



(86) Date de dépôt PCT/PCT Filing Date: 2005/09/27
(87) Date publication PCT/PCT Publication Date: 2006/05/04
(45) Date de délivrance/Issue Date: 2014/07/22
(85) Entrée phase nationale/National Entry: 2007/04/19
(86) N° demande PCT/PCT Application No.: EP 2005/010428
(87) N° publication PCT/PCT Publication No.: 2006/045392
(30) Priorités/Priorities: 2004/10/21 (US60/620,893);
2004/10/22 (US60/621,169)

(51) Cl.Int./Int.Cl. *C12N 15/54* (2006.01),
A01K 67/027 (2006.01), *A61K 31/7088* (2006.01),
A61K 38/45 (2006.01), *A61K 48/00* (2006.01),
A61P 25/28 (2006.01), *C07K 16/40* (2006.01),
C12N 15/63 (2006.01), *C12N 9/12* (2006.01),
C12P 21/02 (2006.01), *C12Q 1/02* (2006.01),
C12Q 1/68 (2006.01), *G01N 33/50* (2006.01)

(72) Inventeurs/Inventors:
MEITINGER, THOMAS, DE;
STROM, TIM, MATTHIAS, DE;
...

(73) Propriétaires/Owners:

(54) Titre : GENE KASPP (LRRKE), PRODUCTION DE CE GENE ET UTILISATION DE CE GENE POUR LA
DETECTION ET LE TRAITEMENT DE TROUBLES NEURODEGENERATIFS

(54) Title: KASPP (LRRKE) GENE, ITS PRODUCTION AND USE FOR THE DETECTION AND TREATMENT OF
NEURODEGENERATIVE DISORDERS

(57) **Abrégé/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.



- (72) **Inventeurs(suite)/Inventors(continued)**: BISKUP, SASKIA, DE; UEFFING, MARIUS, DE; KREMMER, ELISABETH, DE; GLOECKNER, CHRISTIAN J., DE; GASSER, THOMAS, DE; LEITNER, PETRA, DE; ASMUS, FRIEDRICH, DE; PATENGE, NADJA, DE; ZIMPRICH, ALEXANDER, AT; FARRER, MATTHEW J., US; KACHERGUS, JENNIFER M., US; LINCOLN, SARA J., US; HULIHAN, MARY M., US; WSZOLEK, ZIBIGNIEW, US; UITTI, RYAN, US
- (73) **Propriétaires(suite)/Owners(continued)**: EBERHARD KARLS UNIVERSITAET TUEBINGEN, DE; MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH, US; HELMHOLTZ ZENTRUM MUENCHEN DEUTSCHES FORSCHUNGSZENTRUM FUER GESUNDHEIT UND UMWELT (GMBH), DE
- (74) **Agent**: BERESKIN & PARR LLP/S.E.N.C.R.L.,S.R.L.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
4 May 2006 (04.05.2006)

PCT

(10) International Publication Number
WO 2006/045392 A3

(51) International Patent Classification:
C12N 9/12 (2006.01)

(21) International Application Number:
PCT/EP2005/010428

(22) International Filing Date:
27 September 2005 (27.09.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/620,893 21 October 2004 (21.10.2004) US
60/621,169 22 October 2004 (22.10.2004) US

(71) Applicants (for all designated States except US):
GSF-FORSCHUNGSZENTRUM FÜR UMWELT UND GESUNDHEIT GMBH [DE/DE]; Ingolstädter Landstrasse 1, 85764 Neuherberg (DE). **EBERHARD KARLS UNIVERSITÄT TÜBINGEN** [DE/DE]; Österbergstrasse 3, 72024 Tübingen (DE). **MAYO CLINIC** [US/US]; 4500 San Pablo Road, Jacksonville, FL 32224 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **MEITINGER, Thomas** [DE/DE]; Weissenburger Platz 6c, 81667 München (DE). **STROM, Tim, Matthias** [DE/DE]; Adelheidstrasse 23, 80798 München (DE). **BISKUP, Saskia** [DE/DE]; Ettaler Strasse 27, 82061 Neuried (DE). **UEFFING, Marius** [DE/DE]; Auenstrasse 74, 80469 München (DE). **KREMMER, Elisabeth** [DE/DE]; Untere Hauptstrasse 28, 85354 Freising (DE). **GLOECKNER, Christian, J.** [DE/DE]; St.-Ulrich-Strasse 27, 85716 Unterschleissheim (DE). **GASSER, Thomas** [DE/DE]; Gottlieb-Olpp-Strasse 16/5, 72076 Tübingen (DE). **LEITNER, Petra** [DE/DE]; Landhaushöhe 30, 72070 Tübingen (DE). **ASMUS, Friedrich** [DE/DE]; Konrad-Adenauer-Strasse 19, 72072 Tübingen (DE). **PATENGE, Nadja** [DE/DE]; Mömpelgarder Weg 17, 72072 Tübingen (DE). **ZIMPRICH, Alexander** [AT/AT]; Castellezgasse 12/20, 1020 Wien (AT). **FARRER, Matthew, J.** [GB/US]; 1858 Arden Way, Jacksonville, FL 32250 (US).

KACHERGUS, Jennifer, M. [US/US]; 344 Plaza Street, Atlantic Beach, FL (US). **LINCOLN, Sara, J.** [GB/US]; 13425 Foxhaven Drive, Jacksonville, FL 32224 (US). **HULIHAN, Mary, M.** [US/US]; 1505 West Spindrift Circle, Neptune Beach, FL (US). **WSZOLEK, Zibigniew** [US/US]; 3868 Biggin Church Road W., Jacksonville, FL 32224 (US). **UITTI, Ryan** [US/US]; 8052 Shady Grove Road, Jacksonville, FL 32256 (US).

(74) Agent: **BÖSL, Raphael**; Isenbruck Bösl Hörschler Wichmann Huhn, Prinzregentenstr. 68, 81675 München (DE).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
10 August 2006

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: KASPP (LRRKE) GENE, ITS PRODUCTION AND USE FOR THE DETECTION AND TREATMENT OF NEURODEGENERATIVE DISORDERS

(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.

WO 2006/045392 A3

KASPP (LRRK2) Gene, its Production and Use for the Detection and Treatment of Neurodegenerative Disorders

5 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),
10 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.

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
15 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*
20 (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.
25 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.

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".

Recently it has been shown that two large families with autosomal-dominant late-onset parkinsonism (families A and D) are linked to the PARK8-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).

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).

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.

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.

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

- (a) nucleotides 1 to 9104 of SEQ ID NO: 1 or 2;
- (b) nucleotides 1 to 7584 of SEQ ID NO: 1 or 2;
- (c) nucleotides 1 to 7581 of SEQ ID NO: 1 or 2;
- (d) a nucleotide sequence coding for the protein sequence of SEQ ID NO: 1 or 2 or for the
10 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;
- (e) a nucleotide sequence complementary to either of the nucleotide sequences in (a), (b), (c) or (d); and/or
- (f) a nucleotide sequence which hybridizes under high stringency conditions to any of the
15 nucleotide sequences in (a), (b), (c), (d) or (e).

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.

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

- 20 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
25 (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).

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, NY (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 5x SSC [20x: 3M NaCl/0.3M trisodium citrate] or 5x SSPE [20x: 3.6M NaCl/0.2M NaH₂PO₄/0.02M EDTA, pH 7.7]), 5x Denhardt's solution, 1% SDS, and 100 µg/ml denatured salmon sperm DNA. This is followed by several washes with buffer, e.g. in 0.2x 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.

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 Figure 4, Figure 5 and/or Figure 11 and/or the present specification. The boundaries of the exons as specified in Figure 4 are applicable for the nucleotide sequences of SEQ ID NO: 1 and SEQ ID NO: 2.

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.

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

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

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.

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.

5 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
10 $\theta=0$.

In Family D, 34 individuals were typed (10 affecteds and 24 unaffecteds), all affecteds were heterozygous for the 4321C>T (R1441C) 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
15 average age of onset in this family is 65 years according to Wszolek Z. K. et al., Neurology, 62(9), 1619-1622, 2004.

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

20 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 bp away from the exon/intron border. Affected individuals in a further family (469) were found to carry the same mutation as
25 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).

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

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.

- 5 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.

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

The five conserved domains are in detail:

- (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)
15 domain; (4) tyrosine kinase catalytic domain (MAPKKK) and (5) C-terminal WD-40 domain.

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
20 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
25 kinase (Ridley A. J. *Trends Cell. Biol.*, 2001). In particular, the fusion of a Ras-like domain with a MAPKKK domain indicates the function of KASPP/LRRK2 in intramolecular signal transduction. Furthermore, KASPP/LRRK2 should also function as a scaffolding protein like Ksr (Kinase suppressor of Ras). The KASPP/LRRK2 kinase domain shows also similarity to the RIR and Mixed lineage kinases which are part of the TKL- (thyrosine
30 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).

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.

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

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
5 cytoskeletal rearrangements.

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

In contrast to previous reports the so far most common mutation (G2019S; 6055G>A, Hernandez DG et al., Ann Neurol, 57: 453-6, 2005) was not detected in any of the families
10 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
15 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
20 sporadic PD cases in the present population.

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
25 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
30 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.

- 5 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
10 the original defining of the PARK8 locus (Funayama et al., Ann Neurol., 57: 918-21, 2005).

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
15 (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
20 families affected by mutations in the KASPP/LRRK2 gene (Mata et al., Neurosci Lett, 382: 309-311, 2005).

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
25 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)
30 had only slight resting tremor for several years, while his sister (III-3), mother and uncle were affected by severe PD.

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
5 been diagnosed could account for the earlier diagnoses.

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
10 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
15 hallucinations in only the one patient with DLBD-phenotype (Diffuse Lewy Body Disease-phenotype).

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
20 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,
25 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
30 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.

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.

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.

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.

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.

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.

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.

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.

Autopsies performed on affected individuals uniformly demonstrated neuronal loss and
5 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
10 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.

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
15 of parkinsonism.

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
20 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
25 one member with autopsy proven diffuse Lewy body disease. Whether this linkage result reflects variants in the KASPP/LRRK2 gene remains to be determined.

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 bp 3' cDNA probe and found expression in most brain regions, albeit at a very low levels. Two
30 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.

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.

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.

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.

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
5 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
10 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
15 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
20 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
25 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
30 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.

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.

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.

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*,
5 transgenic nematodes, like transgenic *C. elegans*, transgenic fish, like transgenic zebra fish, and transgenic non-human mammals, like transgenic rodents (mice, rats).

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
10 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
15 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)
20 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
25 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.

30 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.

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

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

Q	N	D; E; A; S; T; L; M; K; R
R	K; H	N; Q; S; T; D; E; A
S	A; T; G; N	D; E; R; K
T	A; S; G; N; V	D; E; R; K; I
V	A; L; I	M; T; C; N
W	F; Y; H	L; M; I; V; C
Y	F; W; H	L; M; I; V; C

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.

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

- (a) culturing a cell of the present invention under suitable conditions; and
- (b) isolating the protein produced by the cultured cell.

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:

- (a) contacting said sample with the nucleic acid molecule according to the present invention, and
- (b) detecting the presence of the mutation.

Preferably, the sample is selected from

(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

5 (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;

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, 10 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.

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 15 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 20 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 25 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 30 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. 6,174,670.

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
5 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.

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

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),
15 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
20 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.

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
25 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.

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

- (a) providing at least one nucleic acid molecule or a protein of the present invention,
- (b) providing a test compound, and
- (c) measuring or detecting the influence of the test compound on the expression activity of the nucleic acid molecule or the protein.

5 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.

10 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
15 homogeneous assays are, for example, TR-FRET, FP, ALPHA and gene assays.

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

Methods for preparing such arrays using solid phase chemistry and photolabile protecting groups are disclosed, for example, in US 5,744,305. These arrays can also be brought into
20 contact with test compound or compound libraries and tested for interaction, for example binding or changing conformation.

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
25 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.

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

5 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.

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

The antibody is either polyclonal or monoclonal, preferably it is a monoclonal antibody.
10 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.

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

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),
25 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.

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

Description of the Tables, the Sequences and the Figures:

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.

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

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.

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.

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 ↑ above average of matched healthy controls.

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).

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).

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

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

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

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

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

20 Figure 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.

25 Figure 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.

Figure 3 shows the three across species highly conserved amino acid substitutions R1441C, Y1699C and I1122T.

Figure 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.

Figure 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.

Figure 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 (Figure 6e), which is displayed to demonstrate the same haplotypes in the two families affected by the I2020T mutation, all pedigrees display novel families.

Blackend symbols denote family members with the clinical presentation of PD; “+” denotes a genotyped individual, with “M” for mutation carriers and “wt” for wild-type KASPP/LRRK2. The dotted symbols in Figure 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.

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

Figure 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.

Figures 9 (A) shows a scheme of the KASPP/LRRK2-domain structure and the used Tag-fusion constructs of LRRK2.

5 (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.

10 (C) shows coimmuno-precipitation. Two FLAG tagged LRRK2-baits (full-length and kinase domain) were tested for interaction with HA tagged full length KASPP/LRRK2. The co-precipitated HA-tagged KASPP/LRRK2 was visualized by immuno-blotting (3F10 anti HA, upper panel). A loading control for the bait-constructs is shown in the lower panel (immuno-blot: anti Flag M2). Figure 15 (B) is the same figure as Figure 9
15 (C) in a better shape.

Figure 10 shows the immunofluorescence of different cell structures.

Figure 11 (A) shows cell fractionation of HEK293 cells over expressing KASPP/LRRK2-Flag. The different pellet fractions 700 x g pellet (cell pellet), the 10.000 x g pellet (10K pellet), 160.000 x g pellet (160K pellet)
20 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.

25 (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).

Figure 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.

Figure 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 (700 x g) an organelle pellet (10K pellet), a soluble cytosolic fraction (cytosol) and a microsomal fraction (160k 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.

Figure 14 shows that KASPP/LRRK2-GFP localises to mitochondria, endoplasmic reticulum, Golgi and the microtubular cytoskeleton. HEK293 expressing

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.

Figure 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 Strep/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 Figure 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).

Figure 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:

5 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

10

Example 1: Genetic Analysis

DNA Extraction

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

Sequence analysis

20 Genomic sequences and annotations were obtained from the National Center for Biotechnology Information (NCBI) and University of California Santa Cruz (UCSC). Primers for mutation screening were designed using Primer3 software integrated into script to allow for automated primer design. Exon sequences and exon-intron boundaries were amplified with intronic primers and sequenced them directly by BigDyeTM Terminator Cycle sequencing kit (Applied Biosystems). Between Markers
 25 D12S1692 and D12S85 a total of 29 genes or RNAs were sequenced (see Table 1).

Haplotype analysis

30 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".

Polymorphic repeats were found in intron 5 (caa), intron 20 (atct) and intron 29 (ac).

Linkage analysis

Twopoint LOD scores were calculated using the MLINK program (V 5.10) in its
 5 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.

10 Screening of Mutations and Polymorphisms

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
 15 mass-extension (hME) process for producing primer extension products (Tang K. et
 al., Proc. Natl. Acad. Sci USA, 96, 10016-10020, 1999).

Amplification of KASPP/LRRK2

The complete coding sequence of KASPP/LRRK2 was amplified from human brain
 20 cDNA by using Marathon-Ready™ cDNA (BD Biosciences Clontech). Primers were
 set to amplify three overlapping fragments from exon1-21 (PIf, PIr), 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.

25

Example 2: Northern blot Analysis

Northern blot analysis was performed according to the manufacturers protocols (BD
 Biosciences). For hybridization a KASPP/LRRK2 cDNA fragment was used (bp
 30 6577-7655; corresponding to exon 45-3'UTR.

Example 3: LightCycler Experiments

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 specific PCR products spanning exon 1 to 8, exon 13 to 19 and exon 31 to 39 were quantified using the LightCyclerTM 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 Phosphobillinogen deaminase (h-PBGD) gene, a low-copy housekeeping 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%

15 **Examples of the Second Study**

Subjects and Methods

Subjects

20 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.

DNA of patients with familial and sporadic PD was obtained from our gene bank,
 5 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.

10 Mutational Screening

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
 15 kit (Applied Biosystems) with the same primers and under the same condition as described above.

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

In families with identical mutations haplotype analysis of the KASPP/LRRK2 region
 25 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.

30

DNA extraction from brain tissue

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
 35 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).

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

Clinical investigations

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
10 performed and olfactory function was tested using sniffing sticks (Daum et al., Nervenarzt, 71:643-50, 2000). A neuropsychological test battery sensitive for dementia, concentration, planning, as well as intelligence was chosen (Table 5). To evaluate mood and sensitivity patients were asked to complete the Becks Depressions Inventar (BDI) and the PDQ-39 Parkinson's Disease Quality of Life Questionnaire.

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

Neuroimaging

20 Structural neuroimaging comprised transcranial ultrasound (TCS) and magnet resonance imaging (MRI).

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
25 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 of $>0.24\text{cm}^2$ as markedly hyperechogenic (Berg et al., 1999, supra).

MRI was performed on a Magnetom Avanto 1,5 Tesla, Siemens AG, Germany.

5 Results

Mutational Screening

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 (Figure 6a-f). Four of these are novel missense mutations: R793M; 2378G>T in family DE041 and
10 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.

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
15 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 I2020T mutation (family T10738).

Except for the R793M mutation, which was found in one control person none of the
20 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

Haplotype analysis revealed common haplotypes for the two novel families affected by the
25 R793M mutation as well as for family T10758 and family DE032 affected by the I2020T mutation, indicating common founders for these mutations (Figures 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).

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).

5 Clinical findings

Extensive clinical and neuroimaging examination revealed the features listed in Table 4.

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
10 vary for different mutations.

Common findings in patients with KASPP/LRRK2 mutations

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
15 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).

20 Intrafamily differences in clinical presentation

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 (Figure 6a).

25 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 (Figure 6b).

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

10 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 (Figure 6a).

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 (Figure 6c).

Age of onset

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 of PD was established earlier and also investigation of an additional family member in family DE032 revealed an earlier diagnosis (41 years), while mean age of onset was 54 (48-59 years) in generation I–III (Figure 6e).

Penetrance

25 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.

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 not 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 (Figure 6a).

- 5 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
- 10 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
- 15 argues for incomplete penetrance of this mutation.

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.

20 Phenocopies and simultaneous occurrence of tremor

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.

- In family E an autosomal dominant inheritance of tremor is evident (Figure 6f). Two
- 25 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
- 30 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

5 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
10 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.

15 TCS and MRI findings

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
20 microangiopathy.

Biochemical Characterization of KASPP/LRRK2

Material and Methods

Plasmid and Cloning

25 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

HEK293 cells were cultured in DMEM supplemented with 10% FBS at 37°C and 5% CO₂. For immuno precipitation (IP), tandem affinity purification or cell fractionation experiments cells were transfected with Effectene[®] (Qiagen) and kept under full medium for additional 48 h.

Electrophoresis and Immunoblotting

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. Volkl, 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).

Cell Fractionation

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 x g for 10 min to pellet nuclei, debris, and non-disrupted cells (cell pellet). The supernatant was centrifuged at 10,000 x g for 20 min to obtain the 10K pellet. Cytosol and 160K pellet were prepared by ultracentrifugation of the 10K supernatant (160,000 x g for 1 h).

Carbonate Extraction

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 x 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)

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), 1mM 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 4x in lysis buffer and eluted with SDS-gel sample buffer.

Tandem affinity purification

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 (1x with lysis buffer and 2x with TBS) was done in the microspin columns. Washing solution was removed from the columns by centrifugation (10 s, 2,000 x 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.

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 3x 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

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)

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 3x 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.

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-PTM PVDF membranes (GE Healthcare). Imaging was done on a phosphoimager system (BioRad). Equal loading was ensured by Western blotting analyses (anti-Flag M2).

5 Immunofluorescence

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 100TM for 5 min, blocked with PBS containing 0.1% Tween-20TM and 1% BSA and incubated 3 h at RT with primary antibodies in blocking solution [mouse anti-58K Golgi, 1:100 (Abcam); 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 μ g/ml 4,6-diaminodiphenyl-2-phenylindole (DAPI, Sigma). Coverslips were washed six times with PBS, mounted with FluorSaveTM (Calbiochem) and evaluated by fluorescence microscopy using a Zeiss ApotomeTM 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).

25 Results

KASPP/LRRK2 is a Membrane-associated 280 kD Protein

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.

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).

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.

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

Immunofluorescence microscopy was used to determine the subcellular localisation of GFP-tagged KASPP/LRRK2 transiently expressed in HEK293 cells. After fixation, cells were permeabilised and co-immunolabelled with antibodies specific for distinct subcellular

structures. In HEK293 cells, GFP-tagged KASPP/LRRK2 demonstrated a cytoplasmic distribution (Fig. 314 column 2). Partial co-localization was observed with inner cellular structures, i.e., mitochondria (TOM20), ER (PDI) and Golgi (58K Golgi). In contrast, no overlap was observed with peroxisomes (PMP70). No co-localization was found with the actin cytoskeleton (Phalloidin-Tritc) and intermediate filaments (Vimentin). However, the strongest co-localisation was an overlap with β -tubulin, suggesting an interaction between KASPP/LRRK2 and the microtubular cytoskeleton. Thus, KASPP/LRRK2 is a cytoplasmic protein associated with a subset of inner cellular membranes, i.e., mitochondria, ER and Golgi, and with the microtubular cytoskeleton.

10 Autophosphorylation Levels Between I2020T Mutant and Wildtype KASPP/LRRK2

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.

15 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
20 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

25 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, homodimerisation is required for activity.

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-
30

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.

In a second approach, differently-tagged KASPP/LRRK2 proteins and co-transfected HEK293 cells were utilized with two constructs for expression of HA and the Strep/Flag-tagged KASPP/LRRK2 fusion proteins, with the intention that a certain fraction of cells expressing two different KASPP/LRRK2 fusion proteins would to address the question of dimerisation by co-precipitation experiments. In addition to the full-length LRRK2 protein, a Strep/Flag-tagged version of the kinase domain only was used (Fig. 12). A comparison of purifications with all three tags showed the best results for streptactin, which almost completely precipitates the tagged proteins. Therefore, streptactin was used for precipitation of KASPP/LRRK2 fusion proteins from solubilised cells co-expressing HA and Strep/Flag-tagged KASPP/LRRK2. As shown in Fig. 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}

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

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 COS7 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.

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 (160k pellet).

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

The immunization, generation of hybridoma clones and ELISA for positive clones against peptide #2025 used for the immunization were carried out according to standard protocols. The antibody producing clones were tested for sensitivity and specificity against KASPP/LRRK2. Peptide #2025 with the amino acid sequence "CRMGIKTSEG TPGFRAPEVA RGNVIYNQQA D" represents the kinase domain of KASPP/LRRK2

(amino acids Nos. 2025-2055). This particular peptide was chosen because the homology of the sequences between mouse and human is 100%.

Test Conditions for Sensitivity and Specificity of the Generated Antibodies

A lysate of HEK293 cells overexpressing recombinant KASPP/LRRK2 and a control
5 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
10 adsorption of the antibodies to the membrane was avoided. Thereafter the blots were
incubated with the positively tested hybridoma supernatants for 3 h (ELISA for testing the
affinity to the peptide). The incubation was carried out in a multiscreen chamber (BioRad).
The chamber allows the incubation of a blot with different primary antibodies. The
membranes were taken form the chambers for washing (4 x 5min in TBST buffer). Then.
15 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).

