




BRIEF COMMUNICATION

SORL1 mutation in a Greek family with Parkinson's disease and dementia

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Abstract

Whole exome sequencing and linkage analysis were performed in a three generational pedigree of Greek origin with a broad phenotypic spectrum spanning from Parkinson's disease and Parkinson's disease dementia to dementia of mixed type (Alzheimer disease and vascular dementia). We identified a novel heterozygous c.G1135T (p.G379W) variant in *SORL1* which segregated with the disease in the family. Mutation screening in sporadic Greek PD cases identified one additional individual with the mutation, sharing the same 12.8Mb haplotype. Our findings provide support for *SORL1* mutations resulting in a broad range of additional phenotypes and warrants further studies in neurodegenerative diseases beyond AD.

Introduction

Parkinson's disease (PD) and Alzheimer's disease (AD) are the most common neurodegenerative disorders. Approximately 10% of patients with PD and 1% of patients with AD have a monogenic cause.¹ To date, at least 23 loci and 19 disease-causing genes for parkinsonism have been identified.² The genetic overlap of AD and PD has also been previously studied and potential common single nucleotide polymorphisms (SNPs) associated with both conditions have been identified within *MAPT* and human leukocyte antigen gene regions.³

In this study, we performed whole exome sequencing (WES) and linkage analysis in a three generational family of Greek origin in which affected members presented initially with parkinsonism or dementia.

Methods**Samples**

A large pedigree with seven affected individuals over three consecutive generations presenting with PD and/or dementia of mixed type, and originating from the village

of Rapsani, Greece was included in the study. DNA and clinical data were available for eight individuals (Figure 1). Detailed phenotypic characterization is presented in Table 1. All cases had extensive genetic, metabolic, and imaging investigations carried out that excluded acquired and other inherited causes of PD or dementia. Possible pathogenic structural variants affecting *SNCA*, *PRKN*, *PINK1*, or *DJ1* genes had been excluded with MLPA analysis.⁴ Intronic hexanucleotide repeat expansion in *C9ORF72* was also excluded.⁵ All family members that have been tested carried the *APOE* E3/E3 genotype, thus reducing the possibility of other known genetic factors contributing to the risk of AD. Twenty sporadic Parkinson's disease patients and 50 healthy controls from the same village and a larger cohort with 600 Greek patients and 600 controls were subsequently included in the study for mutation screening. Written informed consent was obtained from all participants according to the declaration of Helsinki. The study was approved by the relevant Institutional Review Boards of the University of Thessaly, Larissa, Greece and University College of London, UK (UCLH: 04/N034).

Genotyping

Genomic DNA was extracted from leukocytes using the AxyPrepBlood Genomic DNA Miniprep Kit (Axygen, USA) as recommended by the manufacturer. All samples from the family and local controls were genotyped according to the manufacturer's instructions using NeuroChip (Illumina, USA) or GSAv2 array (Illumina, USA). After a standard quality control and variant pruning using PLINK 1.9 (<http://pngu.mgh.harvard.edu/purcell/plink/>) variants were further thinned using MapThin (<https://www.staff.ncl.ac.uk/richard.howey/mapthin/>) to 1cM resulting 2916 informative SNPs.

Linkage analysis

The linkage analysis was performed using Merlin.⁶ For parametric analysis, an autosomal disease model with penetrance of 0.0002 (wild type), 0.99 (heterozygous carrier), 1.0 (homozygous carrier), and allele frequency of 0.0002 was used. Haplotypes covering the linked region were generated using Merlin and plotted using HaploPainter.⁷

Whole exome sequencing

Whole exome sequencing (WES) was performed in three affected members of the pedigree (Figure 1: R1F6, R1F9, R1F16_2). DNA library was generated using the TruSeq Exome Enrichment kit (Illumina, San Diego, CA, USA), then sequenced on an Illumina HiSeq 2500. Read

alignment, variant calling, and annotation were done as previously described.^{8,9} ApoE genotype was also assessed separately.

Whole genome sequencing

Given the limitations of exome sequencing, including capture efficiency among others, we decided to pursue whole genome sequencing (WGS) for a more comprehensive analysis. Whole genome sequencing (WGS) was performed in two members of the pedigree (R1F6 and R1F9). The analyses was divided into several separate parts according to the type of variant information obtained from sequencing, CNVs, SVs, and SNP-Indel (Supplementary Data S1)^{8,9}

Prioritization of identified variants

The pathogenicity of the variants identified were classified in accordance with the guidelines of the American College of Medical Genetics and Genomics (ACMG).¹⁰ Only novel or rare variants with a minor allele frequency of <0.01% in public database were included. Heterozygous coding and splicing variants that are predicted deleterious to protein function were prioritized. The Genomic Evolutionary Rate Profiling (GERP) score was based on 100 eutherian mammals alignment (Ensembl release 99).

Sanger sequencing

The variants identified by WES were confirmed by Sanger sequencing. The following primers were used for SORL1 variant: 5'-ATCTGCCTCAGGTTCTCCAA-3' (forward) and 5'-GGCAGGAGAAGGACTCACAG-3' (reverse). Sequencing reactions were performed using the BigDye Terminator 1.1 system (ThermoFisher, Paisley, UK) followed by sequencing using an ABI DNA Analyser (ThermoFisher, Paisley, UK). Electropherograms were analyzed using the Sequencher software package (Gene Codes, MI, USA).

Replication of study in control population

The replication study was performed using Sanger sequencing with the same set of primers as above in a cohort of 600 sporadic PD patients and equal number of age- and gender-matched controls from Greece was performed in order to assess their frequency.

Gene burden analysis

The second release of the Accelerating Medicines Partnership – Parkinson Disease (AMP-PD) initiative (<https://>

TABLE 1. Clinical features of mutation carriers

	RIF6	RIF9	RIF16_2	RIF1	RIF10
Clinical diagnosis	PD	PD	PD	AD	AD
Sex	M	M	M	F	F
Age at onset	50	45	55	65	70
Age at examination	65	67	70	70	83
Symptoms at disease onset:					
Bradykinesia	yes	yes	yes	no	no
Asymmetric tremor	yes	yes	yes	no	no
Symptoms at examination:					
Bradykinesia	yes	yes	yes	no	no
Bilateral tremor	yes	yes	yes	no	no
Rigidity	yes	yes	yes	no	no
Visual hallucinations	no	no	yes	no	yes
Non motor symptoms					
Sleep disorders	yes	no	no	no	no
Autonomic dysfunction	no	yes	no	no	no
constipation	yes	yes	yes	no	no
Olfactory dysfunction	yes	yes	yes	no	yes
Depression	yes	yes	yes	yes	no
Response to levodopa:					
Initial	yes	yes	yes	n/a	n/a
Sustained	yes	yes	yes	n/a	n/a
Motor complications (LID)	yes	yes	yes	n/a	n/a
Sleep disturbances (RBD)	yes	yes	yes	n/a	n/a
Parkinson's Disease Dementia (PDD)	yes	yes	yes	n/a	n/a
Age at onset of PDD	60	60	65	n/a	n/a
Other features					
Ataxia	no	no	no	no	no
Pyramidal signs	no	no	no	no	no
Sensory disturbances	no	no	no	no	no
UPDRS motor score	58	75	64	n/a	n/a
ADDENBROOKE'S COGNITIVE EXAMINATION – ACE-III	74	73	65	50	32
Frontal Assessment Battery (FAB)	8	8	6	n/a	n/a
Cerebrospinal fluid (CSF) biomarkers amyloid- β (A β 42), total tau (T-tau), and phosphorylated tau (P-tau)	n/a	n/a	normal	abnormal/AD profile	Abnormal/AD profile
T-Tau (ng/L)			267	583	634
P-Tau (ng/L)			45	84	75
A β 42 (ng/L)			932	472	546
A β 40 (ng/L)			8753	12853	12456
A β 42/A β 40 ratio			0,106	0,036	0,043
ApoE	E3/E3	E3/E3	E3/E3	E3/E3	E3/E3
Imaging findings					
Brain MRI/CT findings					
Global Brain atrophy	no	no	yes	yes	yes
Vascular leukoencephalopathy	yes	yes	yes	yes	yes
DAT scan	abnormal	abnormal	abnormal	normal	normal

amp-pd.org/) was used in order to study the burden of rare *SORL1* variants. A total of 2,607 PD patients and 3,797 controls were included in the analysis, from the BioFIND study, Harvard Biomarkers Study (HBS), NINDS Parkinson's disease Biomarkers Program (PDBP), Parkinson's Progression Markers Initiative (PPMI), and the NINDS Study of Isradipine as a Disease Modifying

Agent in Subjects With Early Parkinson Disease, Phase 3 (STEADY-PD3). Additional controls were added from the National Institute on Aging (NIA) International Lewy Body Dementia Genetics Consortium Genome Sequencing in Lewy body dementia case-control cohort (LBD).

Quality control procedures of WGS data were performed on individual and variants levels as described by

AMP-PD (<https://amp-pd.org/whole-genome-data>). In addition to filtration procedures performed by AMP-PD, we excluded non-European individuals and filtered by relatedness to remove any second- and first-degree relatives. Variants with a missingness rate of >5% and Hardy-Weinberg equilibrium $<1E-4$ were excluded.

We extracted *SORL1* genotyping data on hg38: chr11:121452313-121633763, and used ANNOVAR to annotate variants.¹¹ To study the burden of rare variants (minor allele