LRP10 genetic variants in familial Parkinson's disease and dementia with Lewy bodies: a genome-wide linkage and sequencing study

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Summary

Background Most patients with Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies do not carry mutations in known disease-causing genes. The aim of this study was to identify a novel gene implicated in the development of these disorders.

Methods Our study was done in three stages. First, we did genome-wide linkage analysis of an Italian family with dominantly inherited Parkinson's disease to identify the disease locus. Second, we sequenced the candidate gene in an international multicentre series of unrelated probands who were diagnosed either clinically or pathologically with Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies. As a control, we used gene sequencing data from individuals with abdominal aortic aneurysms (who were not examined neurologically). Third, we enrolled an independent series of patients diagnosed clinically with Parkinson's disease and controls with no signs or family history of Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies from centres in Portugal, Sardinia, and Taiwan, and screened them for specific variants. We also did mRNA and brain pathology studies in three patients from the international multicentre series carrying disease-associated variants, and we did functional protein studies in in-vitro models, including neurons from induced pluripotent stem-like cells.

Findings Molecular studies were done between Jan 1, 2008, and Dec 31, 2017. In the initial kindred of ten affected Italian individuals (mean age of disease onset 59·8 years [SD 8·7]), we detected significant linkage of Parkinson's disease to chromosome 14 and nominated *LRP10* as the disease-causing gene. Among the international series of 660 probands, we identified eight individuals (four with Parkinson's disease, two with Parkinson's disease dementia, and two with dementia with Lewy bodies) who carried different, rare, potentially pathogenic *LRP10* variants; one carrier was found among 645 controls with abdominal aortic aneurysms. In the independent series, two of these eight variants were detected in three additional Parkinson's disease probands (two from Sardinia and one from Taiwan) but in none of the controls. Of the 11 probands from the international and independent cohorts with *LRP10* variants, ten had a positive family history of disease and DNA was available from ten affected relatives (in seven of these families). The *LRP10* variants were present in nine of these ten relatives, providing independent—albeit limited—evidence of co-segregation with disease. Post-mortem studies in three patients carrying distinct *LRP10* variants showed severe Lewy body pathology. Of nine variants identified in total (one in the initial family and eight in stage 2), three severely affected *LRP10* expression and mRNA stability (1424+5delG, 1424+5G→A, and Ala212Serfs*17, shown by cDNA analysis), four affected protein stability (Tyr307Asn, Gly603Arg, Arg235Cys, and Pro699Ser, shown by cycloheximide-chase experiments), and two affected protein localisation (Asn517del and Arg533Leu; shown by immunocytochemistry), pointing to loss of LRP10 function as a common pathogenic mechanism.

Interpretation Our findings implicate *LRP10* gene defects in the development of inherited forms of α-synucleinopathies. Future elucidation of the function of the LRP10 protein and pathways could offer novel insights into mechanisms, biomarkers, and therapeutic targets.

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Introduction

Parkinson's disease, the most common neuro-degenerative movement disorder, is clinically defined by bradykinesia, resting tremor, muscular rigidity, and a favourable response to levodopa or dopamine-agonist treatment.[1] The pathological hallmarks of Parkinson's disease are loss of nigrostriatal dopaminergic neurons, with intracellular inclusions containing α-synuclein protein (ie, Lewy bodies and Lewy neurites) in surviving neurons.[1] Non-motor manifestations—eg, olfactory, cognitive, psychiatric, sleep, and autonomic disturbances—are nowadays recognised as frequent and relevant features of Parkinson's disease.[1] Cognitive decline progresses into overt dementia in up to 80% of individuals with Parkinson's disease,[2] leading to a diagnosis of Parkinson's disease dementia.[3,4] Furthermore, in patients with dementia with Lewy bodies, severe cognitive disturbances are the initial manifestation, often but not always followed by parkinsonism.[3,4] Dementia with Lewy bodies accounts for roughly 5% of dementia cases in elderly people[5] and is associated with severe and widespread pathological findings of Lewy bodies in the brain.[4,6]

Rare highly penetrant variants in *SNCA*[7,8] and *LRRK2*[8–10] cause hereditary forms of dominantly transmitted, Lewy body-positive Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies. Moreover, common variants in *SNCA*, *LRRK2*, and *GBA* are risk factors for the same disorders.[11–13] Pathological misfolding and aggregation of α-synuclein seems to be central in several neurodegenerative diseases, including Parkinson's disease and dementia with Lewy bodies, collectively termed α-synucleinopathies.[14] These clinical, pathological, and molecular overlaps suggest that Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies are parts of a continuum of Lewy body diseases.[15,16] Yet, in most patients with familial forms of Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies, variants in the above-mentioned genes are not found, suggesting that other causative or predisposing genes remain to be identified. We aimed to identify a novel gene implicated in the development of familial Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies.

Methods

Study participants

Our study was done in three stages. For the first stage, we identified a large Italian family with Parkinson's disease segregating as an autosomal-dominant trait. We examined these family members neurologically and took biological specimens from them.

Research in context

Evidence before this study

We searched PubMed for reports published between database inception and Jan 23, 2018, with the keywords "Parkinson's disease", "Parkinson's disease dementia", "dementia with Lewy bodies", and "genetics". Our search retrieved reports showing strong clinical, pathological, and genetic overlap between Parkinson's disease and dementia with Lewy bodies. Rare, highly penetrant, pathogenic variants in *SNCA* and *LRRK2* are known to be associated with forms of dominantly transmitted, Lewy body-positive Parkinson's disease, Parkinson's disease with dementia, and dementia with Lewy bodies. In most patients, however, variants in these genes are not found, suggesting that other causative or predisposing genes remain to be identified.

Added value of this study

We report, for the first time to our knowledge, *LRP10* as a new gene implicated in the development of familial Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies. We identified genome-wide linkage of Parkinson's disease to chromosome 14 in a large pedigree, and nominated—using whole exome sequencing—*LRP10* as the candidate gene in this locus. We also detected, by Sanger sequencing, additional *LRP10* variants in 11 unrelated families with Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies, with evidence of co-segregation with disease in all pedigrees in which DNA samples from affected relatives were available. We studied brain pathology in three patients carrying different *LRP10* variants, and a severe burden of Lewy pathology was seen. We found that LRP10 protein localises to vesicular structures at endosomes, the trans-Golgi network, and plasma membrane of human induced pluripotent stem cell-derived neurons. Based on the nature of the variants and our functional studies, we conclude that loss of LRP10 function is a common pathogenic mechanism in α-synucleinopathies in the families reported here.

Implications of all the available evidence

Our work shows that *LRP10* variants are implicated in Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies, and are associated with a severe burden of Lewy pathology in the brain. A potential important role is emerging for LRP10 in molecular cascades leading to α-synuclein pathological aggregation and, possibly, its trafficking and spread between brain cells. Our findings warrant future research into the mechanisms of involvement of LRP10 in neurodegeneration. Elucidating further the normal function of the LRP10 protein and its signalling pathways could offer crucial insights into molecular mechanisms of the inherited and sporadic α-synucleinopathies, potentially pointing to novel biomarkers and therapeutic targets.