20 Result

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

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SEQ ID NO: 1

	M A S G S C Q G C E E D E E T L K K	18
	ATG GCT AGT GGC AGC TGT CAG GGG TGC GAA GAG GAC GAG GAA ACT CTG AAG AAG	54
5		
	L I V R L N N V Q E G K Q I E T L V	36
	TTG ATA GTC AGG CTG AAC AAT GTC CAG GAA GGA AAA CAG ATA GAA ACG CTG GTC	108
	Q I L E D L L V F T Y S E R A S K L	54
10	CAA ATC CTG GAG GAT CTG CTG GTG TTC ACG TAC TCC GAG CGC GCC TCC AAG TTA	162
	F Q G K N I H V P L L I V L D S Y M	72
	TTT CAA GGC AAA AAT ATC CAT GTG CCT CTG TTG ATC GTC TTG GAC TCC TAT ATG	216
15	R V A S V Q Q V G W S L L C K L I E	90
	AGA GTC GCG AGT GTG CAG CAG GTG GGT TGG TCA CTT CTG TGC AAA TTA ATA GAA	270
	V C P G T M Q S L M G P Q D V G N D	108
	GTC TGT CCA GGT ACA ATG CAA AGC TTA ATG GGA CCC CAG GAT GTT GGA AAT GAT	324
20		
	W E V L G V H Q L I L K M L T V H N	126
	TGG GAA GTC CTT GGT GTT CAC CAA TTG ATT CTT AAA ATG CTA ACA GTT CAT AAT	378
	A S V N L S V I G L K T L D L L L T	144
25	GCC AGT GTA AAC TTG TCA GTG ATT GGA CTG AAG ACC TTA GAT CTC CTC CTA ACT	432
	S G K I T L L I L D E E S D I F M L	162
	TCA GGT AAA ATC ACC TTG CTG ATA TTG GAT GAA GAA AGT GAT ATT TTC ATG TTA	486
30	I F D A M H S F P A N D E V Q K L G	180
	ATT TTT GAT GCC ATG CAC TCA TTT CCA GCC AAT GAT GAA GTC CAG AAA CTT GGA	540
	C K A L H V L F E R V S E E Q L T E	198
	TGC AAA GCT TTA CAT GTG CTG TTT GAG AGA GTC TCA GAG GAG CAA CTG ACT GAA	594
35		
	F V E N K D Y M I L L S A S T N F K	216
	TTT GTT GAG AAC AAA GAT TAT ATG ATA TTG TTA AGT GCG TCA ACA AAT TTT AAA	648
	D E E E I V L H V L H C L H S L A I	234
40	GAT GAA GAG GAA ATT GTG CTT CAT GTG CTG CAT TGT TTA CAT TCC CTA GCG ATT	702
	P C N N V E V L M S G N V R C Y N I	252
	CCT TGC AAT AAT GTG GAA GTC CTC ATG AGT GGC AAT GTC AGG TGT TAT AAT ATT	756
45	V V E A M K A F P M S E R I Q E V S	270

	GTG GTG GAA GCT ATG AAA GCA TTC CCT ATG AGT GAA AGA ATT CAA GAA GTG AGT	810
	C C L L H R L T L G N F F N I L V L	288
	TGC TGT TTG CTC CAT AGG CTT ACA TTA GGT AAT TTT TTC AAT ATC CTG GTA TTA	864
5	N E V H E F V V K A V Q Q Y P E N A	306
	AAC GAA GTC CAT GAG TTT GTG GTG AAA GCT GTG CAG CAG TAC CCA GAG AAT GCA	918
	A L Q I S A L S C L A L L T E T I F	324
10	GCA TTG CAG ATC TCA GCG CTC AGC TGT TTG GCC CTC CTC ACT GAG ACT ATT TTC	972
	L N Q D L E E K N E N Q E N D D E G	342
	TTA AAT CAA GAT TTA GAG GAA AAG AAT GAG AAT CAA GAG AAT GAT GAT GAG GGG	1026
15	E E D K L F W L E A C Y K A L T W H	360
	GAA GAA GAT AAA TTG TTT TGG CTG GAA GCC TGT TAC AAA GCA TTA ACG TGG CAT	1080
	R K N K H V Q E A A C W A L N N L L	378
	AGA AAG AAC AAG CAC GTG CAG GAG GCC GCA TGC TGG GCA CTA AAT AAT CTC CTT	1134
20	M Y Q N S L H E K I G D E D G H F P	396
	ATG TAC CAA AAC AGT TTA CAT GAG AAG ATT GGA GAT GAA GAT GGC CAT TTC CCA	1188
	A H R E V M L S M L M H S S S K E V	414
25	GCT CAT AGG GAA GTG ATG CTC TCC ATG CTG ATG CAT TCT TCA TCA AAG GAA GTT	1242
	F Q A S A N A L S T L L E Q N V N F	432
	TTC CAG GCA TCT GCG AAT GCA TTG TCA ACT CTC TTA GAA CAA AAT GTT AAT TTC	1296
30	R K I L L S K G I H L N V L E L M Q	450
	AGA AAA ATA CTG TTA TCA AAA GGA ATA CAC CTG AAT GTT TTG GAG TTA ATG CAG	1350
	K H I H S P E V A E S G C K M L N H	468
	AAG CAT ATA CAT TCT CCT GAA GTG GCT GAA AGT GGC TGT AAA ATG CTA AAT CAT	1404
35	L F E G S N T S L D I M A A V V P K	486
	CTT TTT GAA GGA AGC AAC ACT TCC CTG GAT ATA ATG GCA GCA GTG GTC CCC AAA	1458
	I L T V M K R H E T S L P V Q L E A	504
40	ATA CTA ACA GTT ATG AAA CGT CAT GAG ACA TCA TTA CCA GTG CAG CTG GAG GCG	1512
	L R A I L H F I V P G M P E E S R E	522
	CTT CGA GCT ATT TTA CAT TTT ATA GTG CCT GGC ATG CCA GAA GAA TCC AGG GAG	1566
45	D T E F H H K L N M V K K Q C F K N	540
	GAT ACA GAA TTT CAT CAT AAG CTA AAT ATG GTT AAA AAA CAG TGT TTC AAG AAT	1620

	D I H K L V L A A L N R F I G N P G	558
	GAT ATT CAC AAA CTG GTC CTA GCA GCT TTG AAC AGG TTC ATT GGA AAT CCT GGG	1674
5	I Q K C G L K V I S S I V H F P D A	576
	ATT CAG AAA TGT GGA TTA AAA GTA ATT TCT TCT ATT GTA CAT TTT CCT GAT GCA	1728
	L E M L S L E G A M D S V L H T L Q	594
	TTA GAG ATG TTA TCC CTG GAA GGT GCT ATG GAT TCA GTG CTT CAC ACA CTG CAG	1782
10	M Y P D D Q E I Q C L G L S L I G Y	612
	ATG TAT CCA GAT GAC CAA GAA ATT CAG TGT CTG GGT TTA AGT CTT ATA GGA TAC	1836
	L I T K K N V F I G T G H L L A K I	630
15	TTG ATT ACA AAG AAG AAT GTG TTC ATA GGA ACT GGA CAT CTG CTG GCA AAA ATT	1890
	L V S S L Y R F K D V A E I Q T K G	648
	CTG GTT TCC AGC TTA TAC CGA TTT AAG GAT GTT GCT GAA ATA CAG ACT AAA GGA	1944
20	F Q T I L A I L K L S A S F S K L L	666
	TTT CAG ACA ATC TTA GCA ATC CTC AAA TTG TCA GCA TCT TTT TCT AAG CTG CTG	1998
	V H H S F D L V I F H Q M S S N I M	684
	GTG CAT CAT TCA TTT GAC TTA GTA ATA TTC CAT CAA ATG TCT TCC AAT ATC ATG	2052
25	E Q K D Q Q F L N L C C K C F A K V	702
	GAA CAA AAG GAT CAA CAG TTT CTA AAC CTC TGT TGC AAG TGT TTT GCA AAA GTA	2106
	A M D D Y L K N V M L E R A C D Q N	720
30	GCT ATG GAT GAT TAC TTA AAA AAT GTG ATG CTA GAG AGA GCG TGT GAT CAG AAT	2160
	N S I M V E C L L L L G A D A N Q A	738
	AAC AGC ATC ATG GTT GAA TGC TTG CTT CTA TTG GGA GCA GAT GCC AAT CAA GCA	2214
35	K E G S S L I C Q V C E K E S S P K	756
	AAG GAG GGA TCT TCT TTA ATT TGT CAG GTA TGT GAG AAA GAG AGC AGT CCC AAA	2268
	L V E L L L N S G S R E Q D V R K A	774
	TTG GTG GAA CTC TTA CTG AAT AGT GGA TCT CGT GAA CAA GAT GTA CGA AAA GCG	2322
40	L T I S I G K G D S Q I I S L L L R	792
	TTG ACG ATA AGC ATT GGG AAA GGT GAC AGC CAG ATC ATC AGC TTG CTC TTA AGG	2376
	R L A L D V A N N S I C L G G F C I	810
45	AGG CTG GCC CTG GAT GTG GCC AAC AAT AGC ATT TGC CTT GGA GGA TTT TGT ATA	2430

52

	G K V E P S W L G P L F P D K T S N	828
	GGA AAA GTT GAA CCT TCT TGG CTT GGT CCT TTA TTT CCA GAT AAG ACT TCT AAT	2484
	L R K Q T N I A S T L A R M V I R Y	846
5	TTA AGG AAA CAA ACA AAT ATA GCA TCT ACA CTA GCA AGA ATG GTG ATC AGA TAT	2538
	Q M K S A V E E G T A S G S D G N F	864
	CAG ATG AAA AGT GCT GTG GAA GAA GGA ACA GCC TCA GGC AGC GAT GGA AAT TTT	2592
10	S E D V L S K F D E W T F I P D S S	882
	TCT GAA GAT GTG CTG TCT AAA TTT GAT GAA TGG ACC TTT ATT CCT GAC TCT TCT	2646
	M D S V F A Q S D D L D S E G S E G	900
15	ATG GAC AGT GTG TTT GCT CAA AGT GAT GAC CTG GAT AGT GAA GGA AGT GAA GGC	2700
	S F L V K K K S N S I S V G E F Y R	918
	TCA TTT CTT GTG AAA AAG AAA TCT AAT TCA ATT AGT GTA GGA GAA TTT TAC CGA	2754
	D A V L Q R C S P N L Q R H S N S L	936
20	GAT GCC GTA TTA CAG CGT TGC TCA CCA AAT TTG CAA AGA CAT TCC AAT TCC TTG	2808
	G P I F D H E D L L K R K R K I L S	954
	GGG CCC ATT TTT GAT CAT GAA GAT TTA CTG AAG CGA AAA AGA AAA ATA TTA TCT	2862
25	S D D S L R S S K L Q S H M R H S D	972
	TCA GAT GAT TCA CTC AGG TCA TCA AAA CTT CAA TCC CAT ATG AGG CAT TCA GAC	2916
	S I S S L A S E R E Y I T S L D L S	990
30	AGC ATT TCT TCT CTG GCT TCT GAG AGA GAA TAT ATT ACA TCA CTA GAC CTT TCA	2970
	A N E L R D I D A L S Q K C C I S V	1008
	GCA AAT GAA CTA AGA GAT ATT GAT GCC CTA AGC CAG AAA TGC TGT ATA AGT GTT	3024
	H L E H L E K L E L H Q N A L T S F	1026
35	CAT TTG GAG CAT CTT GAA AAG CTG GAG CTT CAC CAG AAT GCA CTC ACG AGC TTT	3078
	P Q Q L C E T L K S L T H L D L H S	1044
	CCA CAA CAG CTA TGT GAA ACT CTG AAG AGT TTG ACA CAT TTG GAC TTG CAC AGT	3132
40	N K F T S F P S Y L L K M S C I A N	1062
	AAT AAA TTT ACA TCA TTT CCT TCT TAT TTG TTG AAA ATG AGT TGT ATT GCT AAT	3186
	L D V S R N D I G P S V V L D P T V	1080
45	CTT GAT GTC TCT CGA AAT GAC ATT GGA CCC TCA GTG GTT TTA GAT CCT ACA GTG	3240
	K C P T L K Q F N L S Y N Q L S F V	1098

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	AAA TGT CCA ACT CTG AAA CAG TTT AAC CTG TCA TAT AAC CAG CTG TCT TTT GTA	3294	
	P E N L T D V V E K L E Q L I L E G	1116	
	CCT GAG AAC CTC ACT GAT GTG GTA GAG AAA CTG GAG CAG CTC ATT TTA GAA GGA	3348	/g
5			fam.38
	N K I S G I C S P L R L K E L K I L	1134	
	AAT AAA ATA TCA GGG ATA TGC TCC CCC TTG AGA CTG AAG GAA CTG AAG ATT TTA	3402	Ex25/g
			Fam.21
	N L S K N H I S S L S E N F L E A C	1152	
10	AAC CTT AGT AAG AAC CAC ATT TCA TCC CTA TCA GAG AAC TTT CTT GAG GCT TGT	3456	
	P K V E S F S A R M N F L A A M P F	1170	
	CCT AAA GTG GAG AGT TTC AGT GCC AGA ATG AAT TTT CTT GCT GCT ATG CCT TTC	3510	
15	L P P S M T I L K L S Q N K F S C I	1188	
	TTG CCT CCT TCT ATG ACA ATC CTA AAA TTA TCT CAG AAC AAA TTT TCC TGT ATT	3564	
	P E A I L N L P H L R S L D M S S N	1206	
20	CCA GAA GCA ATT TTA AAT CTT CCA CAC TTG CGG TCT TTA GAT ATG AGC AGC AAT	3618	
	D I Q Y L P G P A H W K S L N L R E	1224	
	GAT ATT CAG TAC CTA CCA GGT CCC GCA CAC TGG AAA TCT TTG AAC TTA AGG GAA	3672	
	L L F S H N Q I S I L D L S E K A Y	1242	
25	CTC TTA TTT AGC CAT AAT CAG ATC AGC ATC TTG GAC TTG AGT GAA AAA GCA TAT	3726	
	L W S R V E K L H L S H N K L K E I	1260	
	TTA TGG TCT AGA GTA GAG AAA CTG CAT CTT TCT CAC AAT AAA CTG AAA GAG ATT	3780	
30	P P E I G C L E N L T S L D V S Y N	1278	
	CCT CCT GAG ATT GGC TGT CTT GAA AAT CTG ACA TCT CTG GAT GTC AGT TAC AAC	3834	
	L E L R S F P N E M G K L S K I W D	1296	
	TTG GAA CTA AGA TCC TTT CCC AAT GAA ATG GGG AAA TTA AGC AAA ATA TGG GAT	3888	
35	L P L D E L H L N F D F K H I G C K	1314	
	CTT CCT TTG GAT GAA CTG CAT CTT AAC TTT GAT TTT AAA CAT ATA GGA TGT AAA	3942	
	A K D I I R F L Q Q R L K K A V P Y	1332	
40	GCC AAA GAC ATC ATA AGG TTT CTT CAA CAG CGA TTA AAA AAG GCT GTG CCT TAT	3996	
	N R M K L M I V G N T G S G K T T L	1350	
	AAC CGA ATG AAA CTT ATG ATT GTG GGA AAT ACT GGG AGT GGT AAA ACC ACC TTA	4050	
45	L Q Q L M K T K K S D L G M Q S A T	1368	
	TTG CAG CAA TTA ATG AAA ACC AAG AAA TCA GAT CTT GGA ATG CAA AGT GCC ACA	4104	

	V	G	I	D	V	K	D	W	P	I	Q	I	R	D	K	R	K	R	1386
	GTT	GGC	ATA	GAT	GTG	AAA	GAC	TGG	CCT	ATC	CAA	ATA	AGA	GAC	AAA	AGA	AAG	AGA	4158
5	D	L	V	L	N	V	W	D	F	A	G	R	E	E	F	Y	S	T	1404
	GAT	CTC	GTC	CTA	AAT	GTG	TGG	GAT	TTT	GCA	GGT	CGT	GAG	GAA	TTC	TAT	AGT	ACT	4212
	H	P	H	F	M	T	Q	R	A	L	Y	L	A	V	Y	D	L	S	1422
	CAT	CCC	CAT	TTT	ATG	ACG	CAG	CGA	GCA	TTG	TAC	CTT	GCT	GTC	TAT	GAC	CTC	AGC	4266
10	K	G	Q	A	E	V	D	A	M	K	P	W	L	F	N	I	K	A	1440
	AAG	GGA	CAG	GCT	GAA	GTT	GAT	GCC	ATG	AAG	CCT	TGG	CTC	TTC	AAT	ATA	AAG	GCT	4320
	R	A	S	S	S	P	V	I	L	V	G	T	H	L	D	V	S	D	1458
15	CGC	GCT	TCT	TCT	TCC	CCT	GTG	ATT	CTC	GTT	GGC	ACA	CAT	TTG	GAT	GTT	TCT	GAT	4374 Ex31/t
																		fam.D	
	E	K	Q	R	K	A	C	M	S	K	I	T	K	E	L	L	N	K	1476
	GAG	AAG	CAA	CGC	AAA	GCC	TGC	ATG	AGT	AAA	ATC	ACC	AAG	GAA	CTC	CTG	AAT	AAG	4428
20	R	G	F	P	A	I	R	D	Y	H	F	V	N	A	T	E	E	S	1494
	CGA	GGG	TTC	CCT	GCC	ATA	CGA	GAT	TAC	CAC	TTT	GTG	AAT	GCC	ACC	GAG	GAA	TCT	4482
	D	A	L	A	K	L	R	K	T	I	I	N	E	S	L	N	F	K	1512
	GAT	GCT	TTG	GCA	AAA	CTT	CGG	AAA	ACC	ATC	ATA	AAC	GAG	AGC	CTT	AAT	TTC	AAG	4536
25	I	R	D	Q	L	V	V	G	Q	L	I	P	D	C	Y	V	E	L	1530
	ATC	CGA	GAT	CAG	CTT	GTT	GTT	GGA	CAG	CTG	ATT	CCA	GAC	TGC	TAT	GTA	GAA	CTT	4590
	E	K	I	I	L	S	E	R	K	N	V	P	I	E	F	P	V	I	1548
30	GAA	AAA	ATC	ATT	TTA	TCG	GAG	CGT	AAA	AAT	GTG	CCA	ATT	GAA	TTT	CCC	GTA	ATT	4644
	D	R	K	R	L	L	Q	L	V	R	E	N	Q	L	Q	L	D	E	1566
	GAC	CGG	AAA	CGA	TTA	TTA	CAA	CTA	GTG	AGA	GAA	AAT	CAG	CTG	CAG	TTA	GAT	GAA	4698
35	N	E	L	P	H	A	V	H	F	L	N	E	S	G	V	L	L	H	1584
	AAT	GAG	CTT	CCT	CAC	GCA	GTT	CAC	TTT	CTA	AAT	GAA	TCA	GGA	GTC	CTT	CTT	CAT	4752
	F	Q	D	P	A	L	Q	L	S	D	L	Y	F	V	E	P	K	W	1602
	TTT	CAA	GAC	CCA	GCA	CTG	CAG	TTA	AGT	GAC	TTG	TAC	TTT	GTG	GAA	CCC	AAG	TGG	4806
40	L	C	K	I	M	A	Q	I	L	T	V	K	V	E	G	C	P	K	1620
	CTT	TGT	AAA	ATC	ATG	GCA	CAG	ATT	TTG	ACA	GTG	AAA	GTG	GAA	GGT	TGT	CCA	AAA	4860
	H	P	K	G	I	I	S	R	R	D	V	E	K	F	L	S	K	K	1638
45	CAC	CCT	AAG	GGC	ATT	ATT	TCG	CGT	AGA	GAT	GTG	GAA	AAA	TTT	CTT	TCA	AAA	AAA	4914

55

	R K F P K N Y M S Q Y F K L L E K F	1656
	AGG AAA TTT CCA AAG AAC TAC ATG TCA CAG TAT TTT AAG CTC CTA GAA AAA TTC	4968
	Q I A L P I G E E Y L L V P S S L S	1674
5	CAG ATT GCT TTG CCA ATA GGA GAA GAA TAT TTG CTG GTT CCA AGC AGT TTG TCT	5022
	D H R P V I E L P H C E N S E I I I	1692
	GAC CAC AGG CCT GTG ATA GAG CTT CCC CAT TGT GAG AAC TCT GAA ATT ATC ATC	5076
10	R L Y E M P ~~Y~~ F P M G F W S R L I N	1710 <u>EX35</u>
	CGA CTA TAT GAA ATG CCT ~~TAT~~ TTT CCA ATG GGA TTT TGG TCA AGA TTA ATC AAT	5130 /g
		Fam.A
	R L L E I S P Y M L S G R E R A L R	1728
15	CGA TTA CTT GAG ATT TCA CCT TAC ATG CTT TCA GGG AGA GAA CGA GCA CTT CGC	5184
	P N R M Y W R Q G I Y L N W S P E A	1746
	CCA AAC AGA ATG TAT TGG CGA CAA GGC ATT TAC TTA AAT TGG TCT CCT GAA GCT	5238
	Y C L V G S E V L D N H P E S F L K	1764
20	TAT TGT CTG GTA GGA TCT GAA GTC TTA GAC AAT CAT CCA GAG AGT TTC TTA AAA	5292
	I T V P S C R K G C I L L G Q V V D	1782
	ATT ACA GTT CCT TCT TGT AGA AAA GGC TGT ATT CTT TTG GGC CAA GTT GTG GAC	5346
25	H I D S L M E E W F P G L L E I D I	1800
	CAC ATT GAT TCT CTC ATG GAA GAA TGG TTT CCT GGG TTG CTG GAG ATT GAT ATT	5400
	C G E G E T L L K K W A L Y S F N D	1818
30	TGT GGT GAA GGA GAA ACT CTG TTG AAG AAA TGG GCA TTA TAT AGT TTT AAT GAT	5454
	G E E H Q K I L L D D L M K K A E E	1836
	GGT GAA GAA CAT CAA AAA ATC TTA CTT GAT GAC TTG ATG AAG AAA GCA GAG GAA	5508
	G D L L V N P D Q P R L T I P I S Q	1854
35	GGA GAT CTC TTA GTA AAT CCA GAT CAA CCA AGG CTC ACC ATT CCA ATA TCT CAG	5562
	I A P D L I L A D L P R N I M L N N	1872
	ATT GCC CCT GAC TTG ATT TTG GCT GAC CTG CCT AGA AAT ATT ATG TTG AAT AAT	5616
40	D E L E F E Q A P E F L L G D G S F	1890
	GAT GAG TTG GAA TTT GAA CAA GCT CCA GAG TTT CTC CTA GGT GAT GGC AGT TTT	5670
	G S V Y R A A Y E G E E V A V K I F	1908
	GGA TCA GTT TAC CGA GCA GCC TAT GAA GGA GAA GAA GTG GCT GTG AAG ATT TTT	5724
45	N K H T S L R L L R Q E L V V L C H	1926

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	AAT AAA CAT ACA TCA CTC AGG CTG TTA AGA CAA GAG CTT GTG GTG CTT TGC CAC	5778
	L H H P S L I S L L A A G I R P R M	1944
	CTC CAC CAC CCC AGT TTG ATA TCT TTG CTG GCA GCT GGG ATT CGT CCC CGG ATG	5832
5	L V M E L A S K G S L D R L L Q Q D	1962
	TTG GTG ATG GAG TTA GCC TCC AAG GGT TCC TTG GAT CGC CTG CTT CAG CAG GAC	5886
	K A S L T R T L Q H R I A L H V A D	1980
10	AAA GCC AGC CTC ACT AGA ACC CTA CAG CAC AGG ATT GCA CTC CAC GTA GCT GAT	5940
	G L R Y L H S A M I I Y R D L K P H	1998
	GGT TTG AGA TAC CTC CAC TCA GCC ATG ATT ATA TAC CGA GAC CTG AAA CCC CAC	5994
15	N V L L F T L Y P N A A I I A K I A	2016
	AAT GTG CTG CTT TTC ACA CTG TAT CCC AAT GCT GCC ATC ATT GCA AAG ATT GCT	6048
	D Y G ~~E~~ A Q Y C C R M G I K T S E G	2034 Ex41
	GAC TAC GGC ~~ATT~~ GCT CAG TAC TGC TGT AGA ATG GGG ATA AAA ACA TCA GAG GGC	6102 /c
20		fam.32
	T P G F R A P E V A R G N V I Y N Q	2052
	ACA CCA GGG TTT CGT GCA CCT GAA GTT GCC AGA GGA AAT GTC ATT TAT AAC CAA	6156
	Q A D V Y S F G L L L Y D I L T T G	2070
25	CAG GCT GAT GTT TAT TCA TTT GGT TTA CTA CTC TAT GAC ATT TTG ACA ACT GGA	6210
	G R I V E G L K F P N E F D E L E I	2088
	GGT AGA ATA GTA GAG GGT TTG AAG TTT CCA AAT GAG TTT GAT GAA TTA GAA ATA	6264
30	Q G K L P D P V K E Y G C A P W P M	2106
	CAA GGA AAA TTA CCT GAT CCA GTT AAA GAA TAT GGT TGT GCC CCA TGG CCT ATG	6318
	V E K L I K Q C L K E N P Q E R P T	2124
	GTT GAG AAA TTA ATT AAA CAG TGT TTG AAA GAA AAT CCT CAA GAA AGG CCT ACT	6372
35		
	S A Q V F D I L N S A E L V C L T R	2142
	TCT GCC CAG GTC TTT GAC ATT TTG AAT TCA GCT GAA TTA GTC TGT CTG ACG AGA	6426
	R I L L P K N V I V E C M V A T H H	2160
40	CGC ATT TTA TTA CCT AAA AAC GTA ATT GTT GAA TGC ATG GTT GCT ACA CAT CAC	6480
	N S R N A S I W L G C G H T D R G Q	2178
	AAC AGC AGG AAT GCA AGC ATT TGG CTG GGC TGT GGG CAC ACC GAC AGA GGA CAG	6534
45	L S F L D L N T E G Y T S E E V A D	2196
	CTC TCA TTT CTT GAC TTA AAT ACT GAA GGA TAC ACT TCT GAG GAA GTT GCT GAT	6588

