, said method comprising hybridizing under stringent conditions to said nucleic acid molecule a hybridization probe specific for a mutant sequence.

4. The method of claim 1, comprising a step of nucleic acid amplification and/or nucleic acid sequencing.

5. The method of claim 1, wherein the method is or comprises an allele discrimination method selected from the group consisting of allele-specific hybridization, allele-specific primer extension including allele-specific PCR, allele-specific oligonucleotide ligation, allele-specific cleavage of a flap probe and/or allele-specific cleavage using a restriction endonuclease.

6. The method of claim 1, comprising a detection method selected from the group consisting of fluorescence detection, time-resolved fluorescence, fluorescence resonance energy transfer (FRET), fluorescence polarization, colorimetric methods, mass spectrometry, (chemi)luminescence, electrophoretic detection and electrical detection methods.

7. The method of claim 1, wherein the probe or the subject's nucleic acid molecule is attached to a solid support.

8. A method of diagnosing hereditary angioedema type III (HAE III) or a predisposition thereto in a subject being suspected of having developed or of having a predisposition to develop a hereditary angioedema type III or in a subject being suspected of being a carrier for hereditary angioedema type III, the method comprising assessing the presence, amount and/or activity of coagulation factor XII in said subject and including the steps of:

- (a) determining from a biological sample of said subject in vitro, the presence, amount and/or activity of:
 - (i.) a (poly)peptide encoded by the coagulation factor XII gene;
 - (ii.) a substrate of the (poly)peptide of (i); or
 - (iii.) a (poly)peptide processed by the substrate mentioned in (ii);
- (b) comparing said presence, amount and/or activity with that determined from a reference sample; and
- (c) diagnosing, based on the difference between the samples compared in step (b), the pathological condition of a hereditary angioedema type III or a predisposition thereto.

9. The method of claims 1 and 8, wherein the biological sample consists of or is taken from hair, skin, mucosal surfaces, body fluids, including blood, plasma, serum, urine, saliva, sputum, tears, liquor cerebrospinalis, semen, synovial fluid, amniotic fluid, milk, lymph, pulmonary sputum, bronchial secretion, or stool.

10. The method of claim 8, wherein said presence, amount and/or activity is determined by using an antibody or an aptamer, wherein the antibody or aptamer is specific for (a) a

(poly)peptide encoded by the coagulation factor XII gene; (b) a substrate of the (poly)peptide of (a); or (c) a (poly)peptide processed by the substrate mentioned in (b).

11. The method of claim **10**, wherein said antibody or aptamer is specific for a (poly)peptide encoded by the coagulation factor XII gene.

12. The method of claim **8**, wherein the presence, amount and/or activity of the (poly)peptide(s) encoded by the coagulation factor XII gene is determined in (a) a coagulation assay; or in (b) a functional amidolytic assay; or in (c) a mitogenic assay; or in (d) a binding assay measuring binding of a (poly)peptide encoded by the coagulation factor XII gene to a binding partner.

13. A method of identifying a compound modulating coagulation factor XII activity which is suitable as a medicament or a lead compound for a medicament for the treatment and/or prevention of hereditary angioedema type III, the method comprising the steps of:

- (a) in vitro contacting a coagulation factor XII (poly)peptide or a functionally related (poly)peptide with the potential modulator; and
- (b) testing for modulation of coagulation factor XII activity,

wherein modulation of coagulation factor XII activity is indicative of a compound's suitability as a medicament or a lead compound for a medicament for the treatment and/or prevention of hereditary angioedema type III.

14. The method of claim **13**, wherein the coagulation factor XII (poly)peptide of step (a) is present in cell culture or cell culture supernatant or in a subject's sample or purified from any of these sources.

15. The method of claim **13**, wherein said testing is performed by assessing the physical interaction between a coagulation factor XII (poly)peptide and the modulator and/or the effect of the modulator on the function of said coagulation factor XII (poly)peptide.

16. The method of claim **13**, wherein the modulator is an inhibitor of coagulation factor XII activity, selected from the group consisting of:

- (a) an aptamer or inhibitory antibody or fragment or derivative thereof, specifically binding to a coagulation factor XII (poly)peptide and/or specifically inhibiting a coagulation factor XII activity;
- (b) a small molecule inhibitor of coagulation factor XII and/or coagulation factor XII activity; and
- (c) a serine protease inhibitor selected from group (I) consisting of wild-type and modified or engineered proteinaceous inhibitors of serine proteases including C1 esterase inhibitor, antithrombin III, α 2-antiplasmin, α 1-antitrypsin, ovalbumin serpins, and α 2-macroglobulin, or selected from group (II) of Kunitz-type inhibitors including bovine pancreatic trypsin inhibitor.

