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(54) **KASPP (LRRK2) GENE, ITS PRODUCTION AND USE FOR THE DETECTION AND TREATMENT OF NEURODEGENERATIVE DISORDERS**

(75) Inventors: **Thomas Meitinger**, Muenchen (DE); **Tim Matthias Strom**, Muenchen (DE); **Saskia Biskup**, Stuttgart (DE); **Marius Ueffing**, Muenchen (DE); **Elisabeth Kremmer**, Freising (DE); **Christian Johannes Gloeckner**, Rottenburg-Hailfingen (DE); **Thomas Gasser**, Tuebingen (DE); **Petra (Leitner) Herzig**, Basel (CH); **Friedrich Asmus**, Tuebingen (DE); **Nadja Patenge**, Rostock (DE); **Alexander Zimprich**, Wien (AT); **Matthew J. Farrer**, Jacksonville, FL (US); **Jennifer M. Kachergus**, Atlantic Beach, FL (US); **Sara J. Lincoln**, Jacksonville, FL (US); **Mary M. Hulihan**, Atlanta, GA (US); **Zibigniew Wszolek**, Jacksonville, FL (US); **Ryan Uitti**, Jacksonville, FL (US)

(73) Assignees: **Helmholtz Zentrum Muenchen Deutsches Forschungszentrum fuer Gesundheit und Umwelt (GmbH**, Neuherberg (DE); **Mayo Foundation for Medical Education and Research**, Jacksonville, FL (US); **Eberhard Karls Universitaet Tuebingen**, Tuebingen (DE)

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C40B 30/04	(2006.01)
G01N 33/53	(2006.01)

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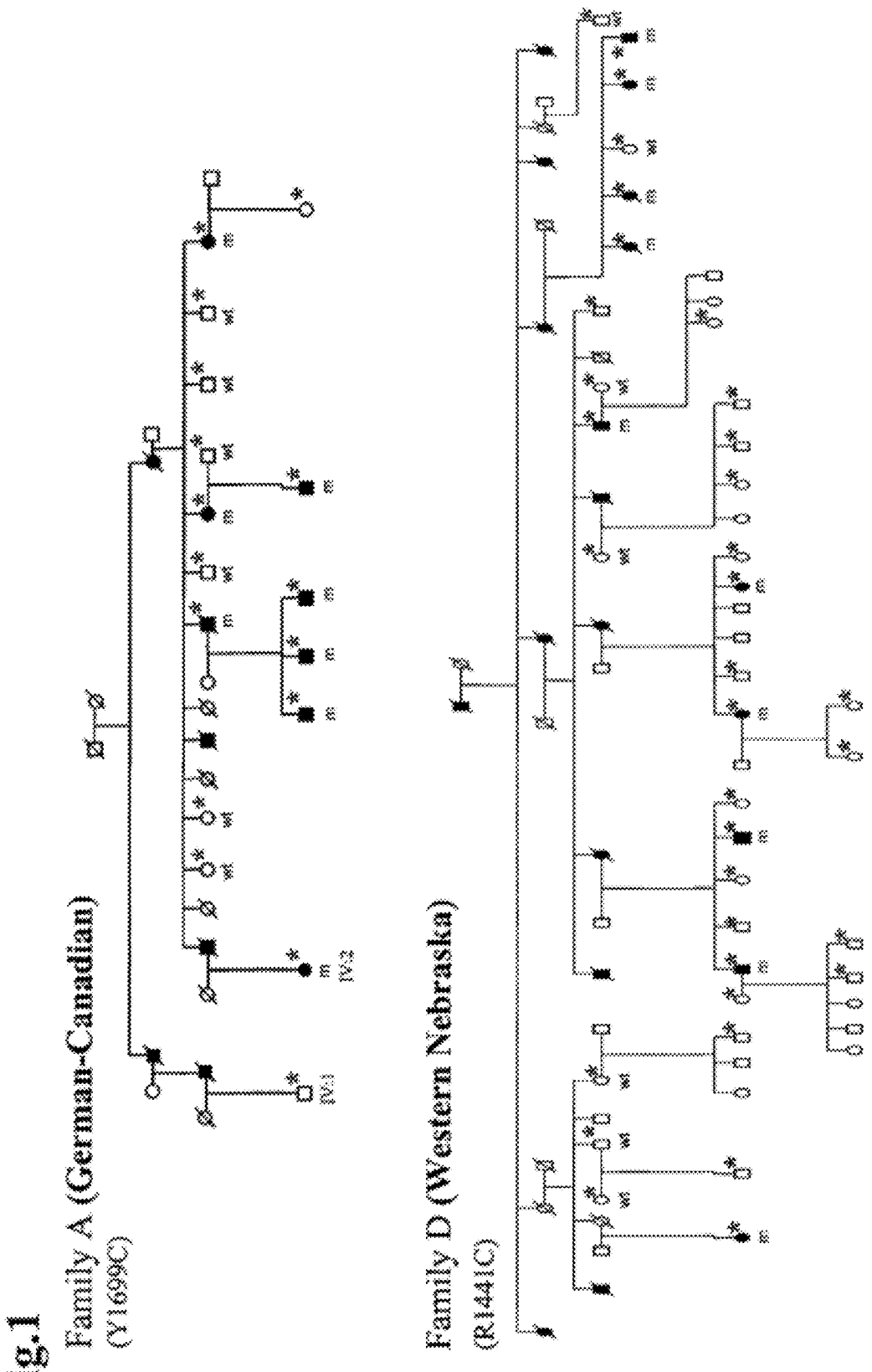
(57) **ABSTRACT**

The present invention refers to a newly discovered gene named KASPP for Kinase Associated with Parkinsonism with Pleiomorphic Pathology or alternatively named LRRK2 for Leucine-Rich Repeat Kinase 2, its production, biochemical characterization and use for the detection and treatment of neurodegenerative disorders, such as Parkinson disease (PD) including, without limitation, sporadic PD, Alzheimer disease (AD), amyotrophic lateral sclerosis (ALS), and other synucleinopathies and/or tauopathy as well as several polymorphisms and mutations in the KASPP/LRRK2 gene segregated with PD.

Fig.1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(Y1699C)
Family D (Western Nebraska)
(R1441C)

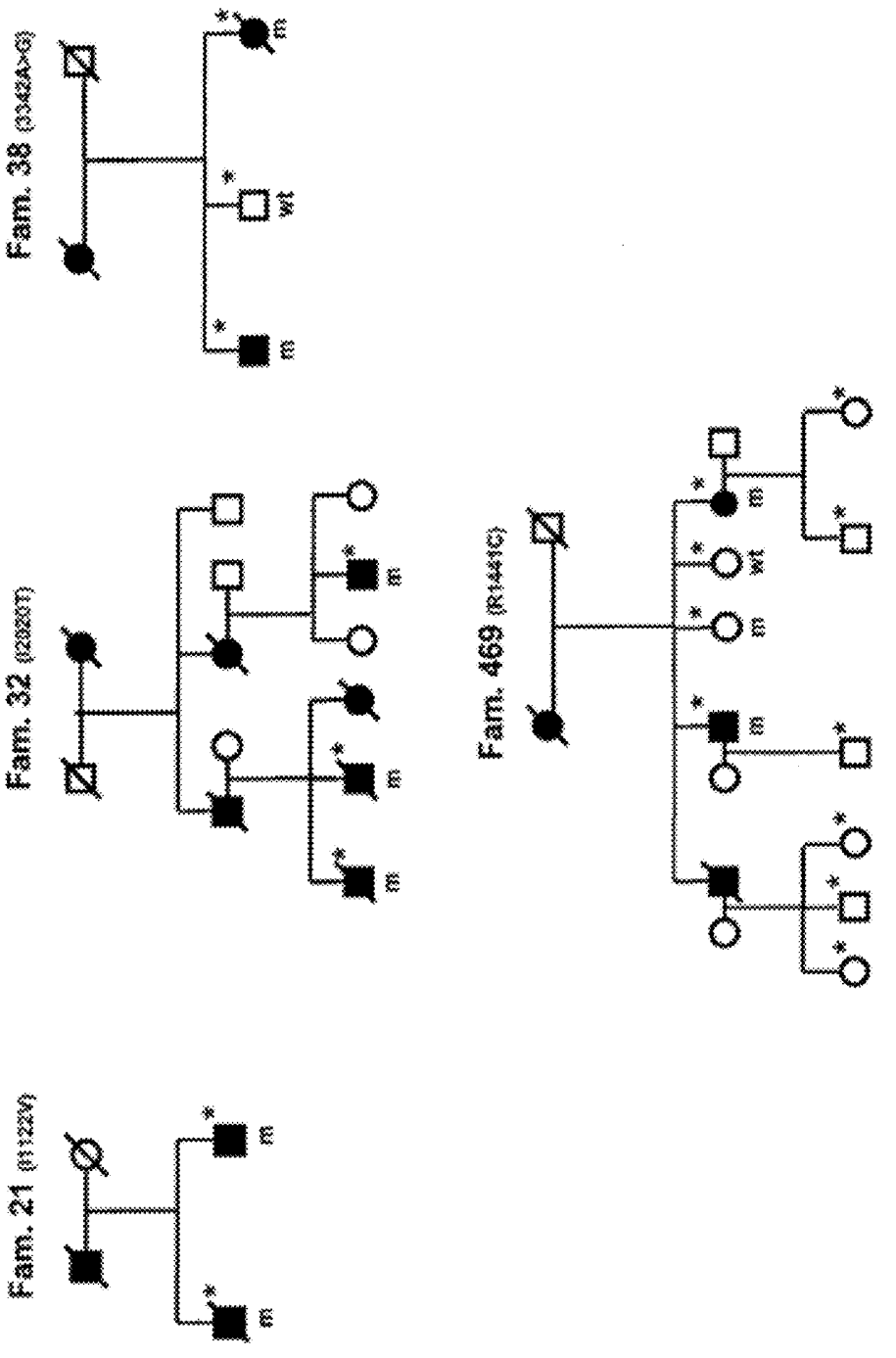
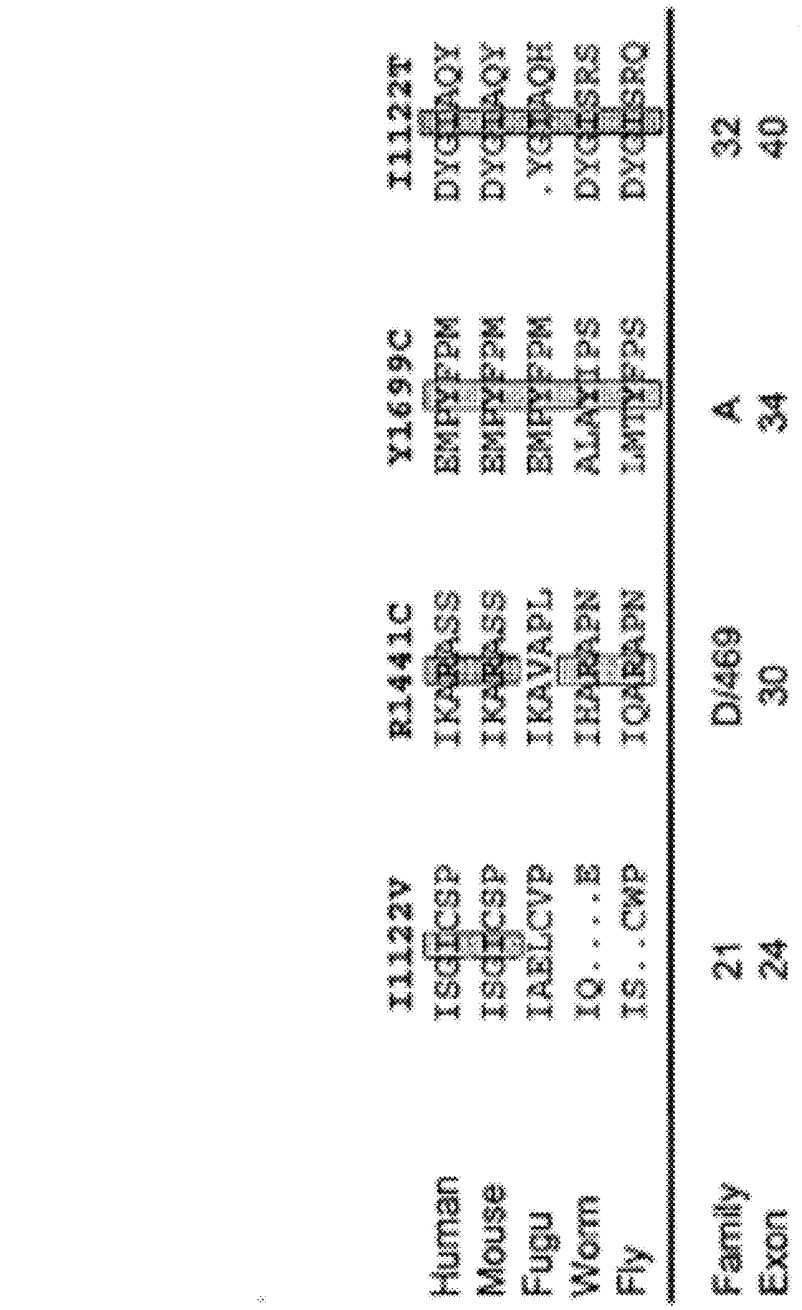


Fig. 2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R1441C
Y1699C
I1122T
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Fugu
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Exon
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24
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30
A
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32
40

Fig.3

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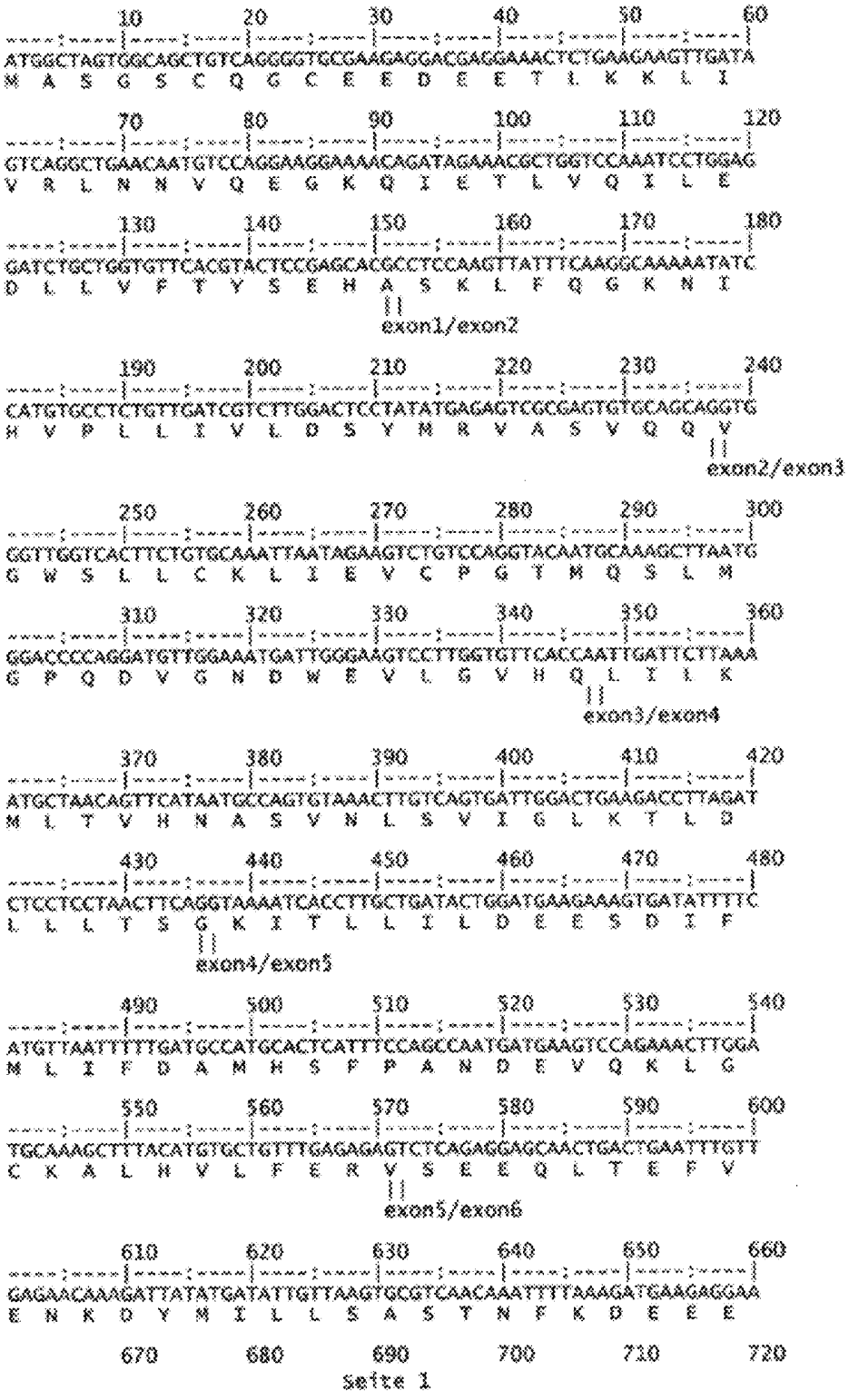
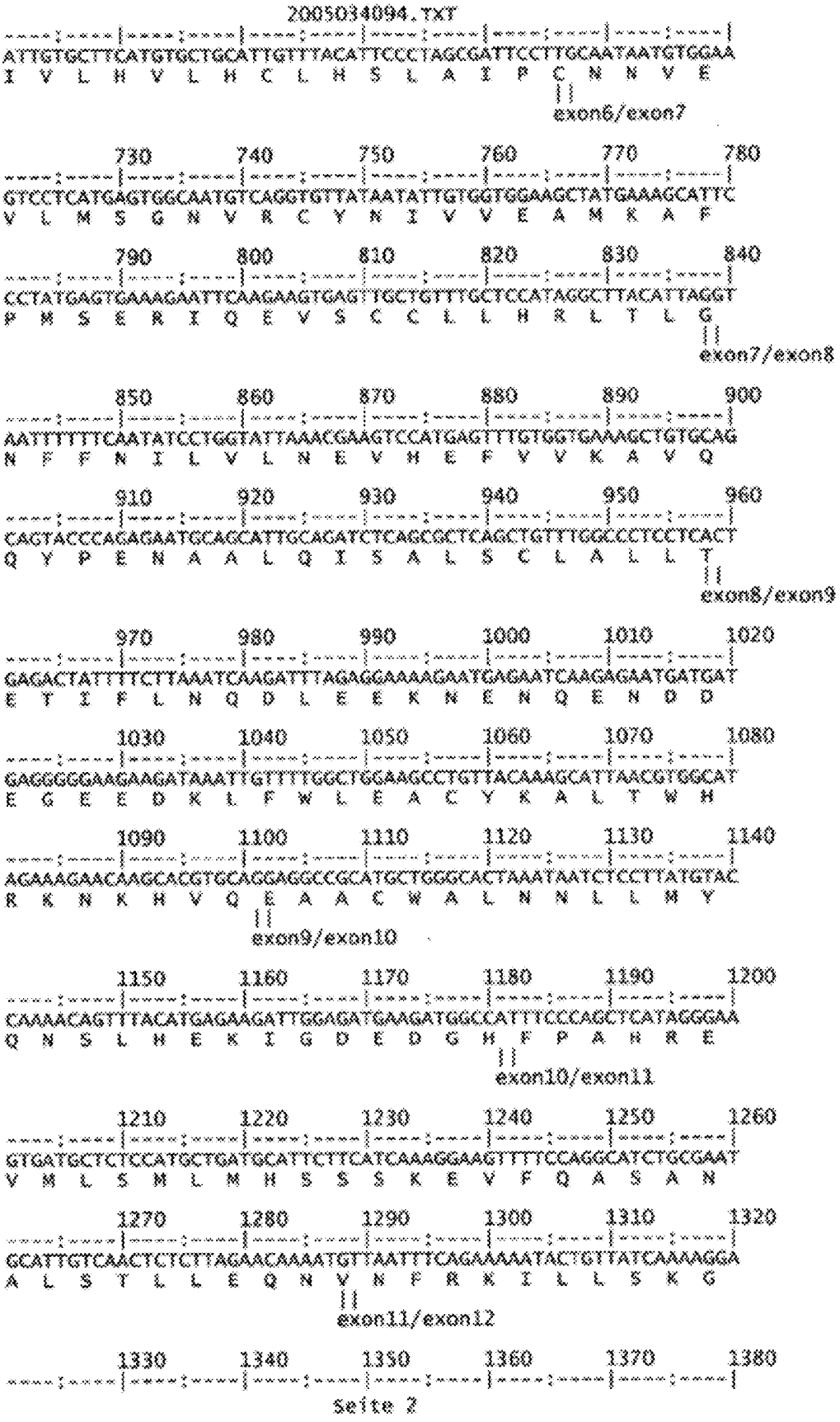
Fig. 4

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V L M S G N V R C Y N I V V E A M K A F
790 800 810 820 830 840
CCTATGAGTGAAAGAATTCAAGAAGTGAGTGCTGTTTGCTCCATAGGCTTACATTAGGT
P M S E R I Q E V S C C L L H R L T L G
exon7/exon8
850 860 870 880 890 900
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N F F N I L V L N E V H E F V V K A V Q
910 920 930 940 950 960
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Q Y P E N A A L Q I S A L S C L A L L T
exon8/exon9
970 980 990 1000 1010 1020
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E T I F L N Q D L E E K N E N Q E N D D
1030 1040 1050 1060 1070 1080
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E G E E D K L F W L E A C Y K A L T W H
1090 1100 1110 1120 1130 1140
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R K N K H V Q E A A C W A L N N L L M Y
exon9/exon10
1150 1160 1170 1180 1190 1200
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Q N S L H E K I G D E D G H F P A H R E
exon10/exon11
1210 1220 1230 1240 1250 1260
GTGATGCTCTCCATGCTGATGCATTCTTCATCAAAGGAAGTTTTCCAGGCATCTGCGAAT
V M L S M L M H S S S K E V F Q A S A N
1270 1280 1290 1300 1310 1320
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A L S T L L E Q N V N F R K I L L S K G
exon11/exon12
1330 1340 1350 1360 1370 1380
Seite 2

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I  H  L  N  V  L  E  L  M  Q  K  H  I  H  S  P  E  V  A  E

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----:----|----:----|----:----|----:----|----:----|----:----|
AGTGGCTGTAAAATGCTAAATCATCTTTTTGAAGGAAGCAACACTTCCCTGGATATAATG
S  G  C  K  M  L  N  H  L  F  E  G  S  N  T  S  L  D  I  M
                                      ||
                                      exon12/exon13

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----:----|----:----|----:----|----:----|----:----|----:----|
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A  A  V  V  P  K  I  L  T  V  M  K  R  H  E  T  S  L  P  V

         1510      1520      1530      1540      1550      1560
----:----|----:----|----:----|----:----|----:----|----:----|
CAGCTGGAGGCGCTTCGAGCTATTTTACATTTTATAGTGCCTGGCATGCCAGAAGAATCC
Q  L  E  A  L  R  A  I  L  H  F  I  V  P  G  M  P  E  E  S
                                         ||
                                         exon13/exon14

         1570      1580      1590      1600      1610      1620
----:----|----:----|----:----|----:----|----:----|----:----|
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R  E  D  T  E  F  H  H  K  L  N  M  V  K  K  Q  C  F  K  N

         1630      1640      1650      1660      1670      1680
----:----|----:----|----:----|----:----|----:----|----:----|
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D  I  H  K  L  V  L  A  A  L  N  R  F  I  G  N  P  G  I  Q
                                   ||
                                   exon14/exon15

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K  C  G  L  K  V  I  S  S  I  V  H  F  P  D  A  L  E  M  L

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----:----|----:----|----:----|----:----|----:----|----:----|
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S  L  E  G  A  M  D  S  V  L  H  T  L  Q  M  Y  P  D  D  Q

         1810      1820      1830      1840      1850      1860
----:----|----:----|----:----|----:----|----:----|----:----|
GAAATTCAGTGTCTGGGTTTAAGTCTTATAGGATACTTGATTACAAAGAAGAATGTGTTC
E  I  Q  C  L  G  L  S  L  I  G  Y  L  I  T  K  K  N  V  F
||
exon15/exon16

         1870      1880      1890      1900      1910      1920
----:----|----:----|----:----|----:----|----:----|----:----|
ATAGGAACTGGACATCTGCTGGCAAAAATTCTGGTTTCCAGCTTATACCGATTTAAGGAT
I  G  T  G  H  L  L  A  K  I  L  V  S  S  L  Y  R  F  K  D

         1930      1940      1950      1960      1970      1980
----:----|----:----|----:----|----:----|----:----|----:----|
GTTGCTGAAATACAGACTAAAGGATTTCAGACAATCTTAGCAATCCTCAAATTGTCAGCA
V  A  E  I  Q  T  K  G  F  Q  T  I  L  A  I  L  K  L  S  A
                     ||
                     exon16/exon17

         1990      2000      2010      2020      2030      2040
----:----|----:----|----:----|----:----|----:----|----:----|
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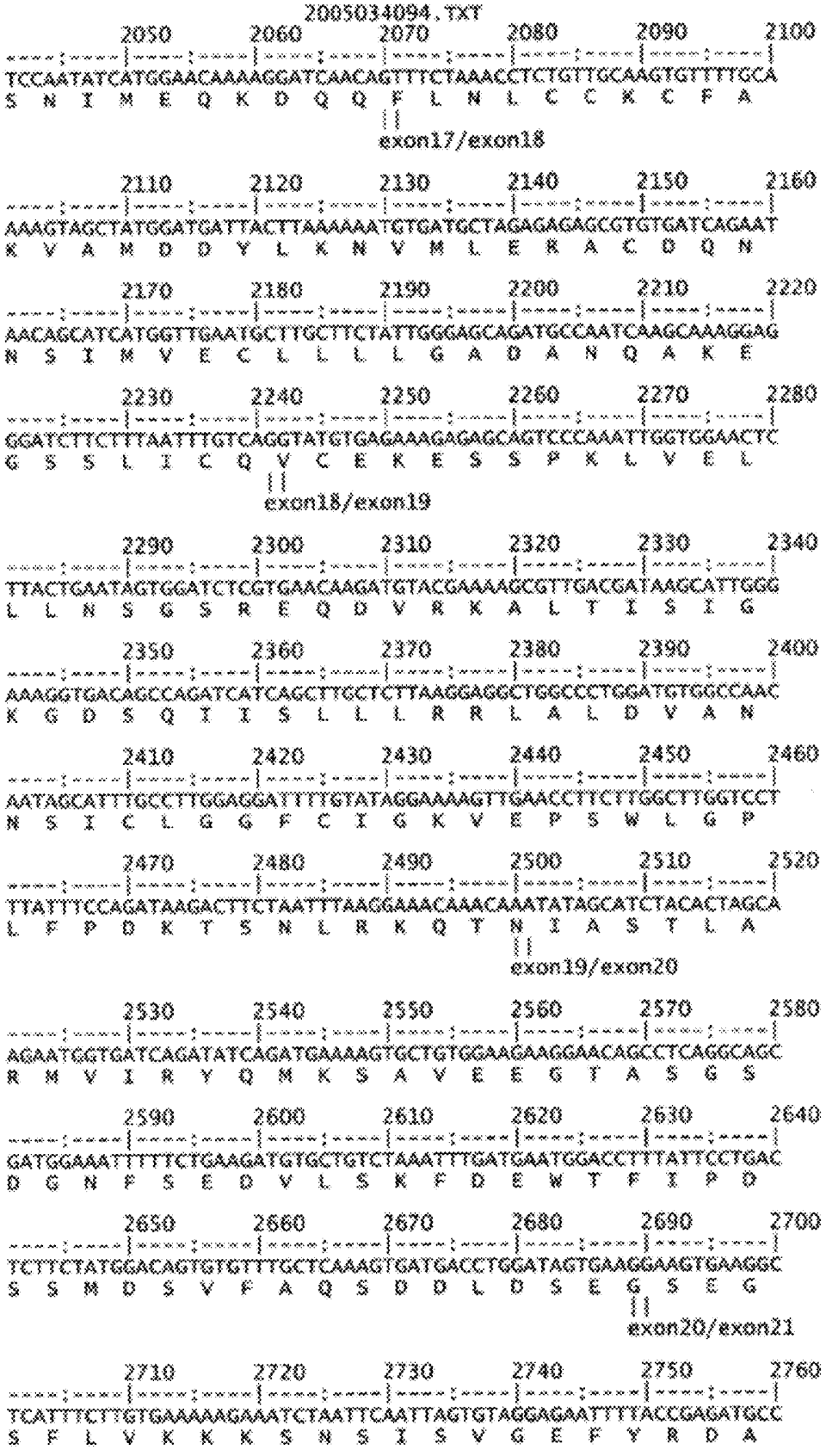
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2170 2180 2190 2200 2210 2220
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N S I M V E C L L L L G A D A N Q A K E
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exon18/exon19
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N S I C L G G F C I G K V E P S W L G P
2470 2480 2490 2500 2510 2520
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exon19/exon20
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2590 2600 2610 2620 2630 2640
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2650 2660 2670 2680 2690 2700
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exon20/exon21
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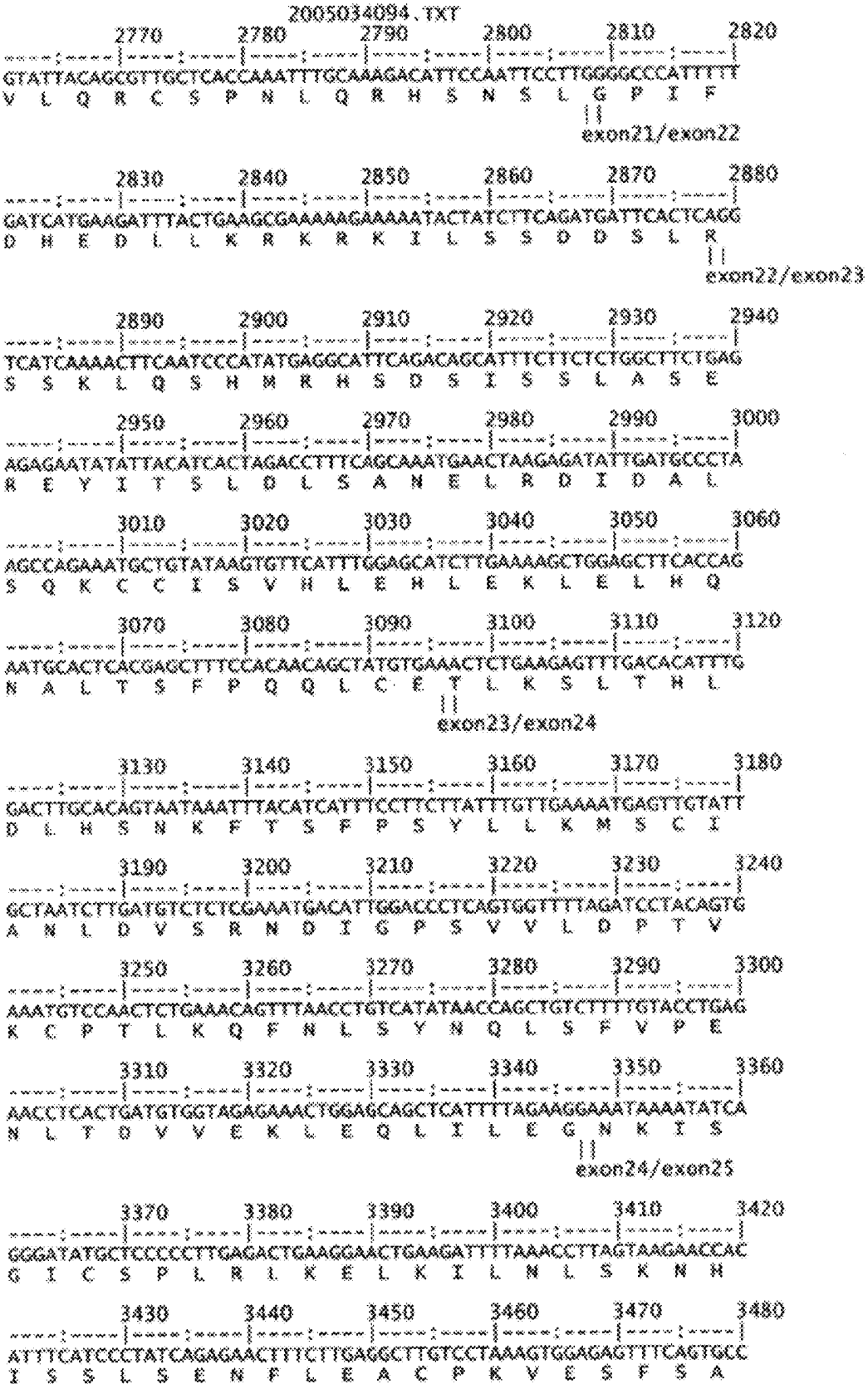
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exon22/exon23
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3010 3020 3030 3040 3050 3060
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S Q K C C I S V H L E H L E K L E L H Q
3070 3080 3090 3100 3110 3120
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exon23/exon24
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3250 3260 3270 3280 3290 3300
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3310 3320 3330 3340 3350 3360
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exon24/exon25
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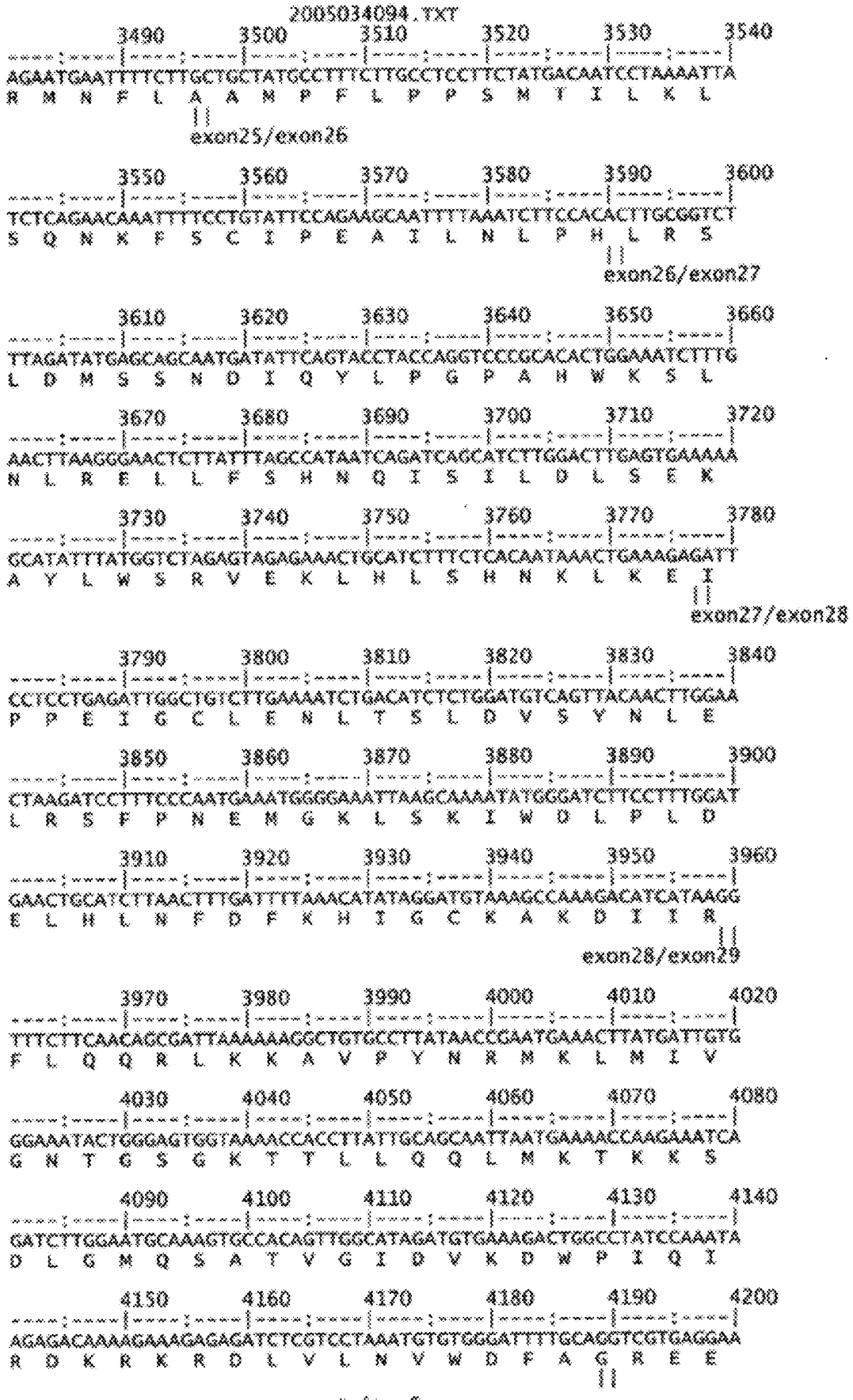
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exon26/exon27
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exon27/exon28
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exon28/exon29
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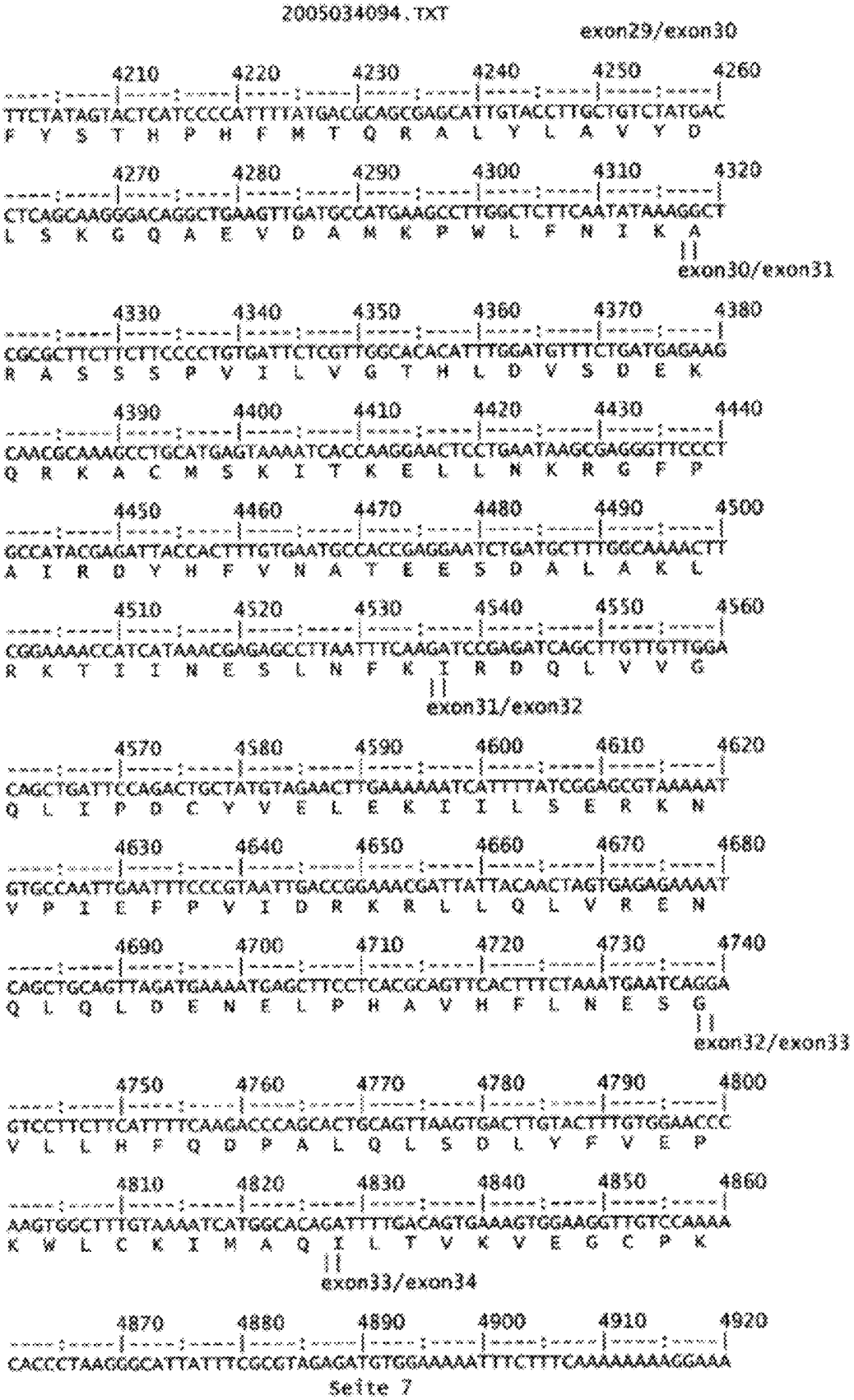
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exon30/exon31
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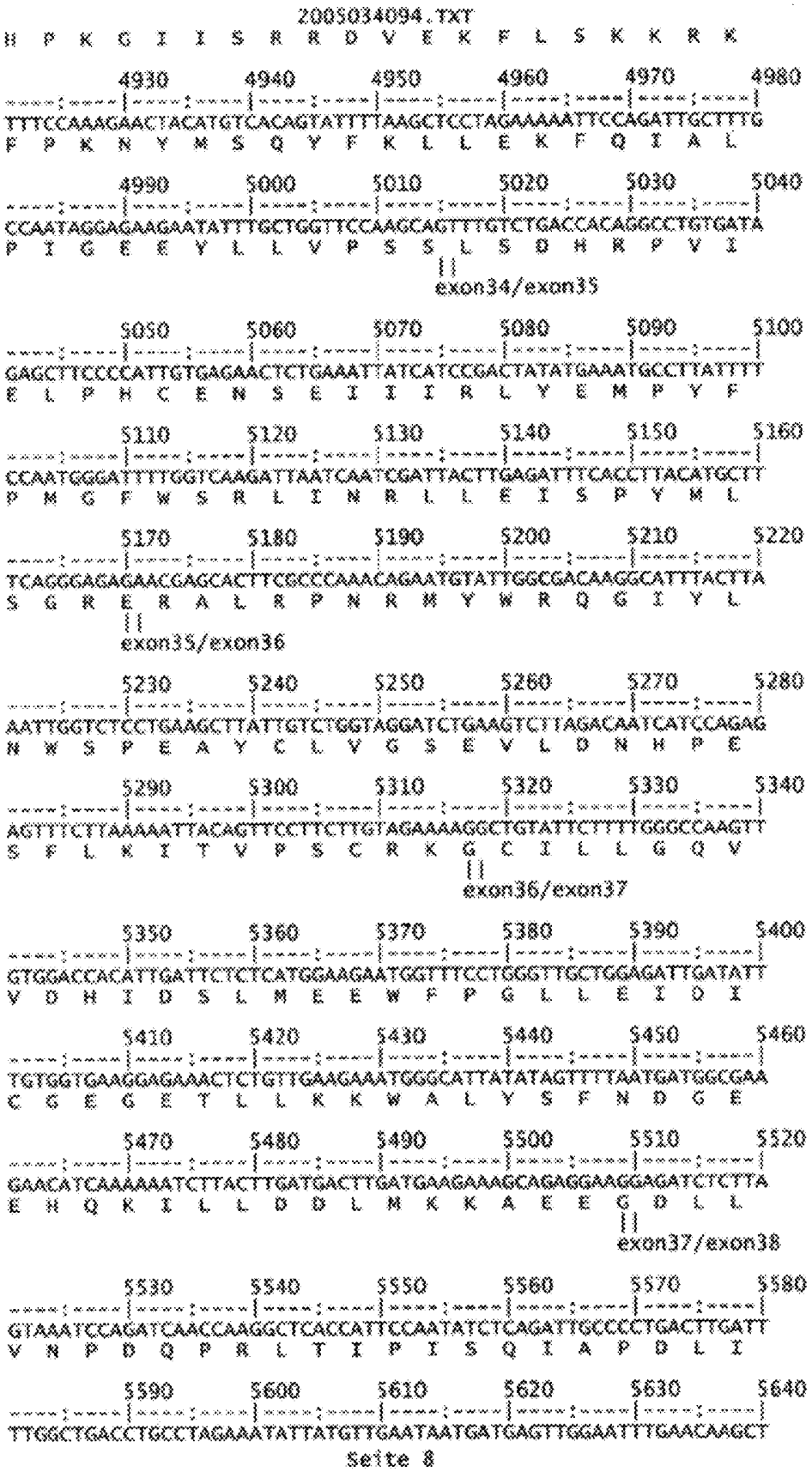
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exon34/exon35
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exon35/exon36
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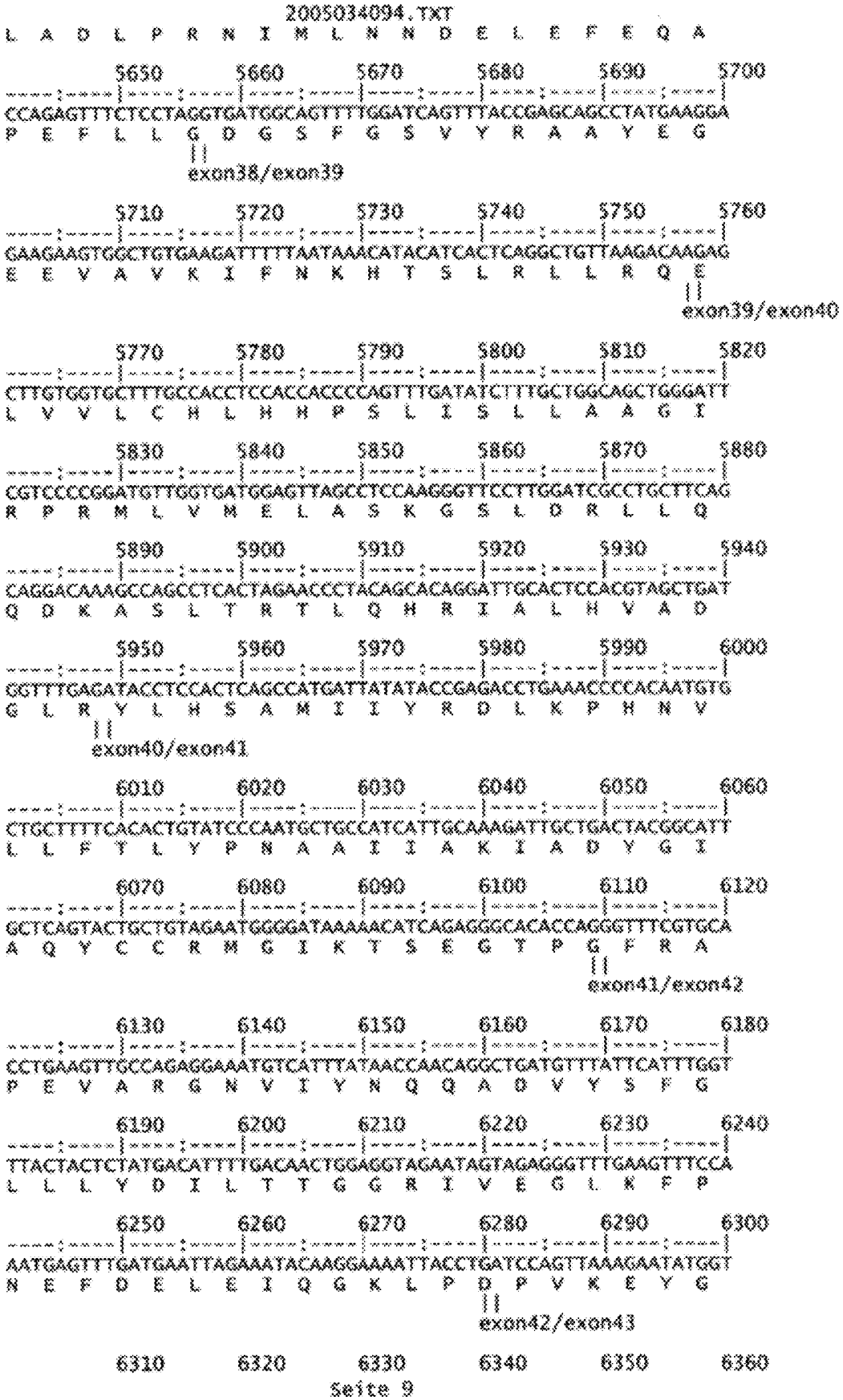
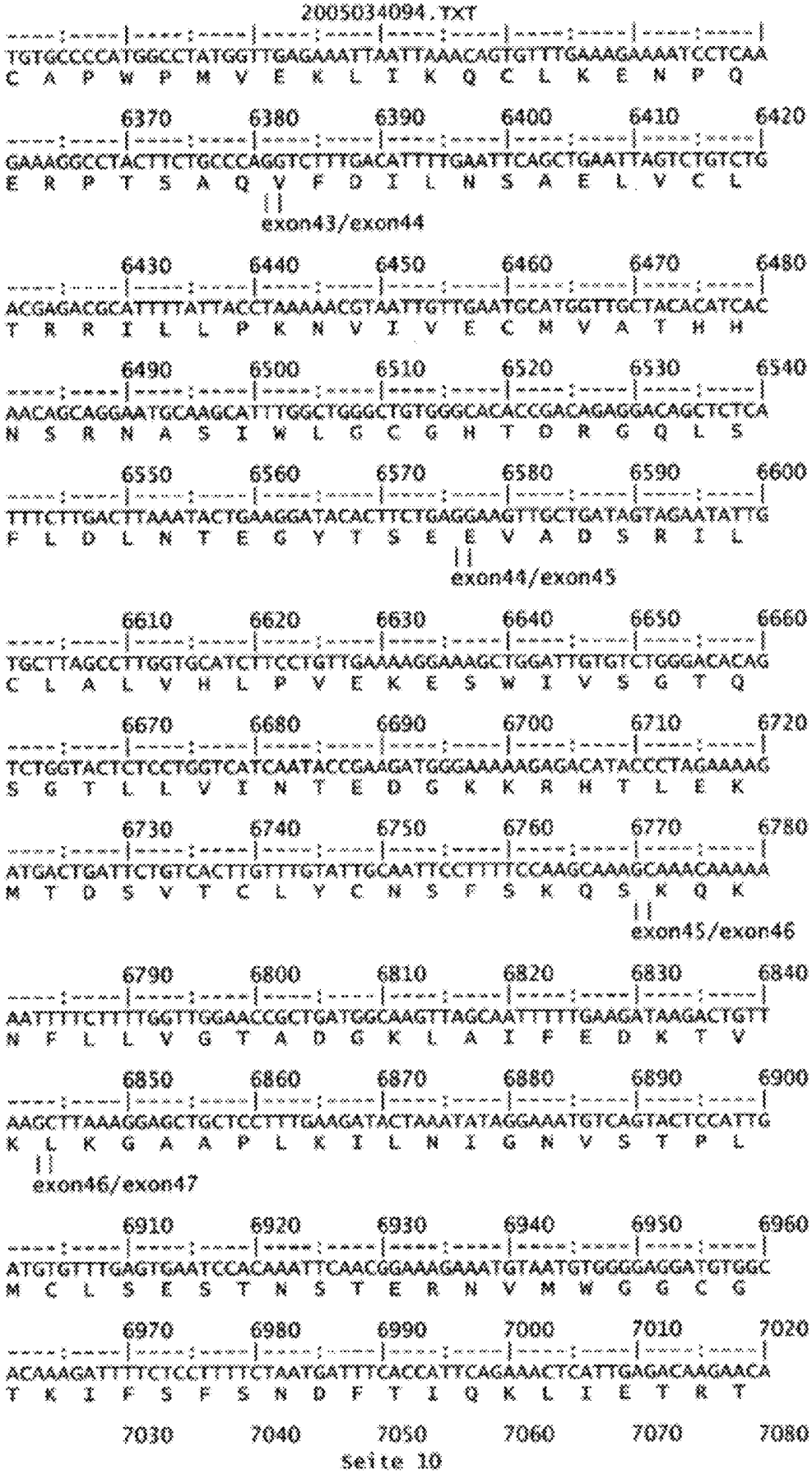
N W S P E A Y C L V G S E V L D N H P E
5290 5300 5310 5320 5330 5340
AGTTTCTTAAAAATTACAGTTCCTTCTTGTAGAAAAGGCTGTATTCTTTTGGGCCAAGTT
S F L K I T V P S C R K G C I L L G Q V
exon36/exon37
5350 5360 5370 5380 5390 5400
GTGGACCACATTGATTCTCTCATGGAAGAATGGTTTCCTGGGTTGCTGGAGATTGATATT
V D H I D S L M E E W F P G L L E I D I
5410 5420 5430 5440 5450 5460
TGTGGTGAAGGAGAAACTCTGTTGAAGAAATGGGCATTATATAGTTTTAATGATGGCGAA
C G E G E T L L K K W A L Y S F N D G E
5470 5480 5490 5500 5510 5520
GAACATCAAAAAATCTTACTTGATGACTTGATGAAGAAAGCAGAGGAAGGAGATCTCTTA
E H Q K I L L D D L M K K A E E G D L L
exon37/exon38
5530 5540 5550 5560 5570 5580
GTAAATCCAGATCAACCAAGGCTCACCATTCCAATATCTCAGATTGCCCCTGACTTGATT
V N P D Q P R L T I P I S Q I A P D L I
5590 5600 5610 5620 5630 5640
TTGGCTGACCTGCCTAGAAATATTATGTTGAATAATGATGAGTTGGAATTTGAACAAGCT


2005034094.TXT
L A D L P R N I M L N N D E L E F E Q A
5650 5660 5670 5680 5690 5700
CCAGAGTTTCTCCTAGGTGATGGCAGTTTTGGATCAGTTTACCGAGCAGCCTATGAAGGA
P E F L L G D G S F G S V Y R A A Y E G
exon38/exon39
5710 5720 5730 5740 5750 5760
GAAGAAGTGGCTGTGAAGATTTTTAATAAACATACATCACTCAGGCTGTTAAGACAAGAG
E E V A V K I F N K H T S L R L L R Q E
exon39/exon40
5770 5780 5790 5800 5810 5820
CTTGTGGTGCTTTGCCACCTCCACCACCCCAGTTTGATATCTTTGCTGGCAGCTGGGATT
L V V L C H L H H P S L I S L L A A G I
5830 5840 5850 5860 5870 5880
CGTCCCCGGATGTTGGTGATGGAGTTAGCCTCCAAGGGTTCCTTGGATCGCCTGCTTCAG
R P R M L V M E L A S K G S L D R L L Q
5890 5900 5910 5920 5930 5940
CAGGACAAAGCCAGCCTCACTAGAACCCTACAGCACAGGATTGCACTCCACGTAGCTGAT
Q D K A S L T R T L Q H R I A L H V A D
5950 5960 5970 5980 5990 6000
GGTTTGAGATACCTCCACTCAGCCATGATTATATACCGAGACCTGAAACCCCACAATGTG
G L R Y L H S A M I I Y R D L K P H N V
exon40/exon41
6010 6020 6030 6040 6050 6060
CTGCTTTTCACACTGTATCCCAATGCTGCCATCATTGCAAAGATTGCTGACTACGGCATT
L L F T L Y P N A A I I A K I A D Y G I
6070 6080 6090 6100 6110 6120
GCTCAGTACTGCTGTAGAATGGGGATAAAAACATCAGAGGGCACACCAGGGTTTCGTGCA
A Q Y C C R M G I K T S E G T P G F R A
exon41/exon42
6130 6140 6150 6160 6170 6180
CCTGAAGTTGCCAGAGGAAATGTCATTTATAACCAACAGGCTGATGTTTATTCATTTGGT
P E V A R G N V I Y N Q Q A D V Y S F G
6190 6200 6210 6220 6230 6240
TTACTACTCTATGACATTTTGACAACTGGAGGTAGAATAGTAGAGGGTTTGAAGTTTCCA
L L L Y D I L T T G G R I V E G L K F P
6250 6260 6270 6280 6290 6300
AATGAGTTTGATGAATTAGAAATACAAGGAAAATTACCTGATCCAGTTAAAGAATATGGT
N E F D E L E I Q G K L P D P V K E Y G
exon42/exon43
6310 6320 6330 6340 6350 6360


2005034094.TXT
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C A P W P M V E K L I K Q C L K E N P Q
6370 6380 6390 6400 6410 6420
GAAAGGCCTACTTCTGCCCAGGTCTTTGACATTTTGAATTCAGCTGAATTAGTCTGTCTG
E R P T S A Q V F D I L N S A E L V C L
exon43/exon44
6430 6440 6450 6460 6470 6480
ACGAGACGCATTTTATTACCTAAAAACGTAATTGTTGAATGCATGGTTGCTACACATCAC
T R R I L L P K N V I V E C M V A T H H
6490 6500 6510 6520 6530 6540
AACAGCAGGAATGCAAGCATTGGCTGGGCTGTGGGCACACCGACAGAGGACAGCTCTCA
N S R N A S I W L G C G H T D R G Q L S
6550 6560 6570 6580 6590 6600
TTTCTTGACTTAAATACTGAAGGATACACTTCTGAGGAAGTTGCTGATAGTAGAATATTG
F L D L N T E G Y T S E E V A D S R I L
exon44/exon45
6610 6620 6630 6640 6650 6660
TGCTTAGCCTTGGTGCATCTTCCTGTTGAAAAGGAAAGCTGGATTGTGTCTGGGACACAG
C L A L V H L P V E K E S W I V S G T Q
6670 6680 6690 6700 6710 6720
TCTGGTACTCTCCTGGTCATCAATACCGAAGATGGGAAAAAGAGACATACCCTAGAAAAG
S G T L L V I N T E D G K K R H T L E K
6730 6740 6750 6760 6770 6780
ATGACTGATTCTGTCACTTGTTTGTATTGCAATTCCTTTTCCAAGCAAAGCAAACAAAAA
M T D S V T C L Y C N S F S K Q S K Q K
exon45/exon46
6790 6800 6810 6820 6830 6840
AATTTTCTTTTGGTTGGAACCGCTGATGGCAAGTTAGCAATTTTTGAAGATAAGACTGTT
N F L L V G T A D G K L A I F E D K T V
6850 6860 6870 6880 6890 6900
AAGCTTAAAGGAGCTGCTCCTTTGAAGATACTAAATATAGGAAATGTCAGTACTCCATTG
K L K G A A P L K I L N I G N V S T P L
exon46/exon47
6910 6920 6930 6940 6950 6960
ATGTGTTTGAGTGAATCCACAAATTCAACGGAAAGAAATGTAATGTGGGGAGGATGTGGC
M C L S E S T N S T E R N V M W G G C G
6970 6980 6990 7000 7010 7020
ACAAAGATTTTCTCCTTTTCTAATGATTTCACCATTCAGAAACTCATTGAGACAAGAACA
T K I F S F S N D F T I Q K L I E T R T
7030 7040 7050 7060 7070 7080

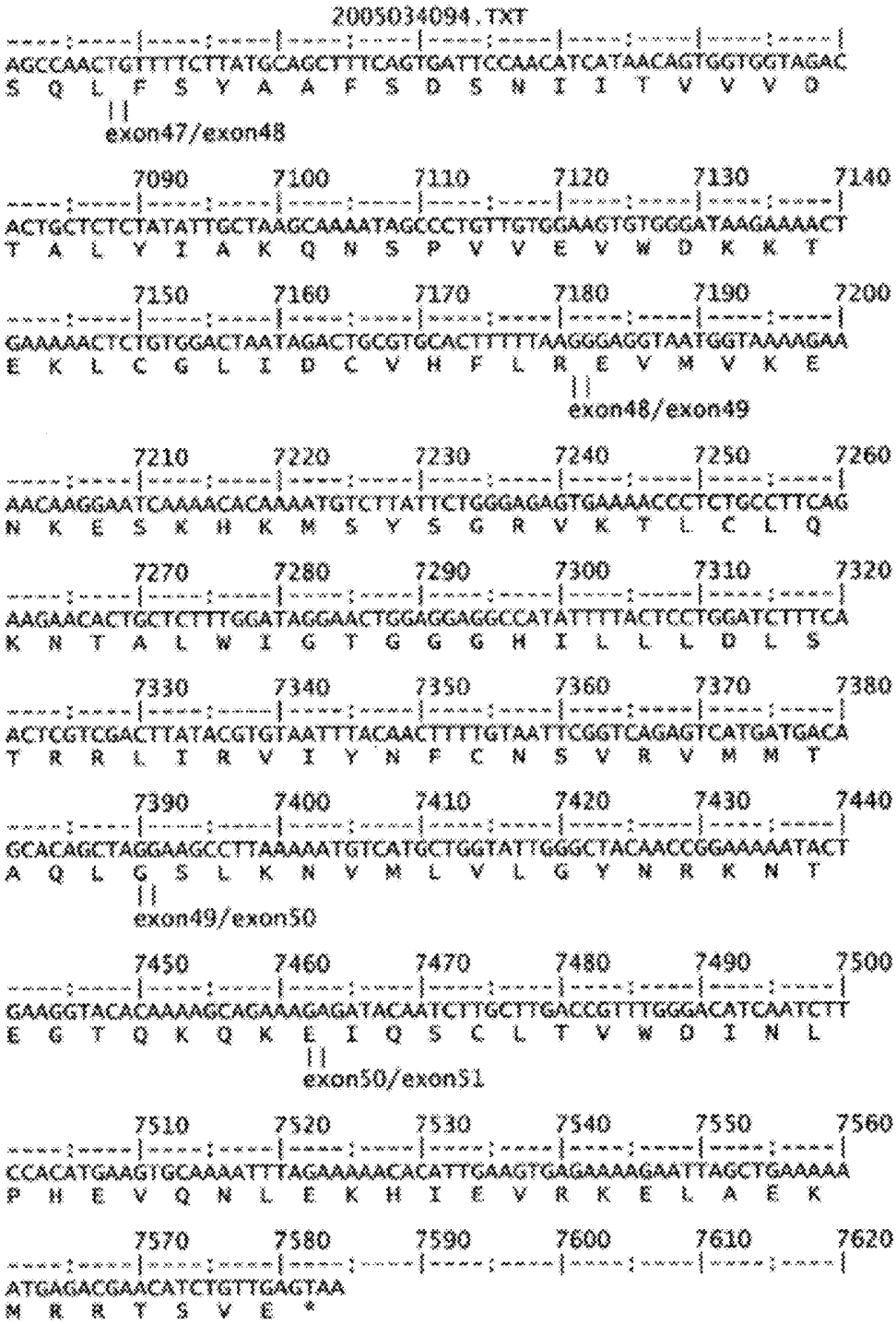
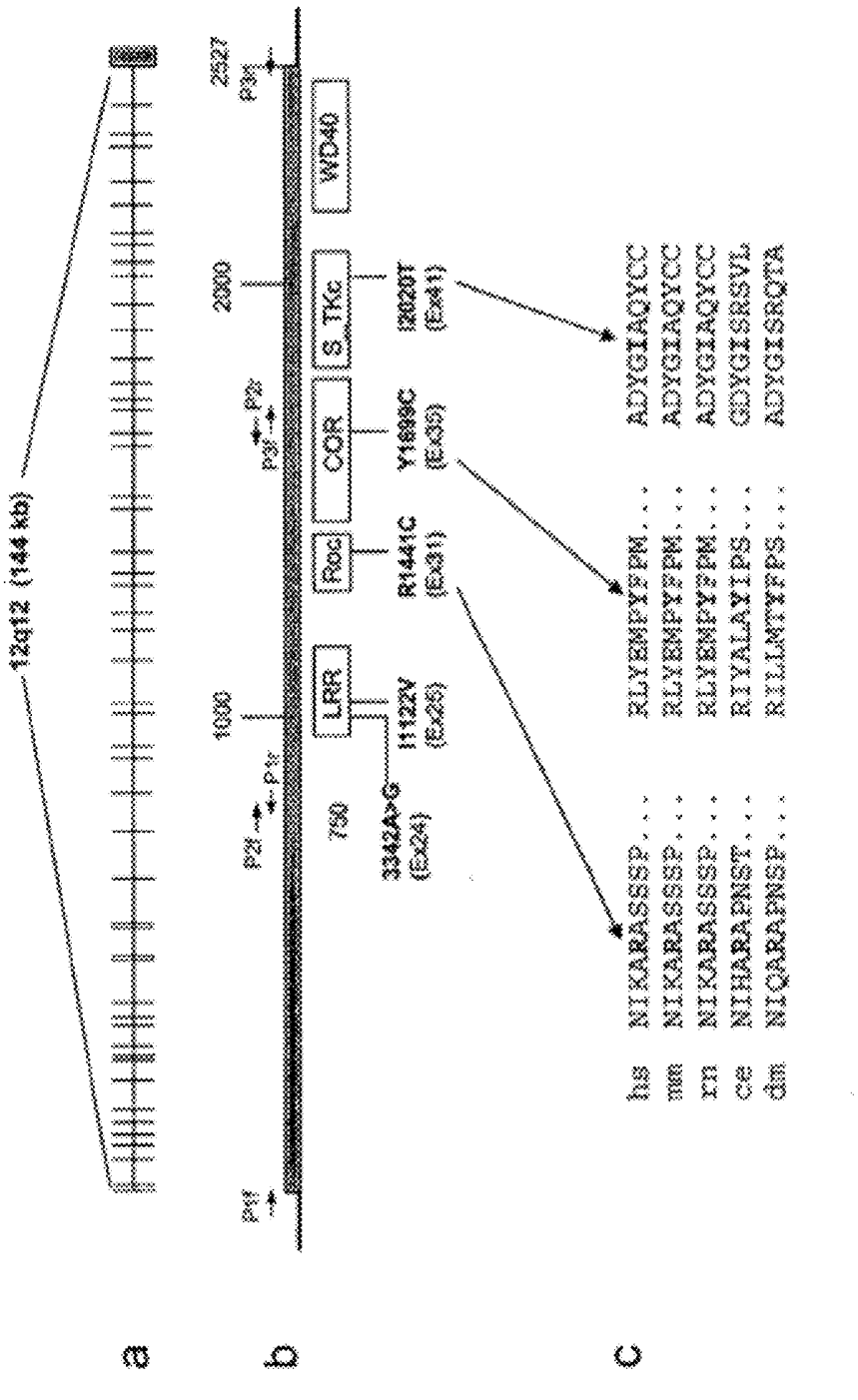