For the second stage, we analysed samples and clinical data that had been obtained from an international series of unrelated probands with Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies between Jan 1, 2000, and Dec 31, 2017. These participants were enrolled from the International Parkinsonism Genetics Network, the Netherlands Brain Bank at the Netherlands Institute of Neuroscience in Amsterdam (selected based on presence of α-synuclein-positive pathology), and the Laboratory of Neuropathology at the University of Bologna in Italy (appendix). We also included as a control whole exome sequencing data from a Dutch study of patients with abdominal aortic aneurysms (unpublished data). Data for neurological diseases were not available in that study.

For the third study stage, we enrolled an independent series of unrelated patients with clinically diagnosed Parkinson's disease and unaffected controls from centres in Portugal, Sardinia, and Taiwan. As controls, we included population-matched series of spouses of individuals with Parkinson's disease or unrelated individuals examined at the same centres with no signs or family history of Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies.

We obtained written informed consent for use of clinical data and biological samples for this study from patients with a clinical diagnosis of Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies (or their next of kin, for patients with dementia) and unaffected relatives. For patients diagnosed pathologically from the Netherlands Brain Bank and the Laboratory of Neuropathology, written informed consent for brain autopsy and use of clinical information and material for research purposes had been obtained previously from the donor or from the next of kin. Participants in the abdominal aortic aneurysms study had provided written informed consent for use of whole exome sequencing data for genetic research. Relevant ethics authorities approved study protocols (appendix).

Procedures

We made clinical diagnoses of Parkinson's disease according to the UK Parkinson's Disease Society Brain Bank criteria.[17] We diagnosed Parkinson's disease dementia in patients developing dementia after 1 year from the onset of Parkinson's disease symptoms.[3] We based our clinical diagnosis of dementia with Lewy bodies on the third report of the Dementia with Lewy Body Consortium.[3]

In the first stage of the study, after confirming the absence of pathogenic mutations in genes causing autosomal-dominant Parkinson's disease (ie, *SNCA*, *LRRK2*, *VPS35*, and *CHCHD2*, as well as *GBA* variants; sequencing and multiplex ligation-dependent probe amplification [MLPA] protocols are reported in the appendix), we did genome-wide single nucleotide polymorphism (SNP) array genotyping in ten affected relatives from the Italian family. We also ran a parametric multipoint linkage analysis, assuming an autosomal-dominant mode of inheritance, and we did copy number analysis with Nexus Copy Number (appendix). We did whole exome sequencing in the index patient. We annotated variants with Annovar (version 2016Feb01)[18] and the Mendelian Clinically Applicable Pathogenicity (M-CAP) score.[19] We then filtered variants located within the linkage interval using the following criteria: (1) the variant being present in the heterozygous state; (2) rarity, defined as a minor allele frequency (MAF) less than 0·1% by the Exome Aggregation Consortium (ExAC), dbSNP, the National Heart, Lung, and Blood Institute's Exome Sequencing Project exome variant server, Genome of the Netherlands (GoNL), and the genome aggregation database (gnomAD); (3) exonic and non-synonymous, or predicted to affect splicing in silico; and (4) pathogenicity, defined as being predicted as pathogenic by at least five of 11 in-silico tools (appendix). This work led to nomination of *LRP10* (low-density lipoprotein receptor-related protein 10) as the candidate disease-causing gene in the Italian family. We used Sanger sequencing for validation and co-segregation analysis in all members of this family for whom DNA was available.

In the second stage of the study, we sequenced the entire *LRP10* open reading frame and exon–intron boundaries in 660 unrelated probands with Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies. We did Sanger sequencing in 659 participants and whole exome sequencing in one. Protocols and primers are detailed in the appendix. We judged of interest variants fulfilling the same criteria mentioned in the first study stage. We used Sanger sequencing for co-segregation analysis when DNA from additional relatives was available. We also searched for *LRP10* variants (entire coding region and exon–intron boundaries) in the whole exome sequencing database of individuals from the abdominal aortic aneurysm study (average *LRP10* depth coverage 100·7 times). We included variants fulfilling the same, above-specified, criteria and compared their frequency with that in our series of 660 patients with Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies.

In stage three of the study, we used high-resolution melting analysis to study three of the identified *LRP10* variants in the independent population-matched series of patients and controls from Portugal, Sardinia, and Taiwan. We also used Sanger sequencing and MLPA to analyse genes causing autosomal-dominant Parkinson's disease (ie, *SNCA*, *LRRK2*, *VPS35*, and *CHCHD2*, and the risk gene *GBA*) in all probands from the second and third study stages in whom *LRP10* variants were identified (appendix).

For pathological analyses, we obtained autopsy tissue blocks from 23 different brain regions in three patients from the international multicentre series of unrelated

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See **Online** for appendix

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probands carrying *LRP10*-disease associated variants (appendix), which we then fixed in formalin, embedded in paraffin, and cut into 8 µm sections. For staining of selected regions, we used haematoxylin and eosin, Congo red, Gallyas silver stain, and immunohistochemistry against α-synuclein, amyloid-β, and hyperphosphorylated tau (appendix). For quantitative mRNA expression studies in human brain tissue of two patients with *LRP10* disease-associated variants, we isolated RNA from the medial temporal gyrus of donors with Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies, and from elderly control donors (appendix). We used standard procedures to isolate DNA and RNA from blood and brain samples. We generated cDNA using total RNA and amplified *LRP10* cDNA fragments and analysed and sequenced them, as described in the appendix.

We studied the effect of the identified, potentially pathogenic variants of *LRP10* on stability of LRP10 protein and subcellular localisation. Details of methods, antibodies, and LRP10 expression constructs are in the appendix. We derived human induced neurons from previously characterised induced pluripotent stem cells (iPSCs),[20] according to published protocols,[21] with minor modifications (appendix). For subcellular localisation, we transfected differentiated neurons with *N*-terminal V5-tagged LRP10 wildtype expression plasmid and processed them further for immunocytochemistry against V5-LRP10, TGOLN2 (also known as TGN46), EEA1, GGA1, and VPS35 (appendix).

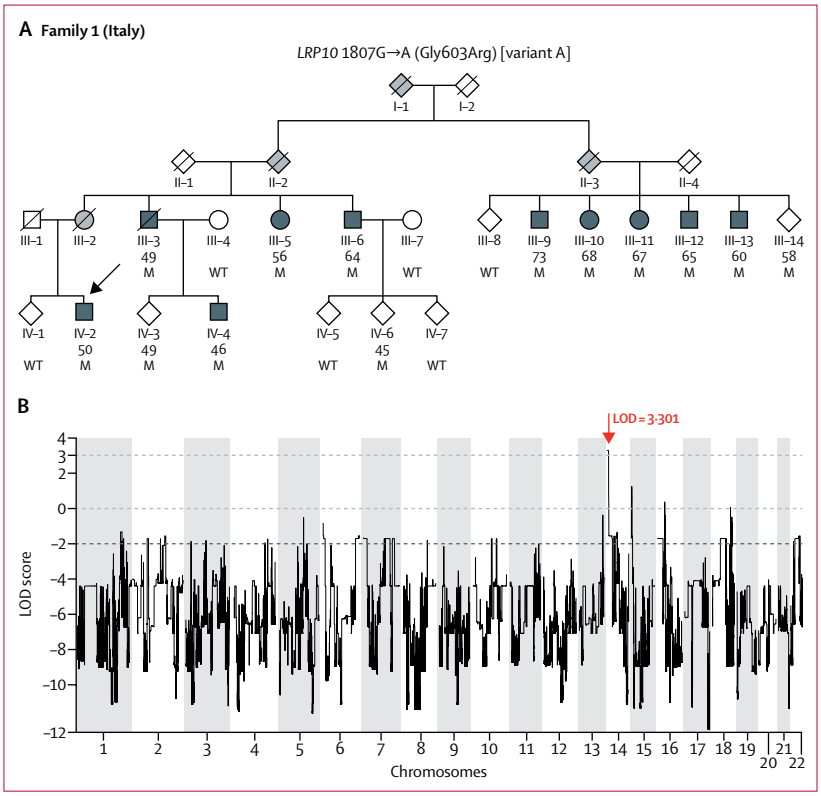


Figure 1: **Pedigree of the Italian family in the first study stage (family 1) and genome-wide linkage analysis**
(A) Pedigree of the *LRP10* 1807G→A (Gly603Arg) variant. Black symbols denote affected individuals; grey symbols indicate individuals reported with Parkinson's disease by history, but not examined personally within this study; diagonal lines indicate deceased individuals; circles indicate women; squares indicate men; diamonds indicate sex-disguised individual. Numbers below individual codes indicate age at symptom onset (for patients) or age at last examination (for living unaffected carriers). The arrow indicates the proband. M=heterozygous *LRP10* Gly603Arg variant carrier. WT=homozygous wildtype individual. (B) Genome-wide linkage analysis in this family yielded significant linkage to a novel locus on chromosome 14p13–q12 (red arrow, LOD score 3·301), spanning 25 Mb from the beginning of the chromosome until the marker rs1950946 (position chr14:0–26,396,221; GRCh37) and containing 243 genes. Dashed lines at LOD scores of –2 and 3 are for ease of reference. LOD=$\log_{10}$ of odds.

Statistical analysis

We describe continuous clinical variables (eg, age at disease onset, age at examination, disease duration) as mean (SD). We did parametric multipoint linkage analysis with the software MERLIN, version 1.1.2,[22] with affected-only analysis. We assumed an autosomal-dominant model of disease inheritance, equal markers allele frequency, a disease allele frequency of 1×10^{-5}, and penetrance of either 0·002 (wildtype), 0·99 (heterozygous carrier), or 0·99 (homozygous carrier). We judged $\log_{10}$ of odds (LOD) scores of 3·3 or higher to be genome-wide significant, according to internationally accepted standards. We compared categorical data using Fisher's exact test. We judged a two-sided p value less than 0·05 to be significant. For stability and subcellular localisation of LRP10 protein, we did statistical analyses with Prism 7 (GraphPad Software, La Jolla, CA, USA; appendix).

Role of the funding source

The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The pedigree of the Italian family in the first stage of the study (family 1) is shown in figure 1A. 13 members of family 1 had Parkinson's disease and one had dementia with Lewy bodies, of whom ten were alive and examined personally by two investigators (EF, GM). 19 relatives in total were included in our study—nine with Parkinson's disease, one with dementia with Lewy bodies, and nine who were unaffected. Mean age at disease onset was 59·8 years (SD 8·7, range 46–73; table). Additional clinical features of family 1 are described in the appendix.

By analysis of the ten members of family 1 with Parkinson's disease or dementia with Lewy bodies, one linkage peak of genome-wide significance (LOD 3·301) was identified on chromosome 14p13–q12 (figure 1B). Copy number aberrations within the linkage interval were not detected by SNP array analysis using Nexus. In the whole exome sequencing analysis of the index case (patient IV-2 in figure 1A), only three heterozygous variants—in the *OR11H12*, *POTEG*, and *LRP10* genes—located within the linkage region had MAF less than 0·1% (appendix). The *OR11H12* and *POTEG* variants were

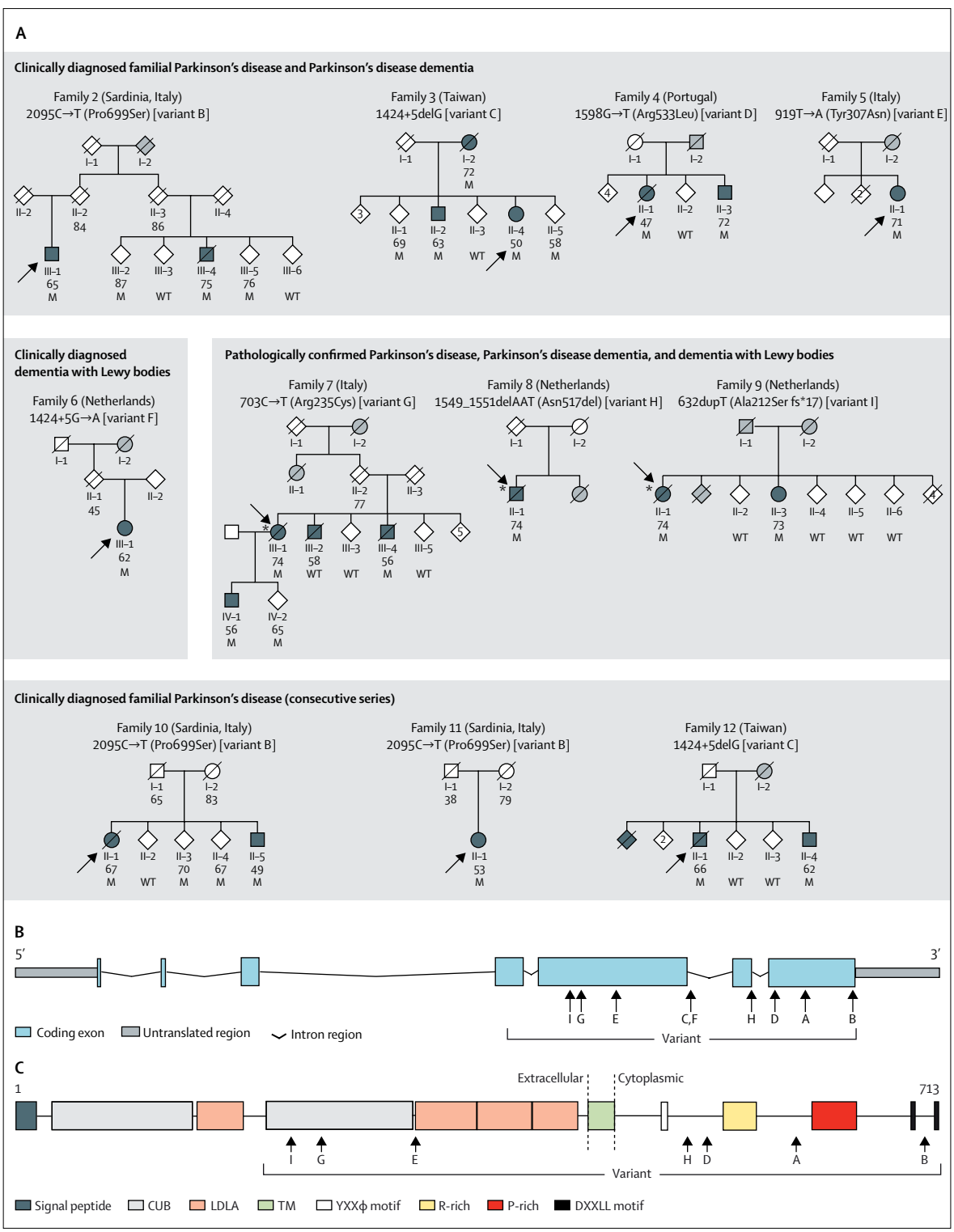
predicted as benign by most of the 11 in-silico tools and had a negative nucleotide conservation score (genomic evolutionary rate profiling [GERP] –0·993 and –0·546, respectively; appendix). However, the *LRP10* variant 1807G→A (Gly603Arg; variant A) was predicted to be damaging by seven of 11 in-silico tools, had a high GERP score (+4·9), and replaced a very conserved residue in the LRP10 protein. Furthermore, *LRP10* was the only one of these three genes with evidence of expression in human brain (appendix).

