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(19) **United States**(12) **Patent Application Publication****Meitinger et al.**(10) **Pub. No.: US 2012/0035072 A1**(43) **Pub. Date: Feb. 9, 2012**(54) **KASPP (LRRK2) GENE, ITS PRODUCTION AND USE FOR THE DETECTION AND TREATMENT OF NEURODEGENERATIVE DISORDERS**

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(21) Appl. No.: **13/219,131**(22) Filed: **Aug. 26, 2011****Related U.S. Application Data**

(62) Division of application No. 11/665,875, filed on May 19, 2010, now Pat. No. 8,029,986, filed as application No. PCT/EP05/10428 on Sep. 27, 2005.

(60) Provisional application No. 60/620,893, filed on Oct. 21, 2004, provisional application No. 60/621,169, filed on Oct. 22, 2004.

Publication Classification

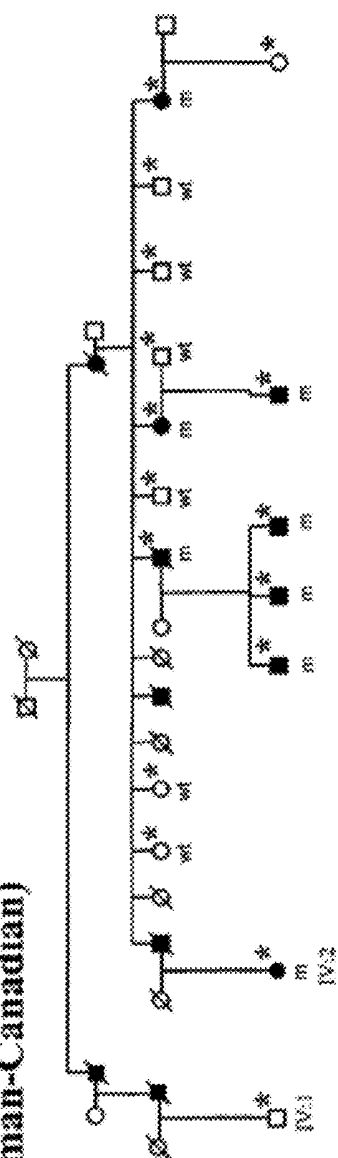
(51) **Int. Cl.**
C12Q 1/68 (2006.01)
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G01N 33/53 (2006.01)

(52) **U.S. Cl.** **506/9**; 436/501; 435/6.11(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.

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Family A (German-Canadian)
(Y1699C)



Family D (Western Nebraska)
(R1441C)

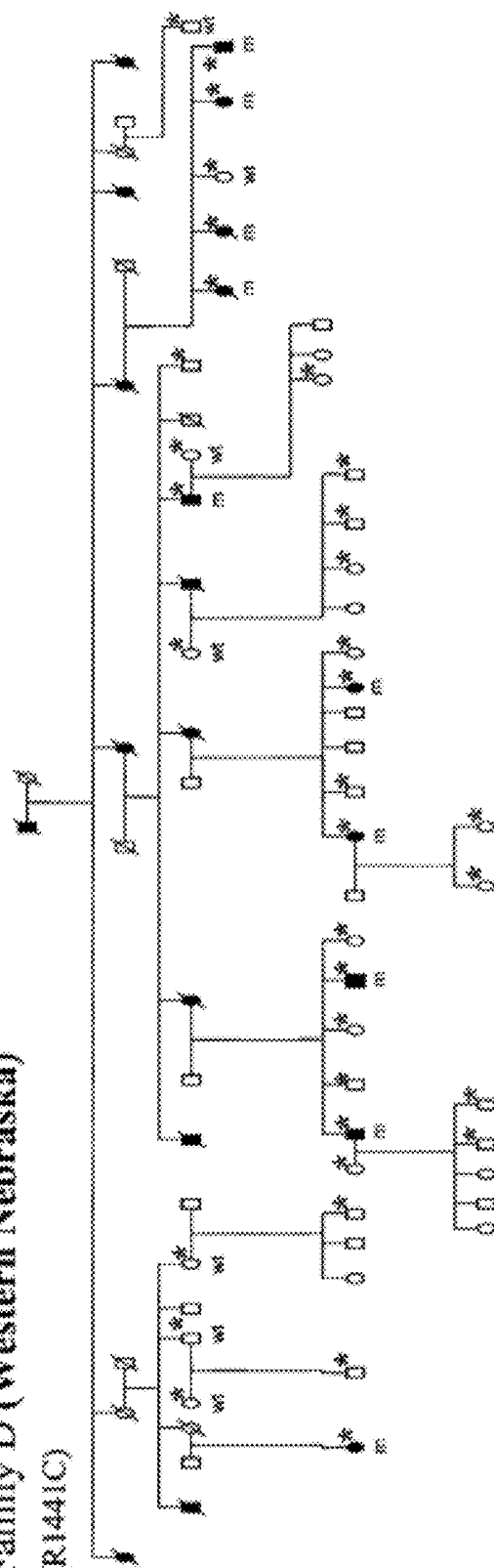


Fig. 2

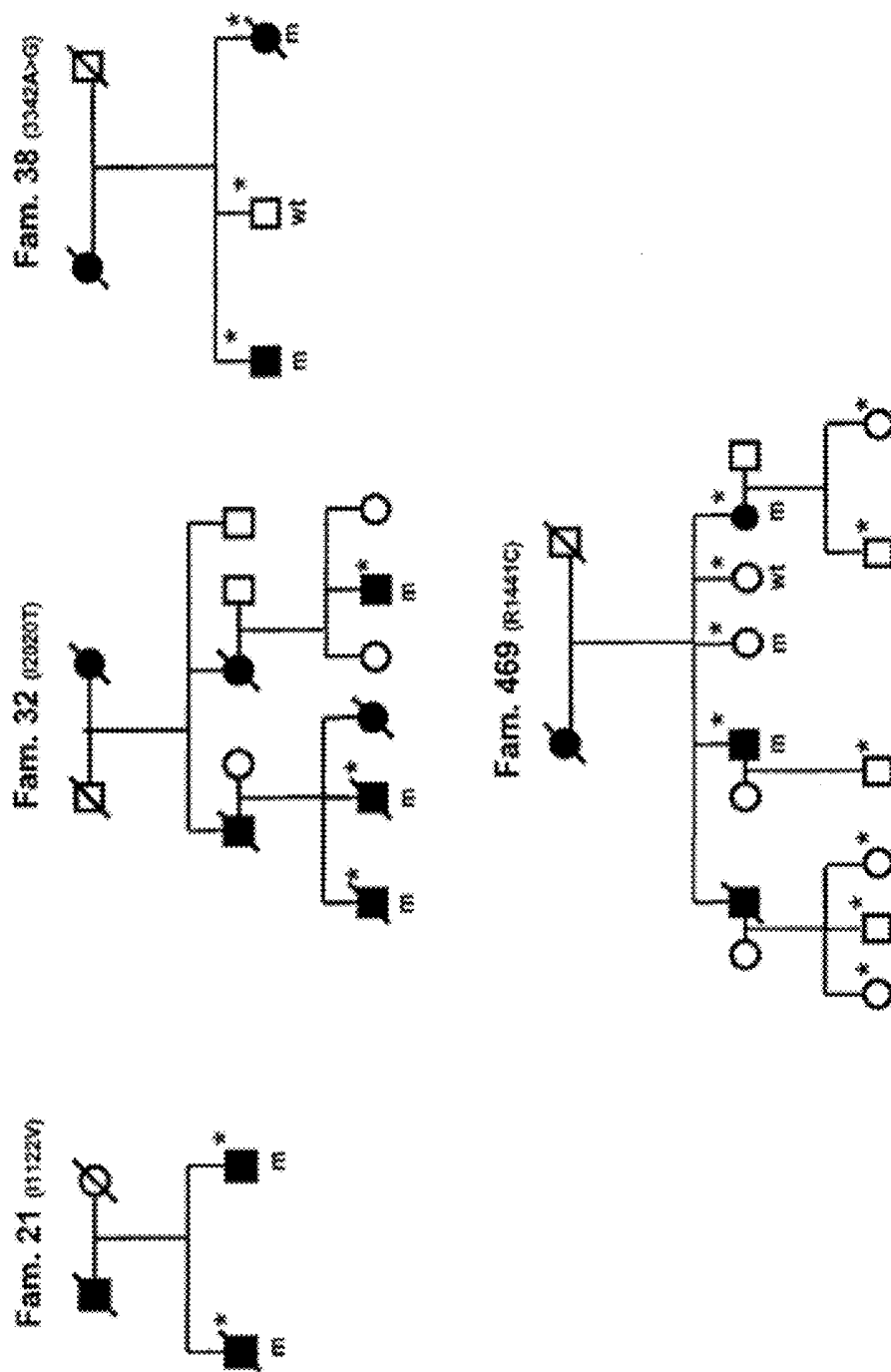


Fig.3

Human	I1122V	R1441C	Y1699C	I1122T
	ISQ IC SP	IKA RA SS	EM PP FPM	DY CT QAQY
Mouse	ISQ IC SP	IKA RA SS	EM PP FPM	DY CT QAQY
Fugu	IAELCVP	IKAVAPL	EM PP FPM	.Y CT QAQH
Worm	IQ...E	IKA RA PN	ALA TI PS	DY CT SRS
Fly	IS...CWP	IQA RA PN	LMT TI FPS	DY CT SRQ
Family	21	D/469	A	32
Exon	24	30	34	40

Fig. 4

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-----:-----:-----:-----:-----:-----:
      10      20      30      40      50      60
ATGGCTAGTGGCAGCTGTCCAGGGTCCGAAGAGGACGAGGAAACTCTGAAGAGTTGATA
M A S G S C Q G C E E D E E T L K K L I

      70      80      90      100     110     120
GTCAGGCTGAACAATGTCCAGGAAGGAAAACAGATAGAAACGCTGGTCCAAATCCTGGAG
V R L N N V Q E G K Q I E T L V Q I L E

      130     140     150     160     170     180
GATCTGCTGGTGTTCACGTACTCCGAGCACGCCCTCCAAGTTATTTCAAGGCCAAAATATC
D L L V F T Y S E H A S K L F Q G K N I
      ||
      exon1/exon2

      190     200     210     220     230     240
CATGTGCCTCTGTTGATCGTCTTGGACTCCTATATGAGAGTCGCGAGTGTGCAGCAGGTG
H V P L L I V L D S Y M R V A S V Q Q V
      ||
      exon2/exon3

      250     260     270     280     290     300
GGTTGGTCACTTCTGTGCAAAATTAATAGAAGTCTGTCCAGGTACAATGCAAAAGCTTAATG
G W S L L C K L I E V C P G T M Q S L M