20050034094.TXT
AGCCAACTGTTTTCTTATGCAGCTTTCAGTGATTCCAACATCATAACAGTGGTGGTAGAC
S Q L F S Y A A F S D S N I I T V V V D
exon47/exon48
7090 7100 7110 7120 7130 7140
ACTGCTCTCTATATTGCTAAGCAAAATAGCCCTGTTGTGGAAGTGTGGGATAAGAAAACT
T A L Y I A K Q N S P V V E V W D K K T
7150 7160 7170 7180 7190 7200
GAAAAACTCTGTGGACTAATAGACTGCGTGCACTTTTTAAGGGAGGTAATGGTAAAAGAA
E K L C G L I D C V H F L R E V M V K E
exon48/exon49
7210 7220 7230 7240 7250 7260
AACAAGGAATCAAAACACAAAATGTCTTATTCTGGGAGAGTGAAAACCCTCTGCCTTCAG
N K E S K H K M S Y S G R V K T L C L Q
7270 7280 7290 7300 7310 7320
AAGAACACTGCTCTTTGGATAGGAACTGGAGGAGGCCATATTTTACTCCTGGATCTTTCA
K N T A L W I G T G G G H I L L L D L S
7330 7340 7350 7360 7370 7380
ACTCGTCGACTTATACGTGTAATTTACAACTTTTGTAATTCGGTCAGAGTCATGATGACA
T R R L I R V I Y N F C N S V R V M M T
7390 7400 7410 7420 7430 7440
GCACAGCTAGGAAGCCTTAAAAATGTCATGCTGGTATTGGGCTACAACCGGAAAAATACT
A Q L G S L K N V M L V L G Y N R K N T
exon49/exon50
7450 7460 7470 7480 7490 7500
GAAGGTACACAAAAGCAGAAAGAGATACAATCTTGCTTGACCGTTTGGGACATCAATCTT
E G T Q K Q K E I Q S C L T V W D I N L
exon50/exon51
7510 7520 7530 7540 7550 7560
CCACATGAAGTGCAAAATTTAGAAAAACACATTGAAGTGAGAAAAGAATTAGCTGAAAAA
P H E V Q N L E K H I E V R K E L A E K
7570 7580 7590 7600 7610 7620
ATGAGACGAACATCTGTTGAGTAA
M R R T S V E *

Fig. 5


a
b
c
12q12 (144 kb)
2527
P3m
2300
1000
750
P1f
P1r
P2f
P2r
P2f
P2r
LRR
Roc
COR
S_TKc
WD40
3342A>G
(Ex24)
I1122V
(Ex26)
R1441C
(Ex31)
Y1699C
(Ex35)
I2020T
(Ex41)
hs NIKARASSSP...
mm NIKARASSSP...
rn NIKARASSSP...
ce NIHARAPNST...
dm NIQARAPNSP...
RLYEMPYFPM...
RLYEMPYFPM...
RLYEMPYFPM...
RIYALAYIPS...
RILLMTYFPS...
ADYGIAQYCC
ADYGIAQYCC
ADYGIAQYCC
GDYGISRSVL
ADYGISRQTA

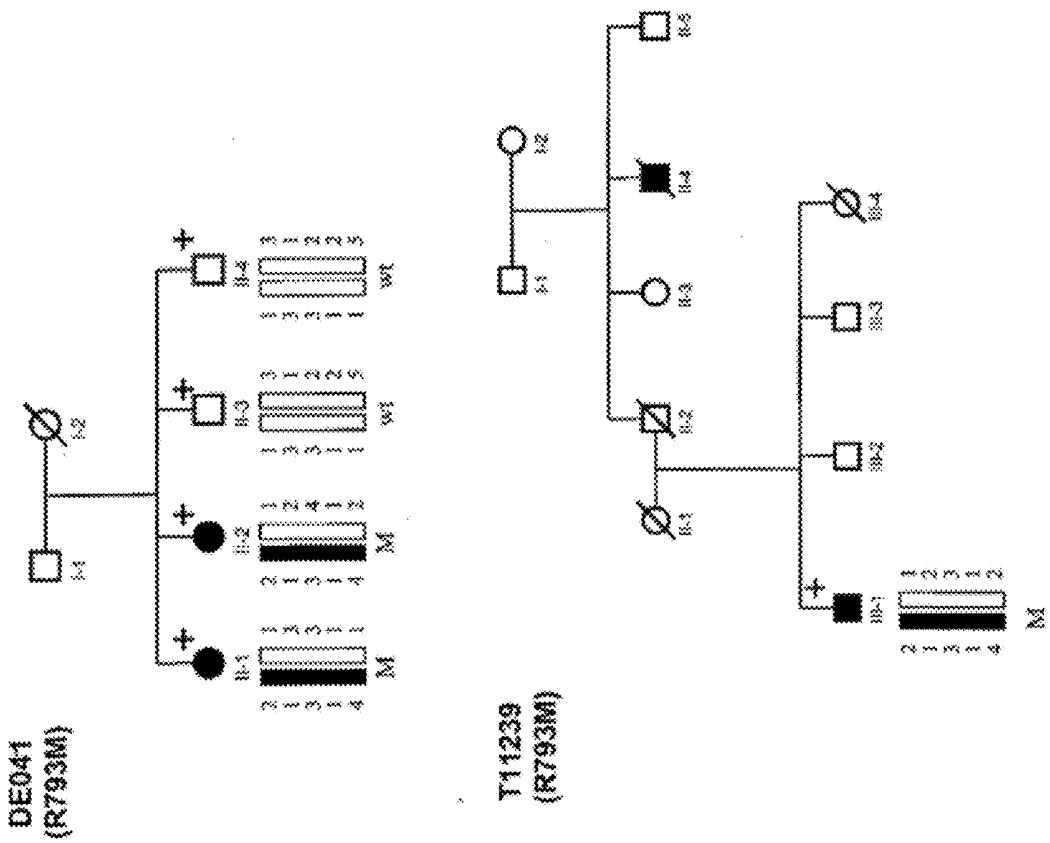


Fig. 6a

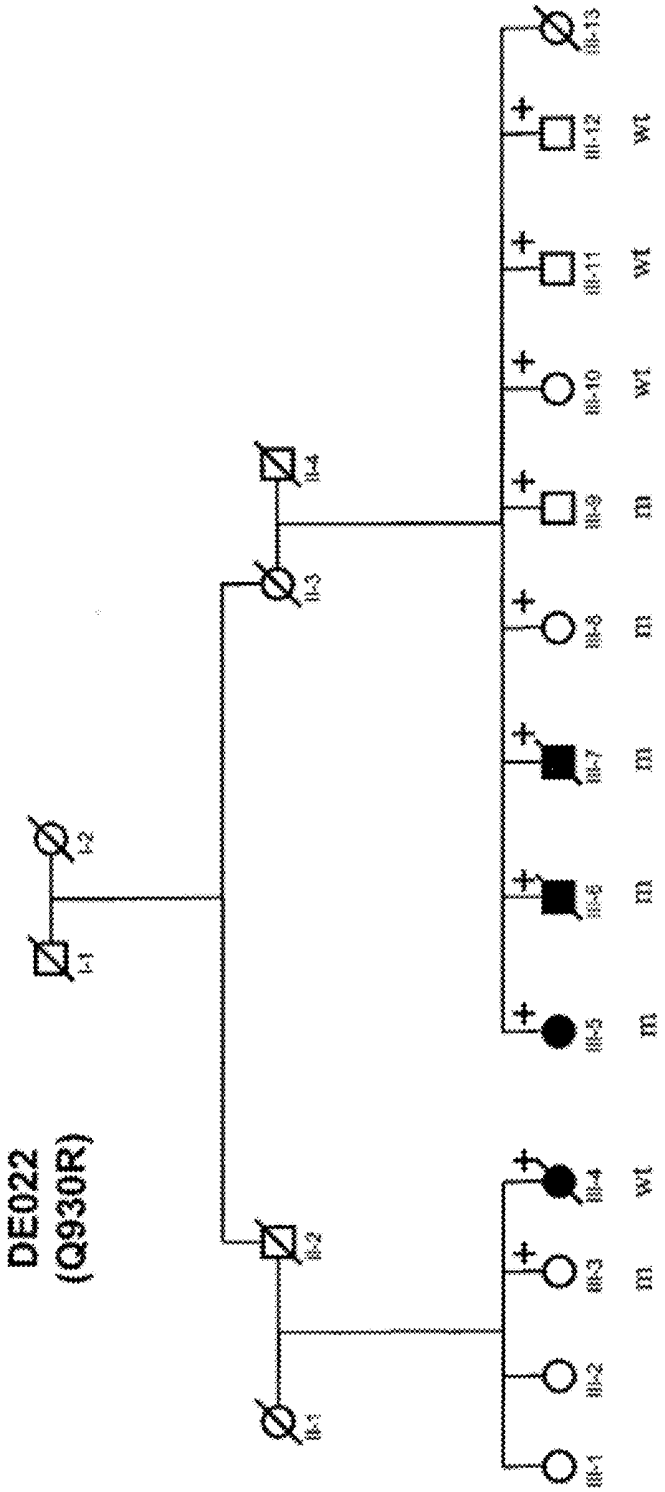


Fig. 6b

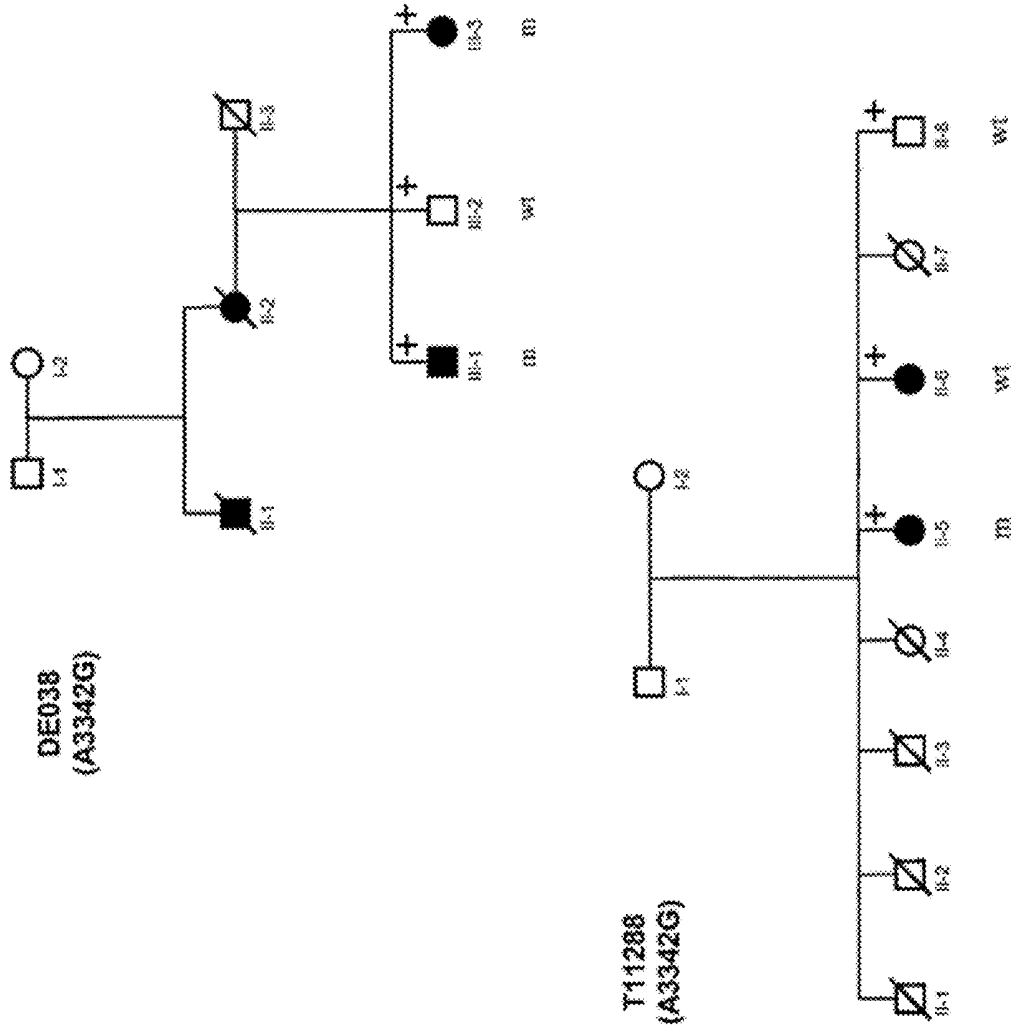
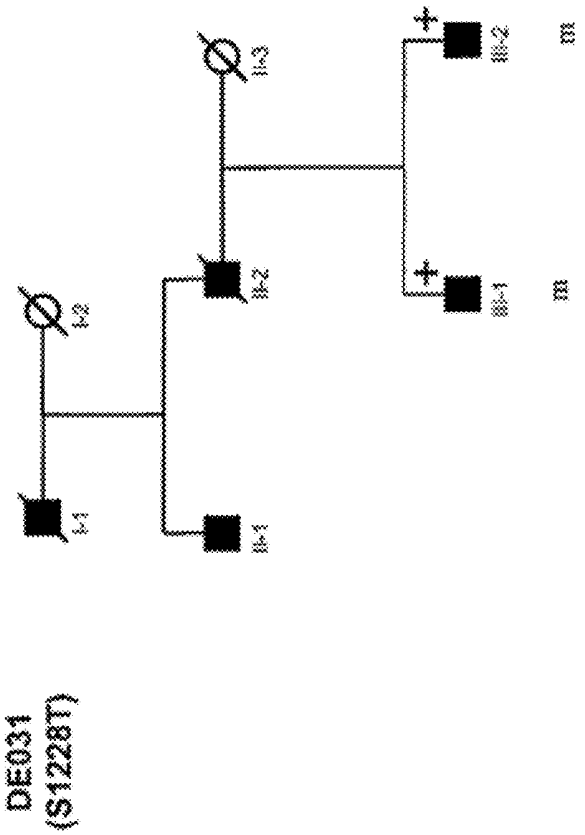


Fig. 6c

Fig. 6d



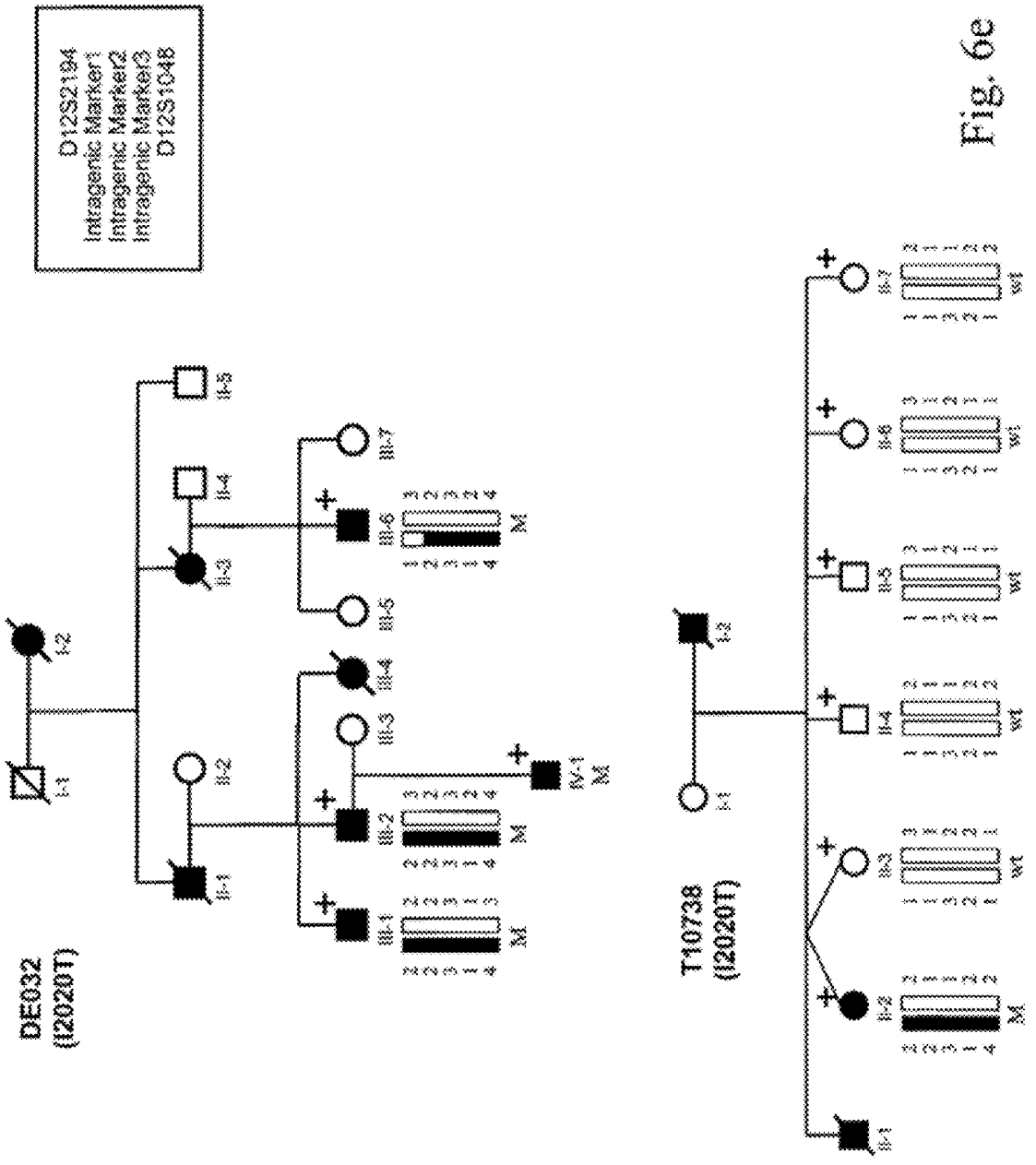

D12S2194
Intragenic Marker1
Intragenic Marker2
Intragenic Marker3
D12S1048
DE032
(I2020T)
T10738
(I2020T)
M

Fig. 6e

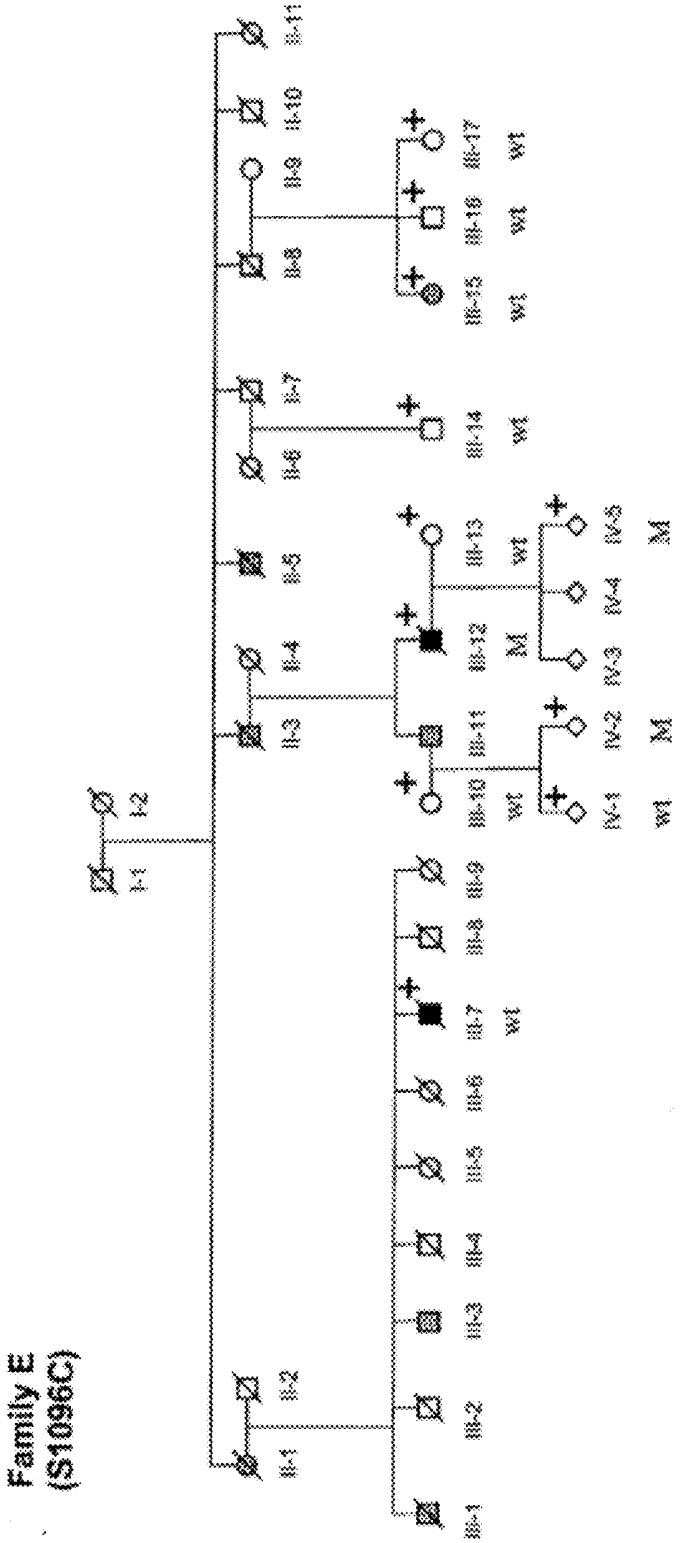

Family E
(S1096C)
I-1
I-2
II-1
II-2
II-3
II-4
II-5
II-6
II-7
II-8
II-9
II-10
II-11
II-12
II-13
II-14
II-15
II-16
II-17
II-18
II-19
III-1
III-2
III-3
III-4
III-5
III-6
III-7
III-8
III-9
III-10
III-11
III-12
III-13
IV-1
IV-2
IV-3
IV-4
WT
M

Fig. 6f

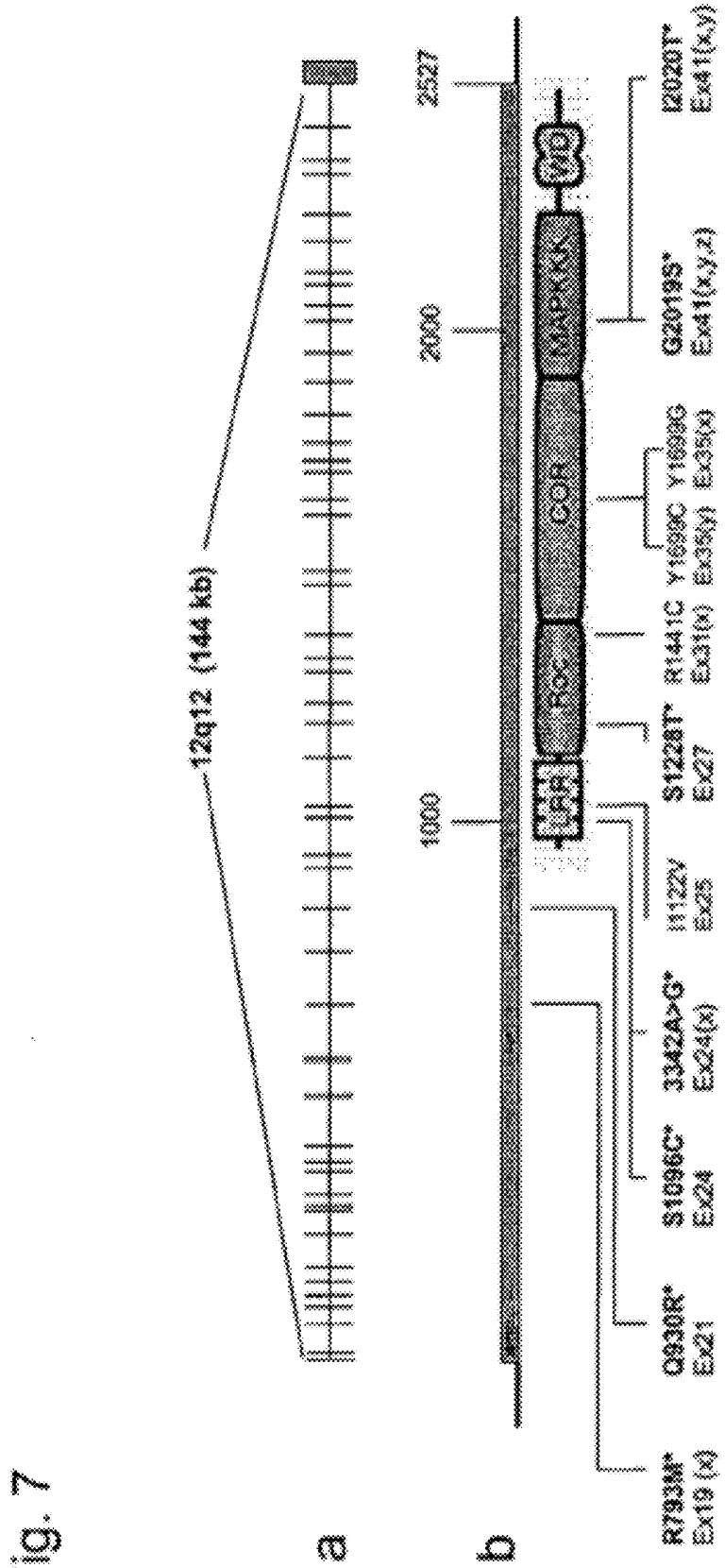

12q12 (144 kb)
a
b
1000
2000
2327
ROC
COR
MAPKKK
R793M*
Ex19 (x)
Q930R*
Ex21
S1096C*
Ex24
3342A>G*
Ex24(x)
I1122V
Ex25
S1228T*
Ex27
R1441C
Ex31(x)
Y1699C
Ex35(y)
Y1699S
Ex35(x)
G2019S*
Ex41(x,y,z)
I2020T*
Ex41(x,y)

Fig. 7

Figure 8

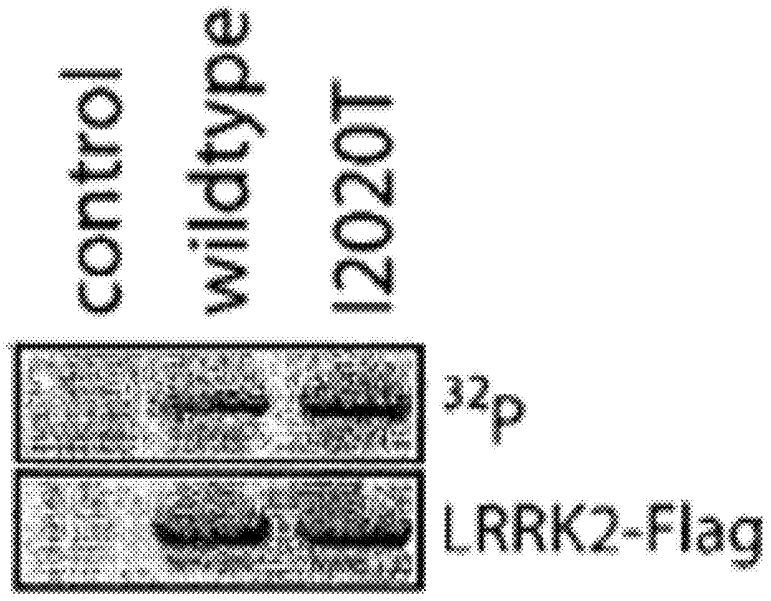


Figure 9

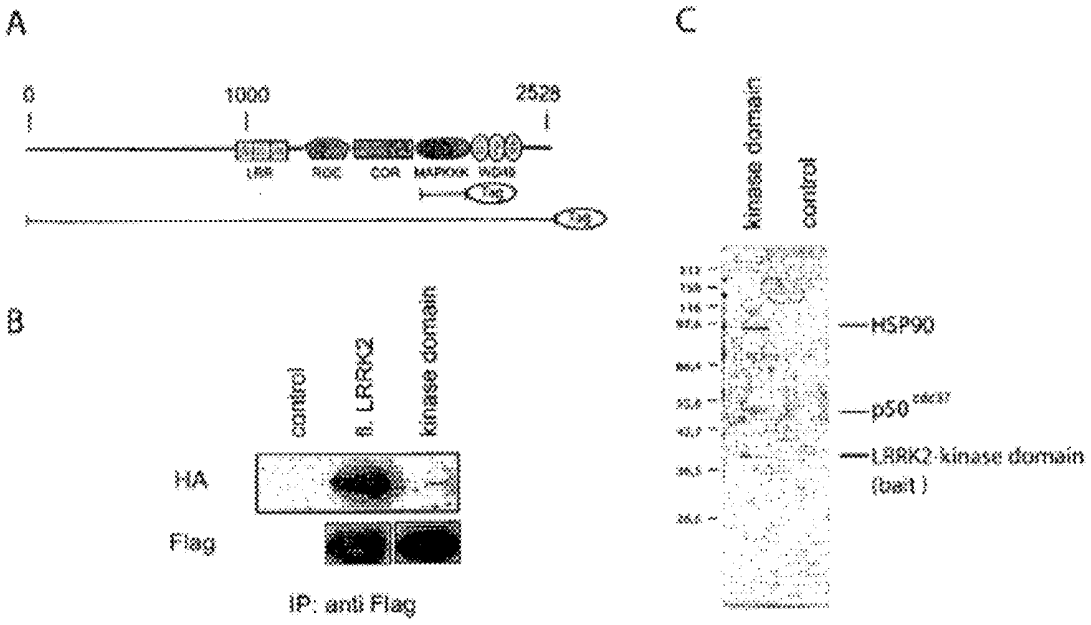

A
0
1000
2529
B
control
e. LRRK2
kinase domain
HA
Flag
IP: anti Flag
C
kinase domain
control
HSP90
p50
LRRK2 kinase domain
(bait)

Figure 10

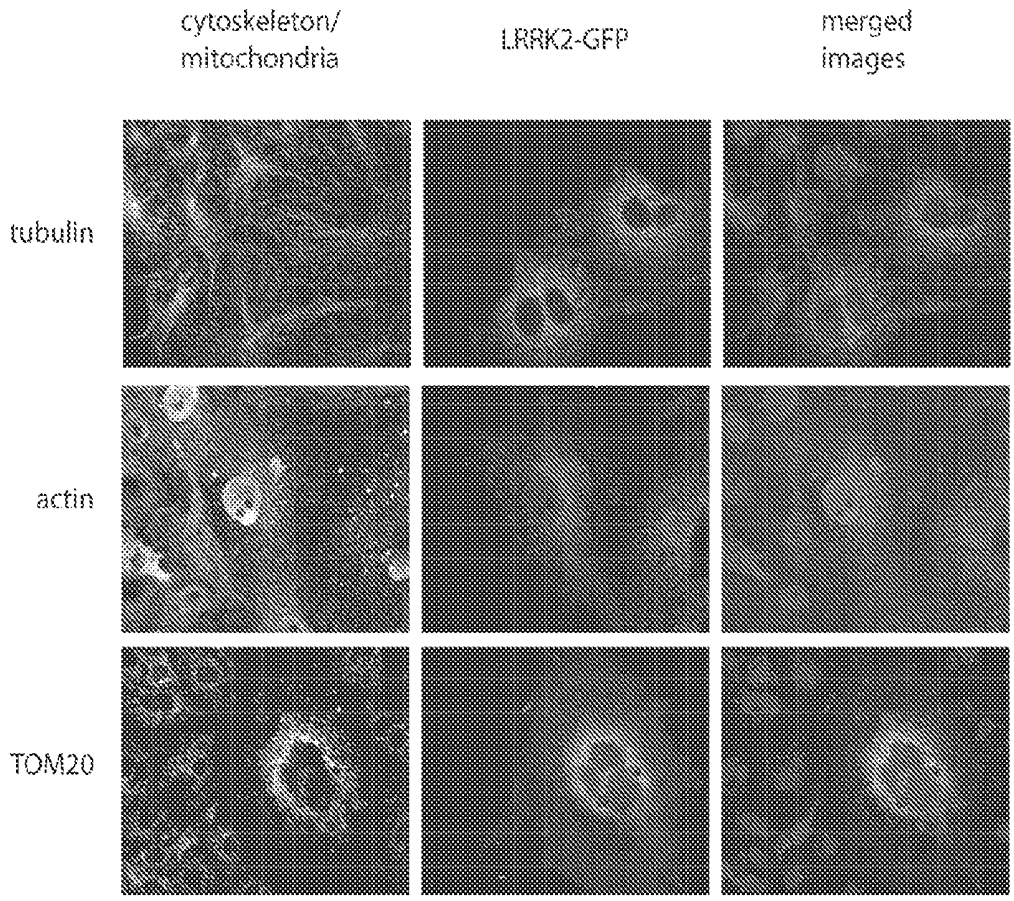

cytoskeleton/
mitochondria
LRRK2-GFP
merged
images
tubulin
actin
TOM20

Figure 11

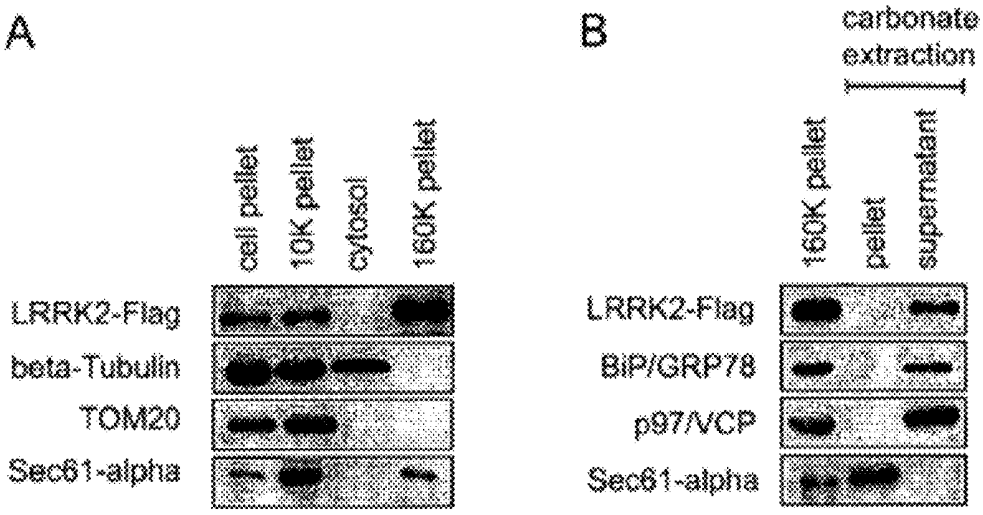

A
cell pellet
10K pellet
cytosol
160K pellet
LRRK2-Flag
beta-Tubulin
TOM20
Sec61-alpha
B
carbonate extraction
160K pellet
pellet
supernatant
LRRK2-Flag
BiP/GRP78
p97/VCP
Sec61-alpha

Figure 12

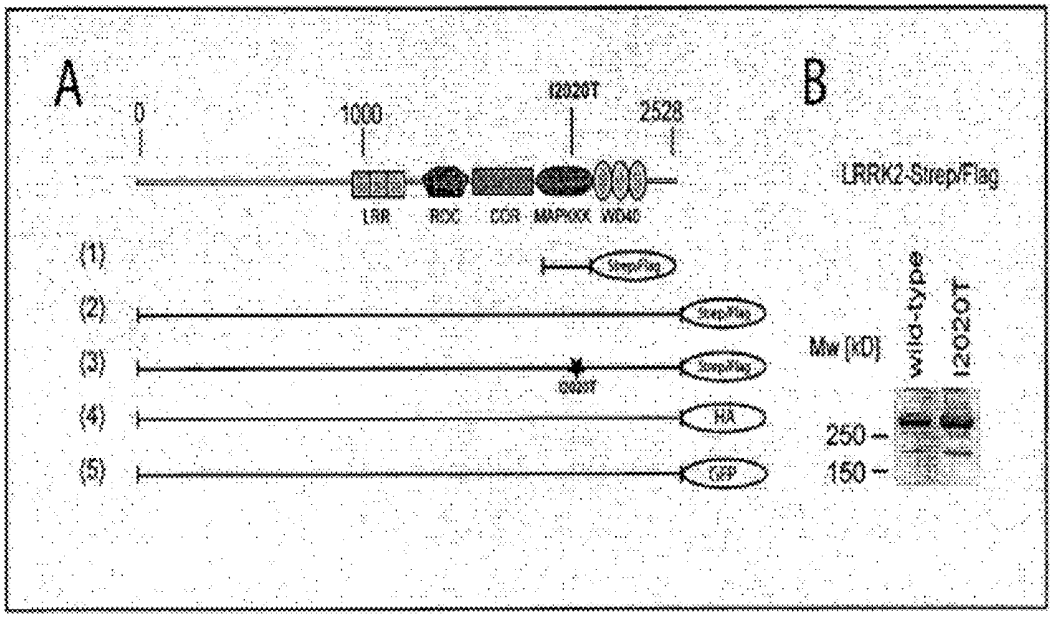

A
0
1000
I2020T
2528
LRR
ROC
COR
MAPKKK
WD40
(1)
(2)
(3)
(4)
(5)
I2020T
HA
GST
B
LRRK2-Strep/Flag
wild-type
I2020T
Mw [kD]
250 –
150 –

Figure 13:

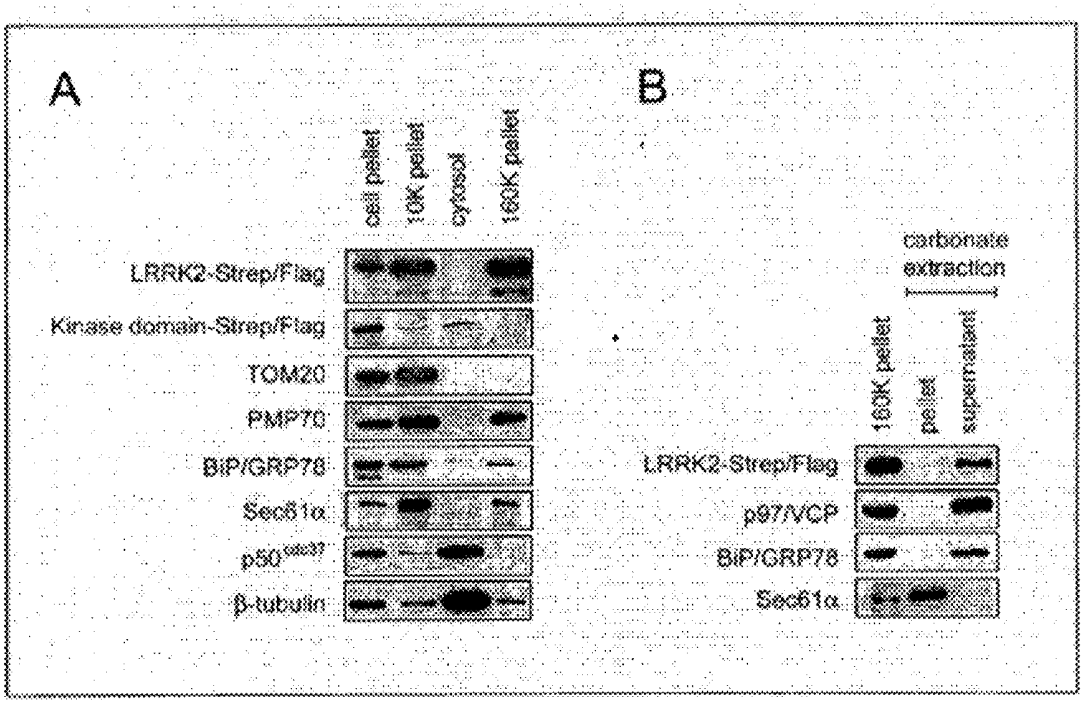

A
cell pellet
10K pellet
cytosol
160K pellet
LRRK2-Strep/Flag
Kinase domain-Strep/Flag
TOM20
PMP70
BiP/GRP78
Sec61α
p50cleaved
β-tubulin
B
carbonate extraction
160K pellet
pellet
supernatant
LRRK2-Strep/Flag
p97/VCP
BiP/GRP78
Sec61α

Figure 14:

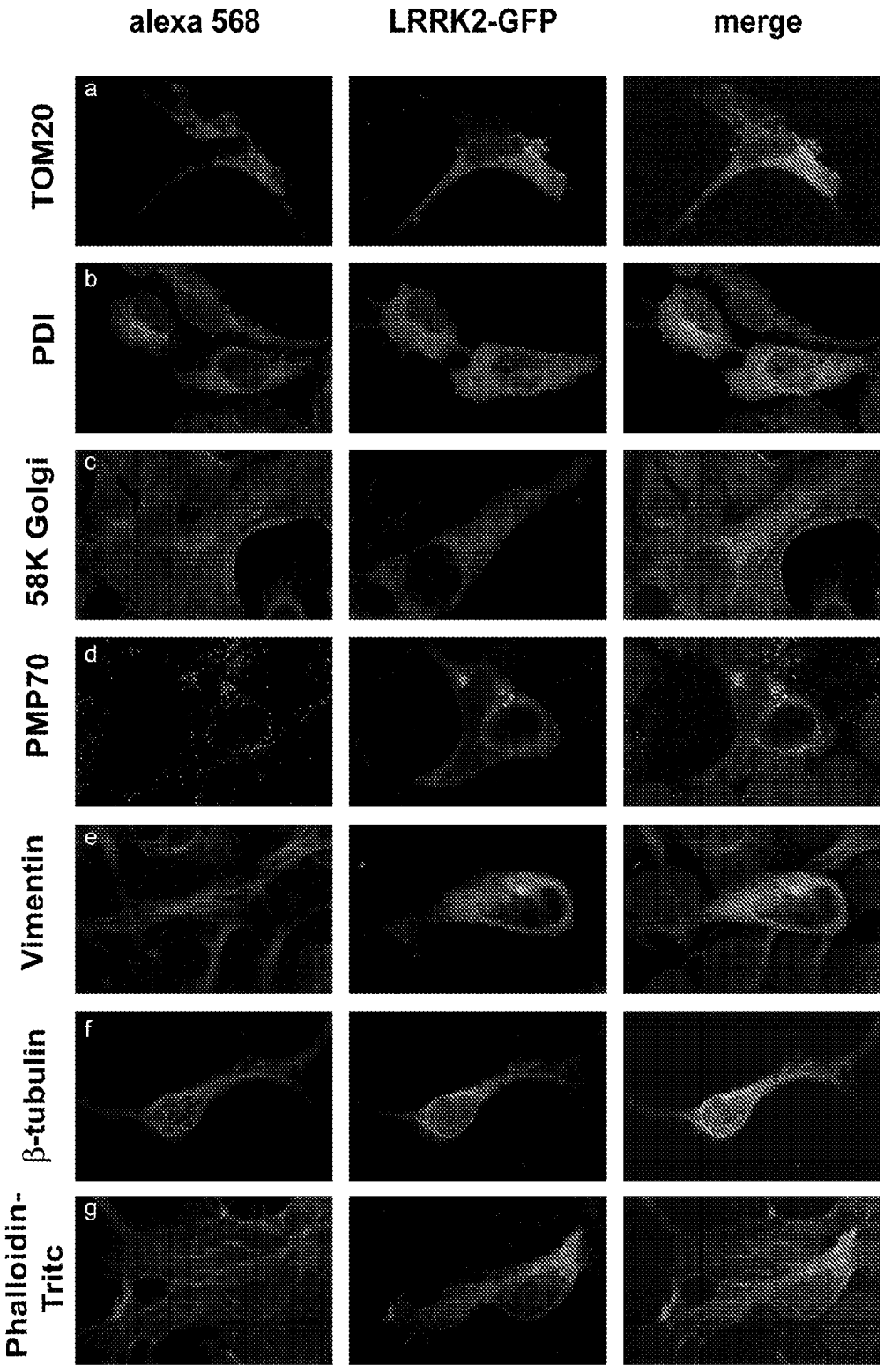

alexa 568
LRRK2-GFP
merge
a
TOM20
b
PDI
c
58K Golgi
d
PMP70
e
Vimentin
f
β-tubulin
g
Phalloidin-Tritc

Figure 15:

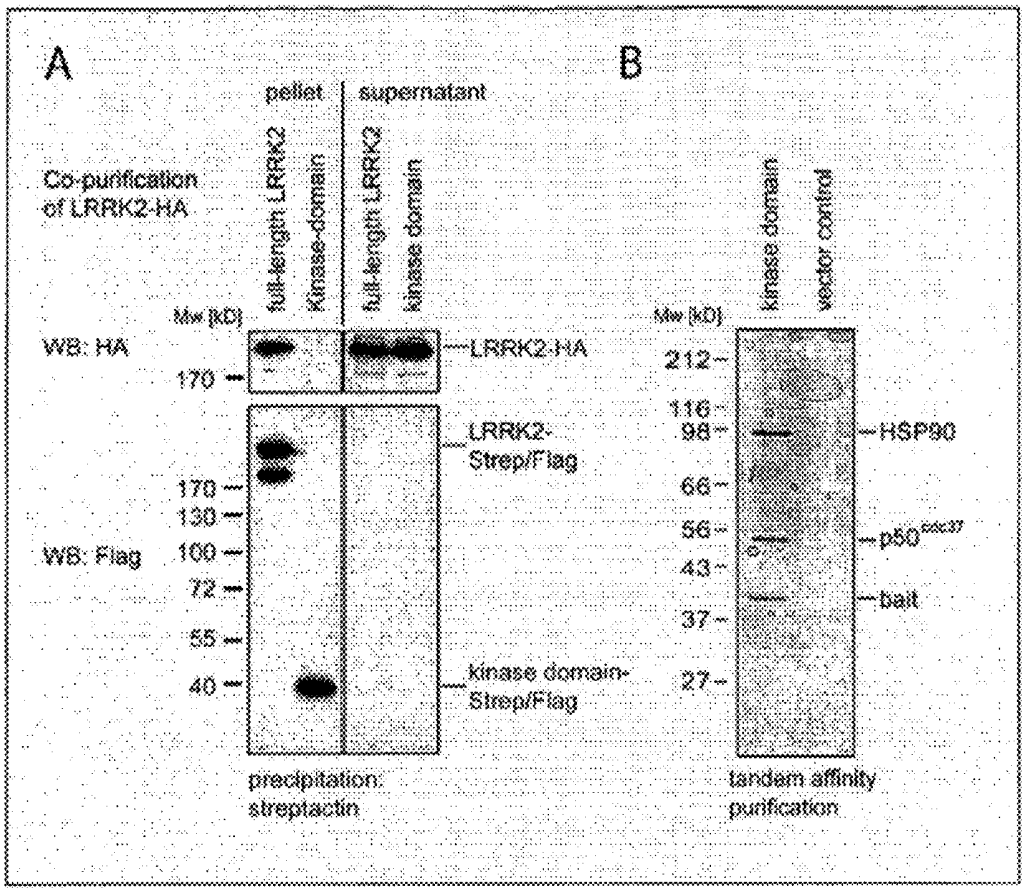
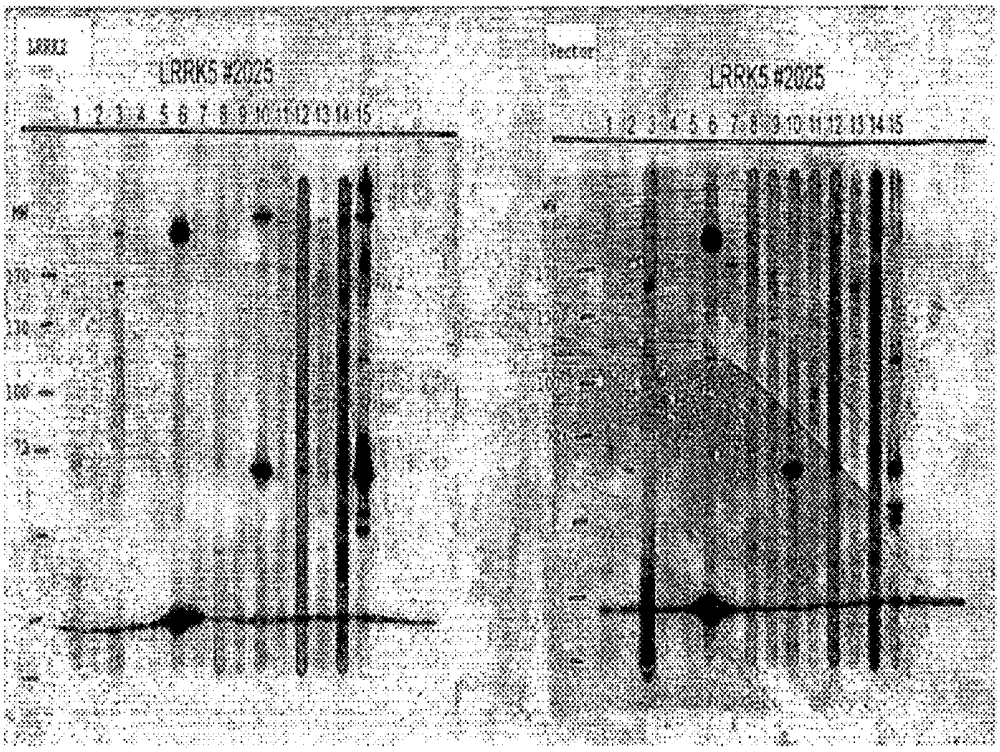

A
B
pellet
supernatant
Co-purification of LRRK2-HA
full-length LRRK2
Kinase-domain
full-length LRRK2
kinase domain
Mw [kD]
WB: HA
170
LRRK2-HA
LRRK2-Strep/Flag
170
130
100
72
55
40
WB: Flag
kinase domain-Strep/Flag
precipitation: streptactin
kinase domain
vector control
Mw [kD]
212
116
98
66
56
43
37
27
HSP90
p50cdc37
bait
tandem affinity purification

Figure 16:


LRRK5 #2025
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Vector
LRRK5 #2025
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

KASPP (LRRK2) GENE, ITS PRODUCTION AND USE FOR THE DETECTION AND TREATMENT OF NEURODEGENERATIVE DISORDERS

[0001] The present invention refers to a newly discovered gene named KASPP for Kinase Associated with Parkinsonism with Pleiomorphic Pathology or alternatively named LRRK2 for Leucine-Rich Repeat Kinase 2, its production, biochemical characterization and use for the detection and treatment of neurodegenerative disorders, such as Parkinson disease (PD) including, without limitation, sporadic PD, Alzheimer disease (AD), amyotrophic lateral sclerosis (ALS), and other synucleinopathies and/or tauopathy as well as several polymorphisms and mutations in the KASPP/LRRK2 gene segregated with PD.

[0002] Parkinson's disease (PD) is the second most neurodegenerative disorder affecting 1-2% of the population aged 65 and older characterized by a progressive loss of dopaminergic neurons of the substantia nigra, associated with the formation of fibrillar aggregates composed of α-synuclein and other proteins (Lewy bodies and Lewy neurites). In most cases, PD occurs as a sporadic disease of unknown etiology, but in rare instances, point mutations or multiplications of the α-synuclein gene can cause autosomal-dominant parkinsonism which resembles the sporadic disease in many aspects. Recessive forms of parkinsonism have been recognized, which are caused by mutations in the genes for parkin (Kitada T. et al., Nature, 392, 605-608, 1998), DJ-1 (Bonifati V. et al., Science, 299, 256-259, 2002) and PINK1 (Valente E. M. et al., Science, 304 (5674), 1158-1160, 2004). Additional loci have been mapped on chromosomes 2p (Gasser T. et al., Nat. Genet., 18, 262-265, 1998), 12cen (Funayama M. et al., Ann. Neurol., 51(3), 296-301, 2002), 1q (Hicks A. A. et al., Ann. Neurol., 52(5), 549-555, 2002), and 2q (Pankratz N. et al., Am. J. Hum. Genet., 72(4), 1053-1057, 2003). In more than 10% of patients with PD one or more relatives are also affected by this disorder (Elbaz et al., Neurology, 52:1876-82, 1999). However, genetic causes are only very rarely found.

[0003] As α-synuclein aggregation is a pathologic feature both in the common sporadic and a dominantly inherited form of PD, and also in other neurodegenerative diseases, such as dementia with Lewy bodies (DLB) and multiple systems atrophy (MSA), those diseases have collectively been called "synucleinopathies". Other forms of parkinsonism are associated with the accumulation of filaments composed of the microtubule associated protein tau (MAPT). Mutations in this gene explain at least a subgroup of families with frontotemporal dementia with parkinsonism (FTDP-17; Ghetti B. et al., "Frontotemporal dementia and parkinsonism linked to chromosome 17 associated with tau gene mutations (FTDP-17)". In: Dickson D. W., "Neurodegeneration: the molecular pathology of dementia and movement disorders" ISN Neuropath Press, Basal, 86-102, 2003), while sporadic cases with tau pathology most commonly present as progressive supranuclear palsy (PSP) or corticobasal degeneration (CBD). Based on the putative central role of the tau protein in these diseases, they have been called "tauopathies".

[0004] Recently it has been shown that two large families with autosomal-dominant late-onset parkinsonism (families A and D) are linked to the PARKS-locus on chromosome 12p11.2-q13.1 (OMIM# 607060), originally mapped in a Japanese family by Funayama et al. (Funayama et al., Ann. Neurol., 51(3), 296-301, 2002; Zimprich A. et al., Am. J. Hum. Genet., 74(1), 11-19, 2004).

[0005] Now, a haplotype analysis refined the candidate region to a 13 Mb interval between flanking markers D12S1692 and D12S85. A total of 29 genes have been sequenced in that region in two patients from each family (see Table 1).

[0006] A whole gene, part of that had previously been deposited under DKFZp434H211 (Accession: XM_058513), has been amplified from human brain cDNA using overlapping primers corresponding to published sequences of various ESTs and mRNAs. Nevertheless, it became clear from cross-species sequence alignments that the DKFZp434H211 clone was incomplete towards the 5'-end.

[0007] Surprisingly, several mutations, including missense mutations and a splice site mutation, have been found in newly discovered large gene coding for a multifunctional protein, which is referred to as Kinase ASsociated with Parkinsonism with Pleiomorphic Pathology, KASPP. KASPP spans a genomic region of 144 Kb and comprises 51 exons and encodes 2527 amino acids (see SEQ ID NOS: 1 and 2 and FIG. **4**). The gene can also be named Leucine-Rich Repeat Kinase 2, LRRK2, because it is the only gene in the human genome encoding a kinase containing leucine rich repeats which was very surprising. KASPP/LRRK2 is a 285 kD protein.

[0008] Therefore, the present invention is directed to an isolated nucleic acid molecule comprising a polynucleotide sequence selected from:

[0009] (a) nucleotides 1 to 9104 of SEQ ID NO: 1 or 2;

[0010] (b) nucleotides 1 to 7584 of SEQ ID NO: 1 or 2;

[0011] (c) nucleotides 1 to 7581 of SEQ ID NO: 1 or 2;

[0012] (d) a nucleotide sequence coding for the protein sequence of SEQ ID NO: 1 or 2 or for the protein sequence of SEQ ID NO: 1 or 2 containing at least one of the mutations depicted in SEQ ID NO: 1 or 2, respectively;

[0013] (e) a nucleotide sequence complementary to either of the nucleotide sequences in (a), (b), (c) or (d); and/or

[0014] (f) a nucleotide sequence which hybridizes under high stringency conditions to any of the nucleotide sequences in (a), (b), (c), (d) or (e).

[0015] SEQ ID NO: 2 is particularly preferred because it reflects the amino acid sequence of human KASPP/LRRK2 and the human nucleotide sequence coding for the human KASPP/LRRK2.

[0016] Examples of polynucleotides selected from paragraph (f), above are the specific mutations, variants and polymorphisms described herein.

[0017] A polymorphism generally is the occurrence of different forms of nucleic acids or proteins in individual organisms or in organisms of the same species, independent of sexual variations. According to the present invention two different polymorphisms have been found for the human KASPP/LRRK2. One shows a variation from cytosine to thymidine at position 635 (c635t) causing a change in the amino acid sequence from serine to leucine (S212L) (see SEQ ID NOS: 4 and 5). The other shows a variation from thymidine to cytosine at position 7190 causing a change in the amino acid sequence from methionine to threonine (M2397T) (see SEQ ID NOS: 6 and 7).

[0018] Examples of high stringency hybridization conditions can be found e.g. in Ausubel, F. M. et al., Current protocols in Molecular Biology, John Wily & Sons, Inc., New

York, N.Y. (1989). In a particular example, a filter, e.g. a nitrocellulose filter, is incubated overnight at 68° C. with a probe in a hybridization solution e.g. containing 50% formamide, high salt (either 5×SSC [20×: 3M NaCl/0.3M trisodium citrate] or 5×SSPE [20×: 3.6M NaCl/0.2M NaH_2PO_4/0.02M EDTA, pH 7.7]), 5× Denhardt's solution, 1% SDS, and 100 μg/ml denatured salmon sperm DNA. This is followed by several washes with buffer, e.g. in 0.2×SSC/0.1% SDS at a temperature selected based on the desired stringency and the melting temperature (Tm) of the DNA hybrid. For example, 68° C. are appropriate for high stringency hybridization.

[0019] The present invention is also directed to a fragment of the inventive nucleic acid molecule specified above containing at least one of the 51 exons and/or coding for at least one of the five domains as specified in FIG. **4**, FIG. **5** and/or FIG. **11** and/or the present specification. The boundaries of the exons as specified in FIG. **4** are applicable for the nucleotide sequences of SEQ ID NO: 1 and SEQ ID NO: 2.

[0020] In addition, the nucleic acid molecule as specified under (f) above may consist of 10 to 50 nucleotides, preferably from 10 to 35 nucleotides, in particular from 20 to 35 nucleotides. Such nucleic acid molecule can be used as a probe or primer for the detection of the polynucleotide of the present invention, in particular for the detection of a mutation thereof as explained in more details below.

[0021] Another example of a fragment is a nucleic acid coding for the immunogenic peptide of SEQ ID NO: 3 and the peptide itself.

[0022] Such fragments may be produced synthetically e.g. by nucleic acid synthesis or by a PCR or TR-PCR reaction.

[0023] Therefore, another embodiment of the present invention is directed to a nucleic acid molecule containing at least one of the mutations depicted in SEQ ID NO: 1 or 2, preferable only one of the mutations depicted in SEQ ID NO: 1 or 2, e.g. the mutation/s at position 2378, 2789, 3287, 3342, 3364, 3683, 4321, 5096 and/or 6059. In addition, the polymorphisms c635t and t7190c are also a specific embodiment of the present invention.

[0024] Surprisingly, is has been discovered that in family A (Y1699C; 5096A>G) and in family D (R1441C; 4321C>T) both mutations segregated with disease in the families (for pedigree structures see FIG. **1**) and were not found in more than 1000 control individuals, nor in 300 sporadic PD-patients.

[0025] In Family A, 16 individuals were typed (8 unaffecteds, 8 affecteds). All affecteds were heterozygous for the mutation and all unaffecteds aged over 60 were wild-type. Individuals IV:1 and IV:2 (family A) were not included in our initial linkage analysis (Zimprich A. et al., 2004, supra), both individuals have now been genotyped. Recalculation of the two-point LOD scores using the mutation as a marker gives maximum LOD scores of 3.78 at θ=0.

[0026] In Family D, 34 individuals were typed (10 affecteds and 24 unaffecteds), all affecteds were heterozygous for the 4321C>T (2.1441C) mutation; out of the 24 clinically unaffected, genotyped individuals, only two were aged over 60 years and were mutation carriers. These individuals are at risk and likely to be presymptomatic given that the average age of onset in this family is 65 years according to Wszolek Z. K. et al., Neurology, 62(9), 1619-1622, 2004.

[0027] To estimate the prevalence of KASPP/LRRK2-mutations among PD-families, one index patient from 44 additional families with PD, 32 consistent with autosomal dominant parkinsonism and 12 affected sib-pairs, were subsequently sequenced.

[0028] Surprisingly, two further miss-sense and one putative splice site mutation have been identified, all in families with typical late onset PD, compatible with a dominant transmission: (I1122V; 3364A>G) in family 21; (I2020T; 6059T>C) in family 32, and (L1114L; 3342A>G) in family 38, which is 6 by away from the exon/intron border. Affected individuals in a further family (469) were found to carry the same mutation as family D (R1441C; 4321C>T). Those two families are not known to be related, nor did they share haplotypes for the closest flanking microsatellite repeat markers D12S2194, D12S1048 or three newly developed intragenic repeat markers, indicating that the mutations are extremely ancient or arose independently (for pedigree structures see FIG. **2**).

[0029] Again, the mutations segregated with the disease in all families and none of them were found in controls. Three of the amino acid substitutions (R1441C, Y1699C and I1122T) are additionally highly conserved across species (see FIG. **3**).

[0030] Screening the entire coding region of the KASPP/LRRK2 gene in a cohort of 53 apparently unrelated families with apparently autosomal mode of inheritance, seven more families with amino acid substitutions or one splice site mutation have been identified. Mutations in the KASPP/LRRK2 gene, therefore, account for 13% of familiar PD in our total cohort.

[0031] In the second study four novel mutations (R793M, Q930R, S1096C and S1228T) have been identified. Therefore, together with the published mutation, until now, 10 missense mutations and one splice site mutation have been described.

[0032] The KASPP/LRRK2 gene consists of 51 exons comprising five conserved domains (see FIGS. **5** and **7**) indicating that it belongs to a recently defined ROCO protein family (Bosgraaf L. et al. Biochim. Biophys. Acta., 1643 (1-3), 5-10, 2003).

[0033] The five conserved domains are in detail:

[0034] (1) N-terminal leucine-rich repeat (LRR) consisting of 12 strands of a 22-28 amino acid motif, present in a tandem array; (2) ROC (Ras of complex) domain indicating the affiliation of the protein to the Ras/GTPase superfamily; (3) COR (C-terminal of Roc) domain; (4) tyrosine kinase catalytic domain (MAPKKK) and (5) C-terminal WD-40 domain.

[0035] Proteins containing LRRs are associated with diverse functions, such as hormone receptor interactions, enzyme inhibition, cell adhesion, cellular trafficking, splicing and substrate binding for ubquitination, a common property involves protein-protein interaction (Kobe B. et al., Curr. Opin. Struct. Biol., 11(6), 725-723, 2001). In particular, the N-terminal LRR and the C-terminal WD-40 propeller structure are assembly points for larger protein complexes. Ras/GTPase domains are involved in the reorganization of the actin cytoskeleton in response to external stimuli. They also have roles in cell transformation by Ras, in cytokinesis, in focal adhesion formation and in the stimulation of stress-activated kinase (Ridley A. J. Trends Cell. Biol, 2001). In particular, the fusion of a Ras-like domain with a MAPKKK domain indicates the function of KASPP/LRRK2 in intramolecular signal transduction. Furthermore, KASPP/LRRK2 should also function as a scaffolding protein like Ksr (Kinase suppressor of Ras). The KASPP/LRRK2 kinase domain shows also similarity to the RIR and Mixed lineage kinases

which are part of the TKL-(thyrosine kinase like) branch of the human kinome indicating an involvement in stress-induced cell signalling and mediation of apoptosis. The COR domain is characteristic for this protein family and shows no significant sequence homology to any domain or protein today. Enzymes with a tyrosine kinase catalytic domain belong to an extensive family of proteins which share a conserved catalytic core common to both serine/threonine and tyrosine protein kinases. They exert their function by catalyzing the transfer of the gamma-phosphate of ATP to tyrosine residues on protein substrates (Hubbard S. R. et al. Annu. Rev. Biochem., 69, 373-398, 2000). The WD40 domain is implicated in signal transduction, pre-mRNA processing and cytoskeleton assembly (Smith T. F. et al., Trends Biochem. Sci., 24(5), 181-185, 1999).

[0036] In view of the present invention three Roco proteins of the Roco-protein family exist now in mammals: LRRK1, DAP-kinase (death associated protein kinase) and KASPP/LRRK2 of the present invention. The mammalian DAP-kinase, for example, should be involved in cytosceletal rearrangements and/or induction of apoptosis dependent on its activity.

[0037] The biochemical analysis of KASPP/LRRK2 and its Parkinson disease-associated variant, I2020T, bearing a mutation located next to the DFG motif (DYG in KASPP/LRRK2) at the beginning of the activation loop of the kinase domain which is highly conserved in almost all MAPKKK, indicates according to the present invention that KASPP/LRRK2 acts as a true protein kinase at cytoskeletal and membraneous structure within the cell. In addition, the found increase (approximately 30-50%) in the kinase activity of the I2020T mutant is consistent with mutations in homologous positions of other kinases like B-Raf associated with cancer (Dibb, N. J. et al. (2004), Nat. Rev. Cancer, 4, 718-727). It is also worth to be noted that this mutation is a dominant feature. In addition to an overall gain in kinase activity, the mutation could also alter substrate specificity. As with oncogenic kinase variants, kinase inhibitors could then be considered as a treatment option. The effectiveness of such therapeutic strategy has been proven with respect to specifically inhibiting the bcr-abl protein kinase within chronic myeloic leukemia (CML) through the kinase inhibitor 2-phenylaminopyrimidine STI571 (Gleevec), a small-molecule tyrosine kinase inhibitor for the treatment of CML (Chalandon, Y. & Schwaller, J., Haematologica, 90, 949-968, 2005).

[0038] In summary, the biochemical analysis of KASPP/LRRK2 and its 12020T mutant according to the present inventions shows that KASPP/LRRK2 shares common features with other MAPKKK, such as autophosphorylation, dimerisation or interaction with kinase specific chaperones. The autokinase activity of the mutant I2020T, localised within the activation loop of the KASPP/LRRK2 kinase domain is increased when compared to wild-type KASPP/LRRK2 according to the present invention. In view of its multimodular structure, KASPP/LRRK2 should be involved in functions as diverse as maintenance of microtubular ultrastructure and dynamics, vesicular trafficking (ER, Golgi compartment) and/or cytoskeletal rearrangements.

[0039] Interestingly, all ten mutations are within these conserved domains (FIG. **7**). The R793M mutation, however, is located in exon 19 which is part of the ancyrin repeat region (amino acid 678-806), that seems to take part in protein-protein interactions.

[0040] In contrast to previous reports the so far most common mutation (G2019S; 6055G>A, Hernandez D G et al., Ann Neurol, 57: 453-6, 2005) was not detected in any of the families investigated but only in one patient with sporadic PD. Moreover, this mutation was found in only one out of 340 patients with sporadic PD. Therefore, the predominance of this mutation (Gilks et al., Lancet, 365: 415-6, 2005; Toft et al., Lancet, 365: 1229-30, 2005) can not be established for all populations. The screening in familial and sporadic PD patients showed frequencies of the G2019S mutation up to 7% in familial and almost 1% in sporadic PD cases, however, no association could be demonstrated of this mutant with the non-mendelian sporadic form of PD in a recent study of the inventors. In the present cohort comprising 340 patients with sporadic PD the novel R793M was additionally found in one sporadic patient. Therefore, KASPP/LRRK2 mutations account for only 0.6% of sporadic PD cases in the present population.

[0041] In three families the specific variation did not cosegregate with one family member each: In family DE022, the Q930R only three of the four family members affected by the disease were mutation carriers (FIG. **6***b*), in family E in fact only one of the two family members with PD phenotype was carrier of the S1096C mutation (FIG. **6***f*), and the splice site mutation cosegregating with PD in one previously investigated family (DE038) was only found in one of the clinically affected sisters (T112888) (FIG. **6***c*). As none of these variations was found in any of the 1200 controls investigated and the splice site variation affected two distinct PD families it is likely that they are causative for the disease, although incomplete penetrance at least in family DE022 could indicate that additional factors may contribute to manifestation of the disease in affected subjects. This may be due to phenocopies in these three families, as the high prevalence of PD in the population makes it well possible that other causes of PD occur in a family affected by KASPP/LRRK2 mutations. Disease phenocopy is not uncommon in PD. It has been described in the original α-synuclein A53T kindred (Polymeropoulos et al., Science, 276: 2045-7, 1997), in a family with the KASPP/LRRK2 G2019S mutation (Hernandez et al., 2005, supra), but also in family D with the KASPP/LRRK2 R1441C mutation.

[0042] Three of the present mutations affect at least two families. For two of these (R793M and I2020T) haplotype analysis revealed a common haplotype indicating a common founder. None of the families was aware of a possible relation to the respective family although the two families harbouring the I2020T mutation lived in the same geographic region. The same mutation has also been described in the Japanese family, who served as the basis for the original defining of the PARK8 locus (Funayama et al., Ann Neurol., 57: 918-21, 2005).

[0043] The R793M mutation, detected in two distinct families with the same haplotype, was also found in one patient with sporadic PD and one control person. Because of technical problems in assessing this CG rich exon call rate of the population screened was low (about 50% in three different tries). Therefore, it may well be, that this mutation is more frequent in apparently sporadic PD patients. Also, the possibility of a polymorphism needs to be taken into account, if this variation was detected in more controls. However, finding of a possible common founder in the two families with the mutation is an indication for a disease related amino-acid substitution. Common founders are also suggested for other

families affected by mutations in the KASPP/LRRK2 gene (Mata et al., Neurosci Lett, 382: 309-311, 2005).

[0044] Mode of inheritance of KASPP/LRRK2 mutations is autosomal dominant. It has been suggested that penetrance of KASPP/LRRK2 mutations is age dependent (DiFonzo et al., Lancet, 365: 412-5, 2005; Toft et al., 2005, supra) accounting for the reduced penetrance in some families. In the present families reduced penetrance was only observed in mutations of exons 19 and 21 located before the highly conserved LRR domain. This indicates that mutations in this region are less severe and have to be associated with other so far unknown factors for disease manifestation. From the splice site mutation of exon 24 onwards, penetrance was complete, although one splice mutation carrier (DE038, III-1) had only slight resting tremor for several years, while his sister (III-3), mother and uncle were affected by severe PD.

[0045] In all families with definite documentation of age of onset an earlier recognitions of first Parkinsonian signs was observed in the younger generations. So far, there are no known pathomechanism that allow the hypothesis of anticipation. Rather, a greater awareness of a possible affliction and a more thorough investigation in families in whom PD has already been diagnosed could account for the earlier diagnoses.

[0046] The clinical presentation of KASPP/LRRK2 mutation carriers varies within families and between families affected by the same mutation. In general the typical phenotype of PD with resting tremor, bradykinesia, rigidity and olfactory dysfunction can be observed. Interestingly, tremor, the main and naming feature of some of the initially described to families (Paisan-Ruiz et al., Neuron, 44: 595-600, 2004) was neither the main initial nor the leading symptom in many of our PD patients. Two patients did not report any resting tremor in their medical history. Rather, the typical pattern of different subtypes known from idiopathic PD could be observed. All patients reported a substantial relief of symptoms after application of dopaminergic treatment, which was hampered by halluzinations in only the one patient with DLBD-phenotype (Diffuse Lewy Body Disease-phenotype).

[0047] In patients with KASPP/LRRK2 mutations a frequent, the patient strongly afflicting symptom seems to be sleeping abnormality. Eight out of 10 patients (80%) reported to suffer from difficulties of either falling a sleep, staying a sleep or both. According to several studies, sleeping disturbancies occur in about 40-75% of PD patients (Lees et al., Clin Neuropharmacol., 11:512-519, 1988; Kumar et al., Mov Disord, 17: 775-781, 2002), but only the minority (about 20%) reports sleeping abnormalities as a problem (Lees et al., 1988, supra). In the present study 80% stated that sleeping disturbances were indeed a problem. More detailed assessment on sleeping behaviour and pattern are to be decided, whether this symptom is more pronounced in KASPP/LRRK2 mutations carriers, possibly indicating an earlier involvement of the respective systems. Postural instability occurs late in the course of the disease. As also described by others (Paisan-Ruiz et al., Ann Neurol., 57:365-72, 2005) dementia is not a common finding in KASPP/LRRK2 associated PD and seems to occur rather late in the disease process. The same holds true for hallucinations in our patient cohort, occurring either late in the disease process or in combination with dementia. In the present cohort, one patient presented with the typical clinical picture of DLBD. Autopsy of one subject with dementia in our first cohort revealed diffuse Lewy Body pathology in one family affected by the Y1699C mutation. Description of the same phenotype in an other patient in this study affected by a different mutation favours the hypothesis that the clinical presentation of DLBD may be caused by the same pathophysiological alterations as the clinical picture of PD. Obviously specific pathophysiological changes (in this case caused by mutations in the KASPP/LRRK2 gene) may lead to the clinical and histopathological entity of both: PD and DLBD.

[0048] In our first study one patient showed mild signs of motor neuron disease. In the second study, however, motor neuron symptoms were neither clinically nor electrophysiologically disclosed in any patient investigated.

[0049] Structural neuroimaging revealed slight to marked atrophy in all 4 patients investigated, although disease duration was only 3-12 years in these and only one was classified as demented (Table 4). This contrasts findings of idiopathic PD, where structural MRI is usually normal and atrophy only occurs with disease progression, usually associated with dementia. The patient with the clinical presentation of DLBD had marked signs of microangiopathy, which may also be causative for an atypical Parkinsonian syndrome. The clinical presentation with fluctuation of vigilance, good response to L-dopa hampered by hypersensitivity and dementia developing over a short period of time, however, makes the diagnosis of DLBD more likely.

[0050] TCS revealed SN hyperechogenicity—the typical sign for idiopathic PD, found in more than 90% of PD patients (Berg et al., 2001, supra; Walter et al., J Neural Transm, 109:191-196, 2002)—on at least one side of KASPP/LRRK2 mutation carriers. Interestingly, SN hyperechogenicity was only moderate in all patients investigated, as opposed to idiopathic PD, where it is marked in 73-79% of the patients. This highly characteristic finding is supposed to be associated with an increase in tissue iron content and possible alterations in iron binding, antedating the manifestation of disease onset (Berg et al., 1999, supra: Berg et al., Neural Transm, 109:191-196, 2002). An only moderate hyperechogenicity of the SN in KASPP/LRRK2 associated PD may argue for a different course of underlying pathomechanisms, which may finally lead to less iron accumulation in KASPP/LRRK2 associated than in idiopathic PD. Similarly, the slower disease progress, documented by less although typically located reduction of F-Dopa uptake in PET examinations (Hernandez et al., 2005, supra) favours the hypothesis of a different course of the disease.

[0051] In conclusion, in two consecutive studies it has been shown that KASPP/LRRK2 mutations account for about 13% of apparently autosomal dominantly inherited PD and sib pairs in the population investigated. Although the phenotype varies within and between families affected by the same mutations it is very similar to the clinical presentation of idiopathic PD. The causal relation between disease manifestation and variation is not equally clear for all variations described. In three families the specific variations did not co-segregate with one family member each affected by the disease. As none of these mutations was found in 1200 control persons, and one variation was found in two distinct PD families phenocopies is indicative.

[0052] Moreover, two patients with the clinical presentation of DLBD should lead to the consideration of KASPP/LRRK2 mutations in families with the simultaneous occurrence of DLBD and PD.

[0053] As already pointed out above mutations have been found in different functional domains but it is unclear which

of them are related to neurodegeneration. However, KASPP/LRRK2 may be central to a range of neurodegenerative processes because our findings show that (i) KASPP/LRRK2-mutations appear to be a numerically important cause of autosomal-dominant parkinsonism (6 independent mutations in 34 families with dominant inheritance) and (ii) affected individuals with KASPP/LRRK2 mutations exhibit strikingly variable pathologic changes, representing aspects of several of the major neurodegenerative diseases.

[0054] Using cell fractionation and carbonate extraction the present invention discloses that KASPP/LRRK2 is associated partially with mitochondria, the cytoskeleton and microsomal membranes, which is an indication that KASPP/LRRK2 is involved in cytoskeletal rearrangements. No KASPP/LRRK2 was found in the cytoplasm. Further, the autokinase activity of KASPP/LRRK2 is not significantly changed in the disease-associated I2020T mutant compared with wild-type, indicating that the autosomal dominant effect is caused by a toxic gain of function rather than loss of function. The I2020T mutation is located next to the conserved motif DFG (DYG in LRRK2) at the beginning of the activation segment of the kinase domain (Ross O. A. & Farrer M. J. Biochem. Soc. Trans., 33, 586-590, 2005) and in the mutation a hydrophobic leucine residue is exchanged by a polar threonine residue. This is also an indication that associated Parkinson's disease is caused by altered substrate specificity or higher KASPP/LRRK2 activity. Homodimerization and association with the HSP90/p50^{cdc37} chaperone-system further indicate that the KASPP/LRRK2 function and activation mechanisms are similar to other MAPKKK. These effects serve as a basis for the development of a suitable screening assay and/or the development of a pharmaceutical or a diagnostic agent as described below in detail.

[0055] Autopsies performed on affected individuals uniformly demonstrated neuronal loss and gliosis in the substantia nigra as the pathological substrate of parkinsonism. However, α-synuclein pathology (Lewy-bodies, LBs) typical for PD was seen only in one case from family D. In another case from this family widespread LB's was more consistent with diffuse Lewy Body disease (DLB). Even more intriguingly, senile plaques and neurofibrillary tangles (NFTs) as well as prominent tau deposits were demonstrated in 3 other brains from both large kindreds. Spinal cord pathology consistent with a diagnosis of amyotrophic lateral sclerosis (ALS) was found in affected members of family A.

[0056] Hence, KASPP/LRRK2 is likely to be central to the aetiology of all neurodegenerative diseases such as PD, Alzheimer disease (AD) and amyotrophic lateral sclerosis (ALS) and pathologies, including synucleinopathy and tauopathy, associated with a clinical phenotype of parkinsonism.

[0057] It has previously been shown that tau and α-synuclein pathologies may be closely linked. Tau-aggregations have been found in the brains of patients carrying pathogenic A53T α-synuclein mutations (Kotzbauer P. T. et al., Exp. Neurol., 187(2), 279-288, 2004; Duda J. E. et al., Acta Neuropathol. Berl., 104, 7-11, 2002) Similarly, the major pathogenic protein aggregating in AD has been shown to promote fibrillization of tau and formation of neurofibrillary tangles in an animal model (Gotz J., Science, 293, 1491-1495, 2001). Interestingly, a genomic region overlapping the PARK8 locus has been identified in a linkage study of familial Alzheimer disease (Scott W. K. et al. Am. J. Hum. Genet., 66(3), 922-932, 2000). Evidence for linkage was derived in a large part from families with at least one member with autopsy proven diffuse Lewy body disease. Whether this linkage result reflects variants in the KASPP/LRRK2 gene remains to be determined.

[0058] The expression pattern of KASPP/LRRK2 was subsequently examined in brain and other tissues. Human brain and multiple tissue Northern blots were hybridized with a 1078 by 3' cDNA probe and found expression in most brain regions, albeit at a very low levels. Two transcripts of about 9 kb and 8 kb, respectively, and multiple bands at lower sizes were found. The two transcript sizes might be explained by alternative splicing and/or the alternative use of polyadenylation sites.

[0059] As low overall expression levels in brain precluded a detailed analysis of its regional distribution by Northern blotting, real-time RT-PCR was done using RNA isolated from adult and fetal whole brain as well as from different brain regions in order to assess quantitative gene expression and alternative splicing. Primers have been designed to generate specific PCR products for exon1-8, exon13-19 and exon 31-39, respectively. Within the same tissue or brain region transcript levels of all three assays showed no significant differences. Consistent expression in most brain regions have been found, slightly higher in putamen, substantia nigra and heart. The highest expression levels were observed in lung. The cDNA analysis, within in multiple tissues, confirms in silico prediction that at least 11 exons may be alternatively spliced. In adult human brain tissue, exon 6 was constitutively expressed within the full length mRNA.

[0060] In view of the above, the present invention is also directed to a vector, preferable an expression vector, containing the nucleic acid molecule of the present invention, to a cell containing the nucleic acid or the vector of the present invention and to a transgenic animal containing the nucleic acid or the vector of the present invention.

[0061] A vector can be a plasmid or phage DNA or any other DNA sequence into which DNA can be inserted to be cloned. The vector can replicate autonomously in a host cell, and can be further characterized by one or a small number of endonuclease recognition sites at which such DNA sequences can be cut in a determinable fashion and into which DNA can be inserted. The vector can further contain a marker suitable for use in the identification of cells transformed with the vector. Markers, for example, are tetracycline resistance genes or ampicillin resistance genes.

[0062] In a further embodiment the vector can be in the form of an expression vector containing an expression cassette which comprises the nucleic acid molecule of the present invention, but preferably also further comprising expression control sequences which are operatively linked to the nucleic acid molecule. The expression control sequences are chosen so that they allow expression of the encoded polypeptide in a host. For example a nucleic acid sequence encoding a polypeptide of the present invention can be isolated and cloned into an expression vector and the vector can then be transformed into a suitable host cell for expression of the polypeptide of the invention. Such a vector can be a plasmid, a phagemid or a cosmid. For example, a nucleic acid molecule of the invention can be cloned in a suitable fashion into prokaryotic or eukaryotic expression vectors, preferably into eukaryotic expression vectors and more preferably into expression vectors allowing expression in a mammalian and in particular in a human cell which is known to a person skilled in the art. Such expression vectors typically comprise at least one promoter and can also comprise a signal for translation initiation of the reading frame encoding the polypeptide and—in the case of prokaryotic expression vectors—a signal for translation termination, while in the case of eukaryotic expression vectors the expression cassette preferably comprises expression signals for transcriptional termination and polyadenylation. Examples of suitable eukaryotic

expression vectors are well known for the person skilled in the art, e.g. for the expression in insect cells via baculovirus vectors, and for expression in mammalian cells, e.g. the SV40 or CMV vectors, the sindbisvirus expression system, or an adenovirus expression system, a Semliki Forest Virus-based expression system, or a lentivirus-based expression system. The molecular biological methods for the production of these expression vectors are also well known to the skilled person, as well as the methods for transfecting host cells and culturing such transfected host cells. In a preferred embodiment the above-mentioned expression control sequences specify induced expression of the polypeptide of the invention, that is induced transcription of the messenger RNA encoding the polypeptide of the invention upon addition or withdrawal of an external signal, such as a small chemical like tetracycline or a hormone like Ecdysone, but the extracellular signal can also be an increase or decrease in temperature or ionizing radiation. Also, inducible expression can be brought about by inducible translation initiation of the messenger RNA or a system in which mRNA stability is controlled in an inducible fashion. Examples of expression control sequences allowing induction of polypeptide production are reviewed in the following publications: the TET-off/TET-on system, suitable for both cell cultures and transgenic animals, but also the expression control system based on Cre-recombinase based methods, predominantly for use in transgenic animals. A further inducible expression system, for use in both cell culture and transgenic animals is based on the insect hormone Ecdysone. Another inducible expression system is the GAL4 system, which has been successfully applied with mice, zebrafish and *Drosophila*, and allows conditional expression at 26-29 degrees, or also a Rapamycin based conditionals expression system. A temperature-sensitive expression system is based on a Sindbis virus expression cassette and predominantly suitable for controlled expression in cell culture systems.

[0063] Another aspect of this invention relates to a cell comprising a nucleic acid or a vector of the present invention. Such a cell can be a mammalian, non-human cell inside or outside of the animal body or a human cell outside of the human body. But it can also be an insect cell, like a drosophila cell, in culture or in the context of a transgenic insect, like a transgenic *Drosophila*. It can also be a nematode cell, like, for example, present in transgenic *C. elegans*. Preferred host cells are mammalian, and particularly human, neuronal cells, microglia cells, astrocytes, oligodendrocytes, fibroblasts, monocytes, and macrophages and other non-neuronal primary cells, which can be kept in primary tissue culture and can be made capable of expressing the polypeptide of the invention by introducing the nucleic acid/or the expression cassette of the invention, for example by transfection with such a nucleic acid or expression cassette. Other means of introducing the nucleic acid and/or the expression cassette of the invention to the above-mentioned primary cells are "gene gun" approaches, mRNA transfer, viral infection, microinjection or liposomal nucleic acid transfer, to name but a few. Other suitable host cells are mammalian cells like HEK cells, HELA cells, PC12 cells, CHO cells, JURKAT cells, mouse 3T3 fibroblasts, mouse hepatoma cells, human neuroblastoma cells, but also established cancer cell lines, particularly neuronal cell lines, of mammalian and particularly of human origin.

[0064] The nucleic acid and/or the expression cassette of the invention can also be introduced into those cells by the above-mentioned nucleic acid transfer methods. Particularly preferred are stably transformed cell lines wherein the expression of the polypeptide of the invention is inducible. Since expression of the polypeptide of the invention may show increased neuropathology, it may be preferred that in such host cells the expression of the polypeptide of the invention is usually very low or off, for example during the generation of a stably transformed cell line, and only for experimental purposes and after establishment of such a stably transformed cell line the expression of the polypeptide of the invention is turned on by addition of a suitable stimulus, like e.g. a hormone like Ecdysone or a small chemical like the antibiotic tetracycline. Again, the above described examples of expression control sequences allowing induction of polypeptide production are suitable for this purpose, like the TET-off/TET-on system, the Cre-recombinase based methods, the Ecdysone system, the GAL4 system, Rapamycin-based systems, or the above described temperature-sensitive expression system based on a Sindbis virus expression cassette.

[0065] Another aspect of the invention relates to transgenic animals which comprise a host cell of the invention. Particularly preferred are transgenic flies, like transgenic *Drosophila*, transgenic nematodes, like transgenic *C elegans*, transgenic fish, like transgenic zebra fish, and transgenic non-human mammals, like transgenic rodents (mice, rats).

[0066] Meanwhile, the generation of transgenic animals are within the general skill of a person skilled in the art. In addition, it is pointed out that under the control of tissue-specific promoters expression could be targeted to the CNS in mice and others. Most tissue-specific to promoters could be used, for example, also in the context of viral vectors. In the following, tissue-specific promoters of rodents are listed. Expression in astrocytes: GFAP-promoter, macrophage colony-stimulating factor (c-fms). Expression in neurons: synapsin promoter, thy-1 promoter, neuron-specific rat enolase promotor (NSE), L7 promoter (Purkinje cells), dopamine beta-hydroxylase (DBH) promoter (predominantly in the peripheral nervous is system), brain dystrophin promoter, calmodulin gene II and III promoter (CaMII, CaMIII), human and murine neurofilament light gene promoter (NF-L), human hypoxanthine phosphoribosyltransferase (hHPRT) promoter, corticotropin-releasing hormone (CRH), T alpha 1 alpha-tubulin promoter, murine low-affinity NGF receptor promoter, hippocalcin gene promoter, olfactory marker protein (OMP) promoter (olfactory neurons), GABA(A) receptor alpha 6 subunit promoter, GABA(A) receptor delta subunit promoter, tyrosine hydroxylase (TH) promoter, mouse vesicular acetylcholine transporter (VAChT), mouse glutamate decarboxylase 65 and mouse glutamate decarboxylase 67 genes promoters, brain-specific promoter of the human FGF1, gonadotropin-releasing hormone (GnRH) promoter, N-methyl-D-aspartate receptor 2A subunit gene promoter; mouse metabotropic glutamate receptor subtype 6 (mGluR6) upstream sequence, Rod photoreceptor cGMP phosphodiesterase (PDE6), human blue opsin promoter and rhodopsin promoter (retina). Neuron-restrictive silencer elements: Neuron-restrictive silencer elements (NRSEs). Expression in oligodendrocytes: MBP (myelin basic protein), proteolipid protein (PLP) promoter.

[0067] Furthermore, the present invention is directed to a protein encoded by a nucleotide sequence of the present invention, in particular a protein containing the amino acid sequence of SEQ ID NO: 1 or 2 or at least one of the mutations depicted in SEQ ID NO: 1 or 2, such as the mutation R793M, Q930R, S1096C, L1114L, I1122V, S1228T, R1441C, Y1699C and/or I2020T.

[0068] It is particularly pointed out that the present invention encompasses also proteins which contain one or more amino acid substitutions in the KASPP/LRRK2 protein but still retains essentially its function. Such amino acid substitutions may be semi-conservative or conservative and more preferably a conservative amino acid residue exchange. In the following table such amino acid substitutions are exemplified.

Amino acid	Conservative substitution	Semi-conservative substitution
A	G; S; T	N; V; C
C	A; V; L	M; I; F; G
D	E; N; Q	A; S; T; K; R; H
E	D; Q; N	A; S; T; K; R; H
F	W; Y; L; M; H	I; V; A
G	A	S; N; T; D; E; N; Q
H	Y; F; K; R	L; M; A
I	V; L; M; A	F; Y; W; G
K	R; H	D; E; N; Q; S; T; A
L	M; I; V; A	F; Y; W; H; C
M	L; I; V; A	F; Y; W; C;
N	Q	D; E; S; T; A; G; K; R
P	V; I	L; A; M; W; Y; S; T; C; F
Q	N	D; E; A; S; T; L; M; K; R
R	K; H	N; Q; S; T; D; E; A
S	A; T; G; N	D; E; R; K
T	A; S; G; N; V	D; E; R; K; I
V	A; L; I	M; T; C; N
W	F; Y; H	L; M; I; V; C
Y	F; W; H	L; M; I; V; C

[0069] For example, changing from A, F, H, I, L, M, P, V, W or Y to C is semi-conservative if the new cysteine remains as a free thiol. Furthermore, the skilled person knows that glycines at sterically demanding positions should not be substituted and that P should not be introduced into parts of the protein which have an alpha-helical or a beta-sheet structure.

[0070] The present invention is also directed to the manufacturing of the proteins by methods already explained above. In short such method comprises

[0071] (a) culturing a cell of the present invention under suitable conditions; and

[0072] (b) isolating the protein produced by the cultured cell.

[0073] Another preferred embodiment of the present invention is directed to a method of detecting a mutation at position 2378, 2789, 3287, 3342, 3364, 3683, 4321, 5096 and/or 6059 in the nucleic acid molecule of SEQ ID NO: 1 or 2 in a sample, the method comprising:

[0074] (a) contacting said sample with the nucleic acid molecule according to the present invention, and

[0075] (b) detecting the presence of the mutation.

[0076] Preferably, the sample is selected from

[0077] (a) a sample, in particular a biopsy, from human tissue or cells, in particular from the brain, in particular putamen or substantia nigra; heart, lung and/or blood lymphocytes; Or

[0078] (b) RNA and/or DNA from a sample, in particular a biopsy, from human tissue or cells, in particular from the brain, in particular putamen or substantia nigra; heart, lung and/or blood lymphocytes;

[0079] In general, the detection can be carried out by Southern blot hybridization, Northern blot hybridization, PCR or RT-PCR including real-time RT-PCR, techniques which are well known to a person skilled in the art. The mutation can also be detected by radiography, fluorescence, chemiluminescence, or any combination thereof. Preferably automated sequencing can be carried out, an electrophoresis method run basically in a capillary (column) combined with fluorescence.

[0080] Other methods for detection and/or quantification of the amount of polynucleotides, i.e. for the methods according to the invention allowing e.g. the determination of the level of expression of a polynucleotide containing a mutation, are real time methods known in the art as the TaqMan® method disclosed in WO92/02638. This method exploits the exonuclease activity of a polymerase to generate a signal. In detail, the (at least one) target nucleic acid component is detected by a process comprising contacting the sample with an oligonucleotide containing a sequence complementary to a region of the target nucleic acid component and a labeled oligonucleotide containing a sequence complementary to a second region of the same target nucleic acid component sequence strand, but not including the nucleic acid sequence defined by the first oligonucleotide, to create a mixture of duplexes during hybridization conditions, wherein the duplexes comprise the target nucleic acid annealed to the first oligonucleotide and to the labeled oligonucleotide such that the 3'-end of the first oligonucleotide is adjacent to the 5'-end of the labeled oligonucleotide. Then this mixture is treated with a template-dependent nucleic acid polymerase having a 5' to 3' nuclease activity under conditions sufficient to permit the 5' to 3' nuclease activity of the polymerase to cleave the annealed, labeled oligonucleotide and release labeled fragments. The signal generated by the hydrolysis of the labeled oligonucleotide is detected and/ or measured. TaqMan® technology eliminates the need for a solid phase bound reaction complex to be formed and made detectable. Other methods include e.g. fluorescence resonance energy transfer (FRET) between two adjacently hybridized probes as used in the LightCycler® format described in U.S. Pat. No. 6,174,670.

[0081] A preferred protocol if the polynucleotide is in form of a transcribed nucleotide is a method where total RNA is isolated, cDNA and, subsequently, cRNA is synthesized and biotin is incorporated during the transcription reaction. The purified cRNA is applied to commercially available arrays which can be obtained e.g. from Affymetrix. The hybridized cRNA is then detected. The arrays are produced by photolithography or other methods known to experts skilled in the art.

[0082] Consequently, the method can be carried out on an array, e.g. in a robotics system or using microfluidics.

[0083] The present invention is also directed to a diagnostic kit containing at least one nucleic acid molecule of the present invention for diagnosing a neuronal disease, in particular a neurodegenerative disorder, especially Parkinson Disease (PD) including, without limitation, sporadic PD, Alzheimer Disease (AD), amyotrophic lateral sclerosis (ALS), synucleinopathy and/or tauopathy, in combination with suitable auxiliaries. Suitable auxiliaries, as used herein, include buffers, enzymes, labelling compounds, and the like. In a preferred embodiment, the nucleic acid molecule contained in the kit is a nucleic acid molecule which is capable of hybridizing to the mRNA corresponding to at least one nucleic acid molecule of the present invention. Preferably, the nucleic acid molecule is attached to a solid support, e.g. a polystyrene microtiter dish, nitrocellulose membrane, glass surface or to non-immobilized particles in solution. Alternatively, the diagnostic kit contains one or more means necessary for automated sequencing.

[0084] The present invention refers also to a pharmaceutical containing at least one nucleic acid molecule or a protein of the present invention which can be used for the prevention or treatment of a neurodegenerative disorder, especially Parkinson disease including, without limitation, sporadic PD, AD, amyotrophic lateral sclerosis (ALS), synucleinopathy and/or tauopathy. Therefore, the nucleic acid molecule or protein of the present invention can also be used for the preparation of a medicament for treating a neurodegenerative disorder as e.g. exemplified above.

[0085] Another embodiment of the present invention is directed to a method for screening a pharmaceutical or diagnostic agent, the method comprising:

[0086] (a) providing at least one nucleic acid molecule or a protein of the present invention,

[0087] (b) providing a test compound, and

[0088] (c) measuring or detecting the influence of the test compound on the expression activity of the nucleic acid molecule or the protein.

[0089] For example, the test compound is provided in the form of a chemical compound library. According to the present invention the term "chemical compound library" refers to a plurality of chemical compounds that have been assembled from any of multiple sources, including chemically synthesized molecules and natural products, or that have been generated by combinatorial chemistry techniques.

[0090] The influence of the test compound can be measured or detected in a heterogeneous or homogeneous assay. As used herein, a heterogeneous assay is an assay which includes one or more washing steps, whereas in a homogeneous assay such washing steps are not necessary. The reagents and compounds are only mixed and measured. Heterogeneous assays are, for example, ELISA, DELFIA, SPA and flashplate assays. Alternative homogeneous assays are, for example, TR-FRET, FP, ALPHA and gene assays.

[0091] The method can also be carried out on an array or using whole cells, e. g. in a robotics system or using microfluidics.

[0092] Methods for preparing such arrays using solid phase chemistry and photolabile protecting groups are disclosed, for example, in U.S. Pat. No. 5,744,305. These arrays can also be brought into contact with test compound or compound libraries and tested for interaction, for example binding or changing conformation.

[0093] Whole cells usually grow at the bottom of multiwell plates and are fixed and permeabilized, blocked and incubated with e.g. a primary (P)-specific antibody against the substrate of interest. Then, e.g. Europium labelled or HRP conjugated secondary antibodies in conjunction with specific chemiluminescent or colorimetric substances, e.g. as described above, are utilized to generate the signal. In combination with the use of a microscope not only the amount of (P)-specific antibodies can be quantified on the single cell level, but also phosphorylation-induced translocations of a substrate or morphological changes of the cells.

[0094] The method can also be carried out in form of a high-through put screening system. In such a system advantageously the screening method is automated and miniaturized, in particular it uses miniaturized wells and microfluidics controlled by a roboter.

[0095] An example for a pharmaceutical or diagnostic agent which can be found by the screening assay of the present invention is an antibody or antibody derivative specifically binding a protein of the present invention.

[0096] Therefore, the present invention is also directed to an antibody or antibody derivative which specifically binds a protein of the present invention.

[0097] The antibody is either polyclonal or monoclonal, preferably it is a monoclonal antibody. The term antibody derivative is understood as also meaning antigen-binding parts of the inventive antibody, prepared by genetic engineering and optionally modified antibodies, such as, for example, chimeric antibodies, humanized antibodies, multifunctional antibodies, bi- or oligospecific antibodies, single-stranded antibodies, F(ab) or $F(ab)_2$ fragments, which are all well known for a person skilled in the art.

[0098] The antibodies of the present invention can also be produced by immunization of a mammal with an immunogenic peptide and/or recombinantly using standard protocols. A particularly preferred immunogenic peptide is the peptide with the amino acid sequence "CRMGIKTSEG TPGFRAPEVA RGNVIYNQQA D" because it represents the kinase domain of KASPP/LRRK2 (amino acids Nos. 2025-2055) and shows a homology between mouse and human of 100%.