17. A method of identifying a compound modulating coagulation factor XII expression and/or secretion which is suitable as a medicament or lead compound for a medicament for the treatment and/or prevention of hereditary angioedema type III, the method comprising the steps of:

- (a) in vitro contacting a cell that expresses or is capable of expressing coagulation factor XII with a potential modulator of expression and/or secretion; and
- (b) testing for altered expression and/or secretion, wherein the modulator is (i) a small molecule compound, an aptamer or an antibody or fragment or derivative thereof, specifically modulating expression and/or

secretion of coagulation factor XII; or (ii) a siRNA or shRNA, a ribozyme, or an antisense nucleic acid molecule specifically hybridizing to a nucleic acid molecule encoding coagulation factor XII or regulating the expression of coagulation factor XII.

18. The method of claims **13** and **17**, wherein coagulation factor XII is a disease-associated mutant of coagulation factor XII.

19. The method of claims **13** and **17**, wherein said modulator is selective for a disease-associated mutant of coagulation factor XII, the method comprising (a) comparing the effect of the modulator on wild-type and disease-associated coagulation factor XII activity or their expression and/or secretion; and (b) selecting a compound which (i) modulates disease-associated coagulation factor XII activity or its expression and/or secretion and which (ii) does not affect wild-type coagulation factor XII activity or its expression and/or secretion.

20. The method of any one of claims **1**, **8**, **13** and **17**, wherein the disease-associated mutant or mutation is:

- (a) a mutant located in the fibronectin type II domain, within the region of amino acid position 1 to 76, and/or a mutation located in the nucleic acid sequence encoding the fibronectin type II domain, within mRNA position 107 to 334;
- (b) a mutant located in the EGF-like domain 1, within the region of amino acid position 77 to 113, and/or a mutation located in the nucleic acid sequence encoding the EGF-like domain 1, within mRNA position 335 to 445;
- (c) a mutant located in the fibronectin type I domain, within the region of amino acid position 114 to 157, and/or a mutation located in the nucleic acid sequence encoding the fibronectin type I domain, within mRNA position 446 to 577;
- (d) a mutant located in the EGF-like domain 2, within the region of amino acid position 158 to 192, and/or a mutation located in the nucleic acid sequence encoding the EGF-like domain 2, within mRNA position 578 to 682;
- (e) a mutant located in the kringle domain, within the region of amino acid position 193 to 276, and/or a mutation located in the nucleic acid sequence encoding the kringle domain, within mRNA position 683 to 934;
- (f) a mutant located in the proline-rich region, within the region of amino acid position 277 to 331, and/or a mutation located in the nucleic acid sequence encoding the proline-rich region, within mRNA position 935 to 1099;
- (g) a mutant located in the region of proteolytic cleavage sites, within the region of amino acid position 332 to 353, and/or a mutation located in the nucleic acid sequence encoding the region of proteolytic cleavage sites, within mRNA position 1100 to 1165;
- (h) a mutant located in the serine protease domain, within the region of amino acid position 354 to 596, and/or a mutation located in the nucleic acid sequence encoding the serine protease domain, within mRNA position 1166 to 1894;
- (i) a mutant located in the signal peptide, within the region of amino acid position -19 to -1, and/or a mutation located in the nucleic acid sequence encoding the signal peptide, within mRNA position 50 to 106;
- (j) a mutation located in the untranslated regions (UTRs) of coagulation factor XII mRNA, within mRNA position 1 to 49 and/or 1895 to 2048;

- (k) a mutation located in an intron of the coagulation factor XII gene; and/or
- (l) a mutation located in a flanking regulatory genomic sequence of the coagulation factor XII gene, within the region encompassing 4000 bp upstream of the transcription initiation site of the coagulation factor XII gene and/or within the region encompassing 3000 bp downstream of the nucleotide sequence representing the 3'-UTR of the coagulation factor XII mRNA.
- 21.** The method of claim **20**, wherein said mutant in (f) is a mutant affecting amino acid residue 309 or 310.
- 22.** The method of claim **21**, wherein said amino acid residue at position 309 is substituted by a basic or positively charged amino acid residue.
- 23.** The method of claim **22**, wherein said basic or positively charged amino acid residue is a lysine or arginine.
- 24.** The method of claims **13** and **17**, comprising the additional step of producing the modulator identified in said methods.
- 25.** The method of claims **1** and **8**, comprising in vitro testing of a sample of a blood donor for determining whether the blood of said donor or components thereof may be used for transfusion to a patient in need thereof, wherein a positive testing indicates a predisposition for hereditary angioedema type III, excluding the transfusion of blood or components thereof from said donor.
- 26.** Use of (a) a (poly)peptide encoded by the coagulation factor XII gene or a fragment thereof, (b) a modulator of coagulation factor XII identified by any of the methods of claims **13** and **17**; (c) a nucleic acid molecule capable of expressing coagulation factor XII or a fragment thereof; and/or (d) a nucleic acid molecule capable of expressing a modulator of coagulation factor XII activity or its expression and/or secretion, for the preparation of a pharmaceutical composition for the treatment and/or prevention of hereditary angioedema type III.
- 27.** The use of claim **26**, wherein said coagulation factor XII or said (poly)peptide is a mutant coagulation factor XII or mutant (poly)peptide or a fragment thereof.
- 28.** The use of claim **27**, wherein said mutant is or is based on:
- a mutant located in the fibronectin type II domain, within the region of amino acid position 1 to 76, and/or a mutation located in the nucleic acid sequence encoding the fibronectin type II domain, within mRNA position 107 to 334;
 - a mutant located in the EGF-like domain 1, within the region of amino acid position 77 to 113, and/or a mutation located in the nucleic acid sequence encoding the EGF-like domain 1, within mRNA position 335 to 445;
 - a mutant located in the fibronectin type I domain, within the region of amino acid position 114 to 157, and/or a mutation located in the nucleic acid sequence encoding the fibronectin type I domain, within mRNA position 446 to 577;
 - a mutant located in the EGF-like domain 2, within the region of amino acid position 158 to 192, and/or a mutation located in the nucleic acid sequence encoding the EGF-like domain 2, within mRNA position 578 to 682
 - a mutant located in the kringle domain, within the region of amino acid position 193 to 276, and/or a mutation located in the nucleic acid sequence encoding the kringle domain, within mRNA position 683 to 934;
- a mutant located in the proline-rich region, within the region of amino acid position 277 to 331, and/or a mutation located in the nucleic acid sequence encoding the proline-rich region, within mRNA position 935 to 1099;
 - a mutant located in the region of proteolytic cleavage sites, within the region of amino acid position 332 to 353, and/or a mutation located in the nucleic acid sequence encoding the region of proteolytic cleavage sites, within mRNA position 1100 to 1165;
 - a mutant located in the serine protease domain, within the region of amino acid position 354 to 596, and/or a mutation located in the nucleic acid sequence encoding the serine protease domain, within mRNA position 1166 to 1894;
 - a mutant located in the signal peptide, within the region of amino acid position -19 to -1, and/or a mutation located in the nucleic acid sequence encoding the signal peptide, within mRNA position 50 to 106;
 - a mutation located in the untranslated regions (UTRs) of coagulation factor XII mRNA, within mRNA position 1 to 49 and/or 1895 to 2048;
 - a mutation located in an intron of the coagulation factor XII gene; and/or
 - a mutation located in a flanking regulatory genomic sequence of the coagulation factor XII gene, within the region encompassing 4000 bp upstream of the transcription initiation site of the coagulation factor XII gene and/or within the region encompassing 3000 bp downstream of the nucleotide sequence representing the 3'-UTR of the coagulation factor XII mRNA.
- 29.** The use of claim **28**, wherein said mutant in (f) is a mutant affecting amino acid residue 309 or 310.
- 30.** The use of claim **29**, wherein said amino acid residue at position 309 is substituted by a basic or positively charged amino acid residue.
- 31.** The use of claim **30**, wherein said basic or positively charged amino acid residue is a lysine or arginine.
- 32.** The use of claim **26**, wherein said modulator is an inhibitor of coagulation factor XII, its activity, its expression and/or its secretion, comprising:
- an aptamer or an inhibitory antibody or fragment or derivative thereof, specifically binding to and/or specifically inhibiting the activity of (i) disease-associated coagulation factor XII or (ii) wild-type and disease-associated coagulation factor XII;
 - a small molecule inhibitor of (i) disease-associated coagulation factor XII and/or disease-associated coagulation factor XII activity; or (ii) wild-type and disease-associated coagulation factor XII and/or wild-type and disease-associated coagulation factor XII activity;
 - a serine protease inhibitor of (i) disease-associated coagulation factor XII or of (ii) wild-type and disease-associated coagulation factor XII selected from a first group consisting of wild-type and modified or engineered proteinaceous inhibitors of serine proteases including C1 esterase inhibitor, antithrombin III, α 2-antiplasmin, α 1-antitrypsin, ovalbumin serpins, and α 2-macroglobulin, or selected from a second group consisting of Kunitz-type inhibitors including bovine pancreatic trypsin inhibitor; or
 - a siRNA or shRNA, a ribozyme or an antisense nucleic acid molecule specifically hybridizing to a nucleic acid molecule encoding coagulation factor XII or regulating the expression of coagulation factor XII, either affecting

(i) disease-associated coagulation factor XII or (ii) wild-type and disease-associated coagulation factor XII.

33. A method of gene therapy in a mammal, characterized by administering an effective amount of a nucleic acid molecule capable of expressing in the mammal:

- (a) siRNA or shRNA, a ribozyme or an antisense nucleic acid molecule specifically hybridizing to a nucleic acid molecule encoding coagulation factor XII or regulating its expression;
- (b) an aptamer or an inhibitory antibody or fragment or derivative thereof, specifically binding coagulation factor XII (poly)peptide;
- (c) coagulation factor XII or a fragment thereof; or
- (d) a serine protease inhibitor selected from group (i) consisting of wild-type and modified or engineered proteinaceous inhibitors of serine proteases including C1 esterase inhibitor, antithrombin III, α 2-antiplasmin, α 1-antitrypsin, ovalbumin serpins, and α 2-macroglobulin, or selected from group (ii) of Kunitz-type inhibitors including bovine pancreatic trypsin inhibitor.

34. A non-human transgenic animal, comprising as a transgene:

- (a) a gene encoding human disease-associated coagulation factor XII;
- (b) (i) a gene encoding human disease-associated coagulation factor XII and (ii) a gene encoding human wild-type coagulation factor XII;
- (c) a nucleic acid molecule causing an altered expression of human coagulation factor XII and a gene encoding human wild-type coagulation factor XII; and/or
- (d) a species-specific coagulation factor XII gene which is specifically altered to contain a human disease-associated mutation.

35. The non-human transgenic animal of claim **34**, additionally expressing siRNA or shRNA, a ribozyme or an antisense nucleic acid molecule specifically hybridizing to said human gene(s) of (a) **34(a)**, (b) **34(b)(i)** or **34(b)**, (c) to the nucleic acid molecule of claim **34(c)**, or (d) to the altered species-specific gene of **34(d)**.

36. The non-human transgenic animal of claim **34**, wherein the animal's native species-specific genes encoding coagulation factor XII are inactivated.

37. Use of the transgenic animal of claim **34**, for screening for compounds for use in the diagnosis, prevention and/or treatment of hereditary angioedema type III.

38. A nucleic acid molecule comprising the human coagulation factor XII nucleotide sequence or a fragment thereof, having a mutation at a position corresponding to position 6927 of GenBank accession no. AF 538691, wherein the wild-type C is substituted by an A or by a G.

39. An oligonucleotide containing at least 8 nucleotides of (a) the mutant nucleotide sequence of claim **38** comprising position 6927, wherein the oligonucleotide contains a nucle-

otide corresponding to mutant position 6927, or the corresponding wild-type sequence of said oligonucleotide or (b) the complementary sequence of (a).

40. A (poly)peptide or a fragment thereof, encoded by the nucleic acid molecule of claim **38**.

41. An antibody or antibody fragment specific for the (poly)peptide of claim **40**.

42. The antibody of claim **41**, which is a monoclonal or polyclonal antibody.

43. A hybridoma producing the monoclonal antibody of claim **42**.

44. A kit for use in diagnosis of hereditary angioedema type III or a susceptibility or predisposition thereto, said kit comprising:

- (a) at least one nucleic acid molecule capable of hybridizing under stringent conditions to a nucleic acid molecule encoding or regulating the expression of coagulation factor XII;
- (b) an antibody or an aptamer specific for coagulation factor XII or a fragment thereof and/or a disease-associated mutant of these;
- (c) a restriction enzyme capable of discriminating between wild-type and disease-associated mutant nucleic acid encoding or regulating the expression of coagulation factor XII;
- (d) a pair of primers complementary to nucleic acid regulating the expression of coagulation factor XII or encoding wild-type and/or disease-associated coagulation factor XII;
- (e) a nucleic acid molecule comprising the human coagulation factor XII nucleotide sequence or a fragment thereof, having a mutation at a position corresponding to position 6927 of GenBank accession no. AF 538691, wherein the wild-type C is substituted by an A or by a G;
- (f) an oligonucleotide containing at least 8 nucleotides of (a) the mutant nucleotide sequence of (e) comprising position 6927, wherein the oligonucleotide contains a nucleotide corresponding to mutant position 6927, or the corresponding wild-type sequence of said oligonucleotide or (b) the complementary sequence of (a);
- (g) a (poly)peptide or a fragment thereof, encoded by the nucleic acid molecule of (e);
- (h) an antibody or antibody fragment specific for the (poly)peptide of (g); and/or
- (i) a hybridoma producing the monoclonal antibody of (h); and optionally instructions for use.

45. The kit of claim **44**, wherein said disease-associated mutant is a mutant as or a mutant as defined in claim **38**.

46. The kit of claim **44**, wherein (a) is a primer pair capable of amplifying exon 9 of human coagulation factor XII gene or a part thereof comprising the mutant position as defined in claim **38** or a probe or pair of probes.

* * * * *

专利名称(译)	检测III型遗传性血管性水肿的方法和试剂盒		
公开(公告)号	US20100324114A1	公开(公告)日	2010-12-23
申请号	US12/245645	申请日	2008-10-03
[标]申请(专利权)人(译)	DEWALD GEORG		
申请(专利权)人(译)	DEWALD GEORG		
当前申请(专利权)人(译)	DEWALD GEORG		
[标]发明人	DEWALD GEORG		
发明人	DEWALD, GEORG		
IPC分类号	A61K31/7105 C12Q1/68 G01N33/50 G01N33/53 C07K2/00 C07K14/00 C07H21/04 A01K67/033 C07K16/18 C12N5/12 A61P9/00 C12Q1/37 G01N33/573		
CPC分类号	C12Q1/37 G01N33/573 Y10T436/143333 G01N2500/00 G01N2800/224 G01N2333/96458		
优先权	2004011790 2004-05-18 EP		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及一种诊断III型遗传性血管性水肿 (HAE III) 或其易感性的方法, 该受试者被怀疑已经发展或具有发展III型遗传性血管性水肿的倾向, 或者被怀疑为a型的受试者。用于遗传性血管性水肿III型的载体, 该方法包括在体外从所述受试者的生物样品中确定调节凝血因子XII的表达或编码凝血因子XII的核酸分子中是否存在疾病相关突变;其中这种突变的存在表明III型遗传性血管性水肿或其易感性。本发明还涉及一种诊断III型遗传性血管性水肿 (HAE III) 或其易感性的方法, 所述受试者被怀疑已经发展或具有发展III型遗传性血管性水肿的倾向, 或者被怀疑是III型遗传性血管性水肿的载体, 该方法包括评估所述受试者中凝血因子XII的存在, 量和/或活性, 并包括以下步骤: (a) 从体外生物样品中测定所述受试者的存在, 数量和活性: (i) 凝血因子XII基因编码的(多)肽; (ii) (i)的(多)肽的底物;或(iii)由(ii)中提到的基质处理的(多)肽; (b) 将所述存在, 数量和/或活动与参考样本确定的存在, 数量和/或活动进行比较; (c) 根据步骤(b)中比较的样品之间的差异, 诊断III型遗传性血管性水肿或其易感性的病理状况。本发明还涉及鉴定调节凝血因子XII活性的化合物的方法, 所述化合物适合作为用于治疗 and/或预防III型遗传性血管性水肿的药物的药物或先导化合物, 该方法包括以下步骤: (a) 体外接触凝血因子XII(多)肽或功能相关的(多)肽与潜在的调节剂; (b) 测试凝血因子XII活性的调节, 其中凝血因子XII活性的调节指示化合物作为治疗 and/或预防III型遗传性血管性水肿的药物的适合性。此外, 本发明涉及基因治疗方法和用于诊断遗传性血管性水肿的试剂盒III型。

```

1      ctattgatct ggactctctg ataggcagct ggaccaacgg acggagccca tgagggtctt
61     gctgctcctg gggttctctg tggtagctt ggagtaacca ctttcgattc caacttggga
121    agcccccaag gagcataagt acaaaactga agagcaacca gtctgtctca ctgtcaacgg
181    gggccgctgc cacttccctc tccagtacca ccggcagctg taaccacaat gtaccaccaa
241    gggccgcca  gcccctcagc cctgggtgctc taaccacccc aactttgattc agaccagcg
301    atggggatac tgtttgagc  ccaagaaagt gaaagaccac tgcagcaaac acagccctgt
361    ccagaaagga gggacctgtg tgaacaatgcc aagcagcccc cactgtctct gtccacaaca
421    cctcaactga aaccactgpc agaaagaga gtctttgag cctcagcttc tccgttttt
481    ccacaagaat gagatatggt atagaactga gcaagcagct gtggccagat gccagtgcga
541    gggctctgat gcccactgpc agcggctgpc cagccagcgc tgcagcacca accgtgctc
361    cctatggggt cgtctgcaag aggtggagg ccacccgctg tgcactgccc cgttgggcta
661    caccgacccc tctgcaagc tggacaccaa ggcagctgc tatgatgcc cgggctcag
721    ctaccgccc  ctggcagaag ccagctctc  gggtagccc  tctcagcgt  gggctcaga
781    ggcacccctac cggaaactga ctgcccagca agcgggaac  tggggactgt  agcccaagc
841    ctttgcctg  aaccggaca  acgacatcc  cccgtggtg  tctgtctga  accgacccc
901    gctagctgg  gagtctgag  actgcccac  ctccagacc  ccaaccagg  cggccctcc
961    gacccegtg  tcccctagc  ttcattgtcc  actcatgccc  ggcagcccg  caccgcccga
      g
1021   gcctcagccc  acgacccgga  ccccgccta  gtcccagacc  ccgggagcct  tggccggcaa
      Thr
      Lys
      Arg
1081   ggggagcag  ccgcttccc  tgaccaggaa  cggcccactg  agctcggggc  agcggctccg
1141   caaagctctg  tcttcgatga  cccgcgtctg  tggcggctg  gtggcgctac  cggggcgca
1201   cccctacatc  gccgcgctgt  actggggcca  cagttctctg  gccggcagcc  tcatcgccc
1261   ctgctgggtg  ctgacggcgg  ctcactgctc  gcaggccggg  ccgcacccc  aggtctgac
1321   ggtggtgctc  ggcaggaac  cccgtaacca  cagctgtgag  ccgtgcca  cgttggcctg
1381   gcctcctac  cgttgaacg  aggcctctc  gccctcagc  taccagcagc  acctggctg
1441   gttgcctct  caggagatg  cgaagcgag  ctgcccctc  ctgtgectt  acctcagcc
1501   gttgtgctg  ccaagcgag  ccagcgacc  ctcagacc  acctctgca  agtggcctg
1561   ctgggcccac  cagttcgagg  gggcgagga  atatcgacc  ttcttcgag  aggcagctt
1621   accgttctc  tccctgagc  gctgctcagc  cccggacgtg  caggatcct  ccatctccc
1681   cggcatgctc  tggcaggg  tctctgagg  cggcacgat  ggtgcccag  gtattctcg
1741   aggcccgctg  gttgtgagg  accaagctg  agagcggcg  ctaccctgc  aaggatcat
1801   cagctgggga  tgggctgtg  gtaaccgaa  caagcaggc  gtctacacc  atgtggccta
1861   ctaccctgcc  tggatccggg  agcaaccgt  tctctgattg  ctcaaggact  catcttccc
1921   tctctgtga  tccgcagtg  agaggtgag  tggggcatg  aaggcaagat  tgttcccat
1981   tcccacgtg  cggccagctc  cgcgcagga  ttggcagaaa  ctcaataag  gtctttgaaa
2041   atgctgag

```