      310     320     330     340     350     360
GGACCCAGGATGTTGGAAATGATTGGGAAGTCCTTGGTGTTCACCAATTGATTCTTAA
G P Q D V G N D W E V L G V H Q L I L K
      ||
      exon3/exon4

      370     380     390     400     410     420
ATGCTAACAGTTTCATAATGCCAGTGTAAACTTGTCAAGTATTGGACTGAAGACCTTAGAT
M L T V H N A S V N L S V I G L K T L D

      430     440     450     460     470     480
CTCCTCCTAACTTCAGGTAAATCACCTTGTGATACTGGATGAAGAAAGTGATATTTTC
L L L T S G K I T L L I L D E E S D I F
      ||
      exon4/exon5

      490     500     510     520     530     540
ATGTTAATTTTGTATGCCATGCACTCATTTCAGCCAATGATGAAGTCCAGAAACTTGG
M L I F D A M H S F P A N D E V Q K L G

      550     560     570     580     590     600
TGCAAAGCTTACATGTGCTGTTTGAGAGAGTCTCAGAGGAGCAACTGACTGAATTTGTT
C K A L H V L F E R V S E E Q L T E F V
      ||
      exon5/exon6

      610     620     630     640     650     660
GAGAACAAAGATTATATGATATTGTTAAGTGGGTCAACAAATTTTAAAGATGAAGAGGAA
E N K D Y M I L L S A S T N F K D E E E

      670     680     690     700     710     720
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-----|-----|-----|-----|-----|-----|-----|
ATTGTGCTTCATGTGCTGCATTGTTTACATTCCCTAGCGATTCCCTGCAATAATGTGGAA
I V L H V L H C L H S L A I P C N N V E
||
exon6/exon7

730 740 750 760 770 780
-----|-----|-----|-----|-----|-----|
GTCCTCATGAGTGGCAATGTCAGGTGTTATAATATTGTGGTGGAAAGCTATGAAAGCATT
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
-----|-----|-----|-----|-----|-----|
CCTATGAGTGAAGAATTCAAGAAGTGAGTGTCTGTTTGTCTCATAGGCTTACATTAGGT
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
-----|-----|-----|-----|-----|-----|
AATTTTTCATATCCTGGTATTAAACGAAGTCCATGAGTTTGTGGTGAAGCTGTGCAG
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
-----|-----|-----|-----|-----|-----|
CAGTACCCAGAGAATGCAGCATTGCAGATCTCAGCGCTCAGCTGTTTGGCCCTCCTCACT
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
-----|-----|-----|-----|-----|-----|
GAGACTATTTTCTTAAATCAAGATTTAGAGGAAAAGAAATGAGAATCAAGAGAATGATGAT
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
-----|-----|-----|-----|-----|-----|
GAGGGGGAAGAAGATAAATTGTTTGGCTGGAAGCCTGTTACAAAGCATTAACGTGGCAT
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
-----|-----|-----|-----|-----|-----|
AGAAAGAACAAGCAGTGCAGGAGGCGCATGCTGGGCACTAAATAATCTCCTTATGTAC
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
-----|-----|-----|-----|-----|-----|
CAAAACAGTTTACATGAGAAGATTGGAGATGAAGATGGCCATTTCAGCTCATAGGGAA
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
-----|-----|-----|-----|-----|-----|
GTGATGCTCTCCATGCTGATGCATTCTTCATCAAAGGAAGTTTTCAGGCATCTGCGAAT
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
-----|-----|-----|-----|-----|-----|
GCATTGTCAACTCTCTAGAACAAAATGTTAATTCAGAAAAATACTGTTATCAAAAGGA
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
-----|-----|-----|-----|-----|-----|

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

-----|-----|-----|-----|-----|-----|
1390 1400 1410 1420 1430 1440
AGTGGCTGTAAATGCTAAATCATCTTTTGAAGGAAGCAACACTTCCCTGGATATAATG
S G C K M L N H L F E G S N T S L D I M

||
exon12/exon13

-----|-----|-----|-----|-----|-----|
1450 1460 1470 1480 1490 1500
GCAGCAGTGGTCCCAAAATACTAACAGTTATGAAACGTCATGAGACATCATTACCAGTG
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
AGCGAGGATACAGAATTTTCATCATAAGCTAAATATGGTTAAAAAACAGTGTTCAGAAAT
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
GATATTCACAACTGGTCTAGCAGCTTTGAACAGGTTCAATTGGAAATCCTGGGATTGAG
D I H K L V L A A L N R F I G N P G I Q

||
exon14/exon15

-----|-----|-----|-----|-----|-----|
1690 1700 1710 1720 1730 1740
AAATGTGGATTAAGTAATTTCTTCTATTGTACATTTTCTGATGCATTAGAGATGTTA
K C G L K V I S S I V H F P D A L E M L

-----|-----|-----|-----|-----|-----|
1750 1760 1770 1780 1790 1800
TCCCTGGAAGGTGCTATGGATTGAGTCTTCACACACTGCAGATGTATCCAGATGACCAA
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
GAAATTCAGTGTCTGGGTTTAAGTCTTATAGGATACTTGATTACAAAGAAGAATGTGTTT
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
ATAGGAACTGGACATCTGCTGGCAAAATCTGGTTTCCAGCTTATACCGATTTAAGGAT
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
GTTGCTGAAATACAGACTAAAGGATTTGAGACAATCTTAGCAATCCTCAAATTTGTCAGCA
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
TCYTTTTCTAAGCTGCTGGTGCATCATTGACTTAGTAATATTCCATCAAATGTCT
S F S K L L V H H S F D L V I F H Q M S

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2050 2060 2070 2080 2090 2100
-----|-----|-----|-----|-----|-----|
TCCAATATCATGGAACAAAAGGATCAACAGTTTCTAAACCTCTGTTGCAAGTGTGTTGCA
S N I M E Q K D Q Q F L N L C C K C F A
||
exon17/exon18

2110 2120 2130 2140 2150 2160
-----|-----|-----|-----|-----|-----|
AAAGTAGCTATGGATGATTACTTAAAAAATGTGATGCTAGAGAGAGCGTGTGATCAGAAT
K V A M D D Y L K N V M L E R A C D Q N

2170 2180 2190 2200 2210 2220
-----|-----|-----|-----|-----|-----|
AACAGCATCATGGTTGAATGCTTGCTTCTATTGGGAGCAGATGCCAATCAAGCAAAGGAG
N S I M V E C L L L L G A D A N Q A K E

2230 2240 2250 2260 2270 2280
-----|-----|-----|-----|-----|-----|
GGATCTTCTTTAATTTGTGAGGTATGTGAGAAAGAGAGCAGTCCCAAATTGGTGGAACTC
G S S L I C Q V C E K E S S P K L V E L
||
exon18/exon19

2290 2300 2310 2320 2330 2340
-----|-----|-----|-----|-----|-----|
TTACTGAATAGTGGATCTCGTGAACAAGATGTACGAAAAGCGTTGACGATAAGCATTGGG
L L N S G S R E Q D V R K A L T I S I G

2350 2360 2370 2380 2390 2400
-----|-----|-----|-----|-----|-----|
AAAGGTGACAGCCAGATCATCAGCTTGCTCTTAAGGAGGCTGGCCCTGGATGTGGCCAAC
K G D S Q I I S L L L R R L A L D V A N

2410 2420 2430 2440 2450 2460
-----|-----|-----|-----|-----|-----|
AATAGCATTTGCCTTGGAGGATTTGTATAGGAAAAGTTGAACCTTCTTGCTTGGTCTCT
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
-----|-----|-----|-----|-----|-----|
TYATTTCAGATAAGACTTCTAATTTAAGGAAACAAACAAATATAGCATCTACACTAGCA
L F P D K T S N L R K Q T N I A S T L A
||
exon19/exon20

2530 2540 2550 2560 2570 2580
-----|-----|-----|-----|-----|-----|
AGAAATGGTGATCAGATATCAGATGAAAAGTGCTGTGGAAGAAGGAACAGCCTCAGGCAGC
R M V I R Y Q M K S A V E E G T A S G S

2590 2600 2610 2620 2630 2640
-----|-----|-----|-----|-----|-----|
GATGGAAATTTTCTGAAGATGTGCTGTCTAAATTTGATGAATGGACCTTTATTCCTGAC
D G N F S E D V L S K F D E W T F I P D

2650 2660 2670 2680 2690 2700
-----|-----|-----|-----|-----|-----|
TCTTCTATGGACAGTGTGTTTGCTCAAAGTGATGACCTGGATAGTGAAGGAAGTGAAGGC
S S M D S V F A Q S D D L D S E G S E G
||
exon20/exon21

2710 2720 2730 2740 2750 2760
-----|-----|-----|-----|-----|-----|
TCATTTCTTGTGAAAAAGAAATCTAATTCAATTAGTGTAGGAGAATTTTACCGAGATGCC
S F L V K K K S N S I S V G E F Y R D A

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2770 2780 2790 2800 2810 2820
-----|-----|-----|-----|-----|-----|
GTATTACAGCGTTGCTCACCAGAAATTTGCAAAGACATTCCAATTCCTTGGGGCCCATTTTT
V L Q R C S P N L Q R H S N S L G P I F
||
exon21/exon22

2830 2840 2850 2860 2870 2880
-----|-----|-----|-----|-----|-----|
GATCATGAAGATTTACTGAAGCGAAAAAGAAAAATACTATCTTCAGATGATTCACCTCAGG
D H E D L L K R K R K I L S S D D S L R
||
exon22/exon23

2890 2900 2910 2920 2930 2940
-----|-----|-----|-----|-----|-----|
TCATCAAAACTTCAATCCCATATGAGGCATTGACACAGCATTTCTTCTCTGGCTTCTGAG
S S K L Q S H M R H S D S I S S L A S E

2950 2960 2970 2980 2990 3000
-----|-----|-----|-----|-----|-----|
AGAGAAATATATTACATCACTAGACCTTTGAGCAATGAACTAAGAGATATTGATGCCCTA
R E Y I T S L D L S A N E L R D I D A L

3010 3020 3030 3040 3050 3060
-----|-----|-----|-----|-----|-----|
AGCCAGAAATGCTGTATAAGTGTTTCAATTTGGAGCATCTTGAAAAGCTGGAGCTTCAACAG
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
-----|-----|-----|-----|-----|-----|
AATGCACTCAGGAGCTTTCCACAACAGCTATGTGAAACTCTGAAGAGTTTGACACATTTG
N A L T S F P Q Q L C E T L K S L T H L
||
exon23/exon24

3130 3140 3150 3160 3170 3180
-----|-----|-----|-----|-----|-----|
GACTTGACAGTAATAAATTTACATCATTTCTTCTTATTTGTTGAAAATGAGTTGTATT
D L H S N K F T S F P S Y L L K M S C I

3190 3200 3210 3220 3230 3240
-----|-----|-----|-----|-----|-----|
GCTAATCTTGATGTCTCTCGAAATGACATTTGGACCTCAGTGGTTTATAGATCCTACAGTG
A N L D V S R N D I G P S V V L D P T V

3250 3260 3270 3280 3290 3300
-----|-----|-----|-----|-----|-----|
AAATGTCCAACCTCTGAAACAGTTTAACTGTATATAACCACTGTCTTTTGTACCTGAG
K C P T L K Q F N L S Y N Q L S F V P E

3310 3320 3330 3340 3350 3360
-----|-----|-----|-----|-----|-----|
AACCTCACTGATGTGGTAGAGAACTGGAGCAGCTCATTTTAGAAGGAAATAAAATATCA
N L T D V V E K L E Q L I L E G N K I S
||
exon24/exon25

3370 3380 3390 3400 3410 3420
-----|-----|-----|-----|-----|-----|
GGGATATGCTCCCTTTGAGACTGAAGGAAGTGAAGATTTAAACCTTAGTAAGAACCAC
G I C S P L R I K E L K I L N L S K N H

3430 3440 3450 3460 3470 3480
-----|-----|-----|-----|-----|-----|
ATTTTCATCCCTATCAGAGAACTTTCTTGAGGCTTGTCTAAAGTGGAGAGTTTCAGTGCC
I S S L S E N F L E A C P K V E S F S A

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3490 3500 3510 3520 3530 3540
-----|-----|-----|-----|-----|-----|
AGAATGAATTTTCTTGCTGCTATGCCTTTCCTGCCTCCTTCTATGACAATCCTAAATTA
R M N F L A A M P F L P P S M T I L K L
||
exon25/exon26

3550 3560 3570 3580 3590 3600
-----|-----|-----|-----|-----|-----|
TCTCAGAACAAATTTTCTGTATTCCAGAAGCAATTTTAAATCTTCCACACTTGGGGTCT
S Q N K F S C I P E A I L N L P H L R S
||
exon26/exon27

3610 3620 3630 3640 3650 3660
-----|-----|-----|-----|-----|-----|
TTAGATATGAGCAGCAATGATATTCACTACCTACCAGGTCCCGCACACTGGAAATCTTTG
L D M S S N D I Q Y L P G P A H W K S L

3670 3680 3690 3700 3710 3720
-----|-----|-----|-----|-----|-----|
AACTTAAGGGAACCTTATTAGCCATAATCAGATCAGCATCTTGGACTTGAGTGAAAAA
N L R E L L F S H N Q I S I L D L S E K

3730 3740 3750 3760 3770 3780
-----|-----|-----|-----|-----|-----|
GCATATTTATGGTCTAGAGTAGAGAACTGCATCTTTCTCACAATAAACTGAAAGAGATT
A Y L W S R V E K L H L S H N K L K E I
||
exon27/exon28

3790 3800 3810 3820 3830 3840
-----|-----|-----|-----|-----|-----|
CCTCCTGAGATTGGCTGTCTTGAAAATCTGACATCTCTGGATGTCAGTTACAACCTGGAA
P P E I G C L E N L T S L D V S Y N L E

3850 3860 3870 3880 3890 3900
-----|-----|-----|-----|-----|-----|
CTAAGATCCTTTCCCAATGAAATGGGGAAATTAAGCAAAATATGGGATCTTCCCTTGGAT
L R S F P N E M G K L S K I W D L P L D

3910 3920 3930 3940 3950 3960
-----|-----|-----|-----|-----|-----|
GAACTGCATCTTAACTTTGATTTTAAACATATAGGATGTAAAGCCAAAGACATCATAAGG
E L H L N F D F K H I G C K A K D I I R
||
exon28/exon29

3970 3980 3990 4000 4010 4020
-----|-----|-----|-----|-----|-----|
TTTCTTCAACAGCGATTAAAAAAGGCTGTGCCTTATAACCGAATGAAACTTATGATTGTG
F L Q Q R L K K A V P Y N R M K L M I V

4030 4040 4050 4060 4070 4080
-----|-----|-----|-----|-----|-----|
GGAAATACTGGGAGTGGTAAAACCACTTATTGCAGCAATTAATGAAAACCAAGAAATCA
G N T G S G K T T L L Q Q L M K T K K S

4090 4100 4110 4120 4130 4140
-----|-----|-----|-----|-----|-----|
GATCTTGGAAATGCAAAGTGCCACAGTTGGCATAGATGTGAAAGACTGGCCTATCCAAATA
D L G M Q S A T V G I D V K D W P I Q I

4150 4160 4170 4180 4190 4200
-----|-----|-----|-----|-----|-----|
AGAGACAAAAGAAAGAGAGATCTGTCCTAAATGTGTGGGATTTTGCAGGTCGTGAGGAA
R D K R K R D L V L N V W D F A G R E E
||

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exon29/exon30

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-----|-----|-----|-----|-----|-----|
4210      4220      4230      4240      4250      4260
TTCTATAGTACTCATCCCCATTTTATGACGCGAGCAGCATTGTACCTTGCTGTCTATGAC
F Y S T H P H F M T Q R A L Y L A V Y D

-----|-----|-----|-----|-----|-----|
4270      4280      4290      4300      4310      4320
CTCAGCAAGGGACAGGCTGAAGTTGATGCCATGAAGCCTTGGCTCTTCAATATAAAGGCT
L S K G Q A E V D A M K P W L F N I K A
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exon30/exon31

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-----|-----|-----|-----|-----|-----|
4330      4340      4350      4360      4370      4380
CGCGCTTCTCTTCCCTGTGATTCTCGTTGGCACACATTGGATGTTTCTGATGAGAAG
R A S S S P V I L V G T H L D V S D E K

-----|-----|-----|-----|-----|-----|
4390      4400      4410      4420      4430      4440
CAACGCAAAGCCTGCATGAGTAAATCACCAAGGAACTCCTGAATAAGCGAGGGTTCCCT
Q R K A C M S K I T K E L L N K R G F P

-----|-----|-----|-----|-----|-----|
4450      4460      4470      4480      4490      4500
GCCATACGAGATTACCACTTTGTGAATGCCACCGAGGAATCTGATGCTTTGGCAAACTT
A I R D Y H F V N A T E E S D A L A K L

-----|-----|-----|-----|-----|-----|
4510      4520      4530      4540      4550      4560
CGGAAAACCATCATAAACGAGAGCCTTAATTTCAAGATCCGAGATCAGCTTGTGTTGGA
R K T I I N E S L N F K I R D Q L V V G
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exon31/exon32

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-----|-----|-----|-----|-----|-----|
4570      4580      4590      4600      4610      4620
CAGCTGATTCAGACTGCTATGTAGAACTTGAAAAATCATTTTATCGGAGCGTAAAAAT
Q L I P D C Y V E L E K I I L S E R K N

-----|-----|-----|-----|-----|-----|
4630      4640      4650      4660      4670      4680
GTGCCAATTGAATTTCCCGTAATTGACCGGAAACGATTATTACAAC TAGTGAGAGAAAAT
V P I E F P V I D R K R L L Q L V R E N

-----|-----|-----|-----|-----|-----|
4690      4700      4710      4720      4730      4740
CAGCTGCAGTTAGATGAAAATGAGCTTCCTCACGCAGTTCACCTTCTAAATGAATCAGGA
Q L Q L D E N E L P H A V H F L N E S G
```

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exon32/exon33

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-----|-----|-----|-----|-----|-----|
4750      4760      4770      4780      4790      4800
GTCCTTCTTCATTTTCAAGACCCAGCACTGCAGTTAAGTGACTTGTACTTTGTGGAACCC
V L L H F Q D P A L Q L S D L Y F V E P

-----|-----|-----|-----|-----|-----|
4810      4820      4830      4840      4850      4860
AAGTGGCTTTGTAAAATCATGGGCACAGATTTTGACAGTGAAAGTGGAAGGTTGTCCAAA
K W L C K I M A Q I L T V K V E G C P K
```

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exon33/exon34

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-----|-----|-----|-----|-----|-----|
4870      4880      4890      4900      4910      4920
CACCCCTAAGGGCATTATTTCCGCTAGAGATGTGGAAAAATTTCTTTCAAAAAAAGGAAA
```


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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

-----|-----|-----|-----|-----|-----|

CCAGAGTTTCTCCTAGGTGATGGCAGTTTGGATCAGTTTACCGAGCAGCCTATGAAGGA

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

-----|-----|-----|-----|-----|-----|

GAAGAAGTGGCTGTGAAGATTTTAAATAACATACATCACTCAGGCTGTAAAGACAAGAG

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

-----|-----|-----|-----|-----|-----|

CTTGTGGTGTCTTGGCCACCTCCACCACCCAGTTTGATATCTTTGCTGGCAGCTGGGATT

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

-----|-----|-----|-----|-----|-----|

CGTCCCCGGATGTGGTGTGGAGTTAGCCTCCAAGGGTTCCTTGGATCGCCTGCTTCAG

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

-----|-----|-----|-----|-----|-----|

GGTTTGAGATACCTCCACTCAGCCATGATTATATACCGAGACCTGAAACCCACAAATGTG

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

-----|-----|-----|-----|-----|-----|

CTGCTTTTCACTGTATCCCAATGCTGCCATCATTGCAAAGATTGCTGACTACGGCATT

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

-----|-----|-----|-----|-----|-----|

GCTCAGTACTGCTGTAGAAATGGGGATAAAAACATCAGAGGGCACACCAGGGTTTCGTGCA

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

-----|-----|-----|-----|-----|-----|

TTACTACTCTATGACATTTTGACAACCTGGAGGTAGAATAGTAGAGGGTTTGAAGTTTCCA

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

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-----|-----|-----|-----|-----|-----|-----|-----|
TGTGCCCCATGGCCTATGGTTGAGAAATTAATTAAACAGTGTGTTGAAAGAAAATCCTCAA
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
-----|-----|-----|-----|-----|-----|-----|-----|
GAAAGGCCTACTTCTGCCAGGTCTTTGACATTTTGAATTCAGCTGAATTAGTCTGTCTG
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
-----|-----|-----|-----|-----|-----|-----|-----|
AACAGCAGGAATGCAAGCATTTGGCTGGGCTGTGGGCACACCGACAGAGGACAGCTCTCA
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
-----|-----|-----|-----|-----|-----|-----|-----|
TGCTTAGCCTTGGTGCATCTTCTGTTGAAAAGGAAAGCTGGATTGTGTCTGGGACACAG
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
-----|-----|-----|-----|-----|-----|-----|-----|
TCTGGTACTCTCCTGGTCAATACCGAAGATGGGAAAAGAGACATACCTTAGAAAAG
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
-----|-----|-----|-----|-----|-----|-----|-----|
ATGACTGATTCTGTCACTTGTTTGATTGCAATTCCTTTTCCAAGCAAAGCAAACAAAA
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
-----|-----|-----|-----|-----|-----|-----|-----|
AATTTCTTTTGGTTGGAACCGCTGATGGCAAGTTAGCAATTTTGAAGATAAGACTGTT
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
-----|-----|-----|-----|-----|-----|-----|-----|
ATGTGTTTGAGTGAATCCACAAATTCAACGGAAAGAAATGTAATGTGGGAGGATGTGGC
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
-----|-----|-----|-----|-----|-----|-----|-----|
ACAAAGATTTTCCTTTTCTAATGATTTCAACATTCAGAAACTATTGAGACAAGAACA
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
-----|-----|-----|-----|-----|-----|-----|-----|
seite 10

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```
-----|-----|-----|-----|-----|-----|
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
-----|-----|-----|-----|-----|-----|
ACTGCTCTCTATATTGCTAAGCAAAATAGCCCTGTTGTGGAGTGTGGGATAAGAAAAC
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
-----|-----|-----|-----|-----|-----|
GAAAAACTCTGTGGACTAATAGACTGCGTGCACCTTTTAAGGGAGGTAATGGTAAAGAA
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
-----|-----|-----|-----|-----|-----|
AACAAGGAATCAAAACACAAATGTCTTATTCTGGGAGAGTGAAAACCTCTGCCTTCAG
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
-----|-----|-----|-----|-----|-----|
AAGAACACTGCTCTTTGGATAGGAACTGGAGGAGGCCATATTTACTCCTGGATCTTTCA
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
-----|-----|-----|-----|-----|-----|
ACTCGTCGACTTATACGTGTAATTTACAACCTTTTGTAAATTCGGTCAGAGTCATGATGACA
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
```