[0099] The antibodies and antibody derivatives of the present invention can be used e.g. for the diagnosis and/or prevention and/or treatment of a neuronal disease, in particular a neurodegenerative disorder, especially Parkinson disease (PD) including, without limitation, sporadic PD, Alzheimer disease (AD), amyotrophic lateral sclerosis (ALS), synucleinopathy and/or tauopathy, but also for the identification of other pharmacologically active substances. Particular uses of the antibodies and/or antibody derivatives of the present invention are e.g. in Western blots, immuno precipitation, immuno fluorescence or ELISA.

[0100] The invention will now be further illustrated below with the aid of the Figures, Tables, Sequence Listings and Examples, without being restricted hereto.

DESCRIPTION OF THE TABLES, THE SEQUENCES AND THE FIGURES

[0101] Table 1 shows the 29 genes and its sources which have been sequenced in the candidate region D12S1692-D12S85. "KASPP/LRRK2" is the abbreviation of the new gene of the present invention.

[0102] Table 2 shows primer sequences for haplotype analysis of the second study consisting of two flanking and three intragenic markers.

[0103] Table 3 shows the frequency of the mutations of the second study. Mutational screening was performed in 53 PD families additional to the 34 families of the first study, 337 patients with sporadic PD and 1200 matched controls.

[0104] Table 4 shows the clinical and neuroimaging features of affected members of the families of the second study. Not all subjects could be investigated with the same methods, which is indicated with nd (not done). No change in comparison with normal is indicated with na (no alteration). y year, ~ongoing at the time of examination, B bradykinesia, R rigidity, RT resting tremor. For brief evaluation of olfaction a sniffing test consisting of 8 different odours (/8) was used.

[0105] Table 5 shows the neuropsychological assessment of the second study. Tests applied for intelligence (LPS-K), executive function (Tower of London), interference (CWIT), dementia CERAD 1-8; concentration (D2) as well as mood (BDI) and quality of life (PDQ-39. ↓ performance below, ~average and I above average of matched healthy controls.

[0106] SEQ ID NO: 1 shows the nucleotide sequence and the amino acid sequence of a KASPP/LRRK2 including the sites of the particular mutations found in the specified families (bold face).

[0107] SEQ ID NO: 2 shows the nucleotide sequence and the amino acid sequence of human KASSP/LRRK2 including the sites of the particular mutations found in the specified families (bold face).

[0108] SEQ ID NO: 3 shows the amino acid sequence of the peptide used for the production of monoclonal antibodies against KASSP/LRRK2.

[0109] SEQ ID NO: 4 shows the relevant section of the amino acid sequence of the S212L polymorphism of human KASSP/LRRK2. The variation is shown in bold face.

[0110] SEQ ID NO: 5 shows the relevant section of the nucleic acid sequence of the c634t polymorphism of human KASSP/LRRK2. The variation is shown in bold face.

[0111] SEQ ID NO: 6 shows the relevant section of the amino acid sequence of the M2397T polymorphism of human KASSP/LRRK2. The variation is shown in bold face.

[0112] SEQ ID NO: 7 shows the relevant section of the amino acid sequence of the t7190c polymorphism of human KASSP/LRRK2. The variation is shown in bold face.

[0113] FIG. **1** shows the pedigree structure of the two largest families: A (German-Canadian), D (Western Nebraska) and with mutations. Blackened symbols denote affected family members; asterisks (*): individuals typed for the mutation, m: mutation carrier and wt: wildtype. To protect the confidentiality of these results, the genotypes of some unaffected individuals of families A and D are not shown.

[0114] FIG. **2** shows pedigree structure of smaller families with mutations. Blackened symbols denote affected family members; asterisks (*): individuals typed for the mutation, m: mutation carrier and wt: wildtype. To protect the confidentiality of these results, the genotypes of some unaffected individuals of family 469 are not shown.

[0115] FIG. **3** shows the three across species highly conserved amino acid substitutions R1441C, Y1699C and I1122T.

[0116] FIG. **4** shows the nucleotide and amino acid sequence of KASPP/LRRK2 as well as the location of the exons 1-51. The vertical lines mark the last and the first nucleotides of the exon, respectively.

[0117] FIG. **5** shows a: exon positions; b: schematic drawing of KASPP/LRRK2 with domains, primer positions for cDNA amplifikation and positions of mutations; and c: Protein alignment of three mutations conserved among hs: *Homo sapiens,* mm: *Mus musculus,* rn: *Rattus norvegicus,* ce: *C. elegans* and dm: *Drosophila melanogaster.*

[0118] FIG. **6** *a-f* show pedigree structures of families with KASPP/LRRK2 mutation. Except of family DE038 which is shown to demonstrate cosegregation in the first family investigated and DE032 (FIG. **6***e*), which is displayed to demonstrate the same haplotypes in the two families affected by the I2020T mutation, all pedigrees display novel families. Blackend symbols denote family members with the clinical presentation of PD; "+" denotes a genotyped individual, with "M" for mutation carriers and "wt" for wild-type KASPP/LRRK2. The dotted symbols in FIG. **6***f* denote family members with the clinical presentation of tremor. To protect confidentiality the genotype of some unaffected family members are not shown. Moreover, the gender of individuals in the youngest generation of family E is disguised.

[0119] FIG. **7** shows a: exon positions; and b: schematic drawing of KASPP/LRRK2 with domains and positions of mutations.

[0120] FIG. **8** shows the autophosphorylation of wild type and mutated KASPP/LRRK2. In the upper panel an autoradiogram of an SDS-PAGE blotted onto PVDF membranes is shown. γ-^{32}P-ATP incorporation (1 h) into KASPP/LRRK2 wildtype and I2020T mutant is visualized using a phosphoimaging system. A loading control by immunoblotting with anti FLAG M2 is shown in the lower panel. All samples shown are form the same experiment and were separated on the same gel.

[0121] FIGS. **9** (A) shows a scheme of the KASPP/LRRK2-domain structure and the used Tag-fusion constructs of LRRK2. (B) shows SDS gel separation of associated proteins co-isolated by tandem affinity purification of KASPP/LRRK2-kinase domain (lane 1) and a vector control (lane 2). The proteins were visualized by colloidal coomassie staining. (C) shows coimmuno-precipitation. Two FLAG tagged LRRK2-baits (full-length and kinase domain) were tested for interaction with HA tagged full length KASPP/LRRK2. The co-precipitated HA-tagged KASPP/LRRK2 was visualized by immuno-blotting (3F10 anti HA, upper panel). A loading control for the bait-constructs is shown in the lower panel (immuno-blot: anti Flag M2). FIG. **15** (B) is the same figure as FIG. **9** (C) in a better shape.

[0122] FIG. **10** shows the immunofluorescence of different cell structures.

[0123] FIG. **11** (A) shows cell fractionation of HEK293 cells over expressing KASPP/LRRK2-Flag. The different pellet fractions 700×g pellet (cell pellet), the 10.000×g pellet (10K pellet), 160.000×g pellet (160K pellet) and the cytosolic fraction (160K supernatant) are shown. The localization of LRRK2-Flag is shown in the first lane. The localization of specific markers for the cytoskeleton (beta-tubulin), mitochondrial membranes (Tom20) and the endoplasmic reticulum membrane (Sec61-alpha) are shown below. (B) shows carbonate extraction of the 160K pellet fraction. The starting material is shown in column 1, the pellet fraction in column 2 and the supernatant fraction in column 3. LRRK2-flag is shown in the first lane. For quality control of the extraction, immuno-blots for several ER-marker proteins have been provided: a luminal ER marker (BiP/GRP78, a 78 kD glucose regulated protein), a peripheral cytosolic ER-associated marker (p97, VCP) and an integral ER membrane marker (Sec61-alpha).

[0124] FIG. **12** (A) shows a further overview of KASPP/LRRK2-domain structure and constructs. The kinase domain of human KASPP/LRRK2 (1), the full-length LRRK2 (2) and a disease associated LRRK2 mutant 12020T (3) were cloned in frame into a modified pcDNA3.0, containing a C-terminal affinity tag. The I2020T mutation is localised, as marked, in the kinase domain of KASPP/LRRK2. Additionally, wildtype KASPP/LRRK2 was C-terminally tagged with a Hemagglutinin (HA) epitope (4) and a GFP (green fluorescent protein)-tag (5). (B) shows LRRK2-tag and LRRK2 I2020T-tag constructs expressing an approximately 280 kD protein in HEK293 cells, visualised by Western-blotting after SDS-PAGE.

[0125] FIG. **13** shows KASPP/LRRK2 appearing in the particulate fractions upon subcellular fractionation and is associated with membranes. (A) shows KASPP/LRRK2 cosediments with membranes. HEK293 cells were fractionated into a cell pellet (700×g) an organelle pellet (10K pellet), a soluble cytosolic fraction (cytosol) and a microsomal fraction (160 k pellet). The fractions were analysed by SDS-PAGE and Western blotting with antibodies against the Flag-tag, TOM20 (mitochondria), PMP70, (peroxisomes), BiP/GRP78, (ER lumen), Sec61α (ER membrane), p50^{cdc37} (cytosol) and β-tubulin (microtubules). (B) shows an alkaline

extraction of KASPP/LRRK2. The 160K pellet was treated with 100 mM sodium carbonate. Membrane and soluble fraction (pellet and supernatant, respectively) were separated by centrifugation and analysed as in (A) using antibodies against the integral ER membrane protein Sec61α, the cytosolic ER-associated protein p97/VCP and the luminal ER protein BiP/GRP78.

[0126] FIG. **14** shows that KASPP/LRRK2-GFP localises to mitochondria, endoplasmic KASPP/LRRK2-GFP (GFP fluorescence shown in the middle panel) were immunostained for a) mitochondria (TOM20), b) endoplasmic reticulum (PDI), c) Golgi (58K Golgi) d) peroxisomes (PMP70), e) intermediate filaments (vimentin), f) microtubular cytoskeleton (β-tubulin) and g) Phalloidin-Tritc (actin cytoskeleton). The right panel depicts digitally merged images taken from the same micrograph section and merges green fluorescence (GFP), red alexa 568 staining (specific markers) and nuclear staining with DAPI.

[0127] FIG. **15** shows that KASPP/LRRK2 dimerises and interacts with HSP90 and $p50^{cdc37}$. (A) shows co-purification of differently tagged KASPP/LRRK2-constructs: HA-tagged full-length KASPP/LRRK2 was tested for its ability to interact with two different tagged KASPP/LRRK2 baits (a full-length and a kinase domain only construct). The constructs were co-expressed transiently in HEK293 cells prior to cell-lysis and purification. The result of the co-purification of HA-tagged KASPP/LRRK2 with the Step/Flag-tagged baits is shown in the upper left panel (pellet). The co-precipitated HA-tagged KASPP/LRRK2 was visualised by Western blotting (3F10 anti-HA). (B) is the same figure as as FIG. **9** (C) in a better shape. Controls: In order to demonstrate equal expression of KASPP/LRRK2-HA a Western blot (anti-HA) of the supernatants is shown (upper right panel). An equal loading of purified bait-proteins was ensured by Western blotting (anti-tag, lower left panel). Purification efficiency of Strep/Flag-tagged baits was determined by Western blotting of the depleted supernatants: after their affinity binding to the beads, no detectable bait protein remains in the supernatants (lower right panel).

[0128] FIG. **16** shows western blots of HEK293 cells with different hybridoma supernatants. The first western blot shows the result of a lysate of HEK293 cells which overexpress KASPP/LRRK2 (named "LRRK2"). The other western blot shows the result of a lysate of HEK293 cells transfected with an empty vector (named "vector"). Clones:

1) 4E1	2) 4A11	3) 3G3	4) 7F1	5) 2H8
6) 2D7	7) 4A8	8) 2B5	9) 4G11	10) 3G6
11) 4G11	12) 3D9	13) 4H11	14) 4F12	15) 4B7.

EXAMPLES OF THE FIRST STUDY

Example 1

Genetic Analysis

DNA Extraction

[0129] Genomic DNA from peripheral blood lymphocytes was extracted using standard protocols and after obtaining informant's consent from all participating family members.

Sequence analysis

[0130] Genomic sequences and annotations were obtained from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) and University of California Santa Cruz (UCSC) (http://genome.ucsc.edu/). Primers for mutation screening were designed using Primer3 software integrated into script to allow for automated primer design (http://ing.gsf.de/ing/ExonPrimer.html). Exon sequences and exon-intron boundaries were amplified with intronic primers and sequenced them directly by BigDye Terminator Cycle sequencing kit (Applied Biosystems). Between Markers D12S1692 and D12S85 a total of 29 genes or RNAs were sequenced (see Table 1).

Haplotype analysis

[0131] Haplotypes were constructed by hand using the repeat markers previously used for linkage analysis (Zimprich, A. et al., 2004, supra). Intragenic markers for haplotype analysis for fam 469 and fam D were established by searching the whole gene for repeat polymorphisms by use of a "tandem repeat finding program" (http://c3.biomath.mssm.edu/trf.html). Polymorphic repeats were found in intron 5 (caa), intron 20 (atct) and intron 29 (ac).

Linkage analysis

[0132] Twopoint LOD scores were calculated using the MLINK program (V 5.10) in its FASTLINK implementation (V4.1P). Phenocopy rate was set at 0.01, penetrance for the heterozygous state and homozygous mutation carriers at 0.90. The allele frequency of the disease causing allele was set at 0.001, as was the frequency of the mutation used as the marker.

Screening of Mutations and Polymorphisms

[0133] For mutations 2000, for polymorphisms at least 1200 control chromosomes from a mixed European descent were screened as controls. In addition 300 patients were screened with sporadic parkinson's disease. Genotyping was performed on a MALDI-TOF mass-spectrometer (Sequenom MassArray system) using the homogeneous mass-extension (hME) process for producing primer extension products (Tang K. et al., Proc. Natl. Acad. Sci USA, 96, 10016-10020, 1999).

Amplifikation of KASPP/LRRK2

[0134] The complete coding sequence of KASPP/LRRK2 was amplified from human brain cDNA by using Marathon-Ready cDNA (BD Biosciences Clontech). Primers were set to amplify three overlapping fragments from exon1-21 (P1f, P1r), from exon 20-35 (p2f, p2r) and exon 34-51 (p3f, p3r) (see FIG. **5**). Sequence information were derived from published the mRNA of DKFZp434H211. PCR products were run on agarose gel to check its length and integrity.

Example 2

Northern blot Analysis

[0135] Northern blot analysis was performed according to the manufacturers protocols (BD Biosciences). For hybridization a KASPP/LRRK2 cDNA fragment was used (bp 6577-7655; corresponding to exon 45-3'UTR.

Example 3

LightCycler Experiments

[0136] mRNAs from different human tissues were purchased from BD Biosciences, (Clontech BD Sciences, Palo Alto, USA) and were reverse transcribed with the Transciptor First Strand cDNA Synthesis Kit (Roche Applied Sciences, Mannheim, Germany) according to the manufacturers protocol. For real-time amplification of KASPP/LRRK2 three spe-

cific PCR products spanning exon1 to 8, exon13 to 19 and exon 31 to 39 were quantified using the

[0137] LightCycler Instrument (Roche Applied Sciences, Mannheim, Germany). Fluorescence-labeled hybridization probes providing maximum specificity were used for product detection. Calculation of sample concentrations were performed using the fit-point algorithm. The Phorphobilinogen deaminase (h-PBGD) gene, a low-copy housekeeping to gene, was used as an external standard and absolutely quantified using the (h-PBGD Housekeeping Gene Set, Roche-Applied Science). Relative transcript levels were calculated as ratios of Park8/PBGD normalized to adult whole brain adult expression as 100%

Examples of the Second Study

Subjects and Methods

Subjects

[0138] DNA of 51 index patients from PD families compatible with an autosomal dominant mode of inheritance of PD or with a mode of inheritance that could not be assigned to a typical Mendelian trait, as well as two affected sib pairs were analyzed for mutations in the KASPP/LRRK2 gene. Clinical diagnosis was based on published criteria (Hughes et al., J Neurol Neurosurg Psychiatry; 55:181-4, 1992) and severity of the disease was rated according to the Unified Parkinson's Disease Rating Scale (UPDRS) (Fahn et al., Recent Developments in Parkinson's Disease. New York: Macmillan,153-163, 1987) and Hoehn and Yahr staging. In one family (family E) typical Parkinsonian features were only found in one member (III-11), while all other affected family members presented primarily with postural tremor. Moreover, all novel and known mutations were investigated in a cohort of 337 patients with apparently sporadic PD (204 male, 133 female, mean age 53±13 years) and a cohort of 1200 subjects without any extrapyramidal disorders matched for age±5 years and sex. Allele frequency of the polymorphism N551K; 1653C>G was investigated in 888 of these control subjects.

[0139] DNA of patients with familial and sporadic PD was obtained from our gene bank, while DNA of control subjects comprised the Kora cohort obtained form the National Research Center of Environment and Health/Munich, Germany. All patients and controls had given informed consent to mutational screenings, which was approved by the local ethical committee.

Mutational Screening

[0140] Genomic DNA was isolated from peripheral blood using standard protocols. Mutational screening in patients of families with autosomal dominant PD was performed for all exons and exon-intron boundaries of the KASPP/LRRK2 gene by direct sequencing of both strands using the BigDye Terminator Cycle sequencing kit (Applied Biosystems) with the same primers and under the same condition as described above.

[0141] Mutational screening in patients with sporadic PD and control subjects was performed using an ABI 7900 Allelic Detection system. As described above genotyping was performed on a MALDI-TOF mass-spectrometer (Sequenom Mass Array system) using the homogeneous mass-extension (hME) process for producing primer extension products.

[0142] In families with identical mutations haplotype analysis of the KASPP/LRRK2 region was performed. Haplotypes were constructed using 5 fluorescent-labeled microsatellite markers, two flanking and three intragenic (Table 2). DNA fragments containing the polymorphic marker sequences were amplified by PCR. Fluorescently labelled PCR products were analyzed on an ABI 3100 automated sequencer with a fluorescence detection system.

DNA extraction from Brain Tissue

[0143] In the large family with only one patient with the clinical picture of PD and many others affected by symptoms resembling essential tremor (family E), blood for DNA extraction was only available of the PD patient. To disclose a possible association of a KASPP/LRRK2 mutation and clinical features of essential tremor DNA was extracted from a microscope slide with paraffin-embedded brain tissue (cerebellum) of one family member with this phenotype (III-7).

[0144] Deparaffinisation was performed using xylene and ethanol followed by a proteinase K digestion. The probe was then purified using phenol/chloroform extraction and finally precipitated with LiCl and Ethanol.

Clinical Investigations

[0145] The index patients of families with mutations in the KASPP/LRRK2 gene were invited for a genetic consultation and clinical and neuroimaging investigations under an approved protocol. After informed consent was given a thorough neurological examination was performed and olfactory function was tested using sniffing sticks (Daum et al., Nervenarzt, 71:643-50, 2000). A neuropsychological test battery sensitive for dementia, concentration, planning, as well as intelligence was chosen (Table 5). To evaluate mood and sensitivity patients were asked to complete the Becks Depressions Inventar (BDI) and the PDQ-39 Parkinson's Disease Quality of Life Questionaire.

[0146] Electrophysiological investigations comprised neurography of the right tibial and sural nerve, and elektromyography of the quadriceps to discern subclinical changes in motor unit potentials and possible abnormal spontaneous activity. Moreover, magnet evoked potentials were performed in all patients without contraindications.

Neuroimaging

[0147] Structural neuroimaging comprised transcranial ultrasound (TCS) and magnet resonance imaging (MRI).

[0148] For TCS a phased-array ultrasound system equipped with a 2.5-MHz transducer with an axial resolution of approximately 0.7 mm and a lateral resolution of about 3 mm (Elegra, Siemens, Erlangen, Germany) was used. The examination was performed through a preauricular acoustic bone window with a penetration depth of 16 cm and a dynamic range of 45 dB as described previously (Berg et al., Ultrasound Med Biol, 25: 901-904, 1999). The SN was identified within the butterfly-shaped structure of the mesencephalic brainstem as clearly as possible, scanning from both temporal bone windows, then the area of hyperechogenic signals in the SN-region was encircled and measured (Berg et al., 1999, supra, Berg et al., J Neurol, 248:684-689, 2001). An area of SN hyperechogenicity $\leqq$0.19 cm^2 was classified as normal, an area >0.19 and $\leqq$0.24 cm^2 as moderately and an are of >0.24 cm^2 as markedly hyperechogenic (Berg et al., 1999, supra).

[0149] MRI was performed on a Magnetom Avanto 1.5 Tesla, Siemens AG, Germany.

Results

Mutational Screening

[0150] Screening the entire coding region of the KASPP/LRRK2 gene of one index patient each from 55 families identified 7 novel families with amino acid substitutions

(FIG. **6***a-f*). Four of these are novel missense mutations: R793M; 2378G>T in family DE041 and to family T11239, Q930R; 2789A>G in family DE022; S1096C; 3287C>G in family E and S1228T; 3683G>C in family DE031. The missense mutation R793M was also found in one patient with sporadic PD and one control person.

[0151] The so far most common amino acid substitution G2019S; 6055G>A was only found in one sporadic PD patient, who showed typical levodopa responsive Parkinson's disease with an age of onset of XX and no additional clinical features. Moreover one additional patient was detected with the already above described splice site mutation 3342A>G (family T11288) and one more family with the above described 12020T mutation (family T10738).

[0152] Except for the R793M mutation, which was found in one control person none of the mutations were found in the control group (Table 3). There was no significant difference in the minor allele frequency of the known N551K; 1653C>G polymorphism between patients with sporadic PD (6.5%) and control subjects (7.3%).

Haplotype Analysis

[0153] Haplotype analysis revealed common haplotypes for the two novel families affected by the R793M mutation as well as for family T10758 and family DE032 affected by the I2020T mutation, indicating common founders for these mutations (FIGS. **6***a* and **6***e*). Although members of family DE032 and T10758 were not aware of common ancestors, they originate from the same geographical area (Baden Wurttemberg, Southern Germany).

[0154] Family T11239 and DE041 were recruited from more distinct geographical areas (Baden Württemberg family T11239 and Hesse family DE041). Members of these families were also not aware of common ancestors. For the A3342G splice site mutation no common haploptype was found in the affected families (DE038 and T11288).

Clinical Findings

[0155] Extensive clinical a neuroimaging examination revealed the features listed in Table 4.

[0156] All patients investigated had typical signs of Parkinson's disease. However, features differed between members within the same family affected by the same mutation as well as between different families with the same mutation. Moreover, penetrance was found to vary for different mutations.

Common Findings in Patients with KASPP/LRRK2 Mutations

[0157] All mutation carriers with clinically apparent PD had the typical Parkinsonian features including bradykinesia, tremor and rigidity. Moreover, all patients experienced substantial relief of symptoms after application of L-dopa, although therapy was complicated in one patient (T11288 II-5) by hallucinations. Estimation of olfactory function by application of 8 sniffing sticks revealed a moderate to severe loss of identification capacity in three of 5 subjects. Postural instability was only found late in the disease course. Hallucinations were reported seldom and only occurred after long disease duration or associated with dementia, whereas sleep disturbances were reported by 80% (Table 4).

Intrafamily Differences in Clinical Presentation

[0158] R793M: Two sisters are affected with a difference of age of onset of 15 years. While at disease onset II-1 had only slight postural tremor on the right side, the initial symptom of II-2 was resting tremor on the left side. An equivalent type of PD developed in II-2 while II-1 showed no resting tremor at all but an akinetic-rigid type of PD (FIG. **6***a*).

[0159] Q930R: Span of age of onset was 21 years among the three members of the same generation affected. Only brother III-7 developed severe dementia and hallucinations after more than 20 years of disease duration (FIG. **6***b*).

[0160] 3342A>G: While sister II-7 of family T11288 presented with typical Parkinsonian features, the clinical picture of early severe dementia, hypersensitivity to dopaminergic halluzinations and daytime sleepiness with fluctuation of vigilance resembled DLBD in II-5. However, mutational analysis revealed the wt allele in II-7. A phenocopy for the more typical PD presentation must therefore be postulated, while the atypical DLBD-type was indeed associated with the 3342A>G splice site mutation. However, the fact that this variation co-segregated with the mutation in the above described family DE038 is an indication for a mutation rather than a benign polymorphism.

Interfamily Differences in Clinical Presentation for the Same Mutation

[0161] R793M: While in III-3 of family T11239 speaking was impossible because of severe tongue dyskinesia, the affected sisters of family 41 did not show any atypical signs except of postural tremor in II-1 (FIG. **6***a*).

[0162] 3342A>G: In family T11288 both sisters and in the reported family DE038 father and III-1 were severely affected by the disease. III-3, however, did not show any Parkinsonian symptoms except of minimal resting tremor of the right thumb for more than 15 years (FIG. **6***c*).

Age of Onset

[0163] Mean age of onset in the novel families was 58±14 years. However, age of onset differed between members of the same family. In offsprings of mutation carriers of the three novel families, in whom clear data of ancestors was available (Table 4) the diagnosis if PD was established earlier and also investigation of an additional family member in family DE032 revealed and earlier diagnosis (41 years), while mean age of onset was 54 (48-59 years) in generation I-III (FIG. **6***e*).

Penetrance

[0164] Combining findings of the second study and the first study a clear autosomal dominant mode of inheritance was found in at least one affected family for the splice site mutation of exon 24, and for the missense mutations of exon 25, exon 27, exon 31, and exon 41. No strong genetic pattern was found in families affected by missense mutations in exon 19, 21 and 24.

[0165] Exon 19; R793M: In family T11239 only the uncle of the index patient was affected, while the father who died at the age of 68 did no show any extrapyramidal sign during life time. In family DE041 two sisters showed typical signs of PD during life time, while none of the parents who died both at the age of 74 showed any Parkinsonian signs (FIG. **6***a*).

[0166] Exon 21, Q930R: Of nine sisters and brothers in family DE022 three were affected by the mutation and had clinical signs of PD, while one other sister and brother, also mutation carrier are not affected by PD at an age of more than 70 years. Neither the mother (II-3), who died at the age of 90 years nor her brother (II-2), who died at 75 years of age showed any Parkinsonian features during life time. The cousin of the affected members of the to family (III-4) displayed signs for typical PD but was not carrier for the Q930R mutation, indicating sporadic PD in this family member.

However, her sister (III-3), was found to have the mutation. Having already reached the age of 77, she has no clinical signs allowing the diagnosis of PD. Both II-3 and II-2 must have been mutation carriers. The fact that none of them and also III-3 have not shown any Parkinsonian features during life time argues for incomplete penetrance of this mutation.

[0167] Exon 24, S1096C: In this large family with an additional tremor phenotype (1f) only III-12 was mutation carrier, affected by PD. One child of his brother, who showed only features of essential tremor but no Parkinsonian symptoms until death at the age of 66 years, is also mutation carrier, indicating incomplete penetrance for this mutation as well.

Phenocopies and Simultaneous Occurrence of Tremor

[0168] One family member with typical PD of DE022, associated with the Q930R mutation and one of the sisters of family T11288, (A3342G splice site mutation of the other sister), again with typical Parkinsonian features had wt alleles, arguing for idiopathic PD in these cases.

[0169] In family E an autosomal dominant inheritance of tremor is evident (FIG. **6***f*). Two family members (III-7 and III-11) showed typical Parkinsonian features during life time, in III-11 the S1096C mutation was detected. As there was no blood available of III-7, DNA extracted from brain tissue was investigated. However, in this patient the C3287G mutation could not be detected, indicating a different cause for Parkinson's disease. Of one of the siblings with a tremor phenotype (III-19, presenting with postural and vocal tremor) also only wild type alleles could be identified. The only family member with a tremor phenotype carrying the S1096C mutation must have been the brother of III-12, as one of his children is also mutation carrier. However, as the mutation could not be detected in III-19 incomplete penetrance of Parkinsonian symptomes in a subject also affected by tremor is more likely than an association of tremor with the mutation in this family.

Neuropsychological Findings

[0170] Of the 5 patients examined in a thorough neuropsychological investigation, three were able to complete the whole test battery. In all three intelligence was above average of a matched control group (LPS-K), suggesting that subtle neuropsychological deficits may well be compensated. Still, all three showed deficits in executive functions (Tower of London) and had high interference scores (CWIT) indicating incapacity to blind overstimulation (Table 5). This pattern is in accordance with neuropsychological deficits in idiopathic PD. The two others investigated were graded as demented. In patient III-3 of family T11239 MMSE was 22. Additionally, severe tongue dystonia prevented accomplishing the CERAD. In patient II-5 of family T11288 with 3342A>G splice site mutation exhaustability and dementia thwarted completing of neuropsychological testing.

TCS and MRI Findings

[0171] TCS: Moderate hyperechogenicity, at least on one side, was found in all but one patient with LRRK2 mutations. Interestingly, none of the patients displayed marked SN hyperechogenicity. MRI showed mild to marked atrophy in the 4 patients investigated (Table 4). The patient with the DLBD phenotype had additionally some evidence for microangiopathy.

Biochemical Characterization of KASPP/LRRK2

Material and Methods

Plasmid and Cloning

[0172] Human KASPP/LRRK2 was cloned via PCR from cDNA which has been generated from lymphoblast mRNA. KASPP/LRRK2 was cloned domain-wise in six fragments. Each fragment was cloned into pcDNA3.0 (Invitrogen) and verified by sequencing. The full length sequence was generated by subsequent fusion of the sub-constructs. The HA and FLAG tag were introduced at the 3' end (c-terminus) of the constructs. The I2020T mutation was introduced into KASPP/LRRK2 by site-directed mutagenesis using the QuikChange® II mutagenesis kit (Stratagene). For fluorescence microscopy, humanised GFP cDNA, derived from pFRED143 (Ludwig, E. et al., J. Virol., 73, 8279-8289, 1999)), was cloned in frame at the 3' end (c-terminus) of KASPP/LRRK2.

Cell Culture

[0173] HEK293 cells were cultured in DMEM supplemented with 10% FBS at 37° C. and 5% CO_2. For immuno precipitation (IP), tandem affinity purification or cell fractionation experiments cells were transfected with Effectene® (Qiagen) and kept under full medium for additional 48 h.

Electrophoresis and Immunoblotting

[0174] For immunoblotting analyses protein samples were separated by SDS-PAGE and transferred onto Hybond-P PVDF membranes (GE Healthcare). After blocking non-specific binding sites with 5% dry milk in TBST (1 h, RT) (25 mM Tris pH 7.4, 150 mM NaCl, 0.1% Tween-20) membranes were incubated overnight at 4° C. with primary antibodies in blocking buffer (mouse anti-Bip/GRP78 (BD), 1:1000; mouse anti-$p50^{cdc37}$ (BD), 1:1000; rat anti-HA 5F10, 1.3 μg/ml; mouse anti-p97/VCP (Progen), 1:1000; rabbit anti-PMP70 (Prof. Dr. A. Völkl, University of Heidelberg, Germany), 1:1000; rabbit anti-Sec61α (Acris), 1:1000; mouse anti-TOM20 (BD), 1:1000; mouse anti β-tubulin (Sigma), 1:2000), washed with TBST and incubated for 1 h with horseradish peroxidase (HRP)-coupled secondary antibodies. Membranes were washed and antibody-antigen complexes were visualized using the ECL+chemiluminescence detection system (GE Healthcare) on Hyperfilms (GE Healthcare). For the HA epitope the monoclonal anti HA 5F10 (Roche) was used in a concentration of 1.3 μg/ml (5% dry milk). For the FLAG epitope, the HRP-coupled monoclonal anti-FLAG M2 antibody (Sigma) was used in a dilution of 1:1000 (5% dry milk) Further antibodies are used with the following dilutions: mouse anti β-Tubulin (Sigma) 1:2000; rabbit anti Sec61-α (Acris) 1:1000; mouse anti TOM20 (BD) 1:1000; mouse anti Bip/GRP78 (BD) 1:1000; mouse anti p97/VCP (ProGen) 1:1000).

[0175] Cell Fractionation

[0176] Cells were harvested via trypsinisation, washed once with cold PBS, resuspended in cold homogenisation buffer (20 mM HEPES pH 7.4, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 250 mM sucrose, protease inhibitor cocktail (Roche)) and homogenized. Homogenates were centrifuged at 700×g for 10 min to pellet nuclei, debris, and non-disrupted cells (cell pellet). The supernatant was centrifuged at 10,000×g for 20 min to obtain

the 10K pellet. Cytosol and 160K pellet were prepared by ultracentrifugation of the 10K supernatant (160,000×g for 1 h).

Carbonate Extraction

[0177] 160K fractions were diluted with 100 mM (final concentration) sodium carbonate pH 11.5 and for 30 min on ice. The suspensions were centrifuged for 1 h at 160,000×g at 4° C. The supernatants were recovered and proteins precipitated with 10% trichloracetic acid. Membrane pellets and precipitated proteins were subjected to SDS-PAGE and Western blotting analyses.

Immuno Precipitation (IP)

[0178] For interaction assays FLAG-tagged KASPP/LRRK2 was lysed for 1 h in lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.5% Nonidet-P40, protease inhibitors (Roche), 1 mM orthovanadate) for 1 h at 4° C. After sedimentation of nuclei (10 min, 10.000 g, 4° C.), the supernatant was incubated with anti FLAG M2 agarose beads (Sigma) for 2 h at 4° C. After incubation, beads were washed 4× in lysis buffer and eluted with SDS-gel sample buffer.

Tandem Affinity Purification

[0179] The tandem affinity purification was done with a c-terminal tandem affinity purification tag consisting of a tandem StrepII tag and a Flag epitope (Strep/Flag-tag). HEK293 cells transiently expressing the Strep/Flag-tagged constructs were lysed in 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.5% Nonidet-P40, protease inhibitors and 1 mM orthovanadate for 1 h at 4° C. Following sedimentation of nuclei, the cleared supernatant was incubated for 2 h at 4° C. with streptactin superflow (IBA). Prior to washing, the lysates with suspended resin were transferred to microspin columns (GE Healthcare). Washing (1× with lysis buffer and 2× with TBS) was done in the microspin columns. Washing solution was removed from the columns by centrifugation (10 s, 2,000×g) after each washing step. Protein baits were eluted with desthiobiotin (2 mM in TBS). The eluates were used for LRRK2 co-precipitation experiments of LRRK2-Strep/Flag constructs vs. LRRK2-HA.

[0180] For MS analysis, a second purification step was added. For this step, the eluates were transferred to anti-Flag M2 agarose (Sigma) and incubated for 2 h at 4° C. The beads were washed 3× with TBS in microspin columns. Proteins were eluted with Flag peptide (Sigma) in PBS at 200 μg/ml peptide. After purification samples were separated by SDS-PAGE and stained with colloidal coomassie according to standard protocols prior to MS identification (Neuhoff, V. et al., Electrophoresis, 9, 255-262, 1988).

Mass Spectrometry

[0181] The proteins were identified by MALDI-MS and MSMS on an AB3700 (Applied Biosystems) instrument. Tryptic in gel proteolysis was done after standard protocols (Shevchenko, A. et al., Anal. Chem. 68, 850-858, 1996). Peptides were spotted on steal targets with the dried droplet method using alpha-cyano-4-hydroxycinnamic acid (Sigma) as matrix (Shevchenko, A. et al., supra). Obtained MS and MS/MS spectra were analysed by GPS explorer software suite (Applied Biosystems).

Kinase Activity Assays (Autophosphorylation Assay)

[0182] For kinase assays (autophoshorylation assays), Strep/Flag-tagged full-length wild-type LRRK2 or LRRK2-I2020T variant were transiently expressed in HEK293 cells (4x14 cm culture dishes per construct, 2×14 cm dishes for the vector control). After cell lysis and removal of the nuclei, the purification of LRRK2 variants was done by immunoprecipitation with anti-Flag M2 agarose. The resin was washed 3× in lysis buffer. The tagged proteins were not eluted, since the kinase assays were directly performed on the resin. Each sample was divided in 4 aliquots and stored in TBS+10% glycerol at −80° C. until use.

[0183] For the kinase assay, one aliquot of each condition (wild-type LRRK2 and LRRK2-I2020T) was divided into three sub-aliquots (1/2, 1/3, 1/6). Each sub-aliquot, as well as one aliquot of the vector control was incubated with 50 μM ATP, 0.3 μCi [γ-^{32}P] ATP in 30 μl assay buffer (25 mM Tris-HCl pH 7.5, 5 mM β-glycerophosphate, 2 mM DTT, 0.1 mM orthovanadate; 10 mM $MgCl_2$; Cell Signaling) for 1 h at 30° C. Reaction was stopped with Laemmli buffer. Protein samples were resolved by SDS-PAGE and transferred onto Hybond-P PVDF membranes (GE Healthcare). Imaging was done on a phosphoimager system (BioRad). Equal loading was ensured by Western blotting analyses (anti-Flag M2).

Immunofluorescence

[0184] HEK293 cells were grown on glass coverslips prior to transfection with GFP-tagged wild-type LRRK2. To avoid cell detachment, coverslips were pre-treated with poly-D-lysine (Sigma) and laminin (Sigma). 48 h post-transfection cells were fixed for 15 min with 4% paraformaldehyde at RT. Fixed cells were permeabilised with PBS containing 0.1% Triton-X 100 for 5 min, blocked with PBS containing 0.1% Tween-20 and 1% BSA and incubated 3 h at RT with primary antibodies in blocking solution [mouse anti-58K Golgi, 1:100 (Abeam); mouse anti-PDI (protein disulfide isomerase), 1:100 (Abcam); rabbit anti-PMP70 (70 kD peroxisomal membrane protein), 1:200; mouse anti-TOM20 (translocase outer mitochondrial membrane protein), 1:500 (BD); mouse anti-β-Tubulin, 1:500 (Sigma); mouse anti-Vimentin, 1:200 (Sigma)]. Coverslips were rinsed six times with PBS and labelled for 1 h with alexa 568-conjugated goat anti-mouse, goat anti-rabbit IgG (Invitrogen) or Phalloidin-TRITC (1:10, 000, Sigma). For nuclear staining the solution also contained 1 μg/ml 4,6-diamnodiphenyl-2-phenylindole (DAPI, Sigma). Coverslips were washed six times with PBS, mounted with FluorSave (Calbiochem) and evaluated by fluorescence microscopy using a Zeiss Apotome equipped with Cy3, FITC and Dapi optical filter sets. The obtained images provide an axial resolution comparable to confocal microscopy (Garini, Y. et al., Curr. Opin. Biotechnol., 16, 3-12, 2005).

Results

[0185] KASPP/LRRK2 is a Membrane-Associated 280 kD Protein

[0186] For functional and biochemical studies, KASPP/LRRK2 was cloned from human cDNA and generated a series of constructs for the expression of Hemagglutinin (HA), Strep/Flag and green fluorescent protein (GFP)-tagged LRRK2 fusion proteins (FIG. **12**A). HEK293 cells that were transiently transfected with c-terminal Strep/Flag-tagged wild-type human KASPP/LRRK2, express a ~280 kD protein recognised by anti-Flag antibody (FIG. **12**B). The observed molecular weight corresponds to that expected for KASPP/LRRK2. An additional weaker signal could be also detected in some cases at ~180 kD that is most likely an N-terminal degradation product of KASPP/LRRK2.

[0187] In order to determine the subcellular localisation of KASPP/LRRK2, two approaches were used: subcellular

fractionation and fluorescence microscopy. For detection of the subcellular distribution of KASPP/LRRK2 in vitro, transfected cells were fractionated by differential centrifugation. The distribution of subcellular organelles in the obtained fractions was then analysed by antibodies specific for mitochondria (TOM20), cytoskeleton (β-tubulin), peroxisomes (PMP70), microsomes (BiP/GRP78, Sec61α) and soluble cytosolic proteins ($p50^{cdc37}$). LRRK2 was found only in membranous fractions (both 10K and 160K pellets), i.e., fractions enriched in mitochondria (10K pellet) and microsomal membranes (160K pellet) but was absent from the cytosol (FIG. **13**A).

[0188] In order to investigate whether KASPP/LRRK2 is a membrane-associated or an integral membrane protein, the 160K pellet was treated with sodium carbonate, pH 11.5 (Fujiki, Y. et al., J. Cell Biol., 93, 97-102, 1982). KASPP/LRRK2, together with other known membrane-associated proteins, the luminal ER marker BiP/GRP78 (78 kD glucose regulated protein) and peripheral cytosolic ER-associated marker VCP (valosin-containing protein), was extracted from microsomal membranes, whereas the integral membrane protein Sec61α was recovered in the membrane pellet (FIG. **13**B). This provides evidence for KASPP/LRRK2 being a membrane-associated protein rather than integrated into membranes.

[0189] Additionally, HEK293 cells that expressed recombinantly Strep/Flag-tagged KASPP/LRRK2 kinase-domain were subjected to subcellular fractionation. In contrast to full-length KASPP/LRRK2, the kinase-domain construct was found in the cytosol, whereas little or no fusion-protein was detected in the particulate fractions (both, 10K and 160K pellet, FIG. **13**A). Thus, the kinase-domain is not implicated in the association of KASPP/LRRK2 to membranous structures.

LRRK2 Co-Localises with Discrete Cytoplasmic Structures

[0190] Immunofluorescence microscopy was used to determine the subcellular localisation of GFP-tagged KASPP/LRRK2 transiently expressed in HEK293 cells. After fixation, cells were permeabilised and co-immunolabelled with antibodies specific for distinct subcellular structures. In HEK293 cells, GFP-tagged KASPP/LRRK2 demonstrated a cytoplasmic distribution (FIG. **314** column 2). Partial co-localization was observed with inner cellular structures, i.e., mitochondria (TOM20), ER (PDI) and Golgi (58K Golgi). In contrast, no overlap was observed with peroxisomes (PMP70). No co-localization was found with the actin cytoskeleton (Phalloidin-Tritc) and intermediate filaments (Vimentin). However, the strongest co-localisation was an overlap with β-tubulin, suggesting an interaction between KASPP/LRRK2 and the microtubular cytoskeleton. Thus, KASPP/LRRK2 is a cytoplasmic protein associated with a subset of inner cellular membranes, i.e., mitochondria, ER and Golgi, and with the microtubular cytoskeleton.

Autophosphorylation Levels Between I2020T Mutant and Wildtype KASPP/LRRK2

[0191] Kinase-domain signatures can be easily detected by bioinformatical tools. Nevertheless, it is necessary to verify the kinase activity of KASPP/LRRK2 by biochemical assays. Furthermore, a comparison of wildtype and mutated KASPP/LRRK2 will contribute to the understanding of the mutation's nature, whether it is a gain- or loss of function mutation.

[0192] The wildtype full length protein vs. the I2020T mutant variant was tested for its ability for auto-phosphorylation. No significant differences in the autophosphorylation levels have been observed between the I2020T variant and the wild-type (FIG. **8**). This confirms that both, KASPP/LRRK2 and the disease-associated mutation I2020T in the kinase domain of KASPP/LRRK2, possess kinase activity. Quantification of autophosphorylation rates revealed an increase in activity of the I2020T mutant compared to wild-type KASPP/LRRK2 of about 30-50%. This finding may be the basis for the development of an appropriate screening assay for modulating compounds, in particular inhibitors, of the increased kinase activity of the I2020T mutant, as e.g. further described herein.

KASPP/LRRK2 Homodimerization

[0193] The kinase domain of LRRK2 is predicted to belong to the class of MAPKKK. A characteristic of such kinases is the formation of dimers. Moreover, for Raf-1 and MLK-3 (mixed lineage kinase 3), one of the closest relatives of LRRK2 in vertebrates, homo-dimerisation is required for activity.

[0194] In a first approach, KASPP/LRRK2 was tested for its ability to interact with itself by co-precipitation experiments. Therefore, tandem Flag-tagged KASPP/LRRK2 baits were co-expressed with HA-tagged full length KASPP/LRRK2. As shown in FIG. **9***a*, the full length KASPP/LRRK2 bait could pull out HA-KASPP/LRRK2 whereas a bait-protein containing only the kinase domain showed no interaction with full length KASPP/LRRK2. Thus, KASPP/LRRK2 interacts with itself indicating formation of homodimers or oligomers of higher order.

[0195] In a second approach, differently-tagged KASPP/LRRK2 proteins and co-transfected HEK293 cells were utilized with two constructs for expression of HA and the Strep/Flag-tagged KASPP/LRRK2 fusion proteins, with the intention that a certain fraction of cells expressing two different KASPP/LRRK2 fusion proteins would to address the question of dimerisation by co-precipitation experiments. In addition to the full-length LRRK2 protein, a Strep/Flag-tagged version of the kinase domain only was used (FIG. **12**). A comparison of purifications with all three tags showed the best results for streptactin, which almost completely precipitates the tagged proteins. Therefore, streptactin was used for precipitation of KASPP/LRRK2 fusion proteins from solubilised cells co-expressing HA and Strep/Flag-tagged KASPP/LRRK2. As shown in FIG. **15**A (lower part) by an anti-Flag antibody, both, the full-length and the KASPP/LRRK2 kinase domain baits were precipitated with the same efficiency. Analysis of the precipitated proteins with the anti-HA antibody showed that only the full-length KASPP/LRRK2 bait could pull out HA-tagged KASPP/LRRK2, whereas the kinase domain did not display any interaction with full-length KASPP/LRRK2 (FIG. **15**A, upper left panel). Thus, only full-length KASPP/LRRK2 interacts with itself forming homodimers or oligomers of higher order.

KASPP/LRRK2 Interaction with HSP90 and its Co-chaperone $p50^{cdc37}$

[0196] To identify proteins which interact with the KASPP/LRRK2 kinase domain tandem affinity purification experiments were performed with a tag system. The purified protein complexes were subjected to SDS page (FIG. **9***b*). The interacting proteins were identified using mass spectrometry. As shown in FIG. **10***b* the isolated kinase domain of KASPP/LRRK2 is associated with HSP90 and its co-chaperone $p50^{cdc37}$. The full-length KASPP/LRRK2, however, binds to HSP90 and $p50^{cdc37}$ to a very low extend. The interaction with the HSP90/$p50^{cdc37}$ chaperone-system is shown for several kinases, including the MAPKKK Raf-1 and MLK-3. In both instances, they do not serve as substrates but associate as chaperones participating in maintenance of proper folding of

the kinase. This experiment is a further evidence that KASPP/LRRK2 possesses kinase activity and is active in transfected cells.

KASPP/LRRK2 Association with Microsomal Membranes

[0197] Information of the localization of KASPP/LRRK2 will help to understand its in vivo function. Using fluorescence microscopy experiments with a c-terminal GFP tagged construct it was shown that KASPP/LRRK2 is cytoplasmic distributed in HEK293 and COST cells (FIG. **10**). By immuno co-staining there was no clear co localisation observed with any cellular structure like cytoskeleton or organelles. However, by co-staining with TOM20 and TIM23 partial overlap with mitochondria was obtained.

[0198] To further analyze the subcellular localization of this protein, a cell fractionation was performed (FIG. **11***a*). Surprisingly, no KASPP/LRRK2 was found in the cytosol. However, high amounts of KASPP/LRRK2 were found in the analyzed membranous fractions (both 10K and 160K pellets), i.e. fractions enriched with mitochondria (10K-pellet) and microsomal membranes (160 k pellet).

[0199] In order to test if KASPP/LRRK2 is an integral membrane or a membrane-associated protein, carbonate extraction of the microsomal membrane fraction (160K pellet) was applied. Since KASPP/LRRK2 could be extracted with carbonate (FIG. **11***b*) it is a membrane-associated protein rather than being integrated into the membrane. Thus, KASPP/LRRK2 is a protein with cytoplasmic but not cytosolic distribution and demonstrates strong association to membranes.

Generation of Monoclonal Antibodies Against KASPP/LRRK2

General

[0200] The immunization, generation of hybridoma clones and ELISA for positive clones against peptide #2025 used for the immunization were carried out according to standard protocols. The antibody producing clones were tested for sensitivity and specificity against KASPP/LRRK2. Peptide #2025 with the amino acid sequence "CRMGIKTSEG TPGFRAPEVA RGNVIYNQQA D" represents the kinase domain of KASPP/LRRK2 (amino acids Nos. 2025-2055). This particular peptide was chosen because the homology of the sequences between mouse and human is 100%.

Test Conditions for Sensitivity and Specificity of the Generated Antibodies

[0201] A lysate of HEK293 cells overexpressing recombinant KASPP/LRRK2 and a control lysate of HEK 293 cells transfected with an empty vector were separated in a PAGE gels (8%). The probe was applied in a broad slot over the whole gel. After electrophoretic separation the separated lysates were transferred on PVDF membranes (western blot procedure). After the transfer the membranes were first blocked with blocking buffer (5% low-fat milk powder, Bio-Rad, in TBS-Tween 20) for 1 h with the effect that unspecific adsorption of the antibodies to the membrane was avoided. Thereafter the blots were incubated with the positively tested hybridoma supernatants for 3 h (ELISA for testing the affinity to the peptide). The incubation was carried out in a multiscreen chamber (BioRad). The chamber allows the incubation of a blot with different primary antibodies. The membranes were taken form the chambers for washing (4×5 min in TBST buffer). Then. the incubation was carried out with a HRP (horse reddish peroxidase) coupled secondary antibody (anti rat IgG) for 1 h. After the incubation the blots were washed with TBST (4 x 10 min). The detection of the antibody reaction was done with the help of chemolumineszenz (ECL+, GE Healthcare) and exposition of a film (hyperfilm, GE Healthcare).

Result

[0202] The results of the above described Western blots are shown in FIG. **16**. Clone 3G6 (No. 10) and 4B7 (No. 15) (peptide #2025) produced a specific signal. A signal was specific if (a) it appeared at the position of the correct molecular weight (280 kD) and (b) it appeared stronger or only for the lysate of the KASSP/LRRK2 overexpressing cells.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 1

<210> SEQ ID NO 1
<211> LENGTH: 9104
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: exon
<222> LOCATION: (1)..(7584)

<400> SEQUENCE: 1

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1               5                   10                  15

aag aag ttg ata gtc agg ctg aac aat gtc cag gaa gga aaa cag ata       96
Lys Lys Leu Ile Val Arg Leu Asn Asn Val Gln Glu Gly Lys Gln Ile
            20                  25                  30

gaa acg ctg gtc caa atc ctg gag gat ctg ctg gtg ttc acg tac tcc      144
Glu Thr Leu Val Gln Ile Leu Glu Asp Leu Leu Val Phe Thr Tyr Ser
        35                  40                  45

gag cgc gcc tcc aag tta ttt caa ggc aaa aat atc cat gtg cct ctg      192
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Glu Arg Ala Ser Lys Leu Phe Gln Gly Lys Asn Ile His Val Pro Leu
    50                  55                  60

ttg atc gtc ttg gac tcc tat atg aga gtc gcg agt gtg cag cag gtg      240
Leu Ile Val Leu Asp Ser Tyr Met Arg Val Ala Ser Val Gln Gln Val
65                  70                  75                  80

ggt tgg tca ctt ctg tgc aaa tta ata gaa gtc tgt cca ggt aca atg      288
Gly Trp Ser Leu Leu Cys Lys Leu Ile Glu Val Cys Pro Gly Thr Met
                85                  90                  95

caa agc tta atg gga ccc cag gat gtt gga aat gat tgg gaa gtc ctt      336
Gln Ser Leu Met Gly Pro Gln Asp Val Gly Asn Asp Trp Glu Val Leu
            100                 105                 110

ggt gtt cac caa ttg att ctt aaa atg cta aca gtt cat aat gcc agt      384
Gly Val His Gln Leu Ile Leu Lys Met Leu Thr Val His Asn Ala Ser
        115                 120                 125

gta aac ttg tca gtg att gga ctg aag acc tta gat ctc ctc cta act      432
Val Asn Leu Ser Val Ile Gly Leu Lys Thr Leu Asp Leu Leu Leu Thr
    130                 135                 140

tca ggt aaa atc acc ttg ctg ata ttg gat gaa gaa agt gat att ttc      480
Ser Gly Lys Ile Thr Leu Leu Ile Leu Asp Glu Glu Ser Asp Ile Phe
145                 150                 155                 160

atg tta att ttt gat gcc atg cac tca ttt cca gcc aat gat gaa gtc      528
Met Leu Ile Phe Asp Ala Met His Ser Phe Pro Ala Asn Asp Glu Val
                165                 170                 175

cag aaa ctt gga tgc aaa gct tta cat gtg ctg ttt gag aga gtc tca      576
Gln Lys Leu Gly Cys Lys Ala Leu His Val Leu Phe Glu Arg Val Ser
            180                 185                 190

gag gag caa ctg act gaa ttt gtt gag aac aaa gat tat atg ata ttg      624
Glu Glu Gln Leu Thr Glu Phe Val Glu Asn Lys Asp Tyr Met Ile Leu
        195                 200                 205

tta agt gcg tca aca aat ttt aaa gat gaa gag gaa att gtg ctt cat      672
Leu Ser Ala Ser Thr Asn Phe Lys Asp Glu Glu Glu Ile Val Leu His
    210                 215                 220

gtg ctg cat tgt tta cat tcc cta gcg att cct tgc aat aat gtg gaa      720
Val Leu His Cys Leu His Ser Leu Ala Ile Pro Cys Asn Asn Val Glu
225                 230                 235                 240

gtc ctc atg agt ggc aat gtc agg tgt tat aat att gtg gtg gaa gct      768
Val Leu Met Ser Gly Asn Val Arg Cys Tyr Asn Ile Val Val Glu Ala
                245                 250                 255

atg aaa gca ttc cct atg agt gaa aga att caa gaa gtg agt tgc tgt      816
Met Lys Ala Phe Pro Met Ser Glu Arg Ile Gln Glu Val Ser Cys Cys
            260                 265                 270

ttg ctc cat agg ctt aca tta ggt aat ttt ttc aat atc ctg gta tta      864
Leu Leu His Arg Leu Thr Leu Gly Asn Phe Phe Asn Ile Leu Val Leu
        275                 280                 285

aac gaa gtc cat gag ttt gtg gtg aaa gct gtg cag cag tac cca gag      912
Asn Glu Val His Glu Phe Val Val Lys Ala Val Gln Gln Tyr Pro Glu
    290                 295                 300

aat gca gca ttg cag atc tca gcg ctc agc tgt ttg gcc ctc ctc act      960
Asn Ala Ala Leu Gln Ile Ser Ala Leu Ser Cys Leu Ala Leu Leu Thr
305                 310                 315                 320

gag act att ttc tta aat caa gat tta gag gaa aag aat gag aat caa     1008
Glu Thr Ile Phe Leu Asn Gln Asp Leu Glu Glu Lys Asn Glu Asn Gln
                325                 330                 335

gag aat gat gat gag ggg gaa gaa gat aaa ttg ttt tgg ctg gaa gcc     1056
Glu Asn Asp Asp Glu Gly Glu Glu Asp Lys Leu Phe Trp Leu Glu Ala
            340                 345                 350

tgt tac aaa gca tta acg tgg cat aga aag aac aag cac gtg cag gag     1104
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Cys Tyr Lys Ala Leu Thr Trp His Arg Lys Asn Lys His Val Gln Glu
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gcc gca tgc tgg gca cta aat aat ctc ctt atg tac caa aac agt tta     1152
Ala Ala Cys Trp Ala Leu Asn Asn Leu Leu Met Tyr Gln Asn Ser Leu
    370                 375                 380

cat gag aag att gga gat gaa gat ggc cat ttc cca gct cat agg gaa     1200
His Glu Lys Ile Gly Asp Glu Asp Gly His Phe Pro Ala His Arg Glu
385                 390                 395                 400

gtg atg ctc tcc atg ctg atg cat tct tca tca aag gaa gtt ttc cag     1248
Val Met Leu Ser Met Leu Met His Ser Ser Ser Lys Glu Val Phe Gln
                405                 410                 415

gca tct gcg aat gca ttg tca act ctc tta gaa caa aat gtt aat ttc     1296
Ala Ser Ala Asn Ala Leu Ser Thr Leu Leu Glu Gln Asn Val Asn Phe
            420                 425                 430

aga aaa ata ctg tta tca aaa gga ata cac ctg aat gtt ttg gag tta     1344
Arg Lys Ile Leu Leu Ser Lys Gly Ile His Leu Asn Val Leu Glu Leu
        435                 440                 445

atg cag aag cat ata cat tct cct gaa gtg gct gaa agt ggc tgt aaa     1392
Met Gln Lys His Ile His Ser Pro Glu Val Ala Glu Ser Gly Cys Lys
    450                 455                 460

atg cta aat cat ctt ttt gaa gga agc aac act tcc ctg gat ata atg     1440
Met Leu Asn His Leu Phe Glu Gly Ser Asn Thr Ser Leu Asp Ile Met
465                 470                 475                 480

gca gca gtg gtc ccc aaa ata cta aca gtt atg aaa cgt cat gag aca     1488
Ala Ala Val Val Pro Lys Ile Leu Thr Val Met Lys Arg His Glu Thr
                485                 490                 495

tca tta cca gtg cag ctg gag gcg ctt cga gct att tta cat ttt ata     1536
Ser Leu Pro Val Gln Leu Glu Ala Leu Arg Ala Ile Leu His Phe Ile
            500                 505                 510

gtg cct ggc atg cca gaa gaa tcc agg gag gat aca gaa ttt cat cat     1584
Val Pro Gly Met Pro Glu Glu Ser Arg Glu Asp Thr Glu Phe His His
        515                 520                 525

aag cta aat atg gtt aaa aaa cag tgt ttc aag aat gat att cac aaa     1632
Lys Leu Asn Met Val Lys Lys Gln Cys Phe Lys Asn Asp Ile His Lys
    530                 535                 540

ctg gtc cta gca gct ttg aac agg ttc att gga aat cct ggg att cag     1680
Leu Val Leu Ala Ala Leu Asn Arg Phe Ile Gly Asn Pro Gly Ile Gln
545                 550                 555                 560

aaa tgt gga tta aaa gta att tct tct att gta cat ttt cct gat gca     1728
Lys Cys Gly Leu Lys Val Ile Ser Ser Ile Val His Phe Pro Asp Ala
                565                 570                 575

tta gag atg tta tcc ctg gaa ggt gct atg gat tca gtg ctt cac aca     1776
Leu Glu Met Leu Ser Leu Glu Gly Ala Met Asp Ser Val Leu His Thr
            580                 585                 590

ctg cag atg tat cca gat gac caa gaa att cag tgt ctg ggt tta agt     1824
Leu Gln Met Tyr Pro Asp Asp Gln Glu Ile Gln Cys Leu Gly Leu Ser
        595                 600                 605

ctt ata gga tac ttg att aca aag aag aat gtg ttc ata gga act gga     1872
Leu Ile Gly Tyr Leu Ile Thr Lys Lys Asn Val Phe Ile Gly Thr Gly
    610                 615                 620

cat ctg ctg gca aaa att ctg gtt tcc agc tta tac cga ttt aag gat     1920
His Leu Leu Ala Lys Ile Leu Val Ser Ser Leu Tyr Arg Phe Lys Asp
625                 630                 635                 640

gtt gct gaa ata cag act aaa gga ttt cag aca atc tta gca atc ctc     1968
Val Ala Glu Ile Gln Thr Lys Gly Phe Gln Thr Ile Leu Ala Ile Leu
                645                 650                 655

aaa ttg tca gca tct ttt tct aag ctg ctg gtg cat cat tca ttt gac     2016
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Lys Leu Ser Ala Ser Phe Ser Lys Leu Leu Val His His Ser Phe Asp
           660                 665                 670

tta gta ata ttc cat caa atg tct tcc aat atc atg gaa caa aag gat     2064
Leu Val Ile Phe His Gln Met Ser Ser Asn Ile Met Glu Gln Lys Asp
        675                 680                 685

caa cag ttt cta aac ctc tgt tgc aag tgt ttt gca aaa gta gct atg     2112
Gln Gln Phe Leu Asn Leu Cys Cys Lys Cys Phe Ala Lys Val Ala Met
    690                 695                 700

gat gat tac tta aaa aat gtg atg cta gag aga gcg tgt gat cag aat     2160
Asp Asp Tyr Leu Lys Asn Val Met Leu Glu Arg Ala Cys Asp Gln Asn
705                 710                 715                 720

aac agc atc atg gtt gaa tgc ttg ctt cta ttg gga gca gat gcc aat     2208
Asn Ser Ile Met Val Glu Cys Leu Leu Leu Leu Gly Ala Asp Ala Asn
                725                 730                 735

caa gca aag gag gga tct tct tta att tgt cag gta tgt gag aaa gag     2256
Gln Ala Lys Glu Gly Ser Ser Leu Ile Cys Gln Val Cys Glu Lys Glu
           740                 745                 750

agc agt ccc aaa ttg gtg gaa ctc tta ctg aat agt gga tct cgt gaa     2304
Ser Ser Pro Lys Leu Val Glu Leu Leu Leu Asn Ser Gly Ser Arg Glu
        755                 760                 765

caa gat gta cga aaa gcg ttg acg ata agc att ggg aaa ggt gac agc     2352
Gln Asp Val Arg Lys Ala Leu Thr Ile Ser Ile Gly Lys Gly Asp Ser
    770                 775                 780

cag atc atc agc ttg ctc tta agg agg ctg gcc ctg gat gtg gcc aac     2400
Gln Ile Ile Ser Leu Leu Leu Arg Arg Leu Ala Leu Asp Val Ala Asn
785                 790                 795                 800

aat agc att tgc ctt gga gga ttt tgt ata gga aaa gtt gaa cct tct     2448
Asn Ser Ile Cys Leu Gly Gly Phe Cys Ile Gly Lys Val Glu Pro Ser
                805                 810                 815

tgg ctt ggt cct tta ttt cca gat aag act tct aat tta agg aaa caa     2496
Trp Leu Gly Pro Leu Phe Pro Asp Lys Thr Ser Asn Leu Arg Lys Gln
           820                 825                 830

aca aat ata gca tct aca cta gca aga atg gtg atc aga tat cag atg     2544
Thr Asn Ile Ala Ser Thr Leu Ala Arg Met Val Ile Arg Tyr Gln Met
        835                 840                 845

aaa agt gct gtg gaa gaa gga aca gcc tca ggc agc gat gga aat ttt     2592
Lys Ser Ala Val Glu Glu Gly Thr Ala Ser Gly Ser Asp Gly Asn Phe
    850                 855                 860

tct gaa gat gtg ctg tct aaa ttt gat gaa tgg acc ttt att cct gac     2640
Ser Glu Asp Val Leu Ser Lys Phe Asp Glu Trp Thr Phe Ile Pro Asp
865                 870                 875                 880

tct tct atg gac agt gtg ttt gct caa agt gat gac ctg gat agt gaa     2688
Ser Ser Met Asp Ser Val Phe Ala Gln Ser Asp Asp Leu Asp Ser Glu
                885                 890                 895

gga agt gaa ggc tca ttt ctt gtg aaa aag aaa tct aat tca att agt     2736
Gly Ser Glu Gly Ser Phe Leu Val Lys Lys Lys Ser Asn Ser Ile Ser
           900                 905                 910

gta gga gaa ttt tac cga gat gcc gta tta cag cgt tgc tca cca aat     2784
Val Gly Glu Phe Tyr Arg Asp Ala Val Leu Gln Arg Cys Ser Pro Asn
        915                 920                 925

ttg caa aga cat tcc aat tcc ttg ggg ccc att ttt gat cat gaa gat     2832
Leu Gln Arg His Ser Asn Ser Leu Gly Pro Ile Phe Asp His Glu Asp
    930                 935                 940

tta ctg aag cga aaa aga aaa ata tta tct tca gat gat tca ctc agg     2880
Leu Leu Lys Arg Lys Arg Lys Ile Leu Ser Ser Asp Asp Ser Leu Arg
945                 950                 955                 960

tca tca aaa ctt caa tcc cat atg agg cat tca gac agc att tct tct     2928
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Ser Ser Lys Leu Gln Ser His Met Arg His Ser Asp Ser Ile Ser Ser
                965                 970                 975

ctg gct tct gag aga gaa tat att aca tca cta gac ctt tca gca aat     2976
Leu Ala Ser Glu Arg Glu Tyr Ile Thr Ser Leu Asp Leu Ser Ala Asn
            980                 985                 990

gaa cta aga gat att gat gcc cta  agc cag aaa tgc tgt  ata agt gtt   3024
Glu Leu Arg Asp Ile Asp Ala Leu  Ser Gln Lys Cys Cys  Ile Ser Val
        995                 1000                1005

cat ttg  gag cat ctt gaa aag  ctg gag ctt cac cag  aat gca ctc      3069
His Leu  Glu His Leu Glu Lys  Leu Glu Leu His Gln  Asn Ala Leu
    1010                1015                1020

acg agc  ttt cca caa cag cta  tgt gaa act ctg aag  agt ttg aca      3114
Thr Ser  Phe Pro Gln Gln Leu  Cys Glu Thr Leu Lys  Ser Leu Thr
    1025                1030                1035

cat ttg  gac ttg cac agt aat  aaa ttt aca tca ttt  cct tct tat      3159
His Leu  Asp Leu His Ser Asn  Lys Phe Thr Ser Phe  Pro Ser Tyr
    1040                1045                1050

ttg ttg  aaa atg agt tgt att  gct aat ctt gat gtc  tct cga aat      3204
Leu Leu  Lys Met Ser Cys Ile  Ala Asn Leu Asp Val  Ser Arg Asn
    1055                1060                1065

gac att  gga ccc tca gtg gtt  tta gat cct aca gtg  aaa tgt cca      3249
Asp Ile  Gly Pro Ser Val Val  Leu Asp Pro Thr Val  Lys Cys Pro
    1070                1075                1080

act ctg  aaa cag ttt aac ctg  tca tat aac cag ctg  tct ttt gta      3294
Thr Leu  Lys Gln Phe Asn Leu  Ser Tyr Asn Gln Leu  Ser Phe Val
    1085                1090                1095

cct gag  aac ctc act gat gtg  gta gag aaa ctg gag  cag ctc att      3339
Pro Glu  Asn Leu Thr Asp Val  Val Glu Lys Leu Glu  Gln Leu Ile
    1100                1105                1110

tta gaa  gga aat aaa ata tca  ggg ata tgc tcc ccc  ttg aga ctg      3384
Leu Glu  Gly Asn Lys Ile Ser  Gly Ile Cys Ser Pro  Leu Arg Leu
    1115                1120                1125

aag gaa  ctg aag att tta aac  ctt agt aag aac cac  att tca tcc      3429
Lys Glu  Leu Lys Ile Leu Asn  Leu Ser Lys Asn His  Ile Ser Ser
    1130                1135                1140

cta tca  gag aac ttt ctt gag  gct tgt cct aaa gtg  gag agt ttc      3474
Leu Ser  Glu Asn Phe Leu Glu  Ala Cys Pro Lys Val  Glu Ser Phe
    1145                1150                1155

agt gcc  aga atg aat ttt ctt  gct gct atg cct ttc  ttg cct cct      3519
Ser Ala  Arg Met Asn Phe Leu  Ala Ala Met Pro Phe  Leu Pro Pro
    1160                1165                1170

tct atg  aca atc cta aaa tta  tct cag aac aaa ttt  tcc tgt att      3564
Ser Met  Thr Ile Leu Lys Leu  Ser Gln Asn Lys Phe  Ser Cys Ile
    1175                1180                1185

cca gaa  gca att tta aat ctt  cca cac ttg cgg tct  tta gat atg      3609
Pro Glu  Ala Ile Leu Asn Leu  Pro His Leu Arg Ser  Leu Asp Met
    1190                1195                1200

agc agc  aat gat att cag tac  cta cca ggt ccc gca  cac tgg aaa      3654
Ser Ser  Asn Asp Ile Gln Tyr  Leu Pro Gly Pro Ala  His Trp Lys
    1205                1210                1215

tct ttg  aac tta agg gaa ctc  tta ttt agc cat aat  cag atc agc      3699
Ser Leu  Asn Leu Arg Glu Leu  Leu Phe Ser His Asn  Gln Ile Ser
    1220                1225                1230

atc ttg  gac ttg agt gaa aaa  gca tat tta tgg tct  aga gta gag      3744
Ile Leu  Asp Leu Ser Glu Lys  Ala Tyr Leu Trp Ser  Arg Val Glu
    1235                1240                1245

aaa ctg  cat ctt tct cac aat  aaa ctg aaa gag att  cct cct gag      3789
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Lys Leu  His Leu Ser His Asn  Lys Leu Lys Glu Ile  Pro Pro Glu
    1250                1255                1260

att ggc  tgt ctt gaa aat ctg  aca tct ctg gat gtc  agt tac aac      3834
Ile Gly  Cys Leu Glu Asn Leu  Thr Ser Leu Asp Val  Ser Tyr Asn
    1265                1270                1275

ttg gaa  cta aga tcc ttt ccc  aat gaa atg ggg aaa  tta agc aaa      3879
Leu Glu  Leu Arg Ser Phe Pro  Asn Glu Met Gly Lys  Leu Ser Lys
    1280                1285                1290

ata tgg  gat ctt cct ttg gat  gaa ctg cat ctt aac  ttt gat ttt      3924
Ile Trp  Asp Leu Pro Leu Asp  Glu Leu His Leu Asn  Phe Asp Phe
    1295                1300                1305

aaa cat  ata gga tgt aaa gcc  aaa gac atc ata agg  ttt ctt caa      3969
Lys His  Ile Gly Cys Lys Ala  Lys Asp Ile Ile Arg  Phe Leu Gln
    1310                1315                1320

cag cga  tta aaa aag gct gtg  cct tat aac cga atg  aaa ctt atg      4014
Gln Arg  Leu Lys Lys Ala Val  Pro Tyr Asn Arg Met  Lys Leu Met
    1325                1330                1335

att gtg  gga aat act ggg agt  ggt aaa acc acc tta  ttg cag caa      4059
Ile Val  Gly Asn Thr Gly Ser  Gly Lys Thr Thr Leu  Leu Gln Gln
    1340                1345                1350

tta atg  aaa acc aag aaa tca  gat ctt gga atg caa  agt gcc aca      4104
Leu Met  Lys Thr Lys Lys Ser  Asp Leu Gly Met Gln  Ser Ala Thr
    1355                1360                1365

gtt ggc  ata gat gtg aaa gac  tgg cct atc caa ata  aga gac aaa      4149
Val Gly  Ile Asp Val Lys Asp  Trp Pro Ile Gln Ile  Arg Asp Lys
    1370                1375                1380

aga aag  aga gat ctc gtc cta  aat gtg tgg gat ttt  gca ggt cgt      4194
Arg Lys  Arg Asp Leu Val Leu  Asn Val Trp Asp Phe  Ala Gly Arg
    1385                1390                1395

gag gaa  ttc tat agt act cat  ccc cat ttt atg acg  cag cga gca      4239
Glu Glu  Phe Tyr Ser Thr His  Pro His Phe Met Thr  Gln Arg Ala
    1400                1405                1410

ttg tac  ctt gct gtc tat gac  ctc agc aag gga cag  gct gaa gtt      4284
Leu Tyr  Leu Ala Val Tyr Asp  Leu Ser Lys Gly Gln  Ala Glu Val
    1415                1420                1425

gat gcc  atg aag cct tgg ctc  ttc aat ata aag gct  cgc gct tct      4329
Asp Ala  Met Lys Pro Trp Leu  Phe Asn Ile Lys Ala  Arg Ala Ser
    1430                1435                1440

tct tcc  cct gtg att ctc gtt  ggc aca cat ttg gat  gtt tct gat      4374
Ser Ser  Pro Val Ile Leu Val  Gly Thr His Leu Asp  Val Ser Asp
    1445                1450                1455

gag aag  caa cgc aaa gcc tgc  atg agt aaa atc acc  aag gaa ctc      4419
Glu Lys  Gln Arg Lys Ala Cys  Met Ser Lys Ile Thr  Lys Glu Leu
    1460                1465                1470

ctg aat  aag cga ggg ttc cct  gcc ata cga gat tac  cac ttt gtg      4464
Leu Asn  Lys Arg Gly Phe Pro  Ala Ile Arg Asp Tyr  His Phe Val
    1475                1480                1485

aat gcc  acc gag gaa tct gat  gct ttg gca aaa ctt  cgg aaa acc      4509
Asn Ala  Thr Glu Glu Ser Asp  Ala Leu Ala Lys Leu  Arg Lys Thr
    1490                1495                1500

atc ata  aac gag agc ctt aat  ttc aag atc cga gat  cag ctt gtt      4554
Ile Ile  Asn Glu Ser Leu Asn  Phe Lys Ile Arg Asp  Gln Leu Val
    1505                1510                1515

gtt gga  cag ctg att cca gac  tgc tat gta gaa ctt  gaa aaa atc      4599
Val Gly  Gln Leu Ile Pro Asp  Cys Tyr Val Glu Leu  Glu Lys Ile
    1520                1525                1530

att tta  tcg gag cgt aaa aat  gtg cca att gaa ttt  ccc gta att      4644
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Ile Leu  Ser Glu Arg Lys Asn  Val Pro Ile Glu Phe  Pro Val Ile
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gac cgg  aaa cga tta tta caa  cta gtg aga gaa aat  cag ctg cag      4689
Asp Arg  Lys Arg Leu Leu Gln  Leu Val Arg Glu Asn  Gln Leu Gln
    1550                1555                1560

tta gat  gaa aat gag ctt cct  cac gca gtt cac ttt  cta aat gaa      4734
Leu Asp  Glu Asn Glu Leu Pro  His Ala Val His Phe  Leu Asn Glu
    1565                1570                1575

tca gga  gtc ctt ctt cat ttt  caa gac cca gca ctg  cag tta agt      4779
Ser Gly  Val Leu Leu His Phe  Gln Asp Pro Ala Leu  Gln Leu Ser
    1580                1585                1590

gac ttg  tac ttt gtg gaa ccc  aag tgg ctt tgt aaa  atc atg gca      4824
Asp Leu  Tyr Phe Val Glu Pro  Lys Trp Leu Cys Lys  Ile Met Ala
    1595                1600                1605

cag att  ttg aca gtg aaa gtg  gaa ggt tgt cca aaa  cac cct aag      4869
Gln Ile  Leu Thr Val Lys Val  Glu Gly Cys Pro Lys  His Pro Lys
    1610                1615                1620

ggc att  att tcg cgt aga gat  gtg gaa aaa ttt ctt  tca aaa aaa      4914
Gly Ile  Ile Ser Arg Arg Asp  Val Glu Lys Phe Leu  Ser Lys Lys
    1625                1630                1635

agg aaa  ttt cca aag aac tac  atg tca cag tat ttt  aag ctc cta      4959
Arg Lys  Phe Pro Lys Asn Tyr  Met Ser Gln Tyr Phe  Lys Leu Leu
    1640                1645                1650

gaa aaa  ttc cag att gct ttg  cca ata gga gaa gaa  tat ttg ctg      5004
Glu Lys  Phe Gln Ile Ala Leu  Pro Ile Gly Glu Glu  Tyr Leu Leu
    1655                1660                1665

gtt cca  agc agt ttg tct gac  cac agg cct gtg ata  gag ctt ccc      5049
Val Pro  Ser Ser Leu Ser Asp  His Arg Pro Val Ile  Glu Leu Pro
    1670                1675                1680

cat tgt  gag aac tct gaa att  atc atc cga cta tat  gaa atg cct      5094
His Cys  Glu Asn Ser Glu Ile  Ile Ile Arg Leu Tyr  Glu Met Pro
    1685                1690                1695

tat ttt  cca atg gga ttt tgg  tca aga tta atc aat  cga tta ctt      5139
Tyr Phe  Pro Met Gly Phe Trp  Ser Arg Leu Ile Asn  Arg Leu Leu
    1700                1705                1710

gag att  tca cct tac atg ctt  tca ggg aga gaa cga  gca ctt cgc      5184
Glu Ile  Ser Pro Tyr Met Leu  Ser Gly Arg Glu Arg  Ala Leu Arg
    1715                1720                1725

cca aac  aga atg tat tgg cga  caa ggc att tac tta  aat tgg tct      5229
Pro Asn  Arg Met Tyr Trp Arg  Gln Gly Ile Tyr Leu  Asn Trp Ser
    1730                1735                1740

cct gaa  gct tat tgt ctg gta  gga tct gaa gtc tta  gac aat cat      5274
Pro Glu  Ala Tyr Cys Leu Val  Gly Ser Glu Val Leu  Asp Asn His
    1745                1750                1755

cca gag  agt ttc tta aaa att  aca gtt cct tct tgt  aga aaa ggc      5319
Pro Glu  Ser Phe Leu Lys Ile  Thr Val Pro Ser Cys  Arg Lys Gly
    1760                1765                1770

tgt att  ctt ttg ggc caa gtt  gtg gac cac att gat  tct ctc atg      5364
Cys Ile  Leu Leu Gly Gln Val  Val Asp His Ile Asp  Ser Leu Met
    1775                1780                1785

gaa gaa  tgg ttt cct ggg ttg  ctg gag att gat att  tgt ggt gaa      5409
Glu Glu  Trp Phe Pro Gly Leu  Leu Glu Ile Asp Ile  Cys Gly Glu
    1790                1795                1800

gga gaa  act ctg ttg aag aaa  tgg gca tta tat agt  ttt aat gat      5454
Gly Glu  Thr Leu Leu Lys Lys  Trp Ala Leu Tyr Ser  Phe Asn Asp
    1805                1810                1815

ggt gaa  gaa cat caa aaa atc  tta ctt gat gac ttg  atg aag aaa      5499
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Gly Glu  Glu His Gln Lys Ile  Leu Leu Asp Asp Leu  Met Lys Lys
    1820                1825                1830

gca gag  gaa gga gat ctc tta  gta aat cca gat caa  cca agg ctc      5544
Ala Glu  Glu Gly Asp Leu Leu  Val Asn Pro Asp Gln  Pro Arg Leu
    1835                1840                1845

acc att  cca ata tct cag att  gcc cct gac ttg att  ttg gct gac      5589
Thr Ile  Pro Ile Ser Gln Ile  Ala Pro Asp Leu Ile  Leu Ala Asp
    1850                1855                1860

ctg cct  aga aat att atg ttg  aat aat gat gag ttg  gaa ttt gaa      5634
Leu Pro  Arg Asn Ile Met Leu  Asn Asn Asp Glu Leu  Glu Phe Glu
    1865                1870                1875

caa gct  cca gag ttt ctc cta  ggt gat ggc agt ttt  gga tca gtt      5679
Gln Ala  Pro Glu Phe Leu Leu  Gly Asp Gly Ser Phe  Gly Ser Val
    1880                1885                1890

tac cga  gca gcc tat gaa gga  gaa gaa gtg gct gtg  aag att ttt      5724
Tyr Arg  Ala Ala Tyr Glu Gly  Glu Glu Val Ala Val  Lys Ile Phe
    1895                1900                1905

aat aaa  cat aca tca ctc agg  ctg tta aga caa gag  ctt gtg gtg      5769
Asn Lys  His Thr Ser Leu Arg  Leu Leu Arg Gln Glu  Leu Val Val
    1910                1915                1920

ctt tgc  cac ctc cac cac ccc  agt ttg ata tct ttg  ctg gca gct      5814
Leu Cys  His Leu His His Pro  Ser Leu Ile Ser Leu  Leu Ala Ala
    1925                1930                1935

ggg att  cgt ccc cgg atg ttg  gtg atg gag tta gcc  tcc aag ggt      5859
Gly Ile  Arg Pro Arg Met Leu  Val Met Glu Leu Ala  Ser Lys Gly
    1940                1945                1950

tcc ttg  gat cgc ctg ctt cag  cag gac aaa gcc agc  ctc act aga      5904
Ser Leu  Asp Arg Leu Leu Gln  Gln Asp Lys Ala Ser  Leu Thr Arg
    1955                1960                1965

acc cta  cag cac agg att gca  ctc cac gta gct gat  ggt ttg aga      5949
Thr Leu  Gln His Arg Ile Ala  Leu His Val Ala Asp  Gly Leu Arg
    1970                1975                1980

tac ctc  cac tca gcc atg att  ata tac cga gac ctg  aaa ccc cac      5994
Tyr Leu  His Ser Ala Met Ile  Ile Tyr Arg Asp Leu  Lys Pro His
    1985                1990                1995

aat gtg  ctg ctt ttc aca ctg  tat ccc aat gct gcc  atc att gca      6039
Asn Val  Leu Leu Phe Thr Leu  Tyr Pro Asn Ala Ala  Ile Ile Ala
    2000                2005                2010

aag att  gct gac tac ggc att  gct cag tac tgc tgt  aga atg ggg      6084
Lys Ile  Ala Asp Tyr Gly Ile  Ala Gln Tyr Cys Cys  Arg Met Gly
    2015                2020                2025

ata aaa  aca tca gag ggc aca  cca ggg ttt cgt gca  cct gaa gtt      6129
Ile Lys  Thr Ser Glu Gly Thr  Pro Gly Phe Arg Ala  Pro Glu Val
    2030                2035                2040

gcc aga  gga aat gtc att tat  aac caa cag gct gat  gtt tat tca      6174
Ala Arg  Gly Asn Val Ile Tyr  Asn Gln Gln Ala Asp  Val Tyr Ser
    2045                2050                2055

ttt ggt  tta cta ctc tat gac  att ttg aca act gga  ggt aga ata      6219
Phe Gly  Leu Leu Leu Tyr Asp  Ile Leu Thr Thr Gly  Gly Arg Ile
    2060                2065                2070

gta gag  ggt ttg aag ttt cca  aat gag ttt gat gaa  tta gaa ata      6264
Val Glu  Gly Leu Lys Phe Pro  Asn Glu Phe Asp Glu  Leu Glu Ile
    2075                2080                2085

caa gga  aaa tta cct gat cca  gtt aaa gaa tat ggt  tgt gcc cca      6309
Gln Gly  Lys Leu Pro Asp Pro  Val Lys Glu Tyr Gly  Cys Ala Pro
    2090                2095                2100

tgg cct  atg gtt gag aaa tta  att aaa cag tgt ttg  aaa gaa aat      6354
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Trp Pro  Met Val Glu Lys Leu  Ile Lys Gln Cys Leu  Lys Glu Asn
    2105                2110                2115

cct caa  gaa agg cct act tct  gcc cag gtc ttt gac  att ttg aat      6399
Pro Gln  Glu Arg Pro Thr Ser  Ala Gln Val Phe Asp  Ile Leu Asn
    2120                2125                2130

tca gct  gaa tta gtc tgt ctg  acg aga cgc att tta  tta cct aaa      6444
Ser Ala  Glu Leu Val Cys Leu  Thr Arg Arg Ile Leu  Leu Pro Lys
    2135                2140                2145

aac gta  att gtt gaa tgc atg  gtt gct aca cat cac  aac agc agg      6489
Asn Val  Ile Val Glu Cys Met  Val Ala Thr His His  Asn Ser Arg
    2150                2155                2160

aat gca  agc att tgg ctg ggc  tgt ggg cac acc gac  aga gga cag      6534
Asn Ala  Ser Ile Trp Leu Gly  Cys Gly His Thr Asp  Arg Gly Gln
    2165                2170                2175

ctc tca  ttt ctt gac tta aat  act gaa gga tac act  tct gag gaa      6579
Leu Ser  Phe Leu Asp Leu Asn  Thr Glu Gly Tyr Thr  Ser Glu Glu
    2180                2185                2190

gtt gct  gat agt aga ata ttg  tgc tta gcc ttg gtg  cat ctt cct      6624
Val Ala  Asp Ser Arg Ile Leu  Cys Leu Ala Leu Val  His Leu Pro
    2195                2200                2205

gtt gaa  aag gaa agc tgg att  gtg tct ggg aca cag  tct ggt act      6669
Val Glu  Lys Glu Ser Trp Ile  Val Ser Gly Thr Gln  Ser Gly Thr
    2210                2215                2220

ctc ctg  gtc atc aat acc gaa  gat ggg aaa aag aga  cat acc cta      6714
Leu Leu  Val Ile Asn Thr Glu  Asp Gly Lys Lys Arg  His Thr Leu
    2225                2230                2235

gaa aag  atg act gat tct gtc  act tgt ttg tat tgc  aat tcc ttt      6759
Glu Lys  Met Thr Asp Ser Val  Thr Cys Leu Tyr Cys  Asn Ser Phe
    2240                2245                2250

tcc aag  caa agc aaa caa aaa  aat ttt ctt ttg gtt  gga acc gct      6804
Ser Lys  Gln Ser Lys Gln Lys  Asn Phe Leu Leu Val  Gly Thr Ala
    2255                2260                2265

gat ggc  aag tta gca att ttt  gaa gat aag act gtt  aag ctt aaa      6849
Asp Gly  Lys Leu Ala Ile Phe  Glu Asp Lys Thr Val  Lys Leu Lys
    2270                2275                2280

gga gct  gct cct ttg aag ata  cta aat ata gga aat  gtc agt act      6894
Gly Ala  Ala Pro Leu Lys Ile  Leu Asn Ile Gly Asn  Val Ser Thr
    2285                2290                2295

cca ttg  atg tgt ttg agt gaa  tcc aca aat tca acg  gaa aga aat      6939
Pro Leu  Met Cys Leu Ser Glu  Ser Thr Asn Ser Thr  Glu Arg Asn
    2300                2305                2310

gta atg  tgg gga gga tgt ggc  aca aag att ttc tcc  ttt tct aat      6984
Val Met  Trp Gly Gly Cys Gly  Thr Lys Ile Phe Ser  Phe Ser Asn
    2315                2320                2325

gat ttc  acc att cag aaa ctc  att gag aca aga aca  agc caa ctg      7029
Asp Phe  Thr Ile Gln Lys Leu  Ile Glu Thr Arg Thr  Ser Gln Leu
    2330                2335                2340

ttt tct  tat gca gct ttc agt  gat tcc aac atc ata  aca gtg gtg      7074
Phe Ser  Tyr Ala Ala Phe Ser  Asp Ser Asn Ile Ile  Thr Val Val
    2345                2350                2355

gta gac  act gct ctc tat att  gct aag caa aat agc  cct gtt gtg      7119
Val Asp  Thr Ala Leu Tyr Ile  Ala Lys Gln Asn Ser  Pro Val Val
    2360                2365                2370

gaa gtg  tgg gat aag aaa act  gaa aaa ctc tgt gga  cta ata gac      7164
Glu Val  Trp Asp Lys Lys Thr  Glu Lys Leu Cys Gly  Leu Ile Asp
    2375                2380                2385

tgc gtg  cac ttt tta agg gag  gta atg gta aaa gaa  aac aag gaa      7209
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Cys Val  His Phe Leu Arg Glu  Val Met Val Lys Glu  Asn Lys Glu
    2390                2395                2400

tca aaa  cac aaa atg tct tat  tct ggg aga gtg aaa  acc ctc tgc      7254
Ser Lys  His Lys Met Ser Tyr  Ser Gly Arg Val Lys  Thr Leu Cys
    2405                2410                2415

ctt cag  aag aac act gct ctt  tgg ata gga act gga  gga ggc cat      7299
Leu Gln  Lys Asn Thr Ala Leu  Trp Ile Gly Thr Gly  Gly Gly His
    2420                2425                2430

att tta  ctc ctg gat ctt tca  act cgt cga ctt ata  cgt gta att      7344
Ile Leu  Leu Leu Asp Leu Ser  Thr Arg Arg Leu Ile  Arg Val Ile
    2435                2440                2445

tac aac  ttt tgt aat tcg gtc  aga gtc atg atg aca  gca cag cta      7389
Tyr Asn  Phe Cys Asn Ser Val  Arg Val Met Met Thr  Ala Gln Leu
    2450                2455                2460

gga agc  ctt aaa aat gtc atg  ctg gta ttg ggc tac  aac cgg aaa      7434
Gly Ser  Leu Lys Asn Val Met  Leu Val Leu Gly Tyr  Asn Arg Lys
    2465                2470                2475

aat act  gaa ggt aca caa aag  cag aaa gag ata caa  tct tgc ttg      7479
Asn Thr  Glu Gly Thr Gln Lys  Gln Lys Glu Ile Gln  Ser Cys Leu
    2480                2485                2490

acc gtt  tgg gac atc aat ctt  cca cat gaa gtg caa  aat tta gaa      7524
Thr Val  Trp Asp Ile Asn Leu  Pro His Glu Val Gln  Asn Leu Glu
    2495                2500                2505

aaa cac  att gaa gtg aga aaa  gaa tta gct gaa aaa  atg aga cga      7569
Lys His  Ile Glu Val Arg Lys  Glu Leu Ala Glu Lys  Met Arg Arg
    2510                2515                2520

aca tct  gtt gag taa gagagaaata ggaattgtct ttggatagga aaattattct    7624
Thr Ser  Val Glu
    2525

ctcctcttgt aaatatttat tttaaaaatg ttcacatgga aagggtactc acatttttg    7684

aaatagctcg tgtgtatgaa ggaatgttat tatttttaat ttaaatatat gtaaaaatac    7744

ttaccagtaa atgtgtattt taaagaacta tttaaaacac aatgttatat ttcttataaa    7804

taccagttac tttcgttcat taattaatga aaataaatct gtgaagtacc taatttaagt    7864

actcatacta aaatttataa ggccgataat tttttgtttt cttgtctgta atggaggtaa    7924

actttatttt aaattctgtg cttaagacag gactattgct tgtcgatttt tctagaaatc    7984

tgcacggtat aatgaaaata ttaagacagt ttcccatgta atgtattcct tcttagattg    8044

catcgaaatg cactatcata tatgcttgta aatattcaaa tgaatttgca ctaataaagt    8104

cctttgttgg tatgtgaatt ctctttgttg ctgttgcaaa cagtgcatct tacacaactt    8164

cactcaattc aaaagaaaac tccattaaaa gtactaatga aaaaacatga catactgtca    8224

aagtcctcat atctaggaaa gacacagaaa ctctctttgt cacagaaact ctctgtgtct    8284

ttcctagaca taatagagtt gtttttcaac tctatgtttg aatgtggata ccctgaattt    8344

tgtataatta gtgtaaatac agtgttcagt ccttcaagtg atatttttat ttttttattc    8404

ataccactag ctacttgttt tctaatctgc ttcattctaa tgcttatatt catcttttcc    8464

ctaaatttgt gatgctgcag atcctacatc attcagatag aaacctttt ttttttcaga    8524

attatagaat tccacagctc ctaccaagac catgaggata aatatctaac acttttcagt    8584

tgctgaagga gaaaggagct ttagttatga tggataaaaa tatctgccac cctaggcttc    8644

caaattatac ttaaattgtt tacatagctt accacaatag gagtatcagg gccaaatacc    8704

tatgtaataa tttgaggtca tttctgcttt aggaaaagta ctttcggtaa attctttggc    8764
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cctgaccagt attcattatt tcagataatt ccctgtgata ggacaactag tacatttaat	8824
attctcagaa cttatggcat tttactatgt gaaaacttta aatttattta tattaagggt	8884
aatcaaattc ttaaagatga aagattttct gtattttaaa ggaagctatg ctttaacttg	8944
ttatgtaatt aacaaaaaaa tcatatataa tagagctctt tgttccagtg ttatctcttt	9004
cattgttact ttgtatttgc aatttttttt accaaagaca aattaaaaaa atgaatacca	9064
tatttaaatg gaataataaa ggtttttaa aaactttaaa	9104

1-31. (canceled)

32. A method of detecting a mutation at position 4321 in the nucleic acid molecule of SEQ ID NO: 1 or 2 in a sample, the method comprising:

(a) contacting a sample with a probe consisting of 10 to 50 nucleotides for the detection of said mutation, wherein said probe hybridizes under high stringency conditions to any of the polynucleotide sequences (i), (ii), (iii), (iv), or (v):

(i) a polynucleotide sequence selected from nucleotides 1 to 9104 of SEQ ID NO: 1 or 2;

(ii) a polynucleotide sequence selected from nucleotides 1 to 7584 of SEQ ID NO: 1 or 2;

(iii) a polynucleotide sequence selected from nucleotides 1 to 7581 of SEQ ID NO: 1 or 2;

(iv) a polynucleotide sequence selected from a nucleotide sequence coding for the protein sequence of SEQ ID NO: 1 or 2 or for the protein sequence of SEQ ID NO: 1 or 2 containing at least one of the mutations depicted in SEQ ID NO: 1 or 2; or

(v) a polynucleotide sequence selected from a nucleotide sequence complementary to any of the nucleotide sequences of (i), (ii), (iii), or (iv):

wherein the polynucleotide sequence codes for the protein sequence of SEQ ID NO. 8 or 9 containing a mutation at position 1441, and wherein said high stringency conditions comprise hybridization at 68° C. in a solution comprising 50% formamide, 5×SSC (Sodium and sodium Citrate buffer) or 5×SSPE (Sodium, Sodium Phosphate, and EDTA buffer at pH 7.7), 5× Denhardt's solution, 1% Sodium Dodecyl Sulfate (SDS), and 100 μg/ml denatured salmon sperm DNA, followed by washing at 68° C. in a buffer comprising 0.2 SSC and 0.1% SDS, and

(b) detecting the presence of the mutation in the nucleic acid of the sample.

33. The method of claim **32**, wherein the sample is selected from

(a) a biopsy from human tissue or cells; or

(b) RNA or DNA from a biopsy from human tissue or cells.

34. The method of claim **32**, wherein the detecting of the mutation comprises Southern blot hybridization, Northern blot hybridization, PCR, RT-PCR, real-time RT-PCR or automated sequencing.

35. The method of claim **32**, wherein the detecting of the mutation comprises radiography, fluorescence, chemiluminescence, or any combination thereof

36. The method of claim **32**, wherein the method is carried out on an array.

37. The method of claim **32**, wherein the method is carried out in a robotics system.

38. The method of claim **32**, wherein the method is carried out using microfluidics.

39. The method of claim **32**, wherein said probe consists of 10 to 35 nucleotides.

40. The method of claim **32**, wherein said probe consists of 20 to 35 nucleotides.

41. The method of claim **33**, wherein said sample is from the brain.

42. The method of claim **41**, wherein said sample is from putamen or substantia nigra.

43. The method of claim **33**, wherein said sample is from heart, lung, or blood lymphocytes.

44. The method of claim **33**, wherein said RNA or DNA is from the brain.

45. The method of claim **44**, wherein said RNA or DNA is from putamen or substantia nigra.

46. The method of claim **33**, wherein said RNA or DNA is from heart, lung, or blood lymphocytes.

47. The method of claim **32**, wherein said method is diagnostic of a neurodegenerative disorder selected from the group consistent of Parkinson disease (PD), sporadic PD, Alzheimer disease (AD), amyotrophic lateral sclerosis (ALS), synucleinopathy, and tauopathy.

* * * * *

专利名称(译)	Kaspp（LRRK2）基因，其产生和用于检测和治疗神经退行性疾病		
公开(公告)号	US20120035072A1	公开(公告)日	2012-02-09
申请号	US13/219131	申请日	2011-08-26
[标]申请(专利权)人(译)	梅约医学教育与研究基金会 EBERHARD卡尔斯UNIV蒂宾根		
申请(专利权)人(译)	亥姆霍兹MUENCHEN DEUTSCHES FORSCHUNGSZENTRUM FUER GESUNDHEIT UND UMWELT（GMBH 梅奥基金会的医学教育和研究 EBERHARD卡尔斯UNIVERSITAET蒂宾根		
当前申请(专利权)人(译)	亥姆霍兹MUENCHEN DEUTSCHES FORSCHUNGSZENTRUM FUER GESUNDHEIT UND UMWELT（GMBH 梅奥基金会的医学教育和研究 EBERHARD卡尔斯UNIVERSITAET蒂宾根		
[标]发明人	MEITINGER THOMAS STROM TIM MATTHIAS BISKUP SASKIA UEFFING MARIUS KREMMER ELISABETH GLOECKNER CHRISTIAN JOHANNES GASSER THOMAS ASMUS FRIEDRICH PATENGE NADJA ZIMPRICH ALEXANDER FARRER MATTHEW J KACHERGUS JENNIFER M LINCOLN SARA J HULIHAN MARY M WSZOLEK ZIBIGNIEW UITTI RYAN		
发明人	MEITINGER, THOMAS STROM, TIM MATTHIAS BISKUP, SASKIA UEFFING, MARIUS KREMMER, ELISABETH GLOECKNER, CHRISTIAN JOHANNES GASSER, THOMAS ASMUS, FRIEDRICH PATENGE, NADJA ZIMPRICH, ALEXANDER FARRER, MATTHEW J. KACHERGUS, JENNIFER M. LINCOLN, SARA J. HULIHAN, MARY M. WSZOLEK, ZIBIGNIEW UITTI, RYAN		
IPC分类号	C12Q1/68 C40B30/04 G01N33/53		
CPC分类号	C12N9/1205 A61P25/28		

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其他公开文献	US8409809
外部链接	Espacenet USPTO

摘要(译)

本发明涉及一种新发现的基因，其命名为KASPP，其用于与具有多形性病理学的帕金森综合征相关的激酶，或者称为LRRK2，用于富含亮氨酸的重复激酶2，其生产，生物化学表征以及用于检测和治疗神经变性疾病如帕金森疾病（PD）包括但不限于散发性PD，阿尔茨海默病（AD），肌萎缩性侧索硬化（ALS）和其它突触核蛋白病和/或tau病变以及在与PD分离的KASPP / LRRK2基因中的几种多态性和突变。

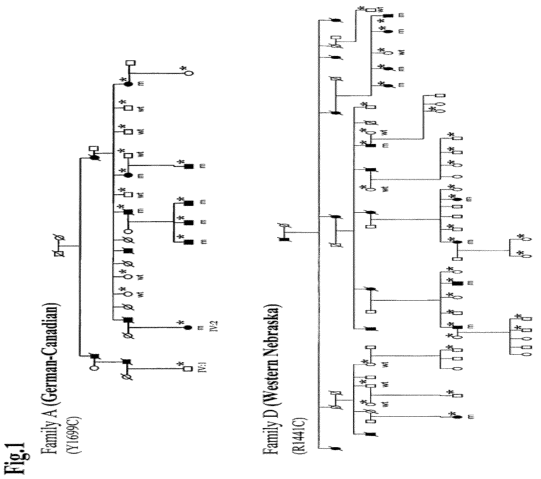

Fig.1