	Affected individuals		Controls		Genetic analyses	*LRP10* pathogenic variant* (family reference; variant reference)
	Total participants (n) and disease characteristics	Mean (SD) age at onset (years)	Controls (n)	Mean (SD) age at examination (years)		
First study stage						
Italian family	10 (9 PD, 1 DLB)	59·8 (8·7)	..	..	Genome-wide SNP array genotyping, genome-wide linkage analysis, WES in the index patient, co-segregation analysis (Sanger sequencing)	1807G→A ([Gly603Arg] family 1; variant A)
Second study stage						
Multicentre series (patients with clinically diagnosed familial PD and PDD)	430 (420 PD, 10 DLB)	54·6 (11·3)	..	..	Sanger sequencing, all *LRP10* exons and exon–intron boundaries	..
Italy (31 centres)	274 (264 PD, 10 PDD)	..	..	..	..	2095C→T ([Pro699Ser] family 2 [Sardinia]; variant B); 919T→A ([Tyr307Asn] family 5; variant E)
UK (1 centre)	45 (PD)	..	..	..	..	..
Portugal (2 centres)	42 (PD)	..	..	..	..	1598G→T ([Arg533Leu] family 4; variant D)
Brazil (4 centres)	37 (PD)	..	..	..	..	..
Taiwan (3 centres)	15 (PD)	..	..	..	..	1424+5delG (family 3; variant C)
Sweden (1 centre)	9 (PD)	..	..	..	..	..
Netherlands (4 centres)	8 (PD)	..	..	..	..	..
Multicentre series (patients clinically diagnosed with DLB)	62	69·6 (9·1)	..	..	Sanger sequencing, all *LRP10* exons and exon–intron boundaries	..
Netherlands (2 centres)	32 (1 possible DLB, 31 probable DLB)	..	..	..	..	1424+5G→A (family 6; variant F)
Italy (2 centres)	30 (2 possible DLB, 28 probable DLB)	..	..	..	..	..
Multicentre series (patients with pathologically confirmed PD, PDD, and DLB)	168 (49 PD, 74 PDD, 45 DLB)	76·9 (7·8)†	..	..	..	..
Netherlands (1 centre)	167 (49 PD, 73 PDD, 45 DLB)	..	..	..	Sanger sequencing, all *LRP10* exons and exon–intron boundaries	1549_1551delAAT ([Asn517del] family 8; variant H); 632dupT ([Ala212Serfs*17] family 9; variant I)
Italy (1 centre)	1 (PDD)	..	..	..	WES	703C→T ([Arg235Cys] family 7; variant G)
Control series (abdominal aortic aneurysm study)	..	..	645 (553 unrelated individuals, 92 relatives)	67·2 (10·1)	WES (average *LRP10* depth coverage >100 times)	451C→T ([Arg151Cys] 1 individual)
Third study stage						
Consecutive series (unrelated patients with PD and controls)						
Italy (Sardinia; 2 centres)	412 (PD)	62·8 (11·0)	242	70·7 (11·7)	HRM	2095C→T ([Pro699Ser] family 10 [familial], family 11 [sporadic]; variant B)
Taiwan (3 centres)	831 (PD)	55·4 (12·1)	431	56·8 (16·3)	HRM	1424+5delG (family 12 [familial]; variant C)
Portugal (2 centres)	223 (PD)	59·2 (11·8)	138	69·0 (7·4)	HRM	..

Details of centres are in the appendix. DLB=dementia with Lewy bodies. HRM=high-resolution melting analysis. PD=Parkinson's disease. PDD=Parkinson's disease dementia. SNP=single nucleotide polymorphism. WES=whole exome sequencing. *National Center for Biotechnology Information reference sequence NM_014045·4; all variants were heterozygous. †Age at death.

Table: **Characteristics of study participants**

LRP10 was nominated as the candidate disease-causing gene in family 1 (NM_014045·4). We confirmed by Sanger sequencing the presence of variant A (1807G→A) in the index case (patient IV-2; appendix) and all nine affected relatives, and variant A was present in three unaffected relatives (patients III-14, IV-3, and IV-6; figure 1A; age at last examination 58 years, 49 years, and 45 years, respectively). Four unaffected



relatives and two spouses did not carry the *LRP10* variant A.

In the second stage of the study, all *LRP10* exons and exon–intron boundaries underwent Sanger sequencing in an international cohort of 660 unrelated probands (table). Of these people, 430 were diagnosed clinically with either familial Parkinson's disease (n=420) or Parkinson's disease dementia (n=10), and 62 were diagnosed clinically with dementia with Lewy bodies; 168 had pathological confirmation of Parkinson's disease (n=49), Parkinson's disease dementia (n=74), or dementia with Lewy bodies (n=45). Eight variants were identified that fulfilled our criteria (variants B to I; table; appendix).

Five of these variants were identified in probands who were diagnosed clinically with either Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies (table). The first variant—2095C→T (Pro699Ser; variant B)—was detected in a Sardinian proband with Parkinson's disease, which was confirmed in a cousin with Parkinson's disease and two unaffected cousins (family 2; figure 2A). A second variant—deletion of guanine at position +5 in *LRP10* intron 5 (1424+5delG; variant C)—was identified in a Taiwanese proband with Parkinson's disease and confirmed in two relatives with Parkinson's disease and two unaffected relatives (family 3; figure 2A). The third variant was 1598G→T (Arg533Leu; variant D) and was identified in a Portuguese proband with Parkinson's disease and a sibling with Parkinson's disease (family 4; figure 2A). In an Italian proband with Parkinson's disease dementia, a fourth variant (919T→A [Tyr307Asn]; variant E) was identified (family 5; figure 2A). In a Dutch proband who had probable dementia with Lewy bodies (family 6; figure 2A), a fifth variant was noted at position +5 in *LRP10* intron 5 (1424+5G→A; variant F). It is noteworthy that the same guanine that was deleted in variant C was replaced by adenine in variant F.

The other three variants were detected in brain DNA samples from the 168 probands with pathological confirmation of disease (table). These variants were characterised as germline by confirming their presence in DNA from blood samples of the same individuals. The sixth variant (703C→T [Arg235Cys]; variant G) was identified in an Italian proband with familial Parkinson's disease dementia (family 7; figure 2A). This patient was diagnosed initially with Parkinson's disease and later developed rapidly progressive dementia. Three affected relatives in family 7 developed a similar neurodegenerative illness, including parkinsonism, dementia, and neurological signs such as supranuclear gaze palsy and ideomotor apraxia (appendix). Variant G was present in two of these affected relatives; the remaining relative, who did not have this variant, had a similar disease (including supranuclear gaze limitation) but had a much longer disease course (roughly 20 years between symptom onset and death) compared with only 8–14 years in the relatives who carried the *LRP10* variant G, and might represent a phenocopy. One other relative in family 7 carried variant G but was unaffected. The seventh variant was an in-frame deletion of three nucleotides (1549_1551delAAT [Asn517del]; variant H) and was identified in a Dutch proband with dementia with Lewy bodies (family 8; figure 2A). The eighth variant was a frameshift (632dupT [Ala212Serfs*17]; variant I) and was detected in a Dutch proband with Parkinson's disease and severe parkinsonism and mild cognitive impairment. This variant I was confirmed in one sibling with dementia but in none of four unaffected siblings (family 9; figure 2A).

Whole exome sequencing data were obtained in the second stage of the study for 645 participants of a Dutch abdominal aortic aneurysm study, for use as a control (table). Only one carrier of an *LRP10* variant that fulfilled our criteria (451C→T [Arg151Cys]) was identified, but the neurological status of this individual was not available. The frequency of *LRP10* variants in our probands of European ancestry (seven carriers in 608 probands [excluding 52 Brazilian and Taiwanese patients]) is significantly higher than that in the abdominal aortic aneurysms series (one carrier; two-sided Fisher's exact test, p=0·0306; appendix).

Three variants identified in the second study stage (variants B, C, and D) underwent high-resolution melting analysis in an independent series of 1466 patients with Parkinson's disease who were unrelated to each other and to people in the previous study stages and 811 controls from centres in Portugal, Sardinia, and Taiwan (third study stage; table). Variant B (2095C→T [Pro699Ser]) was detected in two of 412 patients and none of 242 controls from Sardinia. Of these two probands, one had familial Parkinson's disease, and variant B was also found in one affected and two unaffected siblings (family 10; figure 2A);

Figure 2: **Pedigrees of families carrying *LRP10* variants in the second and third study stages and structure of the *LRP10* gene and LRP10 protein**
(A) Pedigrees of families 2 to 12, carrying *LRP10* variants B to I, respectively. Black symbols denote individuals affected by Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies; grey symbols indicate individuals reported to have Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies by history, but not examined personally within this study; diagonal lines indicate deceased individuals; circles indicate women; squares indicate men; diamonds indicate sex-disguised individuals. Numbers below individual codes indicate age at symptom onset (for patients), age at last examination (for living unaffected carriers), or age at death (for deceased unaffected relatives). The arrows indicate the probands. Numbers within symbols indicate the number of additional people within the same family with that status. M=heterozygous variant carrier. WT=homozygous wildtype individual. *Pathologically proven cases. (B) *LRP10* gene structure. Capital letters indicate the position of the nine identified variants (ie, variant A in figure 1 and variants B to I in figure 2A). (C) LRP10 protein structure. Capital letters indicate the position of the seven coding variants identified (splicing variants C and F are not shown because they affect non-coding regions of the gene). Variant I leads to frameshift and premature protein truncation, and nonsense-mediated mRNA decay (appendix). CUB=complement C1R/C1S, urchin EGF, BMP1. LDLA=low-density lipoprotein receptor class A. TM=transmembrane domain. R-rich=arginine-rich domain. P-rich=proline-rich domain. YXXϕ=a motif of a tyrosine plus two other aminoacids, then an aminoacid with a large bulky hydrophobic side chain. DXXLL=a motif of an aspartic acid, two other aminoacids, then two leucines.

the other proband in whom variant B was found had sporadic Parkinson's disease (family 11; figure 2A). Variant C (1424+5delG) was found in one of 831 patients and none of 431 controls from Taiwan. This proband had familial Parkinson's disease, and variant C was also present in an affected sibling (family 12; figure 2A). Variant D (1598G→T [Arg533Leu]) was not detected in the case-control series from Portugal (223 patients and 138 controls).

Analyses of genes known to cause Parkinson's disease (ie, *SNCA*, *LRRK2*, *VPS35*, and *CHCHD2*, and the risk gene *GBA*) in the 12 probands carrying *LRP10* variants A to I revealed no causative mutations by either Sanger sequencing or MLPA. Only one *GBA* risk variant (508C→T [Arg170Cys]; common nomenclature Arg131Cys) was present in the proband of family 11. The average age at symptom onset (Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies) of all 30 carriers of an *LRP10* variant (ten in family 1 and 20 in families 2–12) was 62·6 years (SD 9·2, range 46–75). Figure 2B shows the location of each of the variants A to I on the *LRP10* gene structure. Figure 2C shows the LRP10 protein structure and the position of the seven coding variants identified in our study (splicing variants C and F are not shown).

Macroscopic examination of brain tissue of three probands showed a pale substantia nigra and locus coeruleus, with only mild atrophy restricted to parietal regions (patient II-1 in family 8; figure 3A), an isolated small amygdala (patient II-1 in family 9; figure 3B), and moderate atrophy in the amygdala and hippocampus (patient III-1 in family 7; figure 3C). Microscopy showed severe loss of neuromelanin-containing neurons in the substantia nigra, and many Lewy bodies and Lewy neurites throughout the brain, compatible with the highest Braak α-synuclein stage (stage 6; appendix). The dorsal motor nucleus of the vagal nerve, locus coeruleus, and substantia nigra showed classic brainstem-type Lewy bodies and Lewy neurites (figure 3). Moreover, crescent-shaped and annular α-synuclein-immunoreactive neuronal inclusions were found in the CA3 region, particularly in the amygdala, similar to previous findings in patients with Parkinson's disease with pathogenic *SNCA* variants.[23,24] In patient II-1 from family 9 (figure 3B), many α-synuclein-positive glial inclusions were present in the substantia nigra and putamen. Alzheimer's disease pathology[25] was of intermediate grade in brain tissue from patient II-1 in family 8 (figure 3A) and was mild in brain tissue from the other two patients.

The two splicing variants—1424+5delG (variant C) and 1424+5G→A (variant F) —were predicted to affect mRNA splicing by five in-silico tools (appendix). Thus, mRNA splicing was assessed in five patients from Taiwanese families carrying variant C (families 3 and 12) and the Dutch proband carrying variant F (family 6). *LRP10* cDNA amplification revealed an identical aberrant pattern in all patients carrying either variant C or F (appendix). Sequencing of the aberrant cDNA species revealed preferential usage of a different upstream splice donor site in exon 5 (position 517–518), resulting in incorporation of a much shorter aberrant exon 5, which missed 907 coding nucleotides (518–1424 in RNA), and at protein level, a frameshift with premature truncation and removal of a large part of the LRP10 protein (Gly173Alafs*34). Sanger sequencing of *LRP10* cDNA in the Dutch proband carrying variant I (632dupT [Ala212Serfs*17]; patient II-1 in family 9) yielded only the wildtype transcript (appendix), suggesting nonsense-mediated mRNA decay. This finding was supported by reduced mRNA levels in brain tissue of this proband (patient II-1) compared with brain tissue from unaffected elderly controls and donors with idiopathic Parkinson's disease and dementia with Lewy bodies (appendix). mRNA expression and stability was normal in the Dutch proband carrying variant H (1549_1551delAAT [Asn517del]; patient II-1 in family 8; appendix).

Functional studies showed that variants E (Tyr307Asn), A (Gly603Arg), G (Arg235Cys), and B (Pro699Ser) reduced LRP10 protein stability significantly compared with wildtype LRP10 (appendix). Variants H (Asn517del) and D (Arg533Leu) did not affect LRP10 protein stability but showed significantly increased surface labelling in cells overexpressing LRP10 (appendix), indicating that these variants affect LRP10 subcellular localisation. In 12-week-old human neuronal cultures, V5-tagged LRP10 localised to vesicular structures, including endosomes, the retromer complex, and the trans-Golgi network, in the neuronal soma and neurites (figure 4). Both in the cell soma and neurites, V5-tagged LRP10 strongly co-localised with the trans-Golgi network marker TGOLN2 (also known as TGN46) and GGA1. Furthermore, in the soma, V5-tagged LRP10 also partly co-localised with the early endosomal marker EEA1 and retromer marker VPS35.

Discussion

During our study, we identified nine rare *LRP10* variants associated with familial Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies. Our findings provide initial evidence for a role of the LRP10 protein in the pathogenesis of neurodegenerative disorders with Lewy body pathology. By studying a large, multi-incident, Parkinson's disease kindred, we nominated *LRP10* as the candidate disease-causing gene. Subsequently, we found 11 probands who each carried one of eight potentially pathogenic variants of *LRP10*, and the same variants were detected in nine of ten affected relatives available for testing, providing independent—albeit limited—evidence of co-segregation with disease.

A further observation is that the same guanine at position 1424+5 in *LRP10* intron 5 was the target of two different alterations (a deletion and a substitution), rare or absent in control databases, and detected in patients with Parkinson's disease or dementia with Lewy bodies from three families and two distant populations. These two variants result in an identical severe aberration at the level

of mRNA splicing. Although small copy number variants within the linkage region and non-coding variants or repeat expansions could have been missed because whole genome sequencing was not done, altogether, our genetic data provide compelling evidence that implicate *LRP10* in the development of Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies.

The identification of *LRP10* variants in some unaffected family members suggests that the penetrance of at least some of these variants is age-related and perhaps incomplete. Three Gly603Arg carriers in family 1 were free from symptoms and clinical signs at age 58 years (III-14), 49 years (IV-3), and 45 years (IV-6), and four Pro699Ser carriers did not manifest disease symptoms or signs at age 87 years (III-2 in family 2), 76 years (III-5 in family 2), 70 years (II-3 in family 10), and 67 years (II-4 in family 10), suggesting a lower penetrance compared with the other *LRP10* variants. This notion would be in line with evidence of reduced penetrance for variants in *SNCA*,[26] and particularly *LRRK2*.[27] Follow-up studies of these and other *LRP10* families to be identified in the future might lead to accurate penetrance estimates. Furthermore, how *LRP10* variants relate to phenotypes varying from typical Parkinson's disease to Parkinson's disease dementia or dementia with Lewy bodies remains currently unknown. Other genetic or non-genetic modifiers are possibly implicated and further studies are warranted.

An abundance of α-synuclein aggregation—in the form of brainstem and cortical Lewy bodies and Lewy neurites—was present in all three brains studied, which were from people who had different *LRP10* variants (Arg235Cys, Asn517del, and Ala212Serfs*), suggesting important involvement of LRP10 in molecular cascades leading to α-synucleinopathy. For most identified *LRP10* variants, we noted a striking effect at the level of transcript expression and transcript stability (1424+5delG, 1424+5G→A, Ala212Serfs*17), protein stability

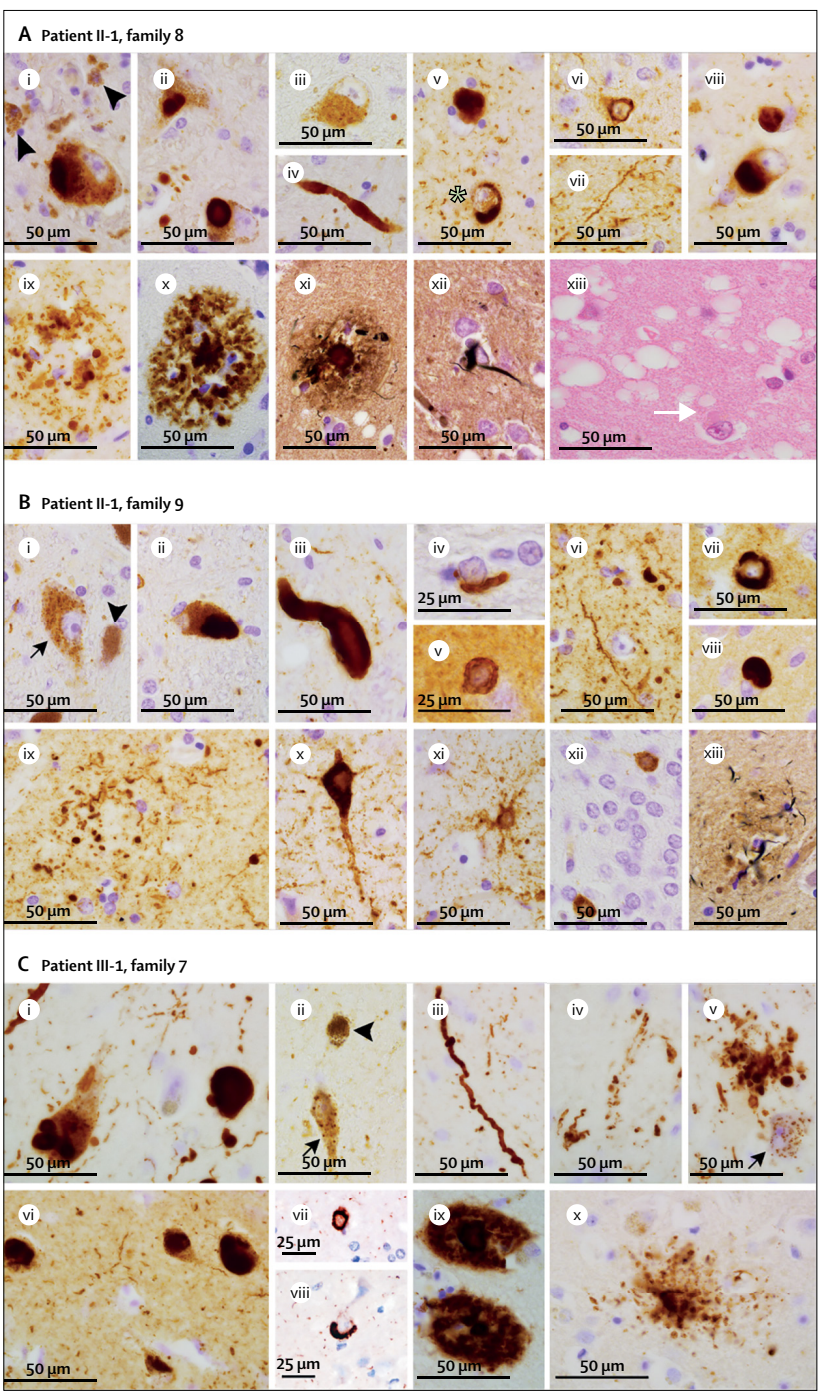


Figure 3: **Brain pathology in three patients with *LRP10* variants**
Brain tissue specimens are shown from (A) patient II-1 in family 8, (B) patient II-1 in family 9, and (C) patient III-1 in family 7. Specimens were stained with haematoxylin and eosin, Congo red, and Gallyas silver stain, and underwent immunohistochemical analysis. (Ai–ix, Bi–ix, Ci–viii) We used immunohistochemistry against α-synuclein (clone KM51) to show α-synuclein protein in brainstem, limbic, and neocortical regions in all three patients. We identified degenerating neuromelanin-containing neurons with loose neuromelanin in substantia nigra (arrowheads in Ai, Bi, Cii); brainstem-type Lewy bodies (Ai–ii, Bii, Ci); granular cytoplasmic staining (Aiii, and black arrows in Bi, Cii, and Cv); bulgy (Aiv, Biii, Ci, Ciii) and thin (Civ) Lewy neurites; globose (Av), crescent-shaped in Av (asterisk) and Cviii, and annular (Avi, Bvii, Cvii) inclusions in neurons; neuritic plaques (Bxii) and Lewy neurites (Avii, Bvi) in the amygdala; and Lewy bodies in neocortical regions (Aviii, Bviii, Cvi). We used immunohistochemistry against α-synuclein (clone KM51) to show glial cytoplasmic inclusions in substantia nigra (Biv) and putamen (Bv); neuritic plaques in amygdala (Bxiii) and substantia nigra (Cv); and cored plaques in neocortex (Aix). We used Gallyas silver stain to show neuritic plaques in neocortex (Axi) and amygdala (Bxiii); and neurofibrillary tangles and threads in temporal regions (Axii, Bx). We used immunohistochemistry against amyloid-β (clone 6f/3d) to show amyloid-β cored plaques in neocortex (Ax, Cix), and immunohistochemistry against hyperphosphorylated tau (clone AT8) to show hyperphosphorylated tau in neurofibrillary tangles and threads in temporal regions (Bx), fuzzy astrocytes in entorhinal and temporal cortex (Bxi, and in patient III-1 in family 7 [data not shown]), ageing-related tau astrogliopathy and coiled bodies in the white matter next to the dentate gyrus (Bxii), and few neocortical neuritic plaques (Cx). We did haematoxylin and eosin staining to show Lewy bodies (white arrow Axiii) and spongiform changes in entorhinal cortex (Axiii). Images Aviii–xiii, Bviii, Bx–xi, Bxiii, Cvi, and Cix–x were taken in the middle temporal gyrus.

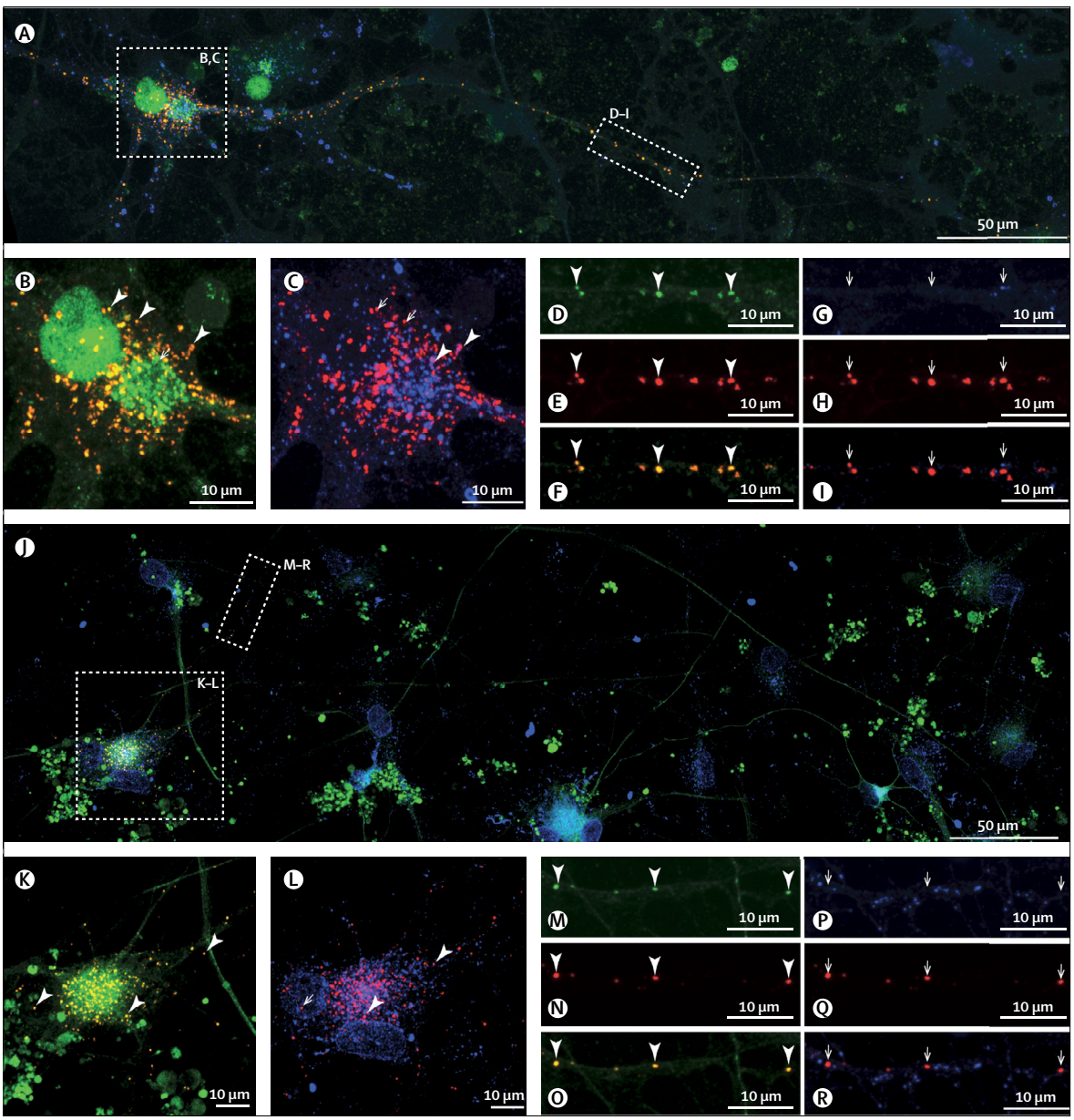


Figure 4: **LRP10 expression studies in iPSC-derived neurons**
Immunocytochemical analysis of 12-week-old induced neurons derived from iPSCs of a donor who did not have Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies. (A) V5-LRP10 transfected neuron, immune-labelled for the presence of V5-LRP10 (red), TGOLN2 (trans-Golgi network; green), and EEA1 (early endosomes; blue). White squares indicate enlarged regions shown in panels B–I. (B) V5-tagged LRP10 (red) co-localises with TGOLN2 (also known as TGN46; green) in the neuronal soma. (C) V5-tagged LRP10 (red) partly co-localises with EEA1 (blue) in the neuronal soma. Purple dots indicated by white arrowheads show co-localisation of LRP10 and EEA1. Red dots indicated by white arrows show lack of co-localisation of LRP10 and EEA1. (D) TGOLN2 (green), (E) V5-tagged LRP10 (red), and (F) merged image in the neurite. (G) EEA1 (blue), (H) V5-tagged LRP10 (red), and (I) merged image in the neurite. (J) V5-LRP10 transfected neurons, immune-labelled for the presence of V5-LRP10 (red), GGA1 (Golgi-associated trafficking proteins; green), and VPS35 (retromer-associated protein; blue). White squares indicate the enlarged regions shown in panels K–R. (K) V5-tagged LRP10 (red) co-localises with GGA1 (green) in the neuronal soma. (L) V5-tagged LRP10 (red) partly co-localises with VPS35 (blue) in the neuronal soma. (M) GGA1 (green), (N) V5-tagged LRP10 (red), and (O) merged image in the neurite. (P) VPS35 (blue), (Q) V5-tagged LRP10 (red), and (R) merged image in the neurite. White arrowheads indicate examples of co-localisation. White arrows indicate lack of co-localisation. iPSC=induced pluripotent stem cell.

(Gly603Arg, Tyr307Asn, Arg235Cys, Pro699Ser), or protein localisation (Arg533Leu, Asn517del), pointing towards loss of LRP10 protein function as a shared pathological mechanism.

Although very little is known about LRP10 protein function, previous studies have shown that LRP10 shuttles between the trans-Golgi network, endosomes, and plasma membrane.[28–31] This finding accords with our

observation that, in iPSC-derived neurons, LRP10 is localised to vesicular structures associated closely with proteins implicated in trafficking between the trans-Golgi network and endosomes, including VPS35 and GGA1. VPS35, which is associated with autosomal-dominant Parkinson's disease,[32,33] regulates LRP10 subcellular localisation[34] and affects α-synuclein aggregation.[35,36] Furthermore, GGA proteins interact with LRP10[28,30] and affect α-synuclein oligomerisation and secretion and ameliorate its toxicity.[37,38] Collectively, these reports and our findings suggest intriguing links between VPS35, GGA proteins, and LRP10 in regulating α-synuclein aggregation, intracellular trafficking, and cell-to-cell transmission.[39]

Our study has some limitations. First, we did not have a second pedigree large enough to yield confirmation of linkage. Second, we enrolled fewer controls from Portugal and Sardinia compared with the number enrolled from Taiwan. Finally, our LRP10 functional studies were based on overexpression and they might not model accurately the behaviour of this protein at physiological expression levels. Despite these limitations, we provide strong evidence for involvement of *LRP10* in the development of inherited forms of Lewy body diseases. Future elucidation of the normal function of LRP10 protein and its signalling pathways might offer crucial insights into the molecular mechanisms of inherited, and perhaps sporadic, α-synucleinopathies, pointing to novel biomarkers and therapeutic targets.

Contributors

MQ designed, undertook, and supervised genetic experiments. WM designed, undertook, and supervised functional experiments. MMG, RM, MMi, ACM, and TZ designed and undertook functional experiments. GJB, DK, EY-D, ES, ABDF, CC, SO, and AMTI designed and undertook genetic experiments. HG and WDJvdB selected Parkinson's disease and dementia with Lewy body donors from the Netherlands Brain Bank cohort for genetic screening. HG, AJR, PP, and WDJvdB assessed neuropathology in brains with *LRP10* pathogenic variants. EF, LJMV, H-CC, MMe, LCG, CC, AT, GCB, JPR, SC, MS, HFC, NV, Y-HW-C, T-HY, AJWB, SEH, MO, PB, DJN, AP, MDM, AJK, EB, GDM, DM-K, JCvS, FJdJ, JJF, GC, C-SL, GM, and PC undertook clinical and genealogical studies and provided clinical data and biological specimens from patients. WFJvI undertook whole exome sequencing. RWWB, DH, and ASI undertook variant calling and bioinformatic analyses on whole exome sequencing data. MG generated, characterised, and provided the induced pluripotent stem cell control line. HG, PP, and WDJvdB wrote sections on clinical and pathological information of the brain donors with *LRP10* pathogenic variants and produced figure 3. MQ and WM analysed and interpreted data and wrote the initial draft of the report. VB provided overall study direction, supervision, and revised the manuscript. All authors critically reviewed the report and approved the final version.

Declaration of interests

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