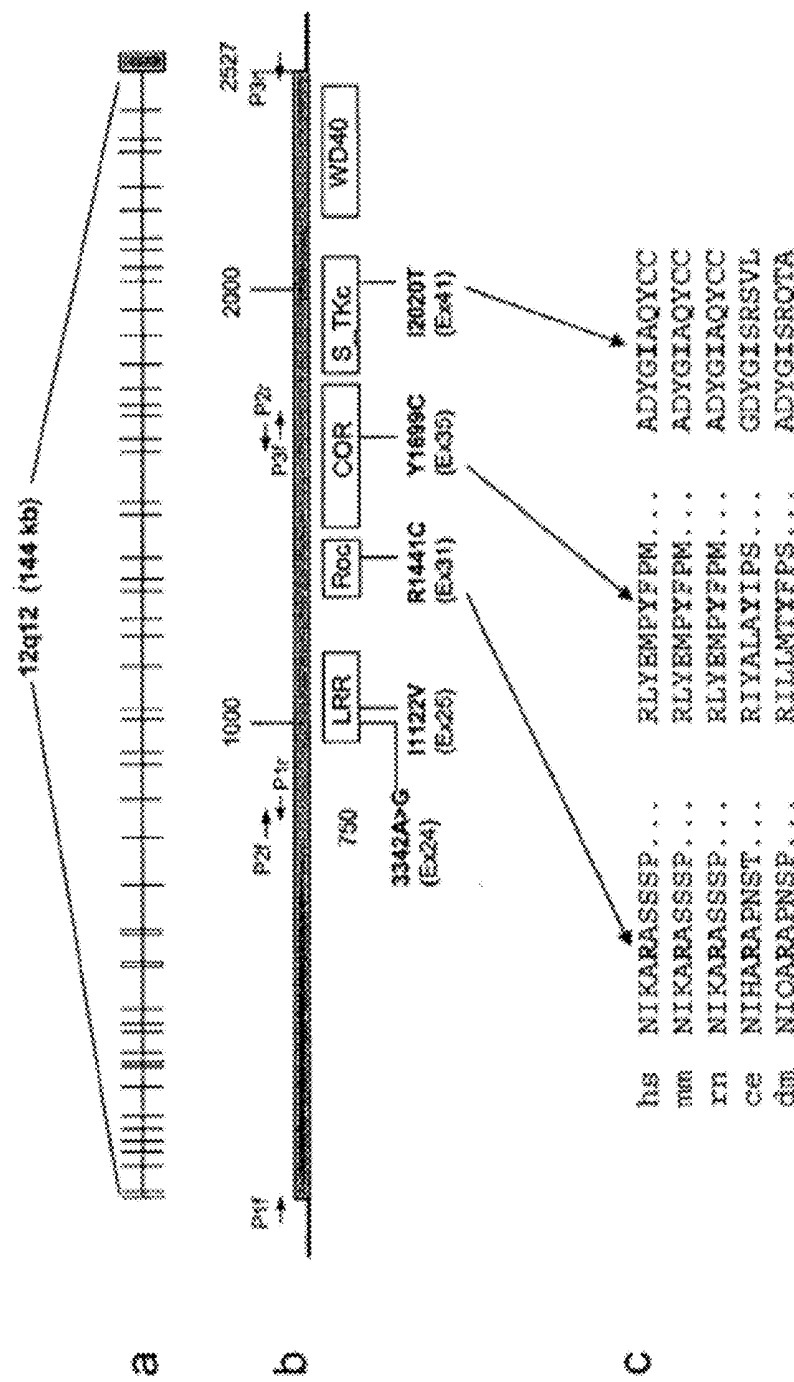
```
7450      7460      7470      7480      7490      7500
-----|-----|-----|-----|-----|-----|
GAAGGTACACAAAAGCAGAAAGAGATACAATCTTGCTTGACCGTTTGGGACATCAATCTT
E G T Q K Q K E I Q S C L T V W D I N L
```

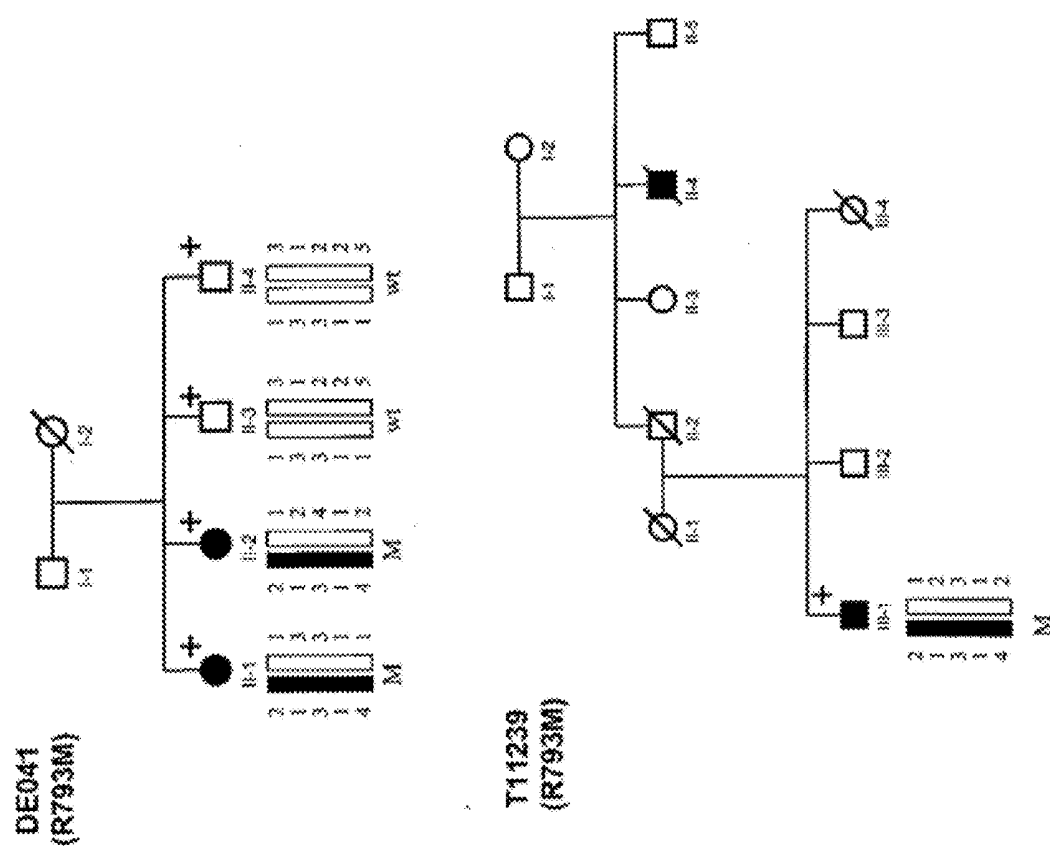
```
||
exon50/exon51
```

```
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
```

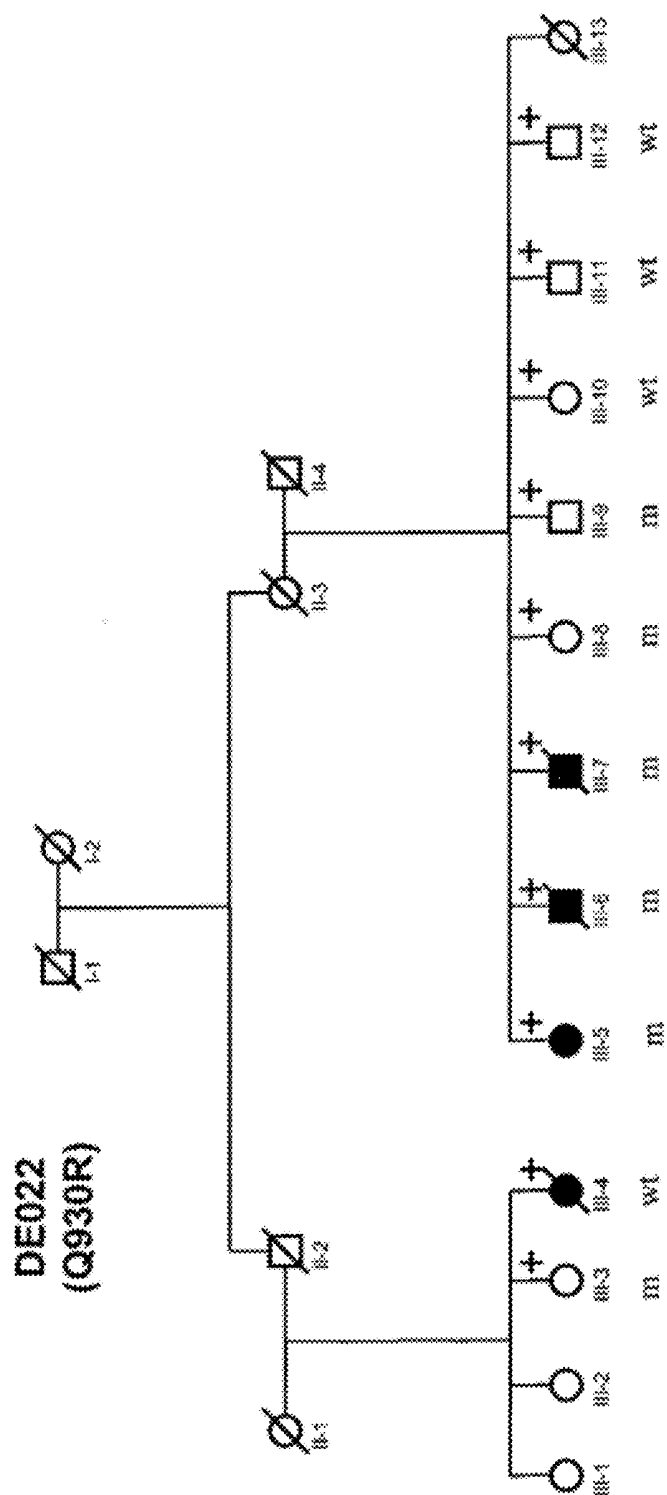
```
7570      7580      7590      7600      7610      7620
-----|-----|-----|-----|-----|-----|
ATGAGACGAACATCTGTTGAGTAA
M R R T S V E *
```

Fig. 5





80



600

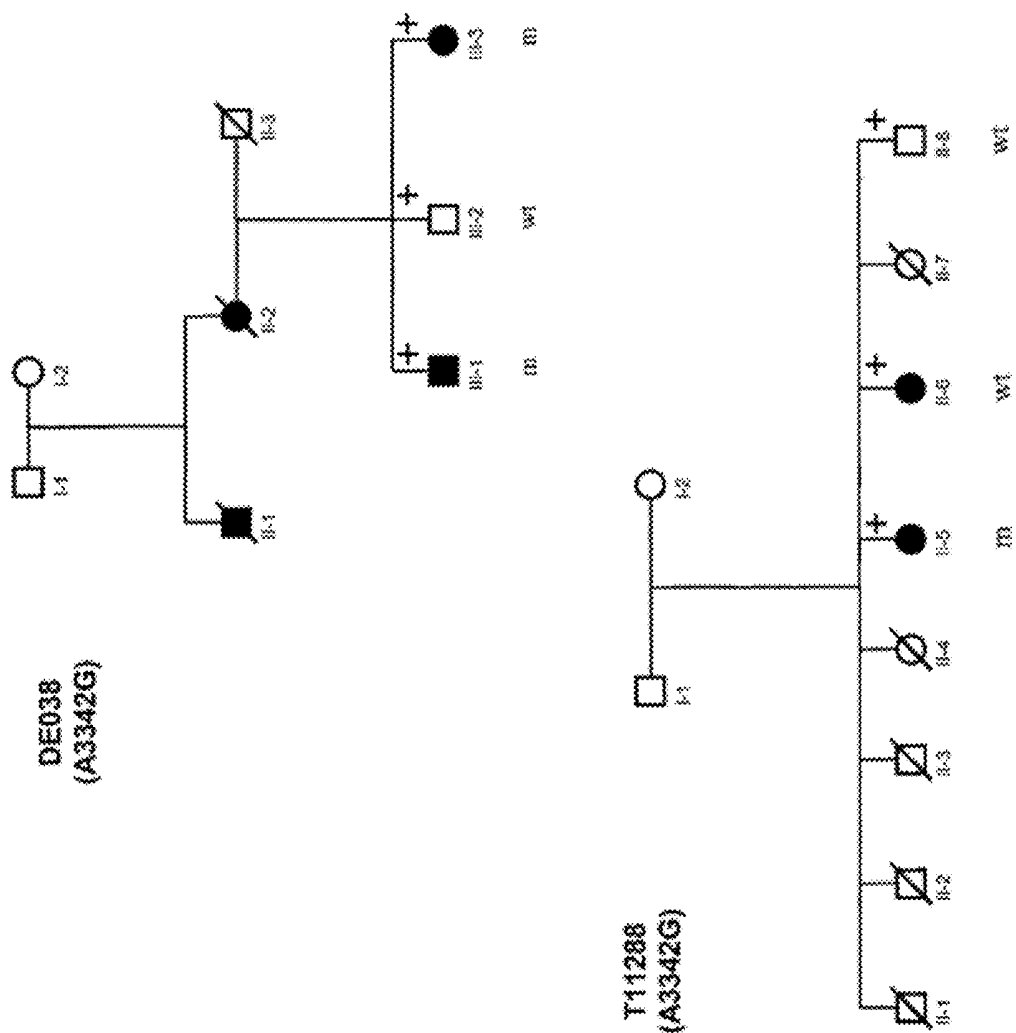
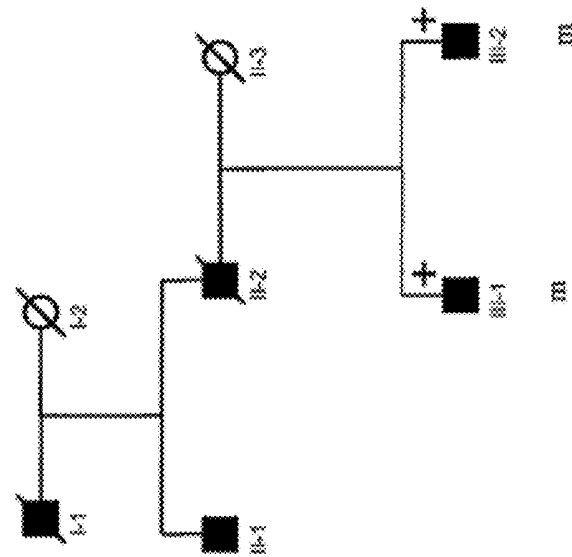
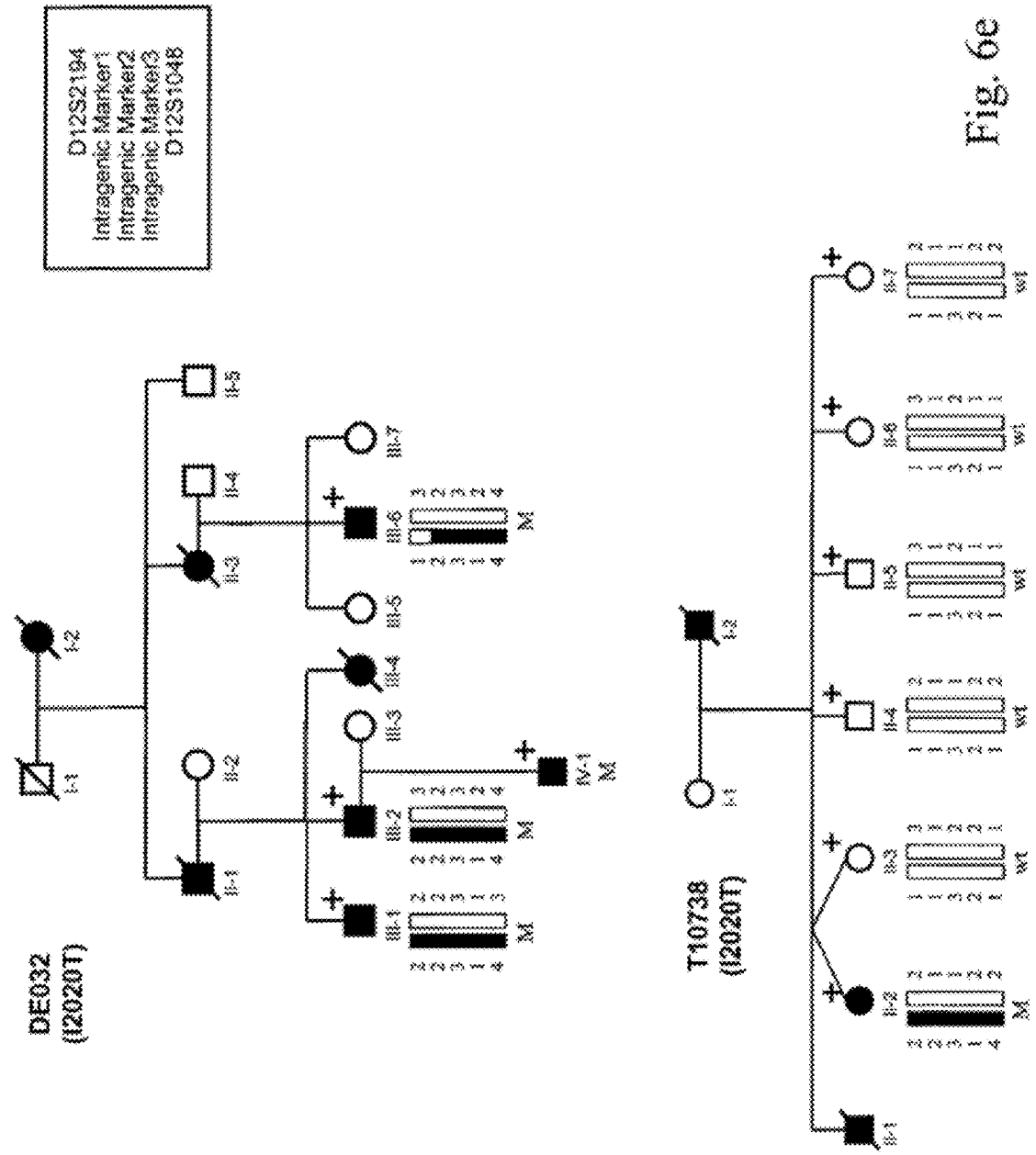


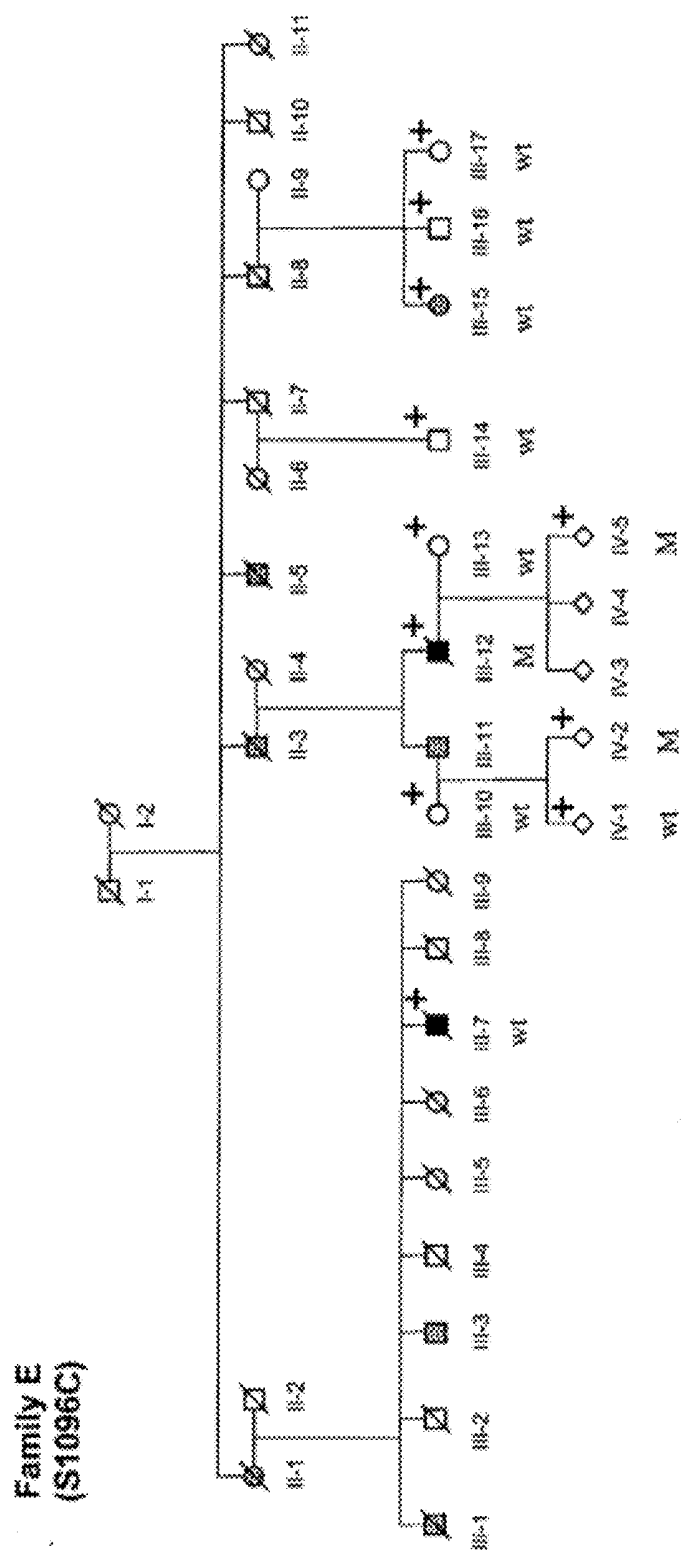
Fig. 6c



DE031
(S1228T)

Fig. 6d





60
61
62

Fig. 7

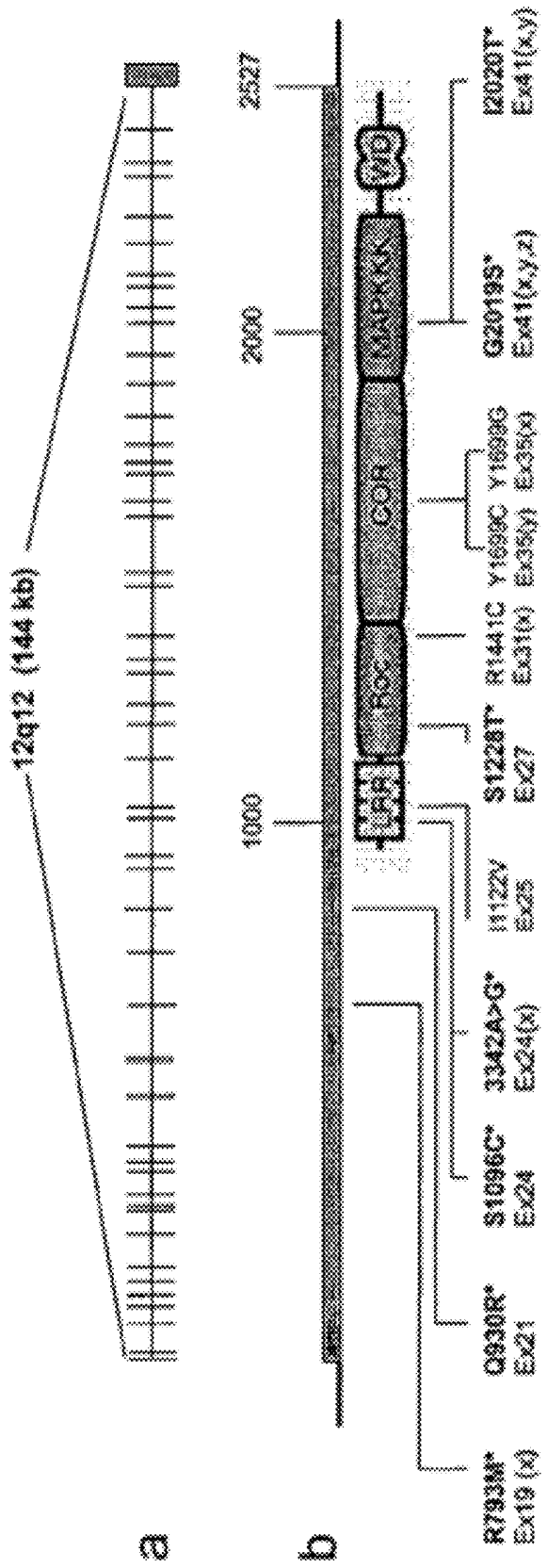


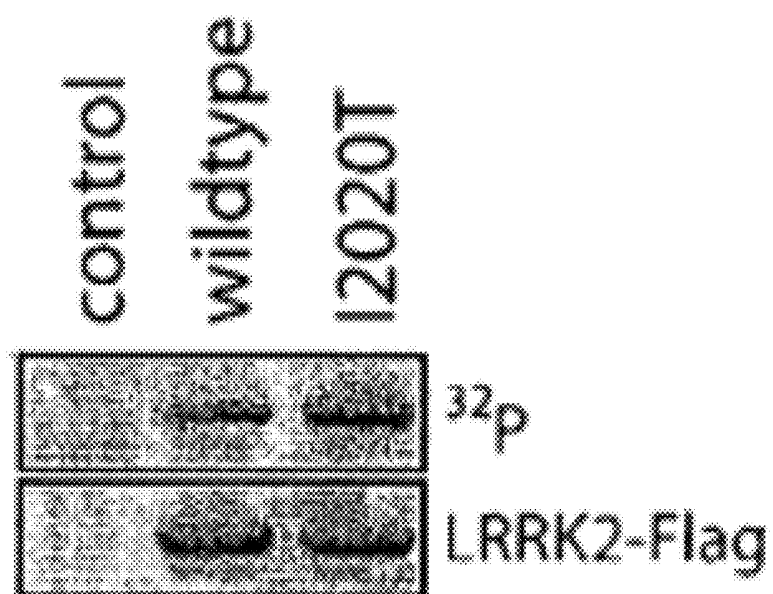
Figure 8

Figure 9

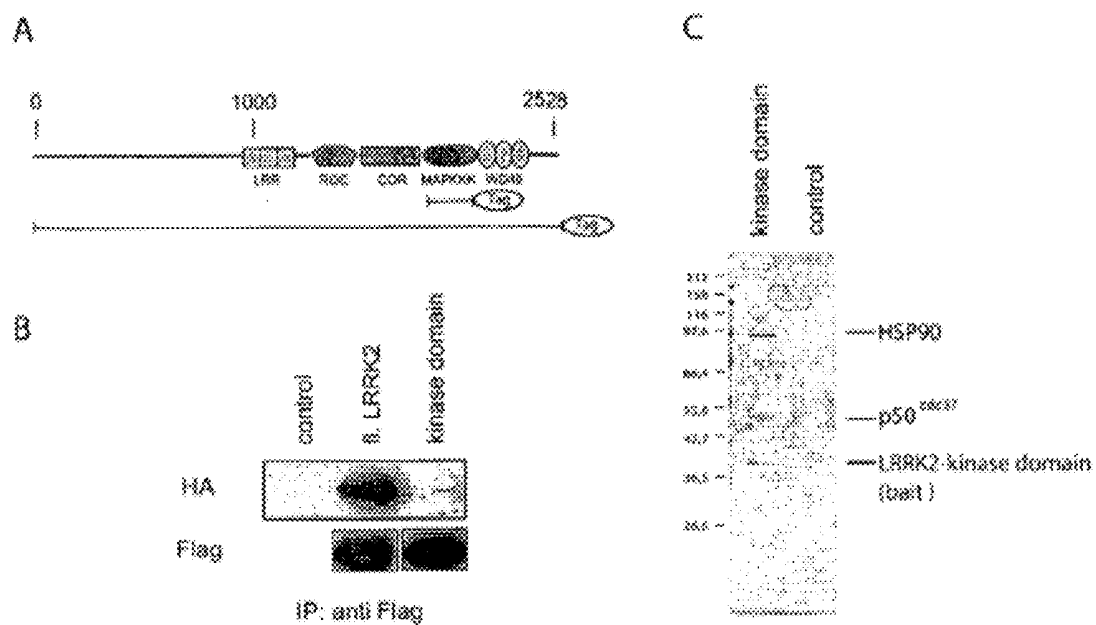


Figure 10

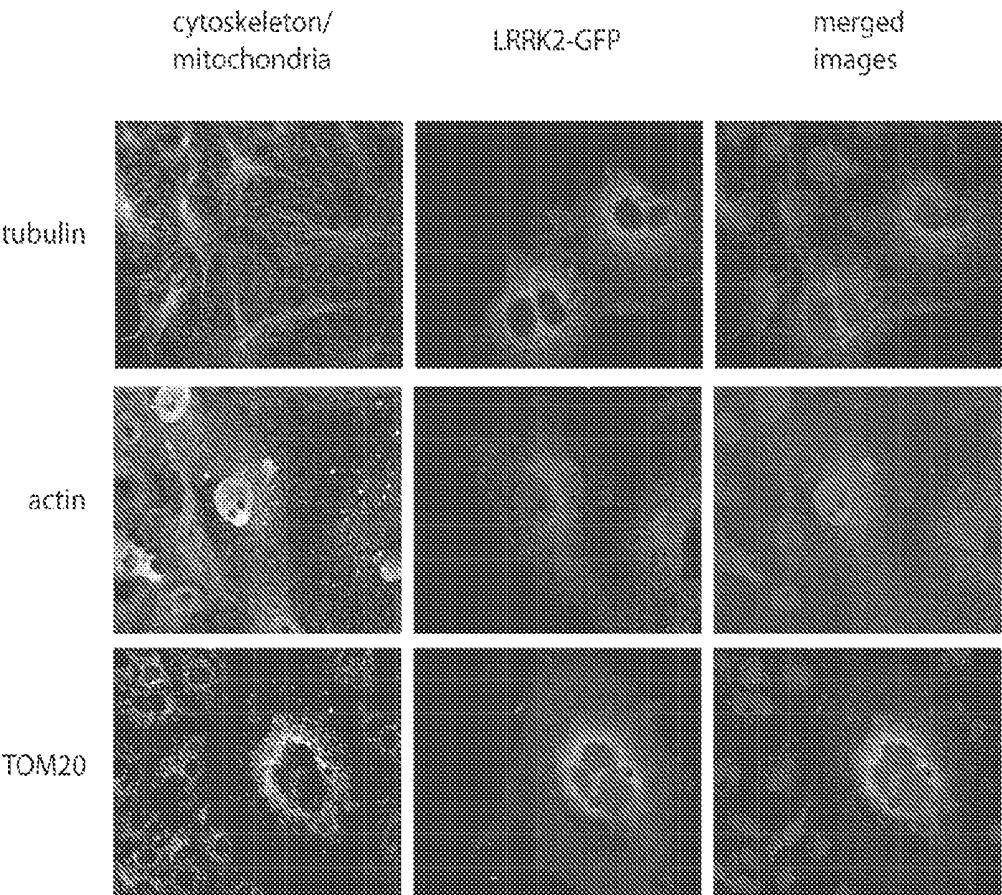


Figure 11

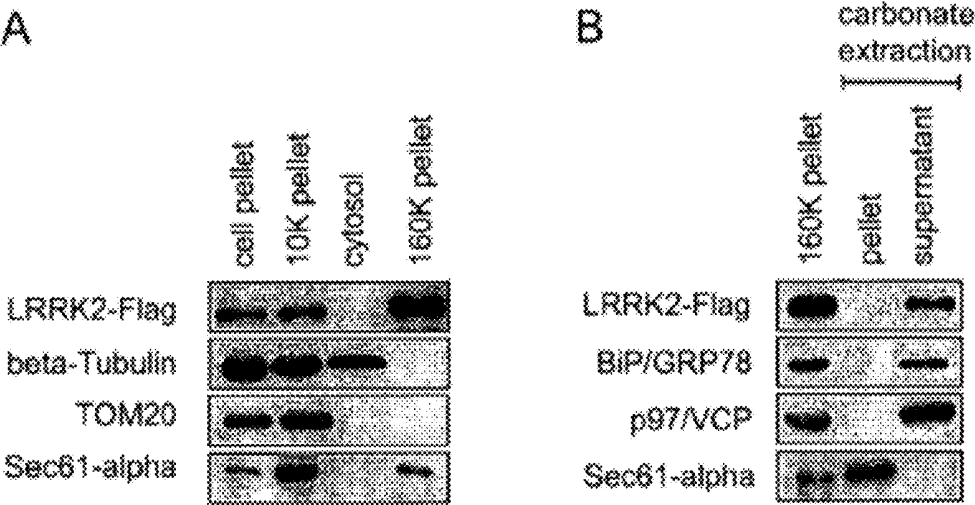


Figure 12

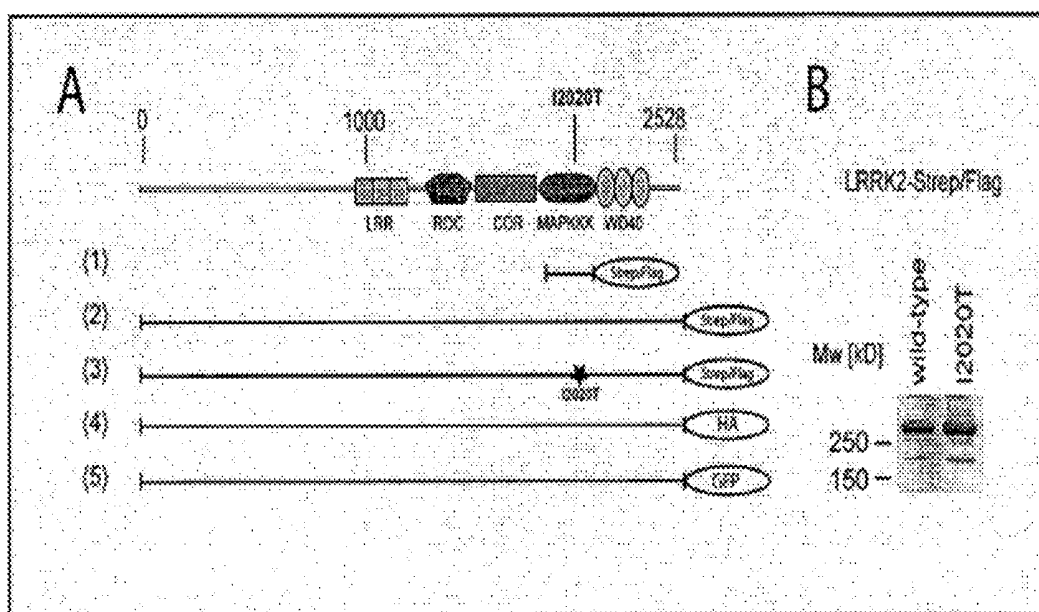


Figure 13:

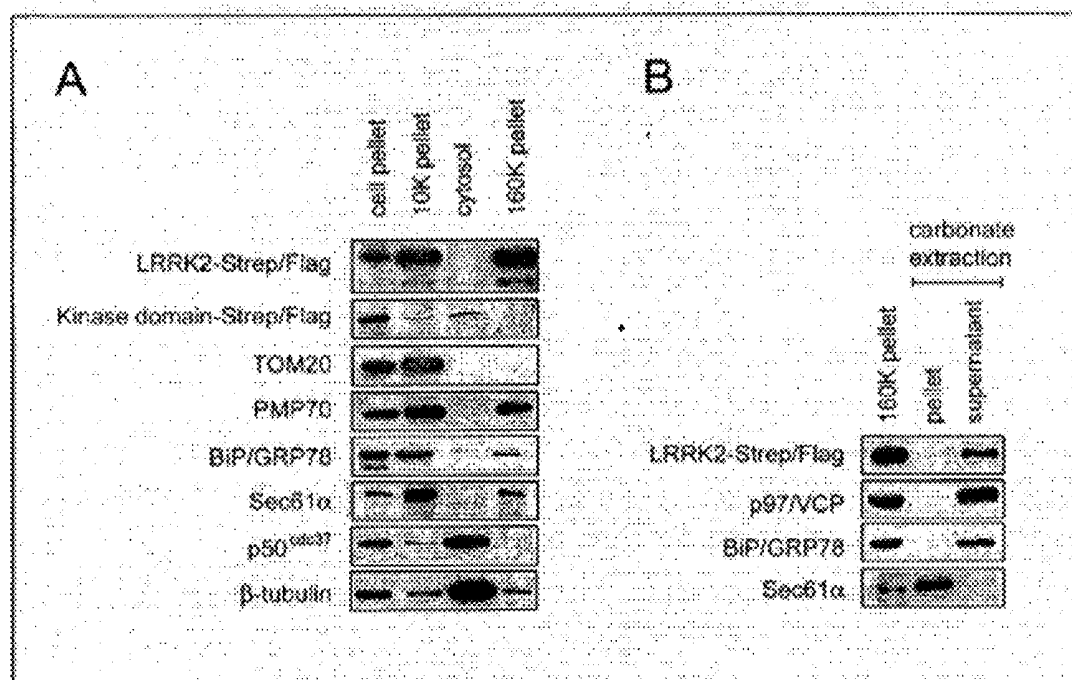


Figure 14:

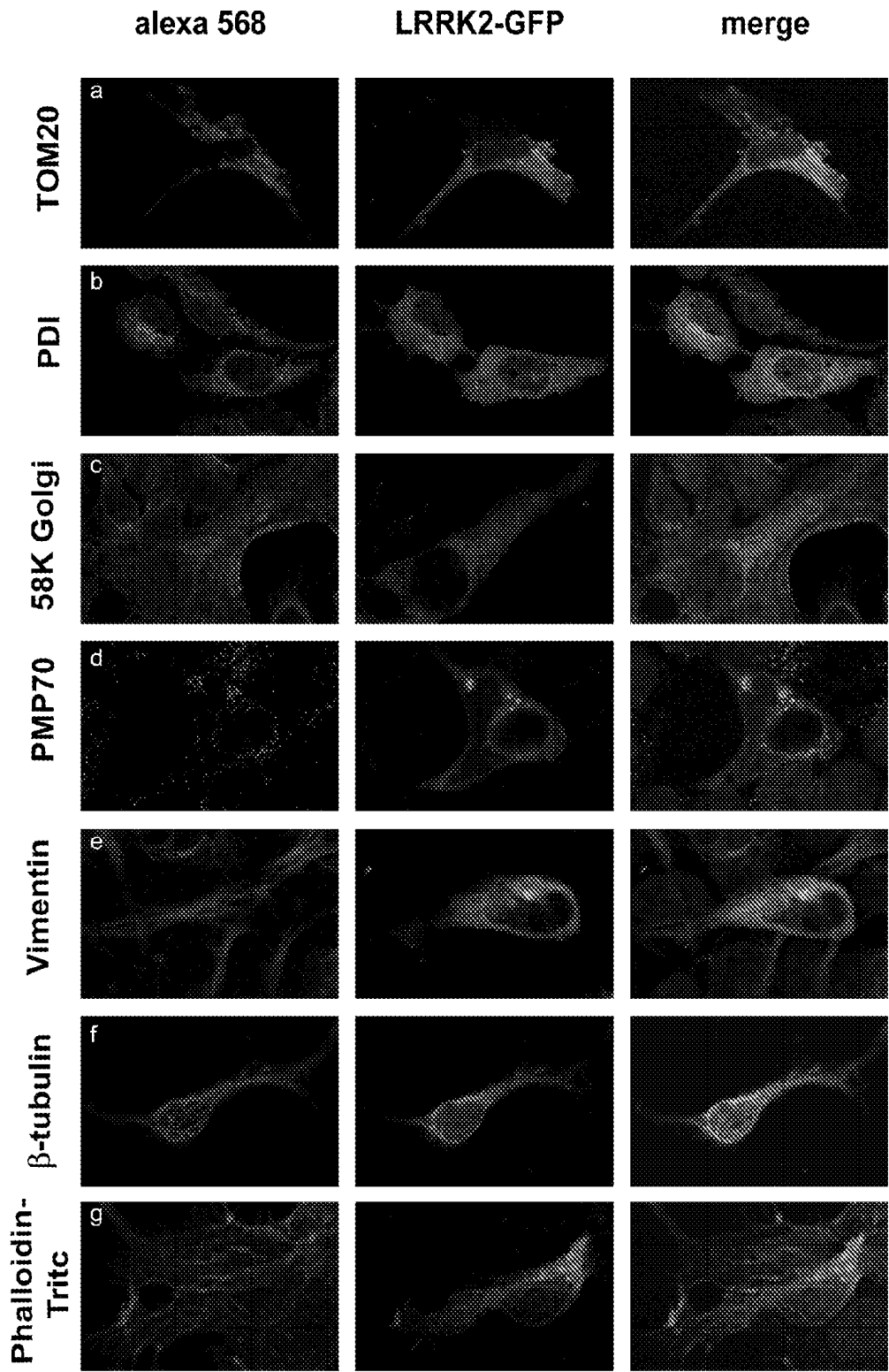


Figure 15:

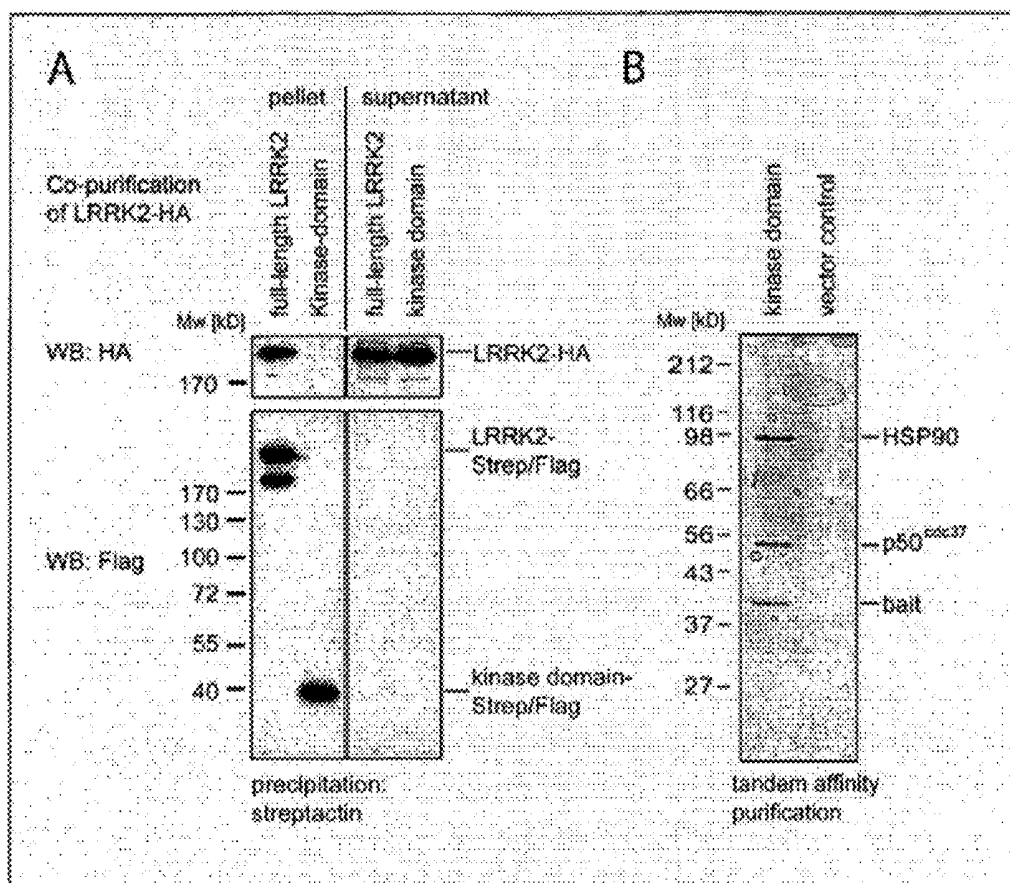
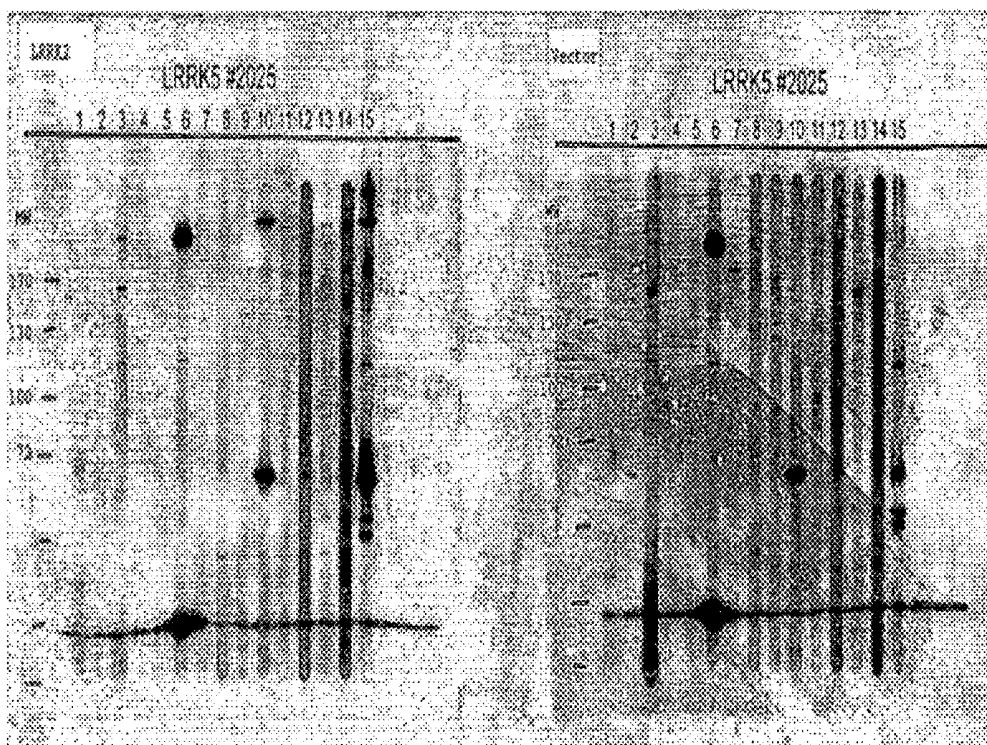


Figure 16:



**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 Wiley & 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 NaH₂PO₄/0.02M 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 (T_m) 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, it 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 $\theta=0$.

[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 addi-

tional 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 familial 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 ubiquitination, 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-(tyrosine 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 cytoskeletal 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 membrane 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 myeloid 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 I2020T 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 ankyrin 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. 6b), in family E in fact only one of the two family members with PD phenotype was carrier of the S1096C mutation (FIG. 6f), 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. 6c). 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 hallucinations 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 disturbances 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 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 promoter (NSE), L7 promoter (Purkinje cells), dopamine beta-hydroxylase (DBH) promoter (predominantly in the peripheral nervous 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; G
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-throughput 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 TPG-FRAPEVA 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 KASPP/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 KASPP/LRRK2.

[0109] SEQ ID NO: 4 shows the relevant section of the amino acid sequence of the S212L polymorphism of human KASPP/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 KASPP/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 KASPP/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 KASPP/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 amplification 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. 6e), which is displayed to demonstrate the same haplotypes in the two families affected by the I2020T mutation, all pedigrees display novel families. Black-end 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. 6f 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 -³²P-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 from 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 coimmunoprecipitation. 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 700xg pellet (cell pellet), the 10.000xg pellet (10K pellet), 160.000xg 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 I2020T (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, wild-type 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 co-sediments with membranes. HEK293 cells were fractionated into a cell pellet (700xg) 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), Sec61 α (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 Sec61 α , 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.gsfc.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 Transcriptor 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 Phosphoribosyl transferase (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 from 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 Beck's Depression Inventory (BDI) and the PDQ-39 Parkinson's Disease Quality of Life Questionnaire.

[0146] Electrophysiological investigations comprised neurography of the right tibial and sural nerve, and electromyography 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 $\leq 0.19 \text{ cm}^2$ was classified as normal, an area >0.19 and $\leq 0.24 \text{ cm}^2$ as moderately and an area $>0.24 \text{ cm}^2$ 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. 6a-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 12020T mutation, indicating common founders for these mutations (FIGS. 6a and 6e). Although members of family DE032 and T10758 were not aware of common ancestors, they originate from the same geographical area (Baden Württemberg, 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 haplotype 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. 6a).

[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. 6b).

[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 hallucinations 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. 6a).

[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. 6c).

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. 6e).

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. 6a).

[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. 6f). 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 symptoms 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% CO₂. For immunoprecipitation (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 MgCl₂, 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% trichloroacetic 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 steel 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 (autophosphorylation 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, 2x14 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 -³²P] 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 MgCl₂; 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 phosphorimager 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-diamidodiphenyl-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. 12A). 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. 12B). 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, Sec61 α) 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. 13A).

[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 Sec61 α was recovered in the membrane pellet (FIG. 13B). 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. 13A). 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-Trite) 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. 9a, 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 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. 15A (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. 15A, 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. 9b). The interacting proteins were identified using mass spectrometry. As shown in FIG. 10b 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. 11a). 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. 11b) 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 TPG-FRAPEVA 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, BioRad, 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 multi-screen chamber (BioRad). The chamber allows the incubation of a blot with different primary antibodies. The membranes were taken from the chambers for washing (4x5 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 KASPP/LRRK2 overexpressing cells.

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Leu	Leu	His	Arg	Leu	Thr	Leu	Gly	Asn	Phe	Phe	Asn	Ile	Leu	Val	Leu	
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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	
	355						360					365				
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	ggt	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	ggg	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	Arg	Arg	Leu	Ala	Leu	Asp	Val	Ala	Asn		
					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	ggg	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	ggg	ccc	gca	cac	tg	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				
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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	ggg	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	
1535 1540 1545	
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	
ggg 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	ggg	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	ggg	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	ggg	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	ggg	tta	cta	ctc	tat	gac	att	ttg	aca	act	gga	ggg	aga	ata	6219
Phe	Gly	Leu	Leu	Leu	Tyr	Asp	Ile	Leu	Thr	Thr	Gly	Gly	Arg	Ile	
2060						2065					2070				
gta	gag	ggg	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	ggg	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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cctgaccagt attcattatt tcagataatt cctgtgata ggacaactag tacatttaat	8824
attctcagaa cttatggcat ttactatgt gaaaacttta aatttattta tattaagggt	8884
aatcaaattc ttaaatgatga aagattttct gtattttaaa ggaagctatg ctttaacttg	8944
ttatgtaatt aacaaaaaaaa tcatatataa tagagctctt tgttcagtg ttatctcttt	9004
caatgttact ttgtatttgc aatttttttt accaaagaca aattaaaaaa atgaatacca	9064
tatttaaatg gaataataaa ggttttttaa 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		
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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。