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	S R I L C L A L V H L P V E K E S W	2214
	AGT AGA ATA TTG TGC TTA GCC TTG GTG CAT CTT CCT GTT GAA AAG GAA AGC TGG	6642
5	I V S G T Q S G T L L V I N T E D G	2232
	ATT GTG TCT GGG ACA CAG TCT GGT ACT CTC CTG GTC ATC AAT ACC GAA GAT GGG	6696
	K K R H T L E K M T D S V T C L Y C	2250
	AAA AAG AGA CAT ACC CTA GAA AAG ATG ACT GAT TCT GTC ACT TGT TTG TAT TGC	6750
10	N S F S K Q S K Q K N F L L V G T A	2268
	AAT TCC TTT TCC AAG CAA AGC AAA CAA AAA AAT TTT CTT TTG GTT GGA ACC GCT	6804
	D G K L A I F E D K T V K L K G A A	2286
15	GAT GGC AAG TTA GCA ATT TTT GAA GAT AAG ACT GTT AAG CTT AAA GGA GCT GCT	6858
	P L K I L N I G N V S T P L M C L S	2304
	CCT TTG AAG ATA CTA AAT ATA GGA AAT GTC AGT ACT CCA TTG ATG TGT TTG AGT	6912
20	E S T N S T E R N V M W G G C G T K	2322
	GAA TCC ACA AAT TCA ACG GAA AGA AAT GTA ATG TGG GGA GGA TGT GGC ACA AAG	6966
	I F S F S N D F T I Q K L I E T R T	2340
	ATT TTC TCC TTT TCT AAT GAT TTC ACC ATT CAG AAA CTC ATT GAG ACA AGA ACA	7020
25	S Q L F S Y A A F S D S N I I T V V	2358
	AGC CAA CTG TTT TCT TAT GCA GCT TTC AGT GAT TCC AAC ATC ATA ACA GTG GTG	7074
	V D T A L Y I A K Q N S P V V E V W	2376
30	GTA GAC ACT GCT CTC TAT ATT GCT AAG CAA AAT AGC CCT GTT GTG GAA GTG TGG	7128
	D K K T E K L C G L I D C V H F L R	2394
	GAT AAG AAA ACT GAA AAA CTC TGT GGA CTA ATA GAC TGC GTG CAC TTT TTA AGG	7182
35	E V M V K E N K E S K H K M S Y S G	2412
	GAG GTA ATG GTA AAA GAA AAC AAG GAA TCA AAA CAC AAA ATG TCT TAT TCT GGG	7236
	R V K T L C L Q K N T A L W I G T G	2430
	AGA GTG AAA ACC CTC TGC CTT CAG AAG AAC ACT GCT CTT TGG ATA GGA ACT GGA	7290
40	G G H I L L L D L S T R R L I R V I	2448
	GGA GGC CAT ATT TTA CTC CTG GAT CTT TCA ACT CGT CGA CTT ATA CGT GTA ATT	7344
	Y N F C N S V R V M M T A Q L G S L	2466
45	TAC AAC TTT TGT AAT TCG GTC AGA GTC ATG ATG ACA GCA CAG CTA GGA AGC CTT	7398

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	K N V M L V L G Y N R K N T E G T Q	2484
	AAA AAT GTC ATG CTG GTA TTG GGC TAC AAC CGG AAA AAT ACT GAA GGT ACA CAA	7452
	K Q K E I Q S C L T V W D I N L P H	2502
5	AAG CAG AAA GAG ATA CAA TCT TGC TTG ACC GTT TGG GAC ATC AAT CTT CCA CAT	7506
	E V Q N L E K H I E V R K E L A E K	2520
	GAA GTG CAA AAT TTA GAA AAA CAC ATT GAA GTG AGA AAA GAA TTA GCT GAA AAA	7560
10	M R R T S V E *	2528
	ATG AGA CGA ACA TCT GTT GAG TAA gagagaaataggaattgtctttggataggaaaattattc	7623
	tctcctcttgtaaataatttttttaaaaatgttcacatggaaagggactcacattttttgaaatagctcgt	7695
15	gtgtatgaaggaatgttatttttttaatttaaatatatgtaaaaatacttaccagtaaatgtgtatttttaa	7767
	agaactattttaaaacacaatgttatattttcttataaataccagttactttcggttcattaattaatgaaaata	7839
	aatctgtgaagtacctaattttaagtactcactactaaaattttataaggccgataattttttgttttcttgtct	7911
20	gtaatggaggtaaactttatttttaattctgtgcttaagacaggactattgcttgctgatttttctagaaat	7983
	ctgcacgggtataatgaaaatattaagacagtttcccatgtaatgtattccttcttagattgcatcgaaatgc	8055
25	actatcatatatgcttgtaaataattcaaataaatttgcaactaataaagtcctttgttggtatgtgaattctc	8127
	tttgttgctggttgcaaacagtgcatcttacacaacttcactcaattcaaaaagaaaactccattaaaagtact	8199
	aatgaaaaaacatgacatactgtcaaagtcctcatatctaggaaagacacagaaactctctttgtcacagaa	8271
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35	tacttggttttctaattctgcttcattctaattgcttatattcatcttttccctaaatttgatgctgcagatc	8487
	ctacatcattcagatagaaaccttttttttttttcagaattatagaattccacagctcctaccaagaccatga	8559
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40	caccctaggcttccaaattataacttaaatgtttacatagcttaccacaataggagtatcagggccaaatac	8703
	ctatgtaataatttgagggtcatttctgcttttaggaaaagtactttcggttaaattctttggccctgaccagta	8775
45	ttcattatttcagataattccctgtgataggacaactagtagacatttaatatctcagaacttatggcatttt	8847

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actatgtgaaaactttaaatatttatattaagggtaatcaaattcttaaagatgaaagattttctgtatt 8919

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5 gtgttatctctttcattggttactttgtatttgcaattttttttaccaaagacaaattaaaaaatgaatacc 9063

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SEQ ID NO: 2

5	M A S G S C Q G C E E D E E T L K K	18
	ATG GCT AGT GGC AGC TGT CAG GGG TGC GAA GAG GAC GAG GAA ACT CTG AAG AAG	54
	L I V R L N N V Q E G K Q I E T L V	36
	TTG ATA GTC AGG CTG AAC AAT GTC CAG GAA GGA AAA CAG ATA GAA ACG CTG GTC	108
10	Q I L E D L L V F T Y S E H A S K L	54
	CAA ATC CTG GAG GAT CTG CTG GTG TTC ACG TAC TCC GAG CAC GCC TCC AAG TTA	162
	F Q G K N I H V P L L I V L D S Y M	72
15	TTT CAA GGC AAA AAT ATC CAT GTG CCT CTG TTG ATC GTC TTG GAC TCC TAT ATG	216
	R V A S V Q Q V G W S L L C K L I E	90
	AGA GTC GCG AGT GTG CAG CAG GTG GGT TGG TCA CTT CTG TGC AAA TTA ATA GAA	270
20	V C P G T M Q S L M G P Q D V G N D	108
	GTC TGT CCA GGT ACA ATG CAA AGC TTA ATG GGA CCC CAG GAT GTT GGA AAT GAT	324
	W E V L G V H Q L I L K M L T V H N	126
	TGG GAA GTC CTT GGT GTT CAC CAA TTG ATT CTT AAA ATG CTA ACA GTT CAT AAT	378
25	A S V N L S V I G L K T L D L L L T	144
	GCC AGT GTA AAC TTG TCA GTG ATT GGA CTG AAG ACC TTA GAT CTC CTC CTA ACT	432
	S G K I T L L I L D E E S D I F M L	162
30	TCA GGT AAA ATC ACC TTG CTG ATA CTG GAT GAA GAA AGT GAT ATT TTC ATG TTA	486
	I F D A M H S F P A N D E V Q K L G	180
	ATT TTT GAT GCC ATG CAC TCA TTT CCA GCC AAT GAT GAA GTC CAG AAA CTT GGA	540
35	C K A L H V L F E R V S E E Q L T E	198
	TGC AAA GCT TTA CAT GTG CTG TTT GAG AGA GTC TCA GAG GAG CAA CTG ACT GAA	594
	F V E N K D Y M I L L S A S T N F K	216
	TTT GTT GAG AAC AAA GAT TAT ATG ATA TTG TTA AGT GCG TCA ACA AAT TTT AAA	648
40	D E E E I V L H V L H C L H S L A I	234
	GAT GAA GAG GAA ATT GTG CTT CAT GTG CTG CAT TGT TTA CAT TCC CTA GCG ATT	702
	P C N N V E V L M S G N V R C Y N I	252
45	CCT TGC AAT AAT GTG GAA GTC CTC ATG AGT GGC AAT GTC AGG TGT TAT AAT ATT	756

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	V V E A M K A F P M S E R I Q E V S	270
	GTG GTG GAA GCT ATG AAA GCA TTC CCT ATG AGT GAA AGA ATT CAA GAA GTG AGT	810
5	C C L L H R L T L G N F F N I L V L	288
	TGC TGT TTG CTC CAT AGG CTT ACA TTA GGT AAT TTT TTC AAT ATC CTG GTA TTA	864
	N E V H E F V V K A V Q Q Y P E N A	306
	AAC GAA GTC CAT GAG TTT GTG GTG AAA GCT GTG CAG CAG TAC CCA GAG AAT GCA	918
10	A L Q I S A L S C L A L L T E T I F	324
	GCA TTG CAG ATC TCA GCG CTC AGC TGT TTG GCC CTC CTC ACT GAG ACT ATT TTC	972
	L N Q D L E E K N E N Q E N D D E G	342
15	TTA AAT CAA GAT TTA GAG GAA AAG AAT GAG AAT CAA GAG AAT GAT GAT GAG GGG	1026
	E E D K L F W L E A C Y K A L T W H	360
	GAA GAA GAT AAA TTG TTT TGG CTG GAA GCC TGT TAC AAA GCA TTA ACG TGG CAT	1080
20	R K N K H V Q E A A C W A L N N L L	378
	AGA AAG AAC AAG CAC GTG CAG GAG GCC GCA TGC TGG GCA CTA AAT AAT CTC CTT	1134
	M Y Q N S L H E K I G D E D G H F P	396
	ATG TAC CAA AAC AGT TTA CAT GAG AAG ATT GGA GAT GAA GAT GGC CAT TTC CCA	1188
25	A H R E V M L S M L M H S S S K E V	414
	GCT CAT AGG GAA GTG ATG CTC TCC ATG CTG ATG CAT TCT TCA TCA AAG GAA GTT	1242
	F Q A S A N A L S T L L E Q N V N F	432
30	TTC CAG GCA TCT GCG AAT GCA TTG TCA ACT CTC TTA GAA CAA AAT GTT AAT TTC	1296
	R K I L L S K G I H L N V L E L M Q	450
	AGA AAA ATA CTG TTA TCA AAA GGA ATA CAC CTG AAT GTT TTG GAG TTA ATG CAG	1350
35	K H I H S P E V A E S G C K M L N H	468
	AAG CAT ATA CAT TCT CCT GAA GTG GCT GAA AGT GGC TGT AAA ATG CTA AAT CAT	1404
	L F E G S N T S L D I M A A V V P K	486
	CTT TTT GAA GGA AGC AAC ACT TCC CTG GAT ATA ATG GCA GCA GTG GTC CCC AAA	1458
40	I L T V M K R H E T S L P V Q L E A	504
	ATA CTA ACA GTT ATG AAA CGT CAT GAG ACA TCA TTA CCA GTG CAG CTG GAG GCG	1512
	L R A I L H F I V P G M P E E S R E	522
45	CTT CGA GCT ATT TTA CAT TTT ATA GTG CCT GGC ATG CCA GAA GAA TCC AGG GAG	1566

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	D	T	E	F	H	H	K	L	N	M	V	K	K	Q	C	F	K	N	540
	GAT	ACA	GAA	TTT	CAT	CAT	AAG	CTA	AAT	ATG	GTT	AAA	AAA	CAG	TGT	TTC	AAG	AAT	1620
	D	I	H	K	L	V	L	A	A	L	N	R	F	I	G	N	P	G	558
5	GAT	ATT	CAC	AAA	CTG	GTC	CTA	GCA	GCT	TTG	AAC	AGG	TTC	ATT	GGA	AAT	CCT	GGG	1674
	I	Q	K	C	G	L	K	V	I	S	S	I	V	H	F	P	D	A	576
	ATT	CAG	AAA	TGT	GGA	TTA	AAA	GTA	ATT	TCT	TCT	ATT	GTA	CAT	TTT	CCT	GAT	GCA	1728
10	L	E	M	L	S	L	E	G	A	M	D	S	V	L	H	T	L	Q	594
	TTA	GAG	ATG	TTA	TCC	CTG	GAA	GGT	GCT	ATG	GAT	TCA	GTG	CTT	CAC	ACA	CTG	CAG	1782
	M	Y	P	D	D	Q	E	I	Q	C	L	G	L	S	L	I	G	Y	612
	ATG	TAT	CCA	GAT	GAC	CAA	GAA	ATT	CAG	TGT	CTG	GGT	TTA	AGT	CTT	ATA	GGA	TAC	1836
15																			
	L	I	T	K	K	N	V	F	I	G	T	G	H	L	L	A	K	I	630
	TTG	ATT	ACA	AAG	AAG	AAT	GTG	TTC	ATA	GGA	ACT	GGA	CAT	CTG	CTG	GCA	AAA	ATT	1890
	L	V	S	S	L	Y	R	F	K	D	V	A	E	I	Q	T	K	G	648
20	CTG	GTT	TCC	AGC	TTA	TAC	CGA	TTT	AAG	GAT	GTT	GCT	GAA	ATA	CAG	ACT	AAA	GGA	1944
	F	Q	T	I	L	A	I	L	K	L	S	A	S	F	S	K	L	L	666
	TTT	CAG	ACA	ATC	TTA	GCA	ATC	CTC	AAA	TTG	TCA	GCA	TCT	TTT	TCT	AAG	CTG	CTG	1998
25	V	H	H	S	F	D	L	V	I	F	H	Q	M	S	S	N	I	M	684
	GTG	CAT	CAT	TCA	TTT	GAC	TTA	GTA	ATA	TTC	CAT	CAA	ATG	TCT	TCC	AAT	ATC	ATG	2052
	E	Q	K	D	Q	Q	F	L	N	L	C	C	K	C	F	A	K	V	702
	GAA	CAA	AAG	GAT	CAA	CAG	TTT	CTA	AAC	CTC	TGT	TGC	AAG	TGT	TTT	GCA	AAA	GTA	2106
30																			
	A	M	D	D	Y	L	K	N	V	M	L	E	R	A	C	D	Q	N	720
	GCT	ATG	GAT	GAT	TAC	TTA	AAA	AAT	GTG	ATG	CTA	GAG	AGA	GCG	TGT	GAT	CAG	AAT	2160
	N	S	I	M	V	E	C	L	L	L	L	G	A	D	A	N	Q	A	738
35	AAC	AGC	ATC	ATG	GTT	GAA	TGC	TTG	CTT	CTA	TTG	GGA	GCA	GAT	GCC	AAT	CAA	GCA	2214
	K	E	G	S	S	L	I	C	Q	V	C	E	K	E	S	S	P	K	756
	AAG	GAG	GGA	TCT	TCT	TTA	ATT	TGT	CAG	GTA	TGT	GAG	AAA	GAG	AGC	AGT	CCC	AAA	2268
40	L	V	E	L	L	L	N	S	G	S	R	E	Q	D	V	R	K	A	774
	TTG	GTG	GAA	CTC	TTA	CTG	AAT	AGT	GGA	TCT	CGT	GAA	CAA	GAT	GTA	CGA	AAA	GCG	2322
	L	T	I	S	I	G	K	G	D	S	Q	I	I	S	L	L	L	R	792
	TTG	ACG	ATA	AGC	ATT	GGG	AAA	GGT	GAC	AGC	CAG	ATC	ATC	AGC	TTG	CTC	TTA	AGG	2376
45																			
	R	L	A	L	D	V	A	N	N	S	I	C	L	G	G	F	C	I	810 DE041

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	AGG CTG GCC CTG GAT GTG GCC AAC AAT AGC ATT TGC CTT GGA GGA TTT TGT ATA	2430 T11239
	G K V E P S W L G P L F P D K T S N	828
	GGA AAA GTT GAA CCT TCT TGG CTT GGT CCT TTA TTT CCA GAT AAG ACT TCT AAT	2484
5	L R K Q T N I A S T L A R M V I R Y	846
	TTA AGG AAA CAA ACA AAT ATA GCA TCT ACA CTA GCA AGA ATG GTG ATC AGA TAT	2538
	Q M K S A V E E G T A S G S D G N F	864
10	CAG ATG AAA AGT GCT GTG GAA GAA GGA ACA GCC TCA GGC AGC GAT GGA AAT TTT	2592
	S E D V L S K F D E W T F I P D S S	882
	TCT GAA GAT GTG CTG TCT AAA TTT GAT GAA TGG ACC TTT ATT CCT GAC TCT TCT	2646
15	M D S V F A Q S D D L D S E G S E G	900
	ATG GAC AGT GTG TTT GCT CAA AGT GAT GAC CTG GAT AGT GAA GGA AGT GAA GGC	2700
	S F L V K K K S N S I S V G E F Y R	918
	TCA TTT CTT GTG AAA AAG AAA TCT AAT TCA ATT AGT GTA GGA GAA TTT TAC CGA	2754
20	D A V L Q R C S P N L Q R H S N S L	936 DE022
	GAT GCC GTA TTA CAG CGT TGC TCA CCA AAT TTG CAA AGA CAT TCC AAT TCC TTG	2808
	G P I F D H E D L L K R K R K I L S	954
25	GGG CCC ATT TTT GAT CAT GAA GAT TTA CTG AAG CGA AAA AGA AAA ATA CTA TCT	2862
	S D D S L R S S K L Q S H M R H S D	972
	TCA GAT GAT TCA CTC AGG TCA TCA AAA CTT CAA TCC CAT ATG AGG CAT TCA GAC	2916
30	S I S S L A S E R E Y I T S L D L S	990
	AGC ATT TCT TCT CTG GCT TCT GAG AGA GAA TAT ATT ACA TCA CTA GAC CTT TCA	2970
	A N E L R D I D A L S Q K C C I S V	1008
	GCA AAT GAA CTA AGA GAT ATT GAT GCC CTA AGC CAG AAA TGC TGT ATA AGT GTT	3024
35	H L E H L E K L E L H Q N A L T S F	1026
	CAT TTG GAG CAT CTT GAA AAG CTG GAG CTT CAC CAG AAT GCA CTC ACG AGC TTT	3078
	P Q Q L C E T L K S L T H L D L H S	1044
40	CCA CAA CAG CTA TGT GAA ACT CTG AAG AGT TTG ACA CAT TTG GAC TTG CAC AGT	3132
	N K F T S F P S Y L L K M S C I A N	1062
	AAT AAA TTT ACA TCA TTT CCT TCT TAT TTG TTG AAA ATG AGT TGT ATT GCT AAT	3186
45	L D V S R N D I G P S V V L D P T V	1080
	CTT GAT GTC TCT CGA AAT GAC ATT GGA CCC TCA GTG GTT TTA GAT CCT ACA GTG	3240

	K C P T L K Q F N L S Y N Q L S F V	1098	FamE
	AAA TGT CCA ACT CTG AAA CAG TTT AAC CTG TCA TAT AAC CAG CTG TCT TTT GTA	3294	
			DE038
5	P E N L T D V V E K L E Q L I L E G	1116	T11288
	CCT GAG AAC CTC ACT GAT GTG GTA GAG AAA CTG GAG CAG CTC ATT TTA GAA GGA	3348	/g
			Fam38
	N K I S G I C S P L R L K E L K I L	1134	
	AAT AAA ATA TCA GGG ATA TGC TCC CCC TTG AGA CTG AAG GAA CTG AAG ATT TTA	3402	Ex25/g
10			Fam21
	N L S K N H I S S L S E N F L E A C	1152	
	AAC CTT AGT AAG AAC CAC ATT TCA TCC CTA TCA GAG AAC TTT CTT GAG GCT TGT	3456	
	P K V E S F S A R M N F L A A M P F	1170	
15	CCT AAA GTG GAG AGT TTC AGT GCC AGA ATG AAT TTT CTT GCT GCT ATG CCT TTC	3510	
	L P P S M T I L K L S Q N K F S C I	1188	
	TTG CCT CCT TCT ATG ACA ATC CTA AAA TTA TCT CAG AAC AAA TTT TCC TGT ATT	3564	
20	P E A I L N L P H L R S L D M S S N	1206	
	CCA GAA GCA ATT TTA AAT CTT CCA CAC TTG CGG TCT TTA GAT ATG AGC AGC AAT	3618	
	D I Q Y L P G P A H W K S L N L R E	1224	
	GAT ATT CAG TAC CTA CCA GGT CCC GCA CAC TGG AAA TCT TTG AAC TTA AGG GAA	3672	
25			
	L L F S H N Q I S I L D L S E K A Y	1242	DE031
	CTC TTA TTT AGC CAT AAT CAG ATC AGC ATC TTG GAC TTG AGT GAA AAA GCA TAT	3726	
	L W S R V E K L H L S H N K L K E I	1260	
30	TTA TGG TCT AGA GTA GAG AAA CTG CAT CTT TCT CAC AAT AAA CTG AAA GAG ATT	3780	
	P P E I G C L E N L T S L D V S Y N	1278	
	CCT CCT GAG ATT GGC TGT CTT GAA AAT CTG ACA TCT CTG GAT GTC AGT TAC AAC	3834	
35	L E L R S F P N E M G K L S K I W D	1296	
	TTG GAA CTA AGA TCC TTT CCC AAT GAA ATG GGG AAA TTA AGC AAA ATA TGG GAT	3888	
	L P L D E L H L N F D F K H I G C K	1314	
	CTT CCT TTG GAT GAA CTG CAT CTT AAC TTT GAT TTT AAA CAT ATA GGA TGT AAA	3942	
40			
	A K D I I R F L Q Q R L K K A V P Y	1332	
	GCC AAA GAC ATC ATA AGG TTT CTT CAA CAG CGA TTA AAA AAG GCT GTG CCT TAT	3996	
	N R M K L M I V G N T G S G K T T L	1350	
45	AAC CGA ATG AAA CTT ATG ATT GTG GGA AAT ACT GGG AGT GGT AAA ACC ACC TTA	4050	

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	L	Q	Q	L	M	K	T	K	K	S	D	L	G	M	Q	S	A	T	1368
	TTG	CAG	CAA	TTA	ATG	AAA	ACC	AAG	AAA	TCA	GAT	CTT	GGA	ATG	CAA	AGT	GCC	ACA	4104
	V	G	I	D	V	K	D	W	P	I	Q	I	R	D	K	R	K	R	1386
5	GTT	GGC	ATA	GAT	GTG	AAA	GAC	TGG	CCT	ATC	CAA	ATA	AGA	GAC	AAA	AGA	AAG	AGA	4158
	D	L	V	L	N	V	W	D	F	A	G	R	E	E	F	Y	S	T	1404
	GAT	CTC	GTC	CTA	AAT	GTG	TGG	GAT	TTT	GCA	GGT	CGT	GAG	GAA	TTC	TAT	AGT	ACT	4212
10	H	P	H	F	M	T	Q	R	A	L	Y	L	A	V	Y	D	L	S	1422
	CAT	CCC	CAT	TTT	ATG	ACG	CAG	CGA	GCA	TTG	TAC	CTT	GCT	GTC	TAT	GAC	CTC	AGC	4266
	K	G	Q	A	E	V	D	A	M	K	P	W	L	F	N	I	K	A	1440
	AAG	GGA	CAG	GCT	GAA	GTT	GAT	GCC	ATG	AAG	CCT	TGG	CTC	TTC	AAT	ATA	AAG	GCT	4320
15	R	A	S	S	S	P	V	I	L	V	G	T	H	L	D	V	S	D	1458
	CGC	GCT	TCT	TCT	TCC	CCT	GTG	ATT	CTC	GTT	GGC	ACA	CAT	TTG	GAT	GTT	TCT	GAT	4374 Ex31/t
																			famD
	E	K	Q	R	K	A	C	M	S	K	I	T	K	E	L	L	N	K	1476
20	GAG	AAG	CAA	CGC	AAA	GCC	TGC	ATG	AGT	AAA	ATC	ACC	AAG	GAA	CTC	CTG	AAT	AAG	4428
	R	G	F	P	A	I	R	D	Y	H	F	V	N	A	T	E	E	S	1494
	CGA	GGG	TTC	CCT	GCC	ATA	CGA	GAT	TAC	CAC	TTT	GTG	AAT	GCC	ACC	GAG	GAA	TCT	4482
25	D	A	L	A	K	L	R	K	T	I	I	N	E	S	L	N	F	K	1512
	GAT	GCT	TTG	GCA	AAA	CTT	CGG	AAA	ACC	ATC	ATA	AAC	GAG	AGC	CTT	AAT	TTC	AAG	4536
	I	R	D	Q	L	V	V	G	Q	L	I	P	D	C	Y	V	E	L	1530
	ATC	CGA	GAT	CAG	CTT	GTT	GTT	GGA	CAG	CTG	ATT	CCA	GAC	TGC	TAT	GTA	GAA	CTT	4590
30	E	K	I	I	L	S	E	R	K	N	V	P	I	E	F	P	V	I	1548
	GAA	AAA	ATC	ATT	TTA	TCG	GAG	CGT	AAA	AAT	GTG	CCA	ATT	GAA	TTT	CCC	GTA	ATT	4644
	D	R	K	R	L	L	Q	L	V	R	E	N	Q	L	Q	L	D	E	1566
35	GAC	CGG	AAA	CGA	TTA	TTA	CAA	CTA	GTG	AGA	GAA	AAT	CAG	CTG	CAG	TTA	GAT	GAA	4698
	N	E	L	P	H	A	V	H	F	L	N	E	S	G	V	L	L	H	1584
	AAT	GAG	CTT	CCT	CAC	GCA	GTT	CAC	TTT	CTA	AAT	GAA	TCA	GGA	GTC	CTT	CTT	CAT	4752
40	F	Q	D	P	A	L	Q	L	S	D	L	Y	F	V	E	P	K	W	1602
	TTT	CAA	GAC	CCA	GCA	CTG	CAG	TTA	AGT	GAC	TTG	TAC	TTT	GTG	GAA	CCC	AAG	TGG	4806
	L	C	K	I	M	A	Q	I	L	T	V	K	V	E	G	C	P	K	1620
	CTT	TGT	AAA	ATC	ATG	GCA	CAG	ATT	TTG	ACA	GTG	AAA	GTG	GAA	GGT	TGT	CCA	AAA	4860
45	H	P	K	G	I	I	S	R	R	D	V	E	K	F	L	S	K	K	1638

	CAC CCT AAG GGC ATT ATT TCG CGT AGA GAT GTG GAA AAA TTT CTT TCA AAA AAA	4914
	R K F P K N Y M S Q Y F K L L E K F	1656
	AGG AAA TTT CCA AAG AAC TAC ATG TCA CAG TAT TTT AAG CTC CTA GAA AAA TTC	4968
5	Q I A L P I G E E Y L L V P S S L S	1674
	CAG ATT GCT TTG CCA ATA GGA GAA GAA TAT TTG CTG GTT CCA AGC AGT TTG TCT	5022
	D H R P V I E L P H C E N S E I I I	1692
10	GAC CAC AGG CCT GTG ATA GAG CTT CCC CAT TGT GAG AAC TCT GAA ATT ATC ATC	5076
	R L Y E M P Y F P M G F W S R L I N	1710 Ex35/g
	CGA CTA TAT GAA ATG CCT TAT TTT CCA ATG GGA TTT TGG TCA AGA TTA ATC AAT	5130 famA
15	R L L E I S P Y M L S G R E R A L R	1728
	CGA TTA CTT GAG ATT TCA CCT TAC ATG CTT TCA GGG AGA GAA CGA GCA CTT CGC	5184
	P N R M Y W R Q G I Y L N W S P E A	1746
	CCA AAC AGA ATG TAT TGG CGA CAA GGC ATT TAC TTA AAT TGG TCT CCT GAA GCT	5238
20	Y C L V G S E V L D N H P E S F L K	1764
	TAT TGT CTG GTA GGA TCT GAA GTC TTA GAC AAT CAT CCA GAG AGT TTC TTA AAA	5292
	I T V P S C R K G C I L L G Q V V D	1782
25	ATT ACA GTT CCT TCT TGT AGA AAA GGC TGT ATT CTT TTG GGC CAA GTT GTG GAC	5346
	H I D S L M E E W F P G L L E I D I	1800
	CAC ATT GAT TCT CTC ATG GAA GAA TGG TTT CCT GGG TTG CTG GAG ATT GAT ATT	5400
30	C G E G E T L L K K W A L Y S F N D	1818
	TGT GGT GAA GGA GAA ACT CTG TTG AAG AAA TGG GCA TTA TAT AGT TTT AAT GAT	5454
	G E E H Q K I L L D D L M K K A E E	1836
	GGC GAA GAA CAT CAA AAA ATC TTA CTT GAT GAC TTG ATG AAG AAA GCA GAG GAA	5508
35	G D L L V N P D Q P R L T I P I S Q	1854
	GGA GAT CTC TTA GTA AAT CCA GAT CAA CCA AGG CTC ACC ATT CCA ATA TCT CAG	5562
	I A P D L I L A D L P R N I M L N N	1872
40	ATT GCC CCT GAC TTG ATT TTG GCT GAC CTG CCT AGA AAT ATT ATG TTG AAT AAT	5616
	D E L E F E Q A P E F L L G D G S F	1890
	GAT GAG TTG GAA TTT GAA CAA GCT CCA GAG TTT CTC CTA GGT GAT GGC AGT TTT	5670
45	G S V Y R A A Y E G E E V A V K I F	1908
	GGA TCA GTT TAC CGA GCA GCC TAT GAA GGA GAA GAA GTG GCT GTG AAG ATT TTT	5724

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	N	K	H	T	S	L	R	L	L	R	Q	E	L	V	V	L	C	H	1926
	AAT	AAA	CAT	ACA	TCA	CTC	AGG	CTG	TTA	AGA	CAA	GAG	CTT	GTG	GTG	CTT	TGC	CAC	5778
5	L	H	H	P	S	L	I	S	L	L	A	A	G	I	R	P	R	M	1944
	CTC	CAC	CAC	CCC	AGT	TTG	ATA	TCT	TTG	CTG	GCA	GCT	GGG	ATT	CGT	CCC	CGG	ATG	5832
	L	V	M	E	L	A	S	K	G	S	L	D	R	L	L	Q	Q	D	1962
	TTG	GTG	ATG	GAG	TTA	GCC	TCC	AAG	GGT	TCC	TTG	GAT	CGC	CTG	CTT	CAG	CAG	GAC	5886
10	K	A	S	L	T	R	T	L	Q	H	R	I	A	L	H	V	A	D	1980
	AAA	GCC	AGC	CTC	ACT	AGA	ACC	CTA	CAG	CAC	AGG	ATT	GCA	CTC	CAC	GTA	GCT	GAT	5940
	G	L	R	Y	L	H	S	A	M	I	I	Y	R	D	L	K	P	H	1998
15	GGT	TTG	AGA	TAC	CTC	CAC	TCA	GCC	ATG	ATT	ATA	TAC	CGA	GAC	CTG	AAA	CCC	CAC	5994
	N	V	L	L	F	T	L	Y	P	N	A	A	I	I	A	K	I	A	2016
	AAT	GTG	CTG	CTT	TTC	ACA	CTG	TAT	CCC	AAT	GCT	GCC	ATC	ATT	GCA	AAG	ATT	GCT	6048
																			DE032
20	D	Y	G	I	A	Q	Y	C	C	R	M	G	I	K	T	S	E	G	2034 T10738
	GAC	TAC	GGC	ATT	GCT	CAG	TAC	TGC	TGT	AGA	ATG	GGG	ATA	AAA	ACA	TCA	GAG	GGC	6102 Ex41/c
																			fam32
	T	P	G	F	R	A	P	E	V	A	R	G	N	V	I	Y	N	Q	2052
	ACA	CCA	GGG	TTT	CGT	GCA	CCT	GAA	GTT	GCC	AGA	GGA	AAT	GTC	ATT	TAT	AAC	CAA	6156
25	Q	A	D	V	Y	S	F	G	L	L	L	Y	D	I	L	T	T	G	2070
	CAG	GCT	GAT	GTT	TAT	TCA	TTT	GGT	TTA	CTA	CTC	TAT	GAC	ATT	TTG	ACA	ACT	GGA	6210
	G	R	I	V	E	G	L	K	F	P	N	E	F	D	E	L	E	I	2088
30	GGT	AGA	ATA	GTA	GAG	GGT	TTG	AAG	TTT	CCA	AAT	GAG	TTT	GAT	GAA	TTA	GAA	ATA	6264
	Q	G	K	L	P	D	P	V	K	E	Y	G	C	A	P	W	P	M	2106
	CAA	GGA	AAA	TTA	CCT	GAT	CCA	GTT	AAA	GAA	TAT	GGT	TGT	GCC	CCA	TGG	CCT	ATG	6318
35	V	E	K	L	I	K	Q	C	L	K	E	N	P	Q	E	R	P	T	2124
	GTT	GAG	AAA	TTA	ATT	AAA	CAG	TGT	TTG	AAA	GAA	AAT	CCT	CAA	GAA	AGG	CCT	ACT	6372
	S	A	Q	V	F	D	I	L	N	S	A	E	L	V	C	L	T	R	2142
	TCT	GCC	CAG	GTC	TTT	GAC	ATT	TTG	AAT	TCA	GCT	GAA	TTA	GTC	TGT	CTG	ACG	AGA	6426
40	R	I	L	L	P	K	N	V	I	V	E	C	M	V	A	T	H	H	2160
	CGC	ATT	TTA	TTA	CCT	AAA	AAC	GTA	ATT	GTT	GAA	TGC	ATG	GTT	GCT	ACA	CAT	CAC	6480
	N	S	R	N	A	S	I	W	L	G	C	G	H	T	D	R	G	Q	2178
45	AAC	AGC	AGG	AAT	GCA	AGC	ATT	TGG	CTG	GGC	TGT	GGG	CAC	ACC	GAC	AGA	GGA	CAG	6534

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	L S F L D L N T E G Y T S E E V A D	2196
	CTC TCA TTT CTT GAC TTA AAT ACT GAA GGA TAC ACT TCT GAG GAA GTT GCT GAT	6588
	S R I L C L A L V H L P V E K E S W	2214
5	AGT AGA ATA TTG TGC TTA GCC TTG GTG CAT CTT CCT GTT GAA AAG GAA AGC TGG	6642
	I V S G T Q S G T L L V I N T E D G	2232
	ATT GTG TCT GGG ACA CAG TCT GGT ACT CTC CTG GTC ATC AAT ACC GAA GAT GGG	6696
10	K K R H T L E K M T D S V T C L Y C	2250
	AAA AAG AGA CAT ACC CTA GAA AAG ATG ACT GAT TCT GTC ACT TGT TTG TAT TGC	6750
	N S F S K Q S K Q K N F L L V G T A	2268
15	AAT TCC TTT TCC AAG CAA AGC AAA CAA AAA AAT TTT CTT TTG GTT GGA ACC GCT	6804
	D G K L A I F E D K T V K L K G A A	2286
	GAT GGC AAG TTA GCA ATT TTT GAA GAT AAG ACT GTT AAG CTT AAA GGA GCT GCT	6858
	P L K I L N I G N V S T P L M C L S	2304
20	CCT TTG AAG ATA CTA AAT ATA GGA AAT GTC AGT ACT CCA TTG ATG TGT TTG AGT	6912
	E S T N S T E R N V M W G G C G T K	2322
	GAA TCC ACA AAT TCA ACG GAA AGA AAT GTA ATG TGG GGA GGA TGT GGC ACA AAG	6966
25	I F S F S N D F T I Q K L I E T R T	2340
	ATT TTC TCC TTT TCT AAT GAT TTC ACC ATT CAG AAA CTC ATT GAG ACA AGA ACA	7020
	S Q L F S Y A A F S D S N I I T V V	2358
30	AGC CAA CTG TTT TCT TAT GCA GCT TTC AGT GAT TCC AAC ATC ATA ACA GTG GTG	7074
	V D T A L Y I A K Q N S P V V E V W	2376
	GTA GAC ACT GCT CTC TAT ATT GCT AAG CAA AAT AGC CCT GTT GTG GAA GTG TGG	7128
	D K K T E K L C G L I D C V H F L R	2394
35	GAT AAG AAA ACT GAA AAA CTC TGT GGA CTA ATA GAC TGC GTG CAC TTT TTA AGG	7182
	E V M V K E N K E S K H K M S Y S G	2412
	GAG GTA ATG GTA AAA GAA AAC AAG GAA TCA AAA CAC AAA ATG TCT TAT TCT GGG	7236
40	R V K T L C L Q K N T A L W I G T G	2430
	AGA GTG AAA ACC CTC TGC CTT CAG AAG AAC ACT GCT CTT TGG ATA GGA ACT GGA	7290
	G G H I L L L D L S T R R L I R V I	2448
45	GGA GGC CAT ATT TTA CTC CTG GAT CTT TCA ACT CGT CGA CTT ATA CGT GTA ATT	7344
	Y N F C N S V R V M M T A Q L G S L	2466

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TAC AAC TTT TGT AAT TCG GTC AGA GTC ATG ATG ACA GCA CAG CTA GGA AGC CTT 7398

 K N V M L V L G Y N R K N T E G T Q 2484
 AAA AAT GTC ATG CTG GTA TTG GGC TAC AAC CGG AAA AAT ACT GAA GGT ACA CAA 7452
 5
 K Q K E I Q S C L T V W D I N L P H 2502
 AAG CAG AAA GAG ATA CAA TCT TGC TTG ACC GTT TGG GAC ATC AAT CTT CCA CAT 7506

 E V Q N L E K H I E V R K E L A E K 2520
 10 GAA GTG CAA AAT TTA GAA AAA CAC ATT GAA GTG AGA AAA GAA TTA GCT GAA AAA 7560

 M R R T S V E * 2528
 ATG AGA CGA ACA TCT GTT GAG TAA gagagaaataggaattgtctttggataggaaaattattc 7623

 15 tctcctcttgtaaataatttatttttaaaaatgttcacatggaaagggactcacattttttgaaatagctcgt 7695

 gtgtatgaaggaatgttatttttttaatttaaatatatgtaaaaatacttaccagtaaatgtgtattttta 7767

 agaactattttaaaacacaatgttatatttcttataaataccagttactttcgttcattaattaatgaaaata 7839
 20 aatctgtgaagtacctaatttaagtactcatactaaaatttataaggccgataattttttgttttcttgtct 7911

 gtaatggaggtaaactttatttttaattctgtgcttaagacaggactattgcttgctgatttttctagaaat 7983

 25 ctgcacggtataatgaaaatattaagacagtttcccatgtaatgtattccttcttagattgcatcgaaatgc 8055

 actatcatatatgcttgtaaataattcaaatgaatttgcactaataaagtcctttgttggtatgtgaattctc 8127

 tttgttgctgttgcaaacagtgcatcttacacaacttcactcaattcaaaagaaaactccattaaaagtact 8199
 30 aatgaaaaaacatgacatactgtcaaagtcctcatatctaggaaagacacagaaactctctttgtcacagaa 8271

 actctctgtgtcttttcttagacataatagagttgtttttcaactctatgtttgaatgtggataccctgaatt 8343

 35 ttgtataattagtgtaaatacagtggttcagtccttcaagtgaatattttttatttttttattcataccactagc 8415

 tacttgttttctaatctgcttcattctaattgcttatattcatcttttccctaaatttgatgatgctgcagatc 8487

 ctacatcattcagatagaaacctttttttttttcagaattatagaattccacagctcctaccaagaccatga 8559
 40 ggataaatatctaacactttttcagttgctgaaggagaaaggagcttttagttatgatggataaaaaatatctgc 8631

 caccctaggcttccaaattataacttaaatgtttacatagcttaccacaataggagtatcagggccaaatac 8703

 45 ctatgtaataatttgaggtcatttctgcttttaggaaaagtactttcggtaaattctttggccctgaccagta 8775

ttcattatttcagataattccctgtgataggacaactagtagtacatttaatatctcagaacttatggcatttt 8847

actatgtgaaaacttttaaattttatttatattaagggtaatcaaattcttaaagatgaaagattttctgtatt 8919

5 ttaaaggaagctatgctttaacttggttatgtaattaacaaaaaatcatatataatagagctctttgttcca 8991

gtgttatctctttcattgttactttgtatgtgcaattttttttaccaaagacaaattaaaaaatgaatacc 9063

atatttaaataagaataataaagggttttttaaaaacttttaa 9104

71

SEQ ID NO: 3

CRMGIKTSEG TPGFRAPEVA RGNVIYNQQA D

5

SEQ ID NO: 4

10 LSALTN

SEQ ID NO: 5

15

ttaagtgcgt taacaaat

20 SEQ ID NO: 6

REVTVK

25

SEQ ID NO: 7

agggaggtaa cggtaaaa

30

Table 1

Gene Symbol	Accession Nr.
PKP2	NM_001005242
ALG10	NM_032834
CPNE8	NM_153634
KIF21A	NM_017641
ABCD2	NM_005164
FLJ40126	NM_173599
SLC2A13	NM_052885
KASPP/LRRK2	
MUC19	AY236870
CNTN1	NM_001843
PDZRN4	NM_013377
LOC283464	BC039145
YAF2	NM_005748
MADP-1	NM_033114
PPHLN1	NM_201438
PRICKLE1	NM_153026
ADAMTS20	NM_025003
DKFZp434G1415	NM_031292
IRAK4	NM_016123
PTK9	NM_002822
DKFZp434K2435	NM_032256
NELL2	NM_006159
PLEKHA9	NM_015899
DKFZp451M105	AL832340
FLJ14711	AK027617
FLJ22820	AK026473
SLC38A1	NM_030674
SLC38A2	NM_018976
SLC38A4	NM_018018

Table 2

Location	Forward	Reverse
D12s2194	F GAGGACTATGATTGCCATGG	R AGGGCATACAAAATGTCCCT
D12s1048	F GGTCTGCTTAGGTCCCTTTT	R AAGGAACCAAGGAGTGGAAG
Intragenic_1	F TTCAGATGTTTGGGGCAAGT	R CATGAAGACTGTGAATGGTTTG
Intragenic_2	F CCAGACAGAAGTCTGAAGGACA	R TCCAAAACAGACAAGAGGTTGA
Intragenic_3	F ATGAAGCCTTGGCTCTTCAA	R TCCCAATTCAAAATTTTAGTGC

5

Table 3

10

Variant	Exon	PD Families (n=55)	Idiopathic PD (n=337)	Controls (n=1200)	Family
R793M; 2378G>T	19	2	1	1	DE041 T11239
Q930R; 2789A>G	21	1	0	0	DE022
S1096C; 3287C>G	24	1	0	0	famE
L1114L; 3342A>G	24	1	0	0	DE038 T11288
S1228T; 3683G>C	27	1	0	0	DE031
G2019S; 6055G>A	41	0	1	0	Hernandez DG et al. (2005)
I2020T; 6059T>C	41	1	0	0	DE032 T10738

Table 4

Family/Patient	T11239	DE041/ II-1//II-2	DE022/ III-5// III-6// III-7	Fam. E	T11288/II-5	DE031/III-1//III-2	T10738/II-2
Mutation	R793M	R793M	Q930R	S1096C	3342A>G	S1228T	I2020T
Age at onset (y)	42 uncle:79	55//70	68//58//47		77	49//49 father: 62 granny: 68	57 father: 59
Disease duration	25 ~	14~//10~	8~//19//30		4~	8~//12~	3~
Initial symptom	B/RT	PT//RT	B,RT//B// B,RT		B	RT//B, RT	B,RT
Response to L- dopa	+	+++	+++//++	+	+	+++	+
Bradykinesia	+	+++	+++//++		+	+++	+
Rigidity	+	+++	+++//++		+	+++	+
Resting Tremor	+	-//+	+++//++		-	+++	+
Postural instability	+	+++	-//+++		+	-//-	-

Sleeping disturbances	+	+//+	+//+//+		+	-//+	+
Long-term complications	+	+//+	+//+//+		-	+//+	-
Hallucinations	+	-//	-//+//+		+	-//	-
Additional findings	Tongue dystonia	-			Dementia L-dopa hypersensitivity		
Sniffing test	2/8	nd	nd	nd	7/8	5/8//5/8	7/8
Electro-neurography	na	nd	nd	nd	na	na//na	Na
Electro-myography	na	nd	nd	nd	na	na//na	Na
Magnet evoked potentials	na	nd	nd	nd	na	na//na	Na
TCS (r;l)	0.21; 0.24	nd	nd	nd	0.24; 0.19	0.23; 0.24// 0.22; 0.24	0.16; 0.17
MRI	nd, because of electrodes	nd	nd	nd	global atrophy, microangiopathy	slightly increased atrophy in both, slight microangiopathic in III-2	Slight fronto-temporally enhanced atrophy

Table 5

Family/Patient	T11239	T11288/II-5	DE031/III-1//	DE031/III-2	T10738/II-2
Mutation	R793M	3342A>G		S1228T	I2020T
LPS-K/ SPM (IQ)	/90↓	75↓↓ /	111↑ /	117↑ /	120↑ /
Tower of London (PR)	xx	-	6↓↓	27↓	xx
CWIT (T-Value INT)	-	(63)	37	41	41
CERAD 1 (Verbal Fluency)	-	6	20~	31↑	36↑
CERAD 2 (Boston Naming Test)	-	12	14~	15~	14~
CERAD 3 (MMSE)	22	24	30~	30~	29~
CERAD 4 (Word List Memory)	-	10	19~	22~	29↑
CERAD 5 (Constructional Praxis)	-	7	10~	10~	11~
CERAD 6.1 (Word List Recall)	-	1	7~	7~	10↑
CERAD 7.1 (Word List Recognition)	-	10	9	10	10
CERAD 8 (Recall of Constructional Praxis)	-	2	10~	13↑	12↑
D2-Test (Concentration)	-	-	38	46	46
BDI	23↑	11	3	9	7.5
PDQ-39 (PDSI)	40.4	25.6	8.8	18.6	23.6

Claims

1. An isolated nucleic acid molecule comprising a polynucleotide sequence selected from:
 - (a) a nucleotide sequence depicted in SEQ ID NO: 2 containing at least one mutation segregating with the occurrence of Parkinson's disease (PD) selected from a G $\rightarrow$ T mutation at position 2378, an A $\rightarrow$ G mutation at position 2789, a C $\rightarrow$ G mutation at position 3287, an A $\rightarrow$ G mutation at position 3342, an A $\rightarrow$ G mutation at position 3364, a G $\rightarrow$ C mutation at position 3683, a C $\rightarrow$ T mutation at position 4321, an A $\rightarrow$ G mutation at position 5096 and/or a T $\rightarrow$ C mutation at position 6059;
 - (b) a nucleotide sequence complementary to the nucleotide sequence in (a); and/or
 - (c) a nucleotide sequence which is capable of hybridizing under high stringency conditions to a nucleotide sequence in (b) and which codes for the protein sequence encoded by the nucleotide sequence depicted in SEQ ID NO: 2 containing at least one mutation segregating with the occurrence of Parkinson's disease (PD) selected from a G $\rightarrow$ T mutation at position 2378, an A $\rightarrow$ G mutation at position 2789, a C $\rightarrow$ G mutation at position 3287, an A $\rightarrow$ G mutation at position 3364, a G $\rightarrow$ C mutation at position 3683, a C $\rightarrow$ T mutation at position 4321, an A $\rightarrow$ G mutation at position 5096 and/or a T $\rightarrow$ C mutation at position 6059 or a nucleotide sequence which is capable of hybridizing under high stringency conditions to the nucleotide sequence in (a) and which is capable of detecting at least one mutation segregating with the occurrence of Parkinson's disease (PD) selected from a G $\rightarrow$ T mutation at position 2378, an A $\rightarrow$ G mutation at position 2789, a C $\rightarrow$ G mutation at position 3287, an A $\rightarrow$ G mutation at position 3342, a A $\rightarrow$ G mutation at position 3364, a G $\rightarrow$ C mutation at position 3683, a C $\rightarrow$ T mutation at position 4321, an A $\rightarrow$ G mutation at position 5096 and/or a T $\rightarrow$ C mutation at position 6059,

wherein high stringency conditions comprise hybridization solutions containing 50% formamide and high salt being either 5x SSC or 5x SSPE, 5x Denhardt's solution, 1% SDS and 100 μ g/ml denatured salmon sperm DNA and washing is in 0.2x SSC/0.1% SDS at 68°C.

2. The nucleic acid molecule according to claim 1 (c) consisting of 10 to 50 nucleotides.
3. The nucleic acid molecule according to claim 1 (c) consisting of 10 to 35 nucleotides.
4. The nucleic acid molecule according to claim 1 (c) consisting of 20 to 35 nucleotides.
5. A vector containing the nucleic acid molecule of any one of claims 1 to 4.
6. A cell containing the nucleic acid of any one of claims 1 to 4 or the vector of claim 5.
7. A protein segregating with the occurrence of Parkinson's disease (PD) encoded by a nucleotide sequence according to claim 1 containing at least one mutation selected from R793M, Q930R, S1096C, I1122V, S1228T, R1441C, Y1699C and/or I2020T.
8. A method of producing the protein according to claim 7, the method comprising:
 - (a) culturing a cell according to claim 6 under suitable conditions; and
 - (b) isolating the protein produced by the cultured cell.
9. A method of detecting a mutation segregating with the occurrence of Parkinson's disease (PD) in the nucleic acid of a sample, the method comprising:
 - (a) contacting said sample with the nucleic acid molecule according to claim 1 or 2 under high stringency conditions, and
 - (b) detecting the presence of at least one mutation in the nucleic acid of the sample by means of Southern blot hybridization, Northern blot hybridization, PCR, RT-PCR, real-time RT-PCR or automated sequencing,

wherein the nucleic acid molecule is capable of detecting the at least one mutation in the nucleic acid of the sample segregating with the occurrence of Parkinson's disease (PD), selected from a G $\rightarrow$ T mutation at position 2378, an A $\rightarrow$ G mutation at position 2789, a C $\rightarrow$ G mutation at position 3287, an A $\rightarrow$ G mutation at position 3342, a A $\rightarrow$ G mutation at position 3364, a G $\rightarrow$ C mutation at position 3683, a C $\rightarrow$ T mutation at position 4321, an A $\rightarrow$ G mutation at position 5096 and/or a T $\rightarrow$ C mutation at position 6059,

wherein high stringency conditions comprise hybridization solutions containing

50% formamide and high salt being either 5x SSC or 5x SSPE, 5x Denhardt's solution, 1% SDS and 100 µg/ml denatured salmon sperm DNA and washing is in 0.2x SSC/0.1% SDS at 68°C.

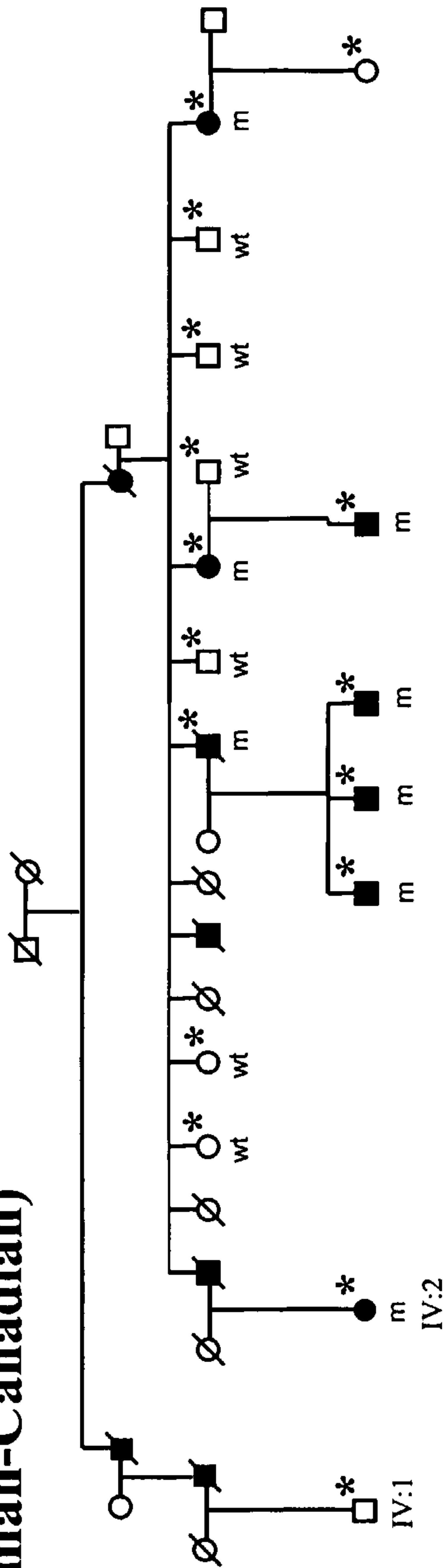
10. The method of claim 9, wherein the sample is selected from
 - (a) a biopsy from human tissues or cells; or
 - (b) RNA and/or DNA from a biopsy from human tissue or cells.
11. The method of claim 10 wherein the sample is from brain, heart, lung and/or blood lymphocytes.
12. The method of any one of claims 9-11, wherein the nucleic acid molecule according to claim 1 (c) consists of 10 to 50 nucleotides.
13. The method of any one of claims 9-11, wherein the nucleic acid molecule according to claim 1 (c) consists of 10 to 35 nucleotides.
14. The method of any one of claims 9-11, wherein the nucleic acid molecule according to claim 1 (c) consists of 20 to 35 nucleotides.
15. The method of any one of the claims 9 to 14, wherein the detecting of the mutation comprises radiography, fluorescence, chemiluminescence, or any combination thereof.
16. The method of any one of the claims 9 to 15, wherein the method is carried out on an array.
17. The method of any one of the claims 9 to 16, wherein the method is carried out in a robotics system.
18. The method of any one of the claims 9 to 17, wherein the method is carried out using microfluidics.
19. A diagnostic kit containing at least one nucleic acid molecule as defined in claim 1 for diagnosing Parkinson disease (PD) wherein the nucleic acid molecule is capable of detecting a mutation segregating with the occurrence of Parkinson's disease (PD), selected from a G → T mutation at position 2378, an A → G mutation at position 2789, a C → G mutation at position 3287, an A → G mutation at position 3342, a A → G mutation at position 3364, a G → C mutation at position 3683, a C → T mutation at position 4321, an A → G mutation at position 5096 and/or a T → C mutation at position 6059 and instructions for use of the kit.
20. The diagnostic kit of claim 19, wherein the nucleic acid molecule according to

claim 1 (c) consists of 10 to 50 nucleotides.

21. The diagnostic kit of claim 19, wherein the nucleic acid molecule according to claim 1 (c) consists of 10 to 35 nucleotides.
22. The diagnostic kit of claim 19, wherein the nucleic acid molecule according to claim 1 (c) consists of 20 to 35 nucleotides.
23. A method for screening a pharmaceutical or diagnostic agent, the method comprising:
providing at least one nucleic acid molecule as defined in any one of claims 1 to 4 or a protein as defined in claim 7,
providing a test compound, and
measuring or detecting the influence of the test compound on the expression of the nucleic acid molecule or the activity of the protein.
24. The method according to claim 23, wherein the test compound is provided in the form of a chemical compound library.
25. The method according to claim 23 or 24, wherein the influence of the test compound is measured or detected in a heterogeneous or homogeneous assay.
26. The method according to any one of the claims 23 to 25, wherein the method is carried out on an array.
27. The method according to any one of the claims 23 to 26, wherein the method is carried out using whole cells.
28. The method according to any one of the claims 23 to 26, wherein the method is carried out in a robotics system.
29. The method according to any one of the claims 23 to 28, wherein the method is carried out using microfluidics.

Fig.1

Family A (German-Canadian)
(Y1699C)



Family D (Western Nebraska)
(R1441C)

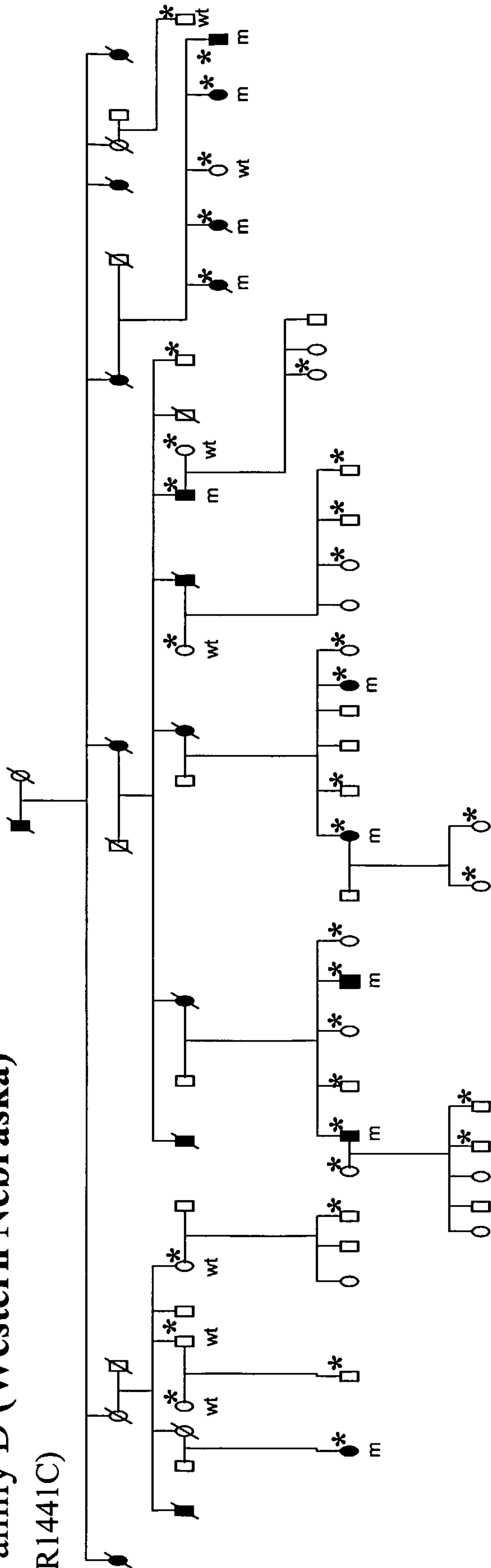


Fig. 2

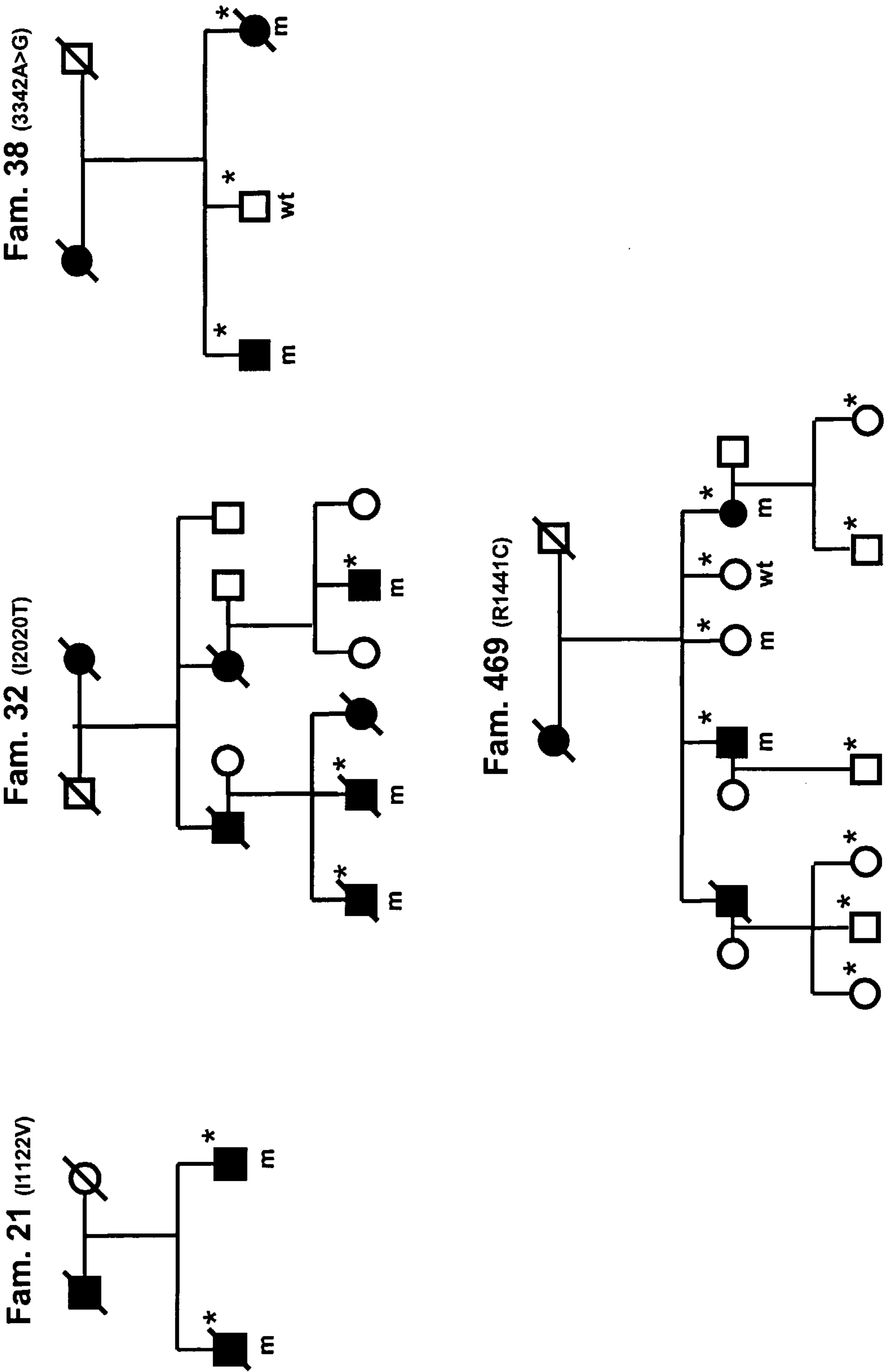


Fig.3

Human	I1122V	R1441C	Y1699C	I1122T
Mouse	ISGICSP	IKARASS	EMPYFPM	DYGTAAQY
Fugu	ISGICSP	IKARASS	EMPYFPM	DYGTAAQY
Worm	IAELCVP	IKAVAPL	EMPYFPM	.YGTAAQH
Fly	IQ...E	IHARAPN	ALAYTIPS	DYGTLSRS
	IS..CWP	IQARAPN	LMTYFPS	DYGTLSRQ
Family	21	D/469	A	32
Exon	24	30	34	40

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Fig. 4

```

      10      20      30      40      50      60
-----|-----|-----|-----|-----|-----|
ATGGCTAGTGGCAGCTGTCAGGGGTGCGAAGAGGACGAGGAAACTCTGAAGAAGTTGATA
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
-----|-----|-----|-----|-----|-----|
GATCTGCTGGTGTTCACGTACTCCGAGCAGCCTCCAAGTTATTTCAAGGCAAAAATATC
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
-----|-----|-----|-----|-----|-----|
GGTTGGTCACTTCTGTGCAAATTAATAGAAGTCTGTCCAGGTACAATGCAAAGCTTAATG
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
-----|-----|-----|-----|-----|-----|
GGACCCCAGGATGTTGGAAATGATTGGGAAGTCCTTGGTGTTCACCAATTGATTCTTAAA
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
-----|-----|-----|-----|-----|-----|
ATGCTAACAGTTCATAATGCCAGTGTAACCTTGTCACTGATTGGACTGAAGACCTTAGAT
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
-----|-----|-----|-----|-----|-----|
CTCCTCCTAACTTCAGGTAAAATCACCTTGCTGATACTGGATGAAGAAAGTGATATTTTC
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
-----|-----|-----|-----|-----|-----|
ATGTTAATTTTGGATGCCATGCACTCATTTCAGCCAATGATGAAGTCCAGAACTTGA
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
-----|-----|-----|-----|-----|-----|
TGCAAAGCTTTACATGTGCTGTTTGAGAGAGTCTCAGAGGAGCAACTGACTGAATTTGTT
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
-----|-----|-----|-----|-----|-----|
GAGAACAAAGATTATATGATATTGTTAAGTGCGTCAACAAATTTTAAAGATGAAGAGGAA
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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-----|-----|-----|-----|-----|-----|-----|
ATTGTGCTTCATGTGCTGCATTGTTTACATTCCCTAGCGATTCCCTTGCAATAATGTGGAA
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
-----|-----|-----|-----|-----|-----|
GTCCTCATGAGTGGCAATGTCAGGTGTTATAATATTGTGGTGAAGCTATGAAAGCATTC
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
-----|-----|-----|-----|-----|-----|
CCTATGAGTGAAAGAATTCAAGAAGTGAGTTGCTGTTTGCTCCATAGGCTTACATTAGGT
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
-----|-----|-----|-----|-----|-----|
AATTTTTCATATCCTGGTATTAACGAAGTCCATGAGTTTGTGGTGAAGCTGTGCAG
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
-----|-----|-----|-----|-----|-----|
GAGACTATTTTCTTAAATCAAGATTTAGAGGAAAAGAATGAGAATCAAGAGAATGATGAT
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
-----|-----|-----|-----|-----|-----|
GAGGGGGAAGAAGATAAATTGTTTGGCTGGAAGCCTGTTACAAAGCATTAAACGTGGCAT
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
-----|-----|-----|-----|-----|-----|
AGAAAGAACAAGCACGTGCAGGAGGCCGCTGCTGGGCACTAAATAATCTCCTTATGTAC
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
-----|-----|-----|-----|-----|-----|
CAAAACAGTTTACATGAGAAGATTGGAGATGAAGATGGCCATTTCCAGCTCATAGGGAA
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
-----|-----|-----|-----|-----|-----|
GTGATGCTCTCCATGCTGATGCATTCTTCATCAAAGGAAGTTTCCAGGCATCTGCGAAT
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
-----|-----|-----|-----|-----|-----|
GCATTGTCAACTCTCTTAGAACAAAATGTTAATTTAGAAAAATACTGTTATCAAAAAGGA
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
AGTGGCTGTAAAATGCTAAATCATCTTTTTGAAGGAAGCAACACTTCCCTGGATATAATG
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
GCAGCAGTGGTCCCCAAAATACTAACAGTTATGAAACGTCATGAGACATCATTACCAAGTG
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
AGGGAGGATACAGAATTTTCATCATAAGCTAAATATGGTTAAAAAACAGTGTTCAGAAGAT
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
GATATTCACAACTGGTCCTAGCAGCTTTGAACAGGTTTCATTGGAAATCCTGGGATTCAG
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
AAATGTGGATTAAAAGTAATTTCTTCTATTGTACATTTTCTGATGCATTAGAGATGTTA
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
TCCCTGGAAGGTGCTATGGATTTCAGTGCTTCACACACTGCAGATGTATCCAGATGACCAA
S L E G A M D S V L H T L Q M Y P D D Q

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

||
exon15/exon16

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

1930 1940 1950 1960 1970 1980
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
GTTGCTGAAATACAGACTAAAGGATTTTCAGACAATCTTAGCAATCCTCAAATTGTCAGCA
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
TCTTTTCTAAGCTGCTGGTGCATCATTATTTGACTTAGTAATATTCCATCAAATGTCT
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
-----|-----|-----|-----|-----|-----|
TCCAATATCATGGAACAAAAGGATCAACAGTTTCTAAACCTCTGTTGCAAGTGTGTTTGCA
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
-----|-----|-----|-----|-----|-----|
GGATCTTCTTTAATTTGTCAGGTATGTGAGAAAGAGAGCAGTCCCAAATTGGTGGAAGTC
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
-----|-----|-----|-----|-----|-----|
TACTGAATAGTGGATCTCGTGAACAAGATGTACGAAAAGCGTTGACGATAAGCATTGGG
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
-----|-----|-----|-----|-----|-----|
AATAGCATTTGCCTTGGAGGATTTTGTATAGGAAAAGTTGAACCTTCTTGGCTTGGTCCT
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
-----|-----|-----|-----|-----|-----|
TTATTTCCAGATAAGACTTCTAATTTAAGGAAACAAACAAATATAGCATCTACACTAGCA
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
-----|-----|-----|-----|-----|-----|
AGAATGGTGATCAGATATCAGATGAAAAGTGCTGTGGAAGAAGGAACAGCCTCAGGCAGC
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
-----|-----|-----|-----|-----|-----|
GTATTACAGCGTTGCTCACCAAATTTGCAAAGACATTCCAATTCCTTGGGGCCCATTTTT
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
-----|-----|-----|-----|-----|-----|
GATCATGAAGATTTACTGAAGCGAAAAAGAAAAATACTATCTTCAGATGATTCACTCAGG
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
-----|-----|-----|-----|-----|-----|
TCATCAAACTTCAATCCCATATGAGGCATTGAGACAGCATTTCTTCTCTGGCTTCTGAG
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
-----|-----|-----|-----|-----|-----|
AGAGAATATATTACATCACTAGACCTTTCAGCAAATGAACTAAGAGATATTGATGCCCTA
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
-----|-----|-----|-----|-----|-----|
AGCCAGAAATGCTGTATAAGTGTTTCATTTGGAGCATCTTGAAAAGCTGGAGCTTCACCAG
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
-----|-----|-----|-----|-----|-----|
AATGCACTCACGAGCTTTCACAACAGCTATGTGAACTCTGAAGAGTTTGACACATTTG
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
-----|-----|-----|-----|-----|-----|
GCTAATCTTGATGTCTCTCGAAATGACATTGGACCCTCAGTGGTTTTAGATCCTACAGTG
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
-----|-----|-----|-----|-----|-----|
AAATGTCCAACCTCTGAAACAGTTTAAACCTGTCATATAACCAGCTGTCTTTGTACCTGAG
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
-----|-----|-----|-----|-----|-----|
AACCTCACTGATGTGGTAGAGAACTGGAGCAGCTCATTTTGAAGGAAATAAAATATCA
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
-----|-----|-----|-----|-----|-----|
GGGATATGCTCCCCCTTGAGACTGAAGGAACTGAAGATTTTAAACCTTAGTAAGAACCAC
G I C S P L R L K E L K I L N L S K N H

3430 3440 3450 3460 3470 3480
-----|-----|-----|-----|-----|-----|
ATTTTCATCCCTATCAGAGAACTTTCTTGAGGCTTGTCCTAAAGTGGAGAGTTTCAGTGCC
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
AGAATGAATTTTCTTGCTGCTATGCCTTTCTTGCCTCCTTCTATGACAATCCTAAAATTA
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
TCTCAGAACAAATTTTCTGTATTCCAGAAGCAATTTTAAATCTTCCACACTTGCAGTCT
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
TTAGATATGAGCAGCAATGATATTTCAGTACCTACCAGGTCCCGCACACTGGAAATCTTTG
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
AACTTAAGGGAAGTCTTATTTAGCCATAATCAGATCAGCATCTTGGACTTGAGTGAAAAA
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
CCTCCTGAGATTGGCTGTCTTGAAAATCTGACATCTCTGGATGTCAGTTACAACCTTGGA
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
CTAAGATCCTTTCCCAATGAAATGGGGAAATTAAGCAAAATATGGGATCTTCCTTTGGAT
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
GAACTGCATCTTAACCTTTGATTTTAAACATATAGGATGTAAAGCCAAAGACATCATAAGG
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
GGAAATACTGGGAGTGGTAAAACACCTTATTGCAGCAATTAATGAAAACCAAGAAATCA
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
GATCTTGAATGCAAAGTGCCACAGTTGGCATAGATGTGAAAGACTGGCCTATCCAAATA
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
AGAGACAAAAGAAAGAGAGATCTCGTCCTAAATGTGTGGGATTTTGCAGGTCGTGAGGAA
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

4210 4220 4230 4240 4250 4260
-----|-----|-----|-----|-----|-----|
TTCTATAGTACTCATCCCCATTTTATGACGCAGCGAGCATTGTACCTTGCTGTCTATGAC
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

||
exon30/exon31

4330 4340 4350 4360 4370 4380
-----|-----|-----|-----|-----|-----|
CGCGCTTCTTCTTCCCCTGTGATTCTCGTTGGCACACATTTGGATGTTTCTGATGAGAAG
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
-----|-----|-----|-----|-----|-----|
CAACGCAAAGCCTGCATGAGTAAAATCACCAAGGAACTCCTGAATAAGCGAGGGTTCCCT
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
-----|-----|-----|-----|-----|-----|
CGGAAAACCATCATAAACGAGAGCCTTAATTTCAAGATCCGAGATCAGCTTGTTGTTGGA
R K T I I N E S L N F K I R D Q L V V G

||
exon31/exon32

4570 4580 4590 4600 4610 4620
-----|-----|-----|-----|-----|-----|
CAGCTGATTCCAGACTGCTATGTAGAACTTGAAAAAATCATTTTATCGGAGCGTAAAAAT
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
-----|-----|-----|-----|-----|-----|
GTGCCAATTGAATTTCCCGTAATTGACCGGAAACGATTATTACAAGTGTGAGAGAAAAT
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

||
exon32/exon33

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
-----|-----|-----|-----|-----|-----|
AAGTGGCTTTGTAAAATCATGGCACAGATTTTGACAGTGAAAGTGGAAGGTTGTCCAAAA
K W L C K I M A Q I L T V K V E G C P K

||
exon33/exon34

4870 4880 4890 4900 4910 4920
-----|-----|-----|-----|-----|-----|
CACCCTAAGGGCATTATTTTCGCGTAGAGATGTGGAAAAATTTCTTTCAAAAAAAGGAAA

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H P K G I I S R R D V E K F L S K K R K

4930 4940 4950 4960 4970 4980
-----|-----|-----|-----|-----|-----|
TTTCCAAAGAACTACATGTCACAGTATTTTAAGCTCCTAGAAAAATTCCAGATTGCTTTG
F P K N Y M S Q Y F K L L E K F Q I A L

4990 5000 5010 5020 5030 5040
-----|-----|-----|-----|-----|-----|
CCAATAGGAGAAGAATATTTGCTGGTTCCAAGCAGTTTGTCTGACCACAGGCCTGTGATA
P I G E E Y L L V P S S L S D H R P V I

||
exon34/exon35

5050 5060 5070 5080 5090 5100
-----|-----|-----|-----|-----|-----|
GAGCTTCCCCATTGTGAGAACTCTGAAATTATCATCCGACTATATGAAATGCCTTATTTT
E L P H C E N S E I I I R L Y E M P Y F

5110 5120 5130 5140 5150 5160
-----|-----|-----|-----|-----|-----|
CCAATGGGATTTTGGTCAAGATTAATCAATCGATTACTTGAGATTTTACCTTACATGCTT
P M G F W S R L I N R L L E I S P Y M L

5170 5180 5190 5200 5210 5220
-----|-----|-----|-----|-----|-----|
TCAGGGAGAGAACGAGCACTTCGCCCAAACAGAATGTATTGGCGACAAGGCATTTACTTA
S G R E R A L R P N R M Y W R Q G I Y L

||
exon35/exon36

5230 5240 5250 5260 5270 5280
-----|-----|-----|-----|-----|-----|
AATTGGTCTCCTGAAGCTTATTGTCTGGTAGGATCTGAAGTCTTAGACAATCATCCAGAG
N W S P E A Y C L V G S E V L D N H P E

5290 5300 5310 5320 5330 5340
-----|-----|-----|-----|-----|-----|
AGTTTCTTAAAAATTACAGTTCCTTCTTGTAGAAAAGGCTGTATTCTTTTGGGCCAAGTT
S F L K I T V P S C R K G C I L L G Q V

||
exon36/exon37

5350 5360 5370 5380 5390 5400
-----|-----|-----|-----|-----|-----|
GTGGACCACATTGATTCTCTCATGGAAGAATGGTTTCCTGGGTTGCTGGAGATTGATATT
V D H I D S L M E E W F P G L L E I D I

5410 5420 5430 5440 5450 5460
-----|-----|-----|-----|-----|-----|
TGTGGTGAAGGAGAACTCTGTTGAAGAAATGGGCATTATATAGTTTAAATGATGGCGAA
C G E G E T L L K K W A L Y S F N D G E

5470 5480 5490 5500 5510 5520
-----|-----|-----|-----|-----|-----|
GAACATCAAAAAATCTTACTTGATGACTTGATGAAGAAAGCAGAGGAAGGAGATCTCTTA
E H Q K I L L D D L M K K A E E G D L L

||
exon37/exon38

5530 5540 5550 5560 5570 5580
-----|-----|-----|-----|-----|-----|
GTAAATCCAGATCAACCAAGGCTCACCATTCCAATATCTCAGATTGCCCTGACTTGATT
V N P D Q P R L T I P I S Q I A P D L I

5590 5600 5610 5620 5630 5640
-----|-----|-----|-----|-----|-----|
TTGGCTGACCTGCCTAGAAATATTATGTTGAATAATGATGAGTTGGAATTTGAACAAGCT

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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
-----|-----|-----|-----|-----|-----|
CCAGAGTTTCTCCTAGGTGATGGCAGTTTTGGATCAGTTTACCGAGCAGCCTATGAAGGA
P E F L L G D G S F G S V Y R A A Y E G

||
exon38/exon39

5710 5720 5730 5740 5750 5760
-----|-----|-----|-----|-----|-----|
GAAGAAGTGGCTGTGAAGATTTTAATAAACATACATCACTCAGGCTGTTAAGACAAGAG
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
-----|-----|-----|-----|-----|-----|
CTTGTGGTGCTTTGCCACCTCCACCACCCAGTTTGATATCTTTGCTGGCAGCTGGGATT
L V V L C H L H H P S L I S L L A A G I

5830 5840 5850 5860 5870 5880
-----|-----|-----|-----|-----|-----|
CGTCCCCGGATGTTGGTGATGGAGTTAGCCTCCAAGGGTTCCTTGGATCGCCTGCTTCAG
R P R M L V M E L A S K G S L D R L L Q

5890 5900 5910 5920 5930 5940
-----|-----|-----|-----|-----|-----|
CAGGACAAAGCCAGCCTCACTAGAACCCTACAGCACAGGATTGCACTCCACGTAGCTGAT
Q D K A S L T R T L Q H R I A L H V A D

5950 5960 5970 5980 5990 6000
-----|-----|-----|-----|-----|-----|
GGTTTGAGATACCTCCACTCAGCCATGATTATATACCGAGACCTGAAACCCCAATGTG
G L R Y L H S A M I I Y R D L K P H N V

||
exon40/exon41

6010 6020 6030 6040 6050 6060
-----|-----|-----|-----|-----|-----|
CTGCTTTTCACACTGTATCCCAATGCTGCCATCATTGCAAAGATTGCTGACTACGGCATT
L L F T L Y P N A A I I A K I A D Y G I

6070 6080 6090 6100 6110 6120
-----|-----|-----|-----|-----|-----|
GCTCAGTACTGCTGTAGAATGGGGATAAAAACATCAGAGGGCACACCAGGGTTTCGTGCA
A Q Y C C R M G I K T S E G T P G F R A

||
exon41/exon42

6130 6140 6150 6160 6170 6180
-----|-----|-----|-----|-----|-----|
CCTGAAGTTGCCAGAGGAAATGTCATTTATAACCAACAGGCTGATGTTTATTCATTTGGT
P E V A R G N V I Y N Q Q A D V Y S F G

6190 6200 6210 6220 6230 6240
-----|-----|-----|-----|-----|-----|
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
-----|-----|-----|-----|-----|-----|
TGCTTAGCCTTGGTGCATCTTCCTGTTGAAAAGGAAAGCTGGATTGTGTCTGGGACACAG
C L A L V H L P V E K E S W I V S G T Q

6670 6680 6690 6700 6710 6720
-----|-----|-----|-----|-----|-----|
TCTGGTACTCTCCTGGTCATCAATACCGAAGATGGGAAAAAGAGACATACCCTAGAAAAG
S G T L L V I N T E D G K K R H T L E K

6730 6740 6750 6760 6770 6780
-----|-----|-----|-----|-----|-----|
ATGACTGATTCTGTCACTTGTGTTGTATTGCAATTCCTTTTCCAAGCAAAGCAAACAAAAA
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
-----|-----|-----|-----|-----|-----|
AATTTTCTTTTGGTTGGAACCGCTGATGGCAAGTTAGCAATTTTGAAGATAAGACTGTT
N F L L V G T A D G K L A I F E D K T V

6850 6860 6870 6880 6890 6900
-----|-----|-----|-----|-----|-----|
AAGCTTAAAGGAGCTGCTCCTTTGAAGATACTAAATATAGGAAATGTCAGTACTCCATTG
K L K G A A P L K I L N I G N V S T P L

||
exon46/exon47

6910 6920 6930 6940 6950 6960
-----|-----|-----|-----|-----|-----|
ATGTGTTTGAGTGAATCCACAAATTCAACGGAAAGAAATGTAATGTGGGGAGGATGTGGC
M C L S E S T N S T E R N V M W G G C G

6970 6980 6990 7000 7010 7020
-----|-----|-----|-----|-----|-----|
ACAAAGATTTTCTCCTTTTCTAATGATTTCAACATTCAGAACTCATTGAGACAAGAACA
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

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-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
AGCCAAGTGTCTTTCTTATGCAGCTTTTCAGTGATTCCAACATCATAACAGTGGTGGTAGAC
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
ACTGCTCTCTATATTGCTAAGCAAAATAGCCCTGTTGTGGAAGTGTGGGATAAGAAACT
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
GAAAACTCTGTGGACTAATAGACTGCGTGCACTTTTAAAGGGAGGTAATGGTAAAAGAA
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
AACAAGGAATCAAAACACAAAATGTCTTATTCTGGGAGAGTGAAAACCTCTGCCTTCAG
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
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
AAGAACACTGCTCTTTGGATAGGAAGTGGAGGAGGCCATATTTTACTCCTGGATCTTTCA
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

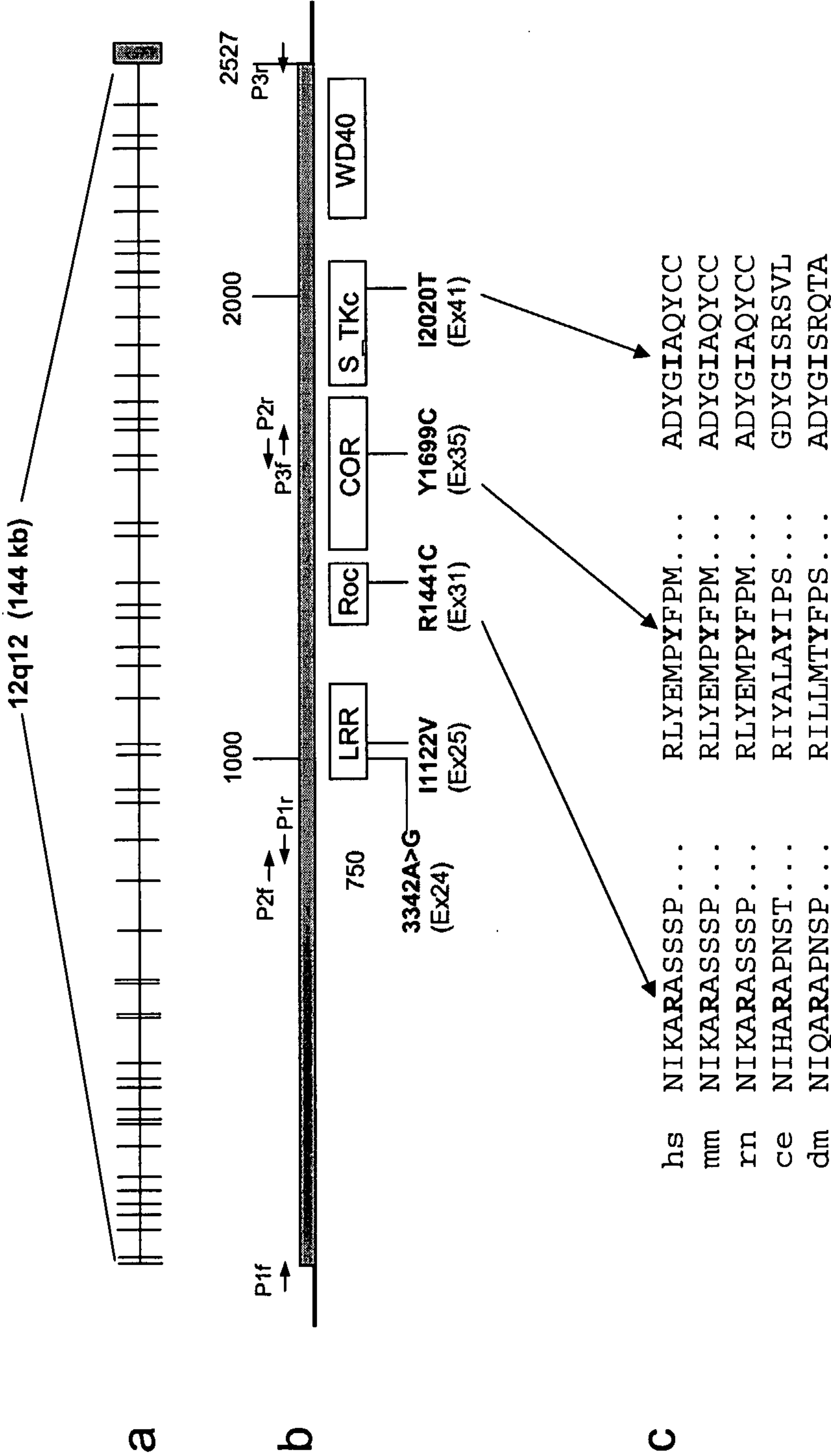
7390 7400 7410 7420 7430 7440
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
GCACAGCTAGGAAGCCTTAAAAATGTCATGCTGGTATTGGGCTACAACCGGAAAAATACT
A Q L G S L K N V M L V L G Y N R K N T
||
exon49/exon50

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

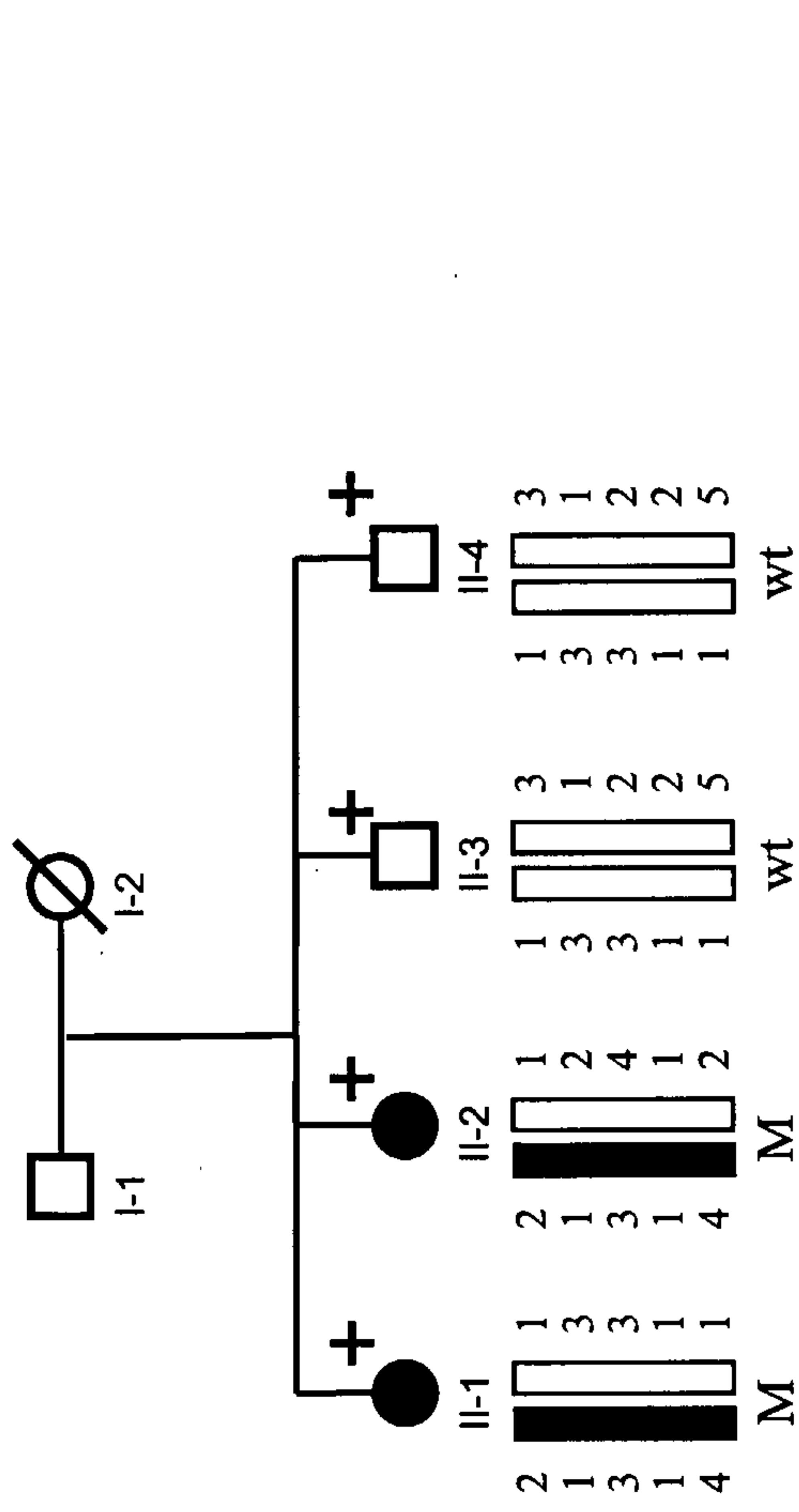
7510 7520 7530 7540 7550 7560
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|
CCACATGAAGTGCAAAATTTAGAAAAACACATTGAAGTGAGAAAAGAATTAGCTGAAAAA
P H E V Q N L E K H I E V R K E L A E K

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

Fig. 5



DE041
(R793M)



T11239
(R793M)

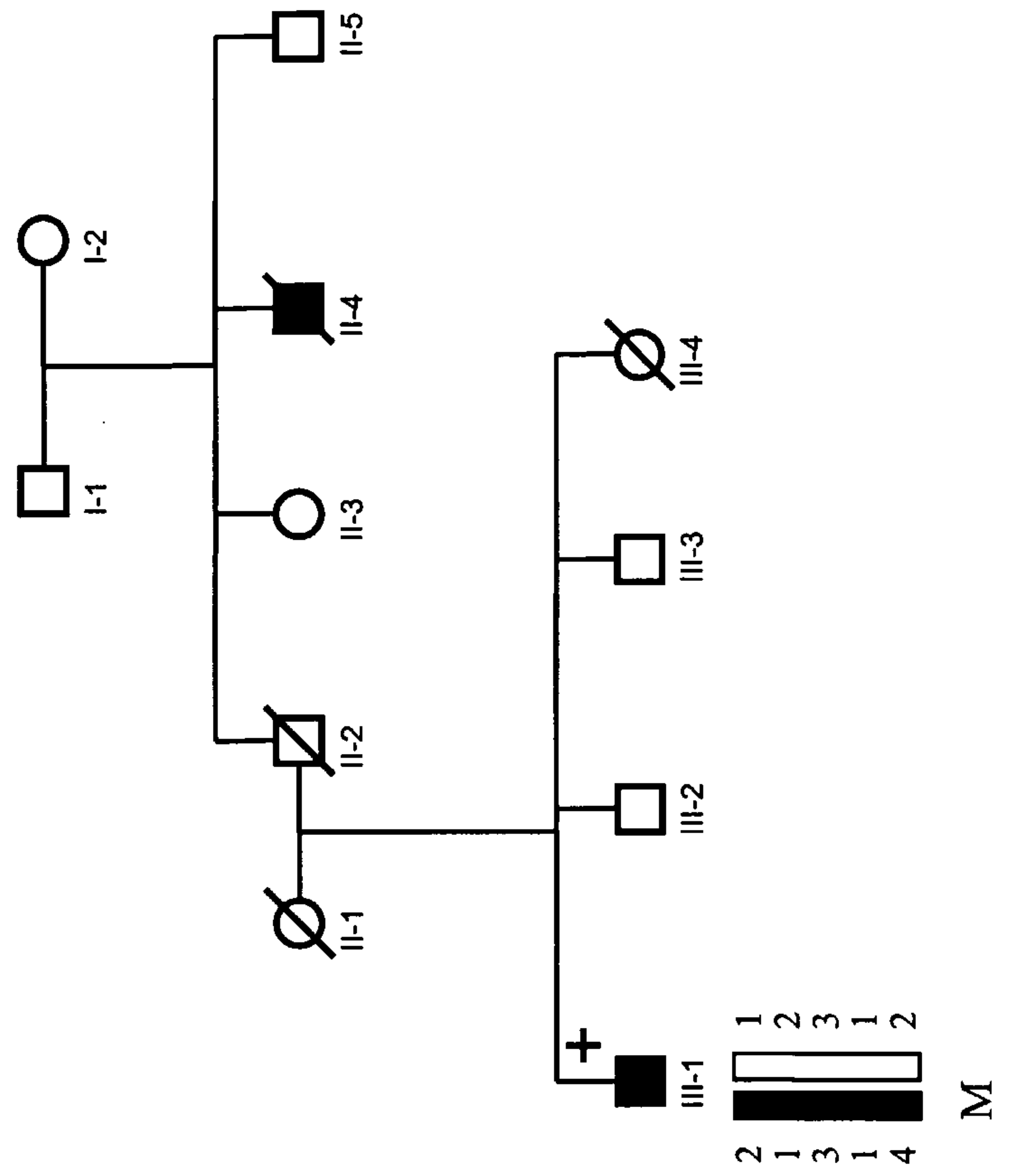


Fig. 6a

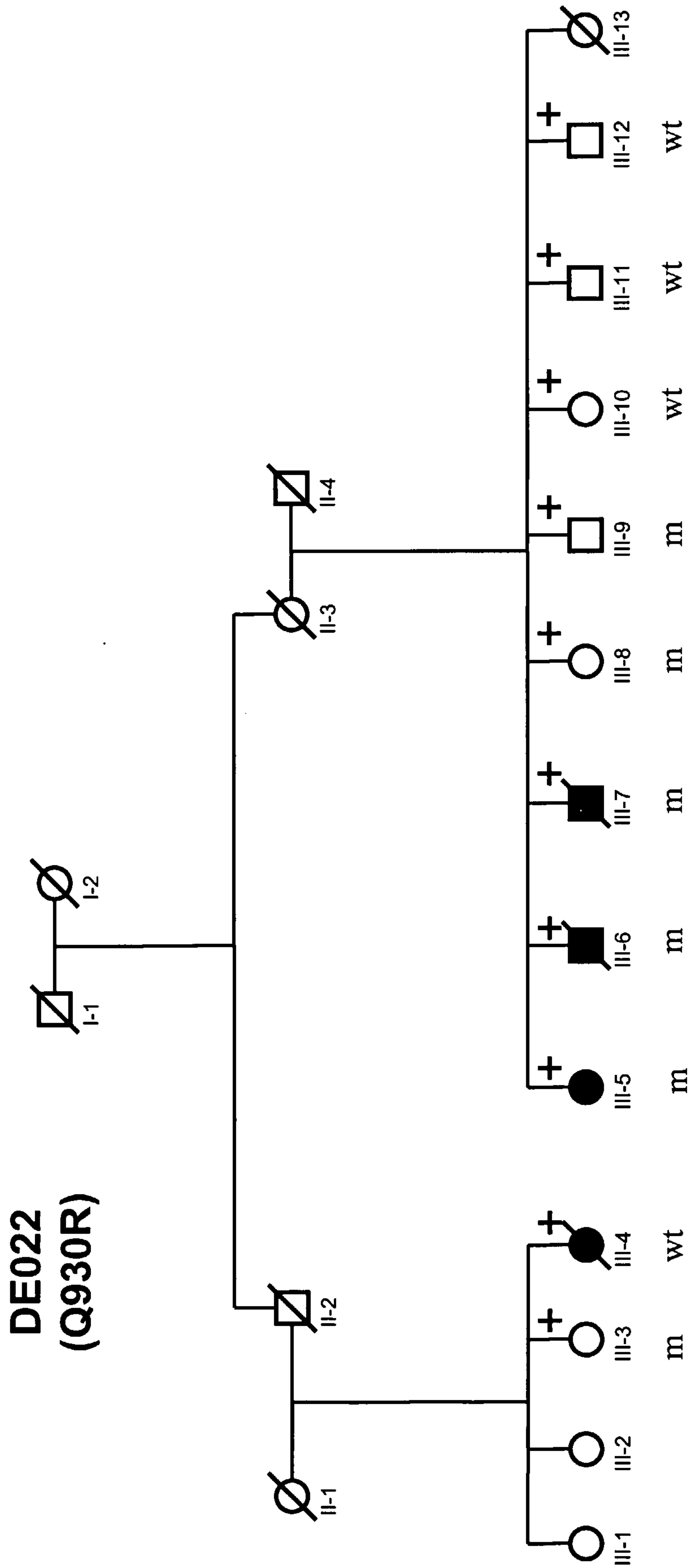


Fig. 6b

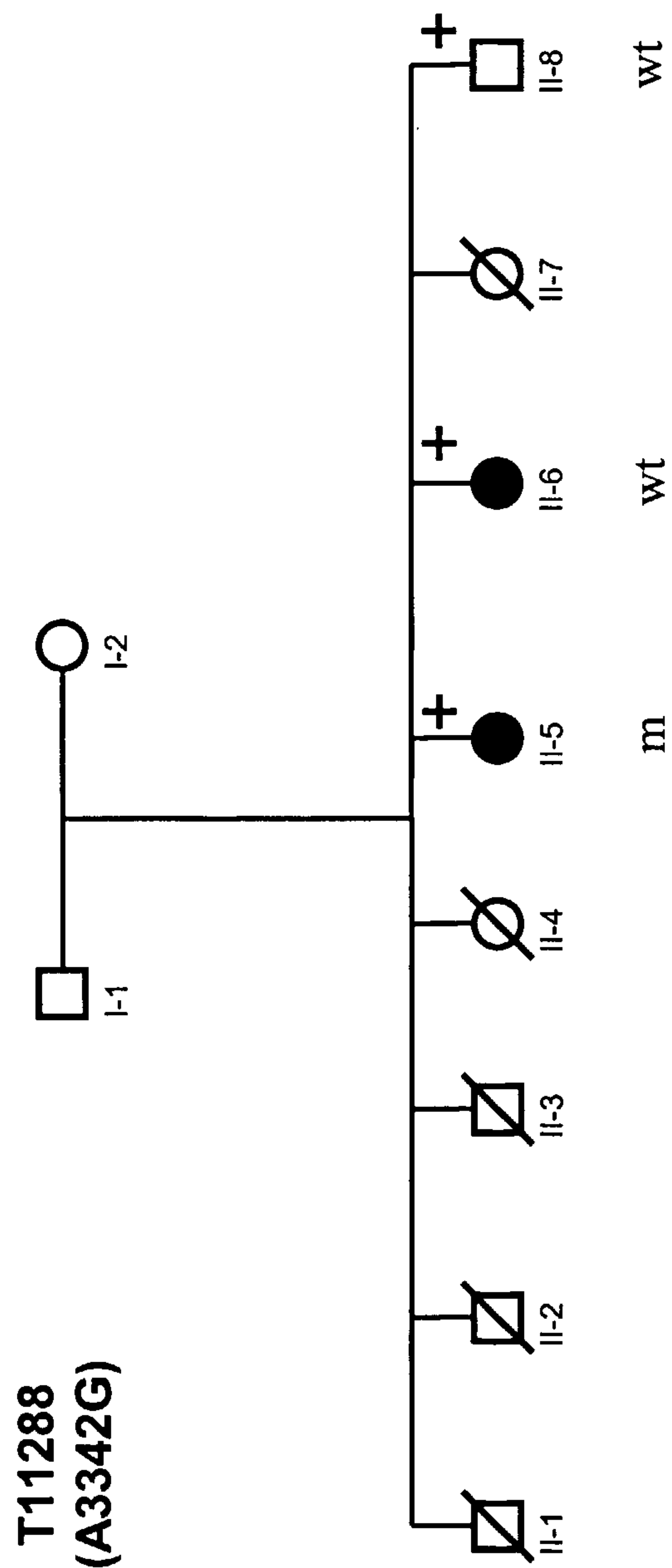
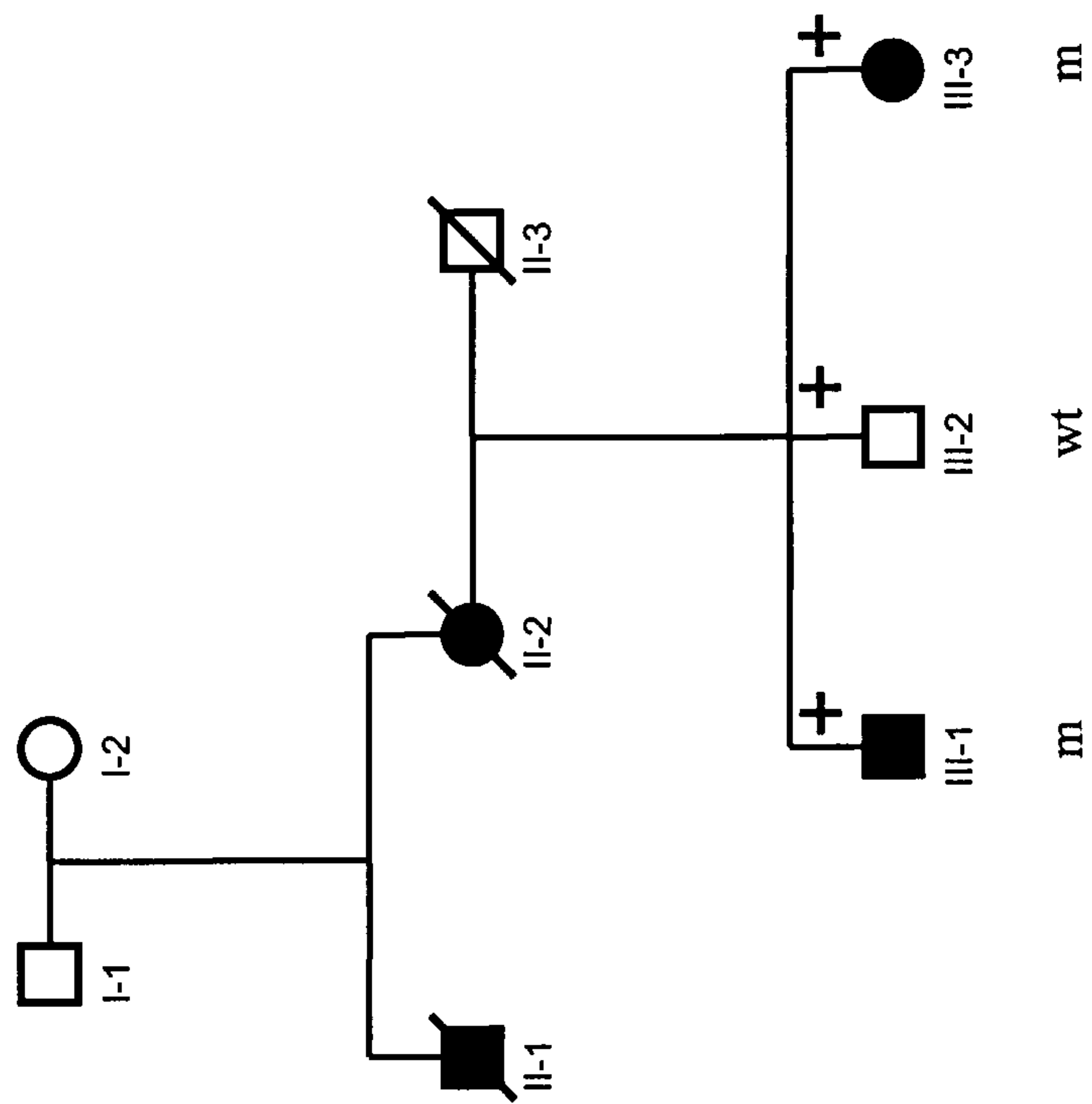


Fig. 6c

DE031
(S1228T)

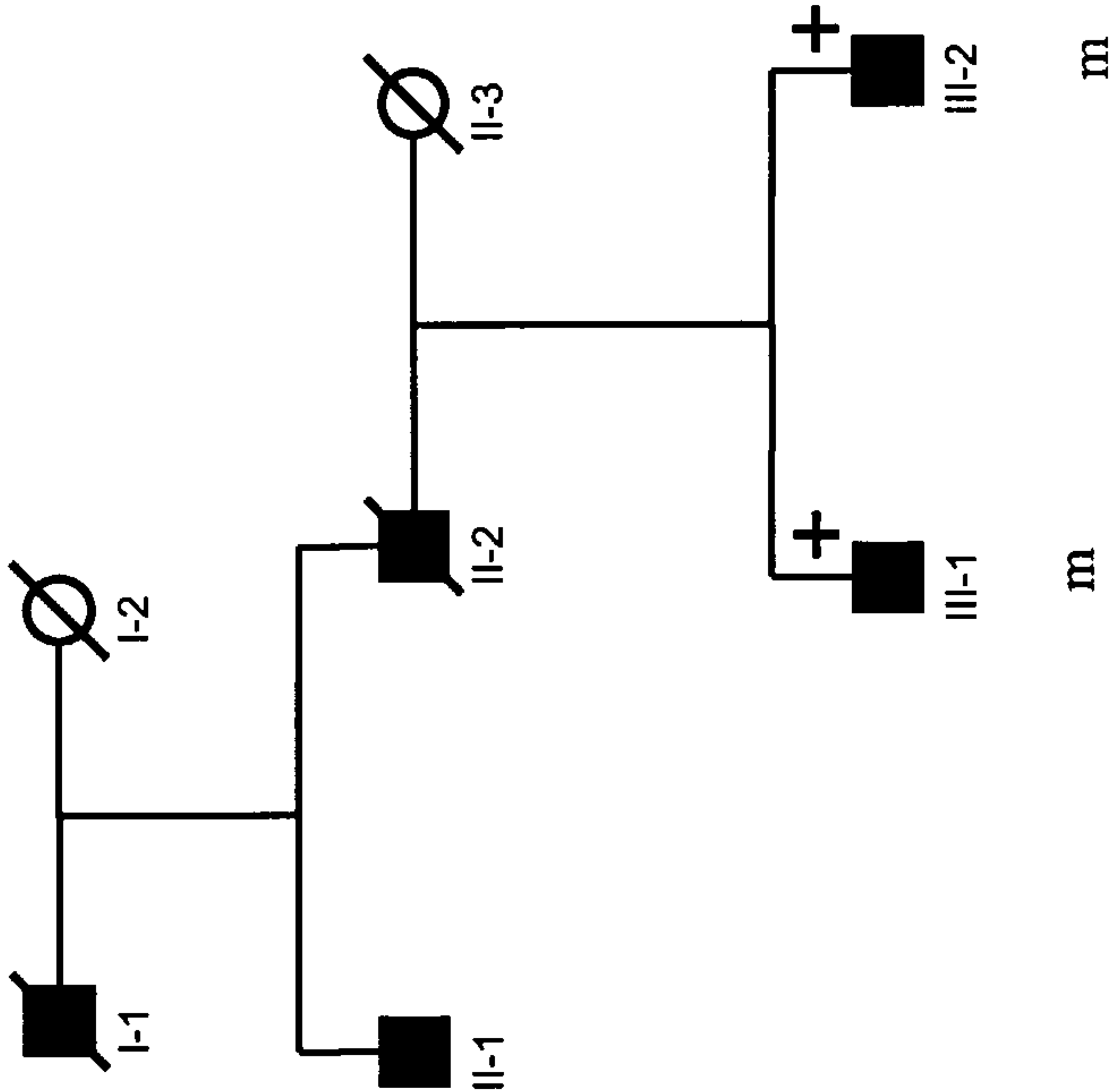


Fig. 6d

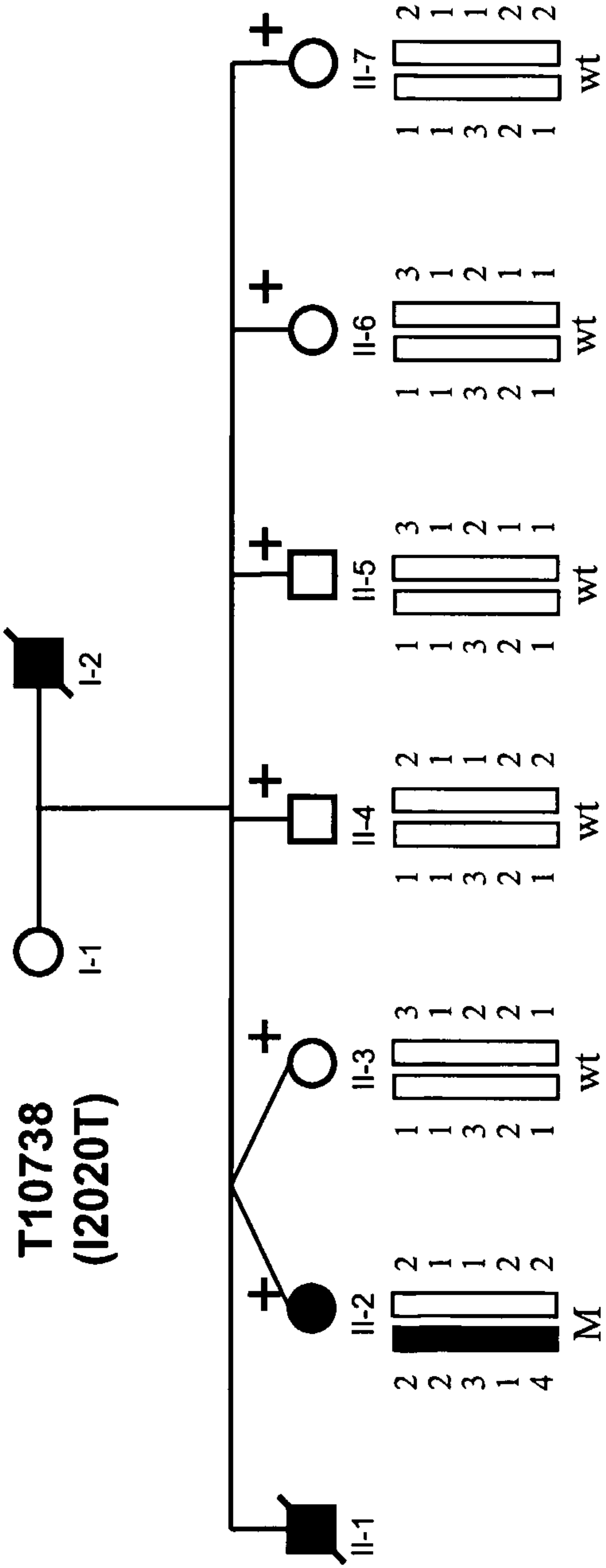
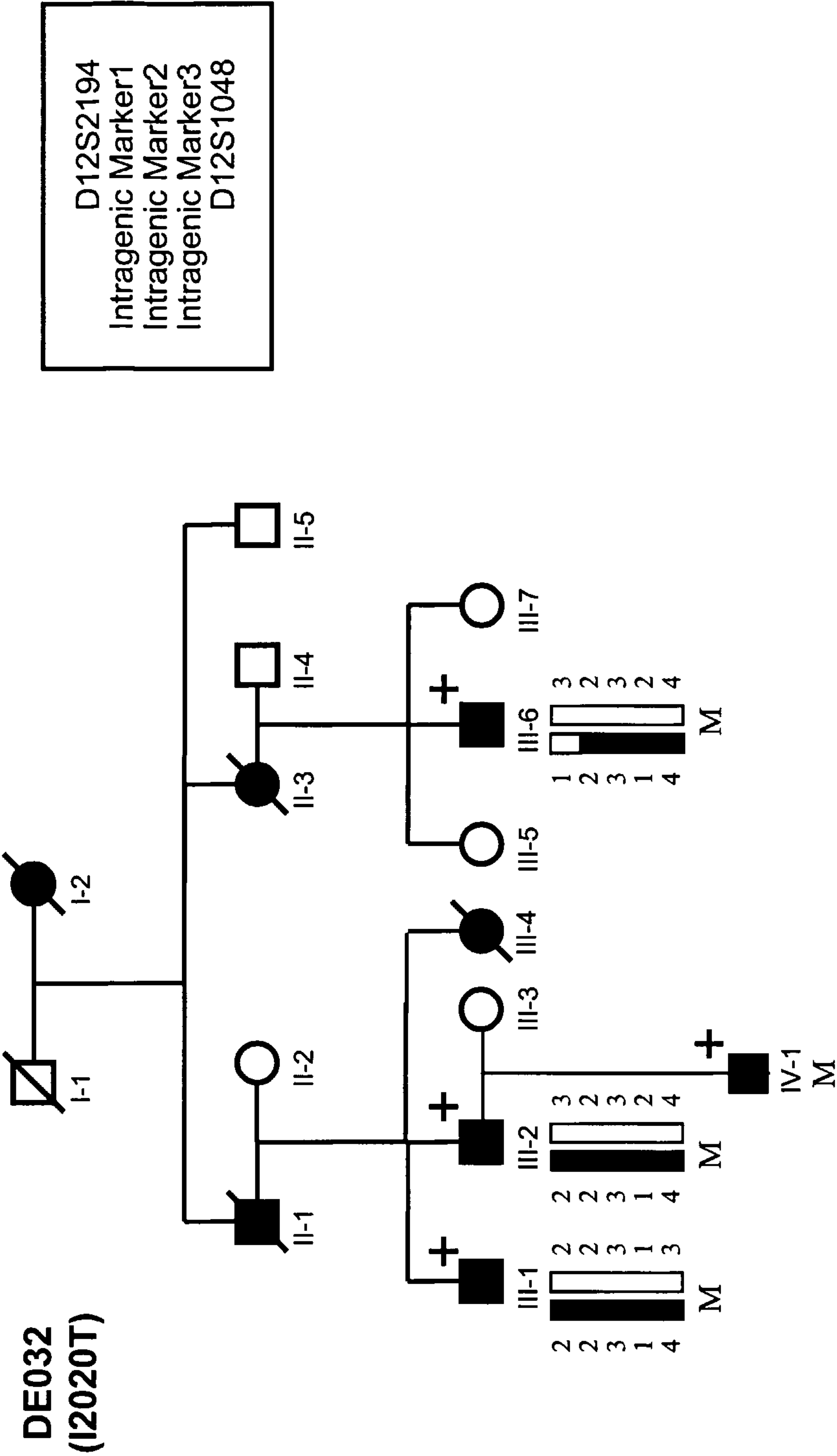


Fig. 6e

Family E
(S1096C)

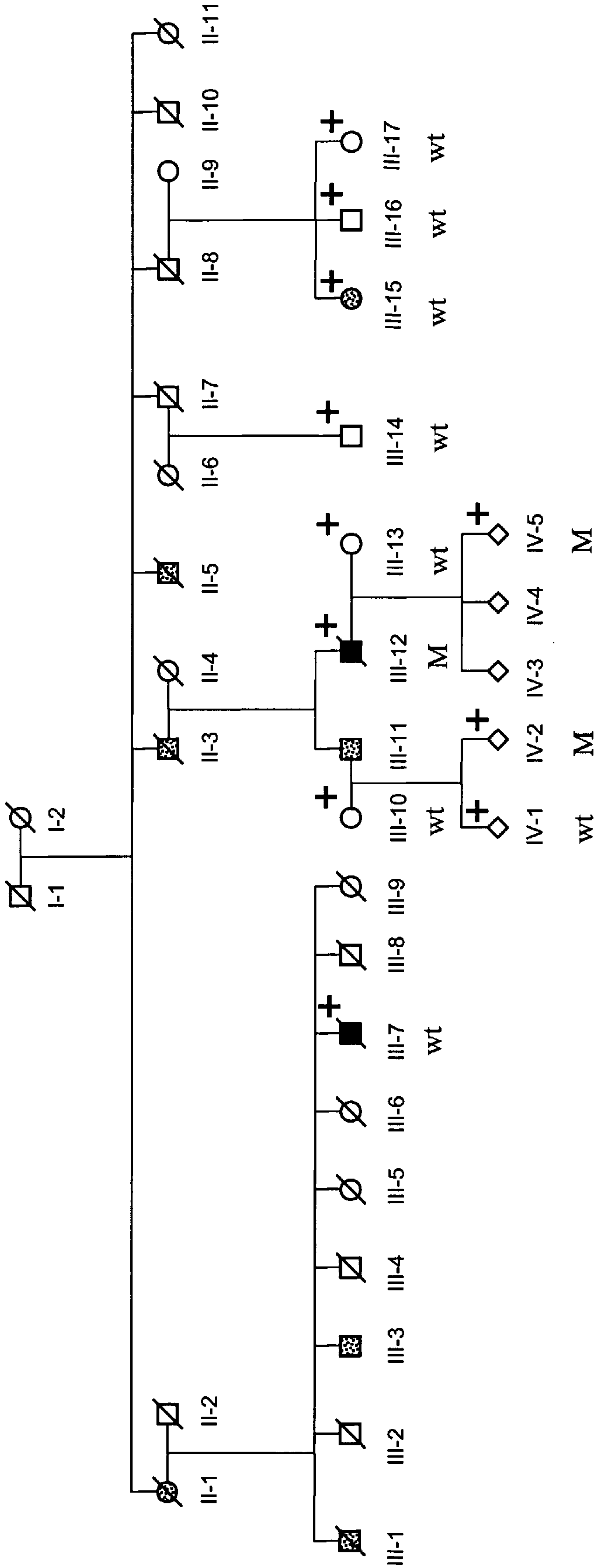


Fig. 6f

Fig. 7

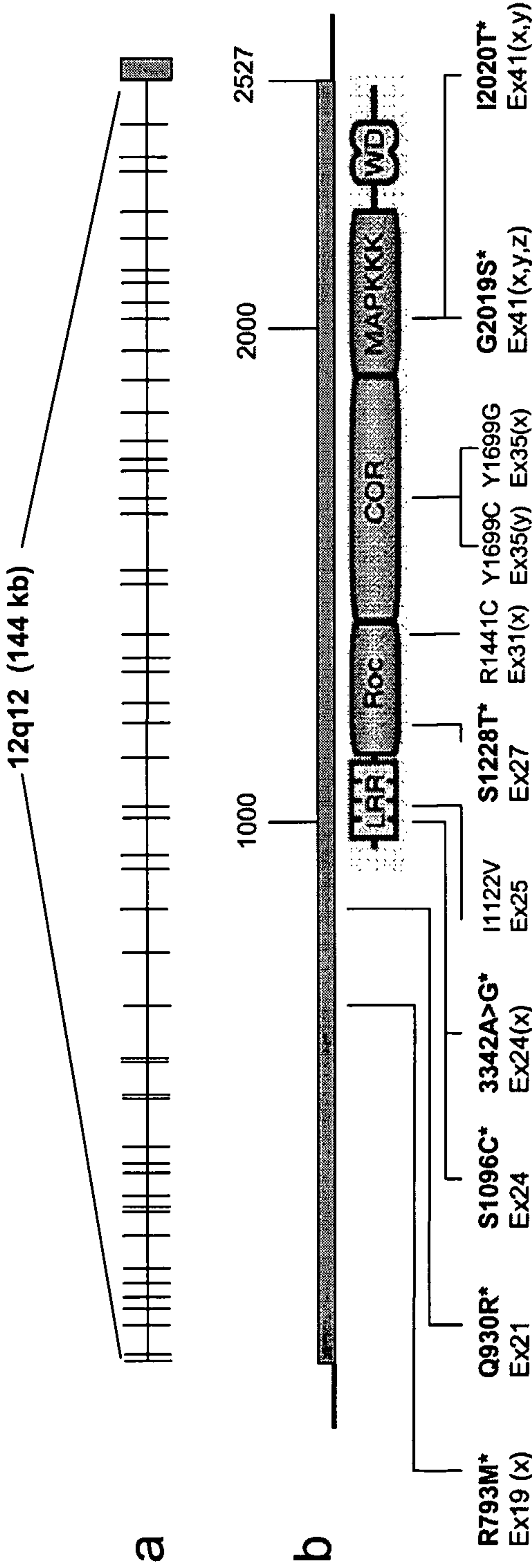


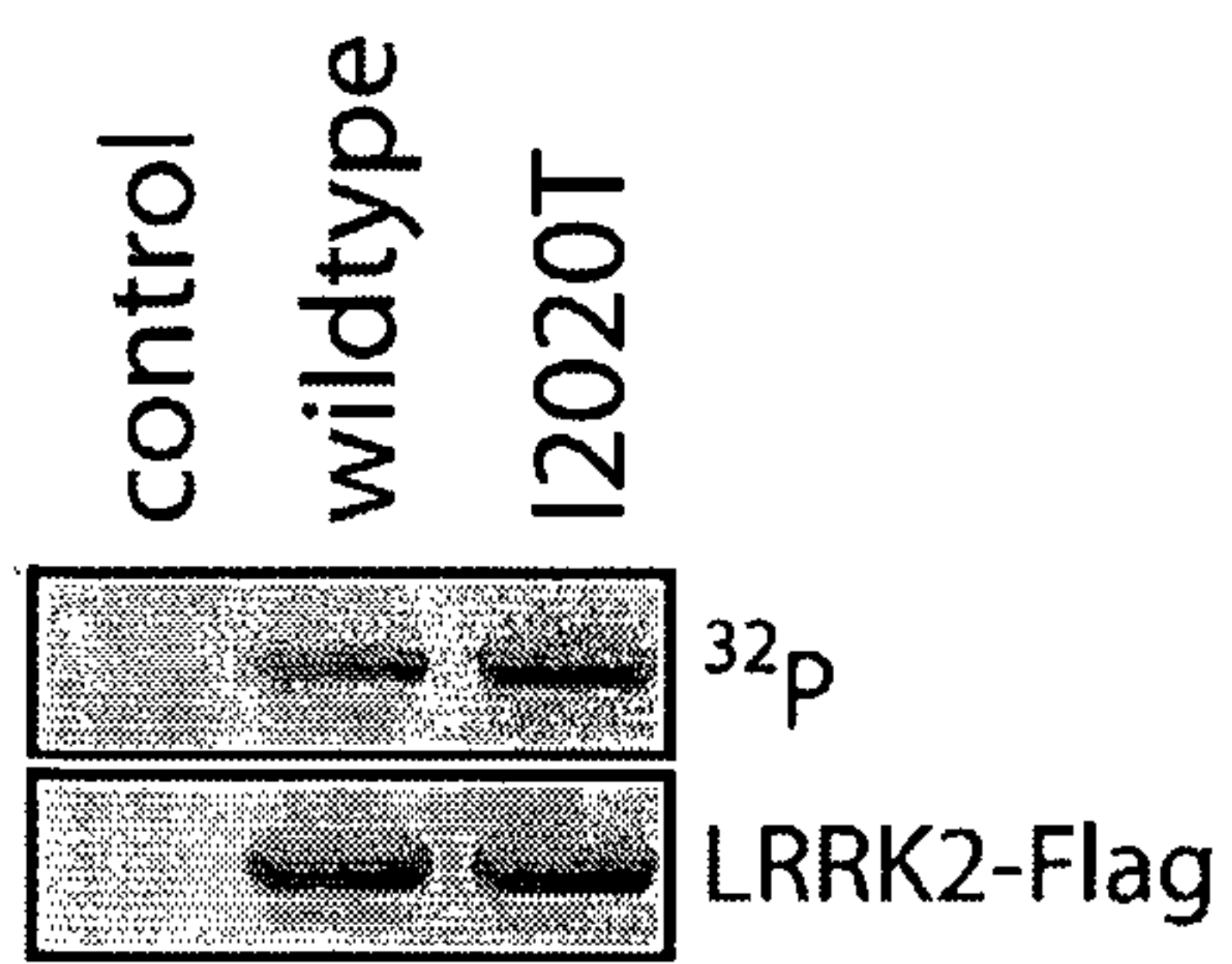
Figure 8

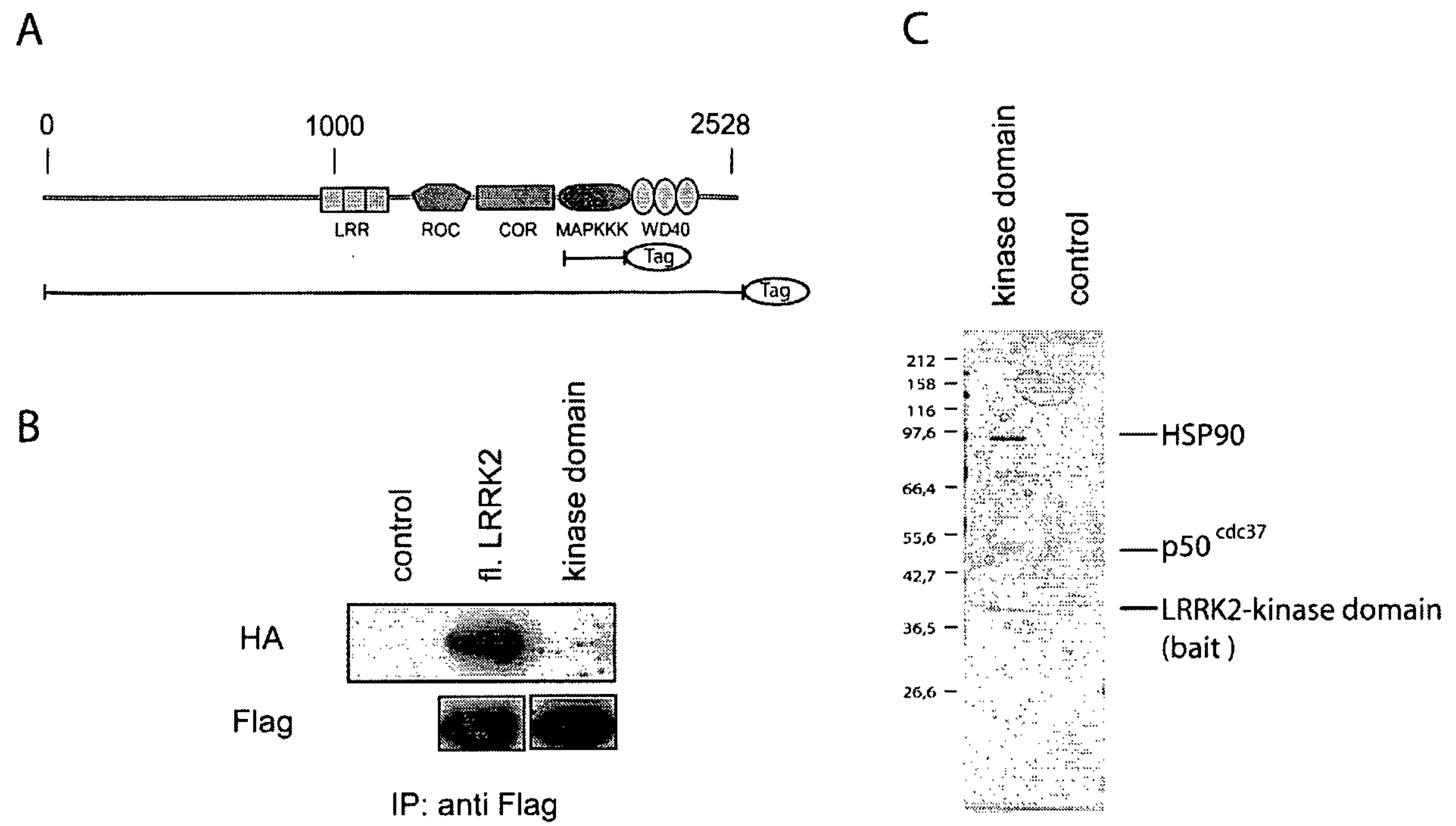
Figure 9

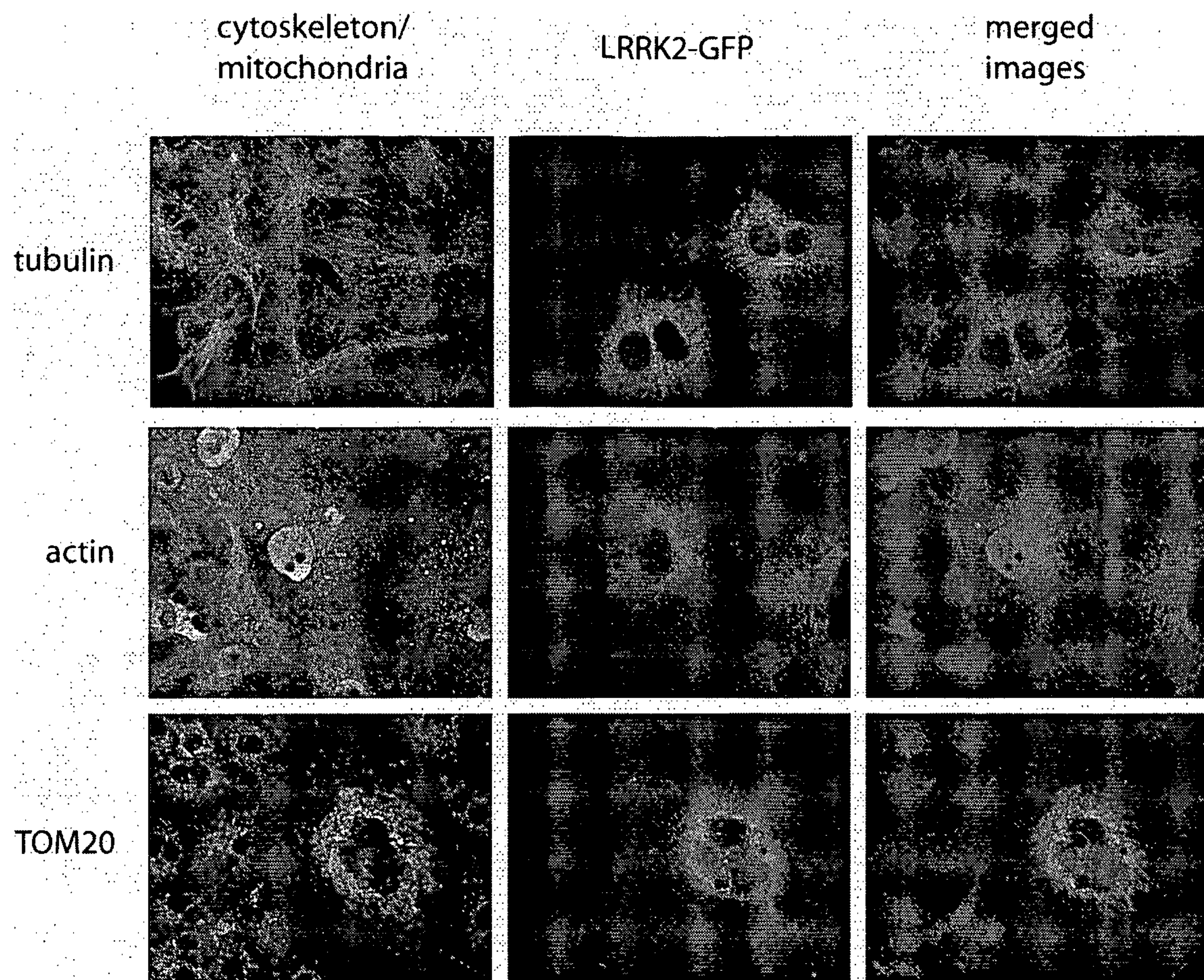
Figure 10

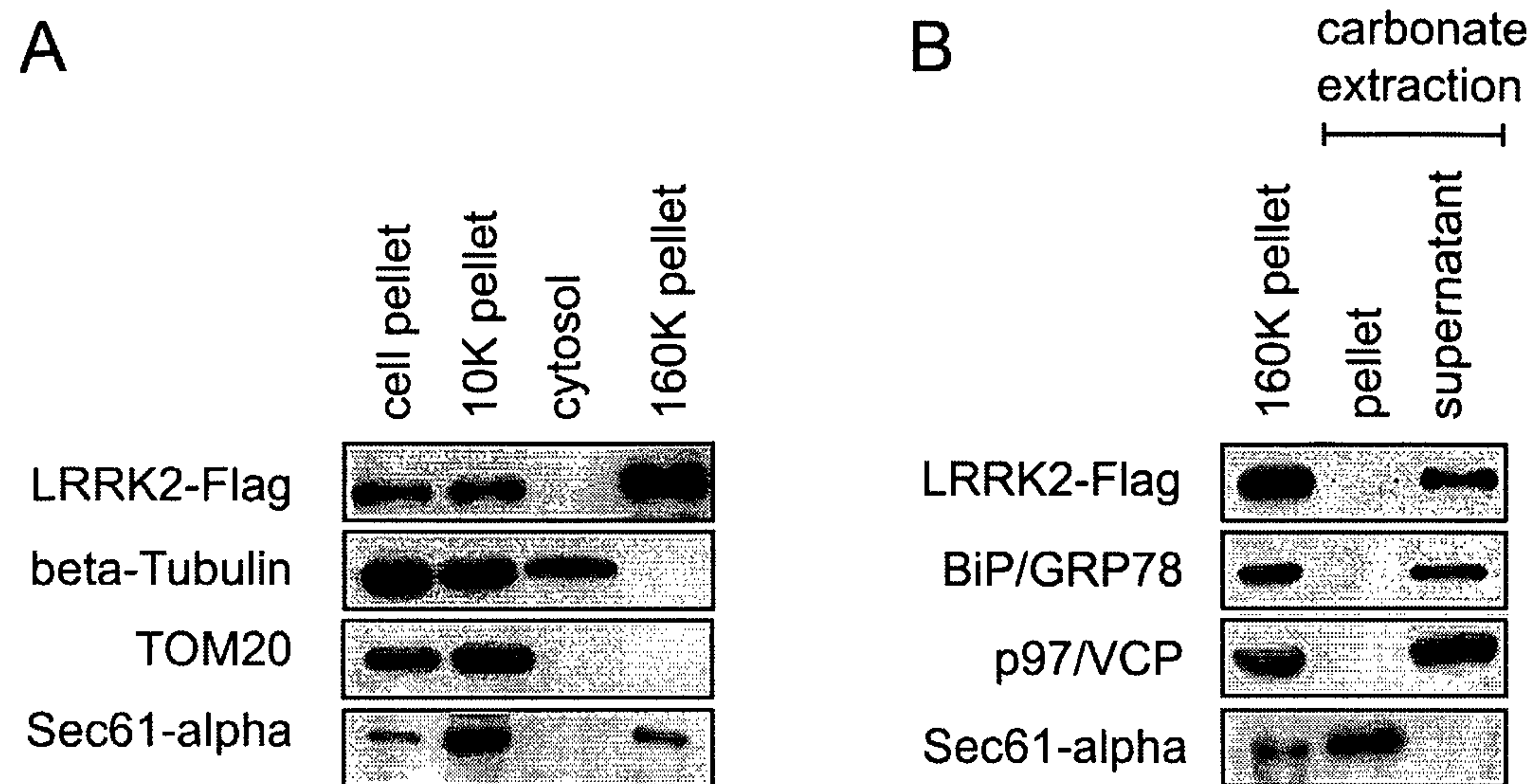
Figure 11

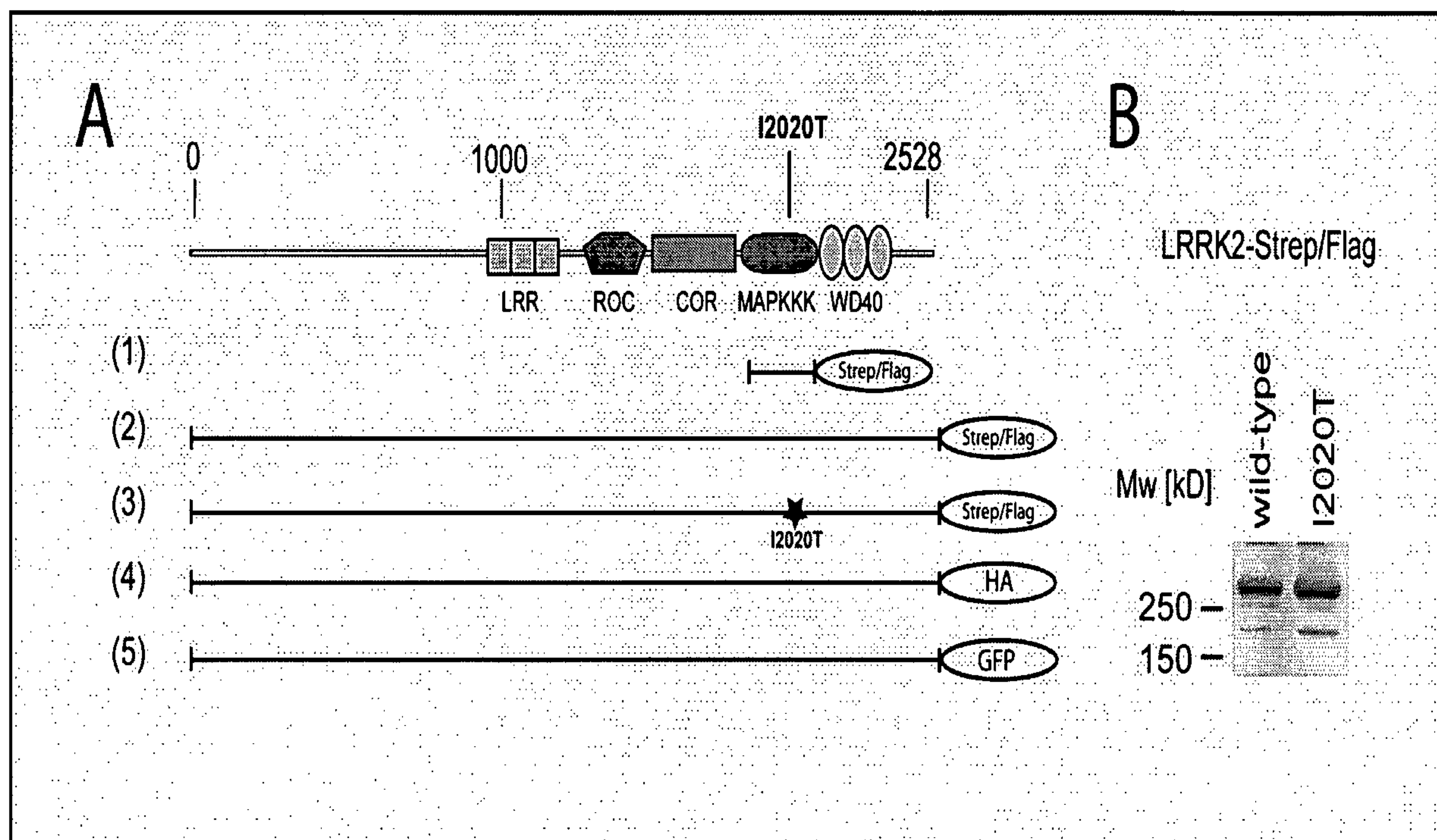
Figure 12

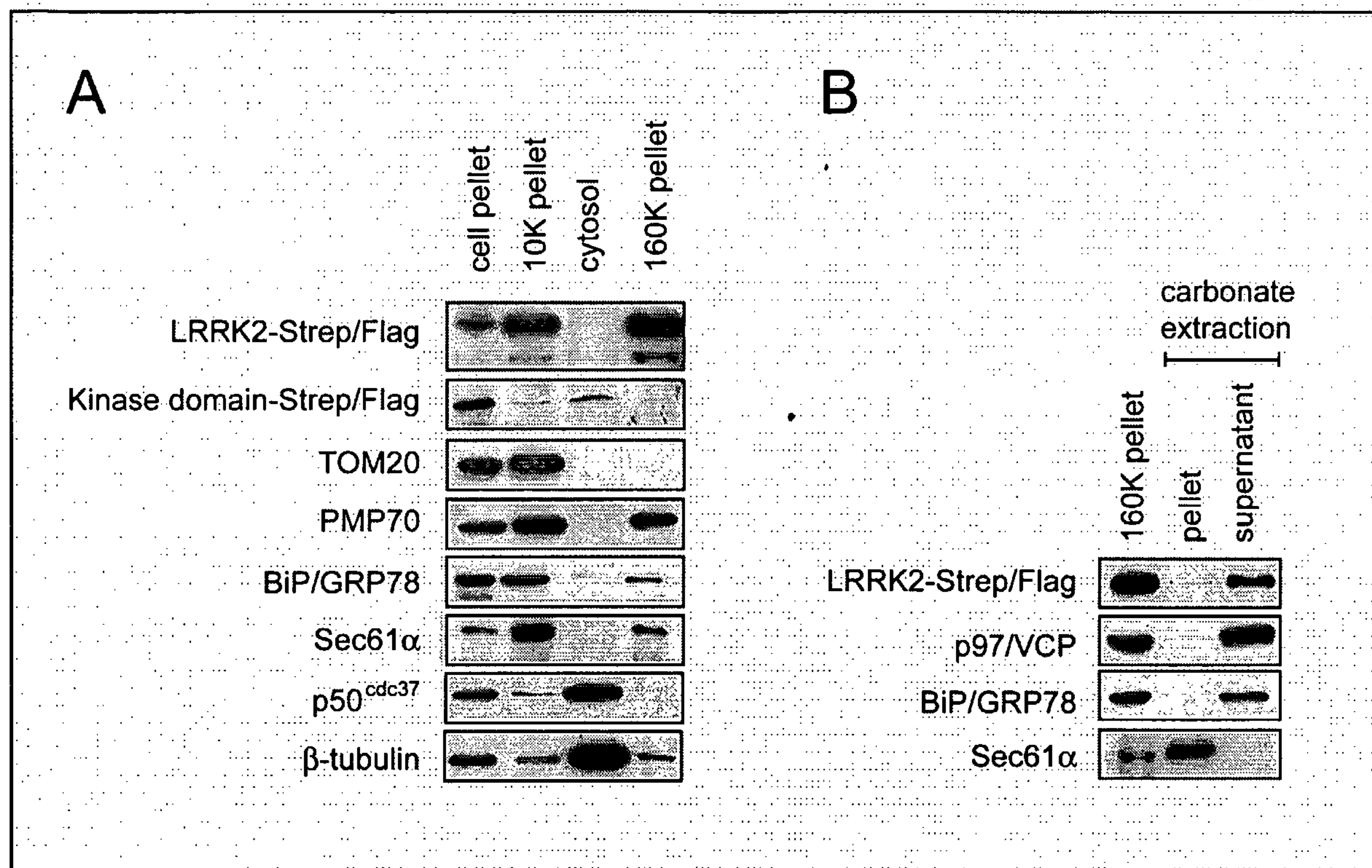
Figure 13:

Figure 14:

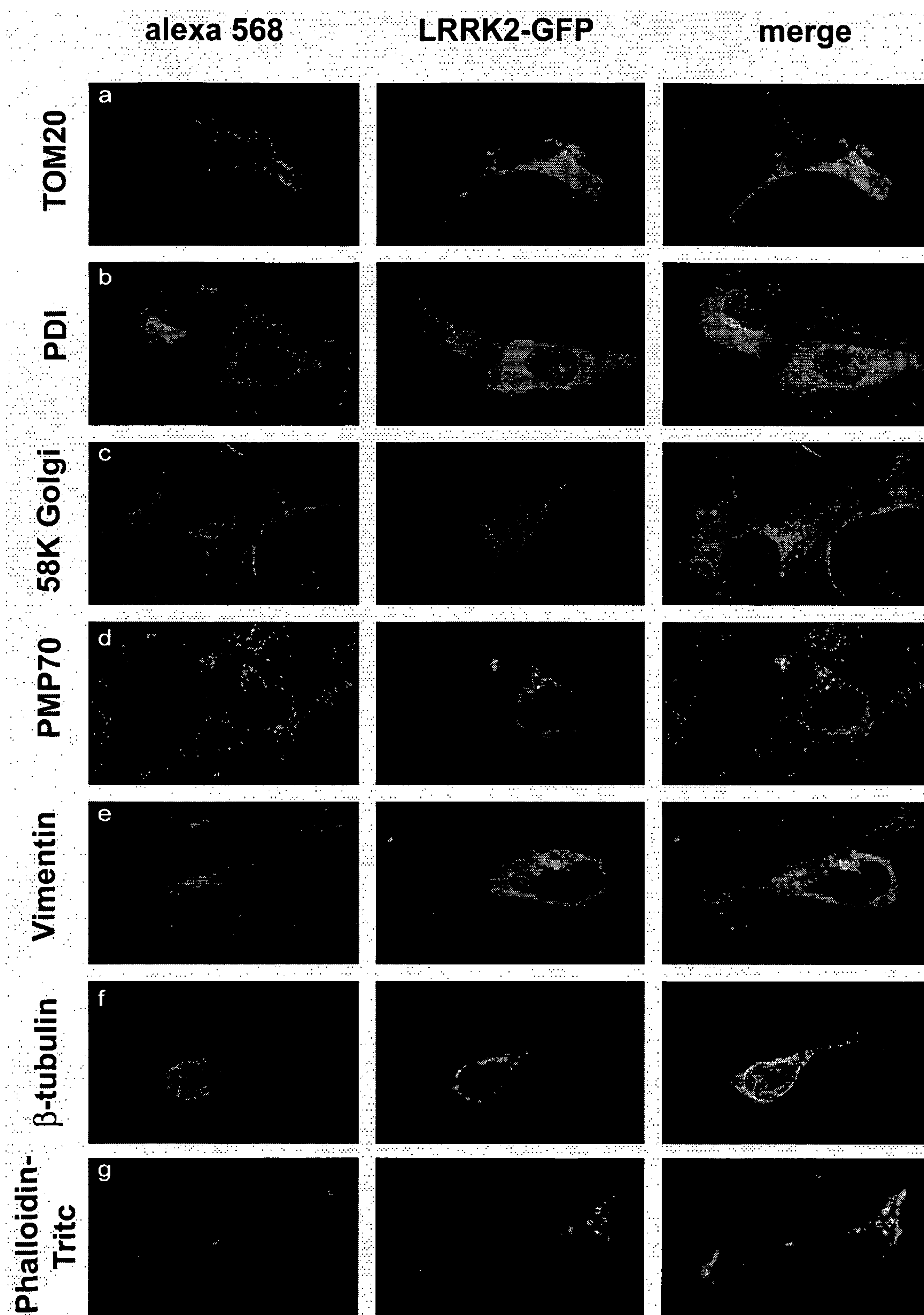


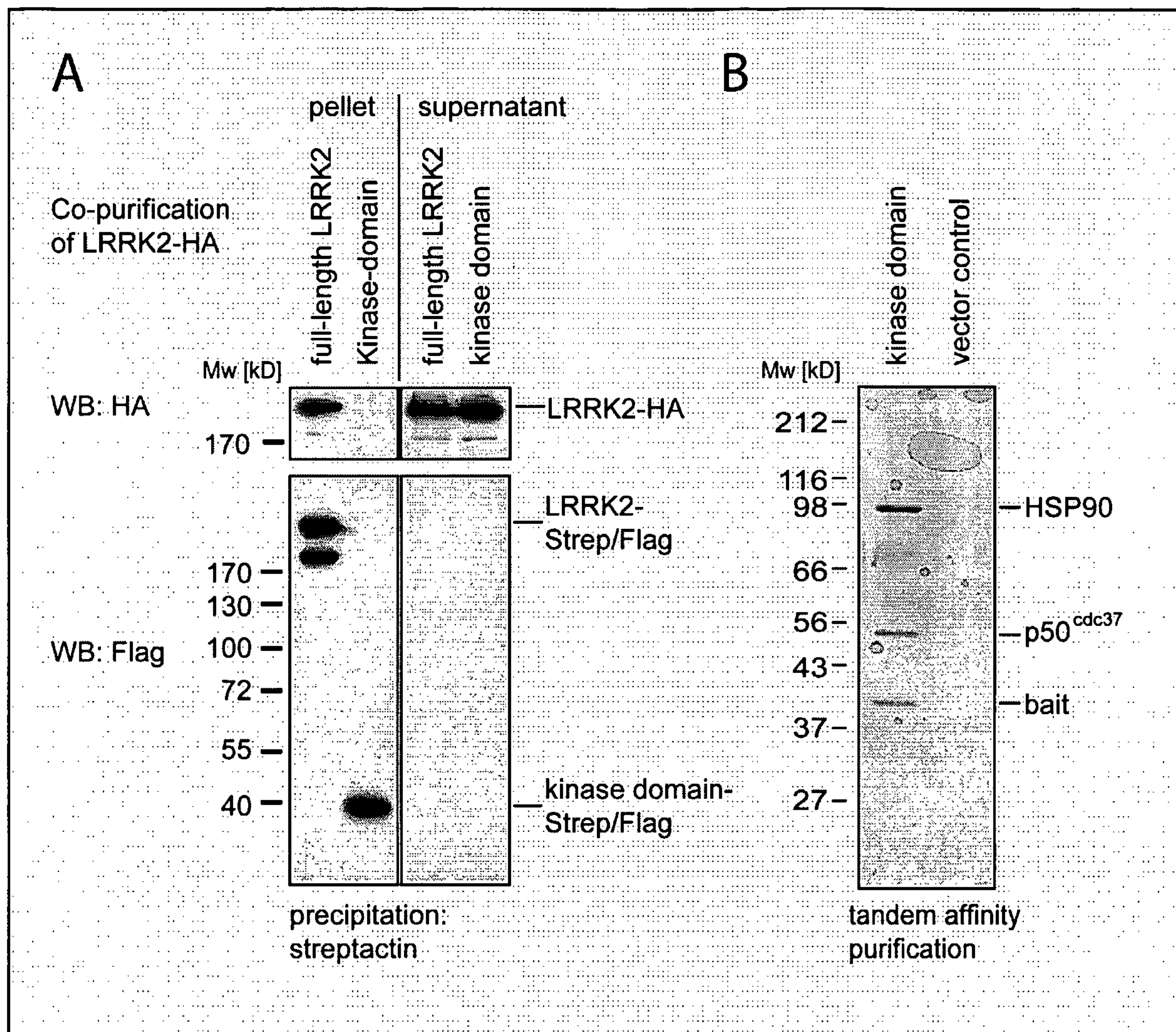
Figure 15:

Figure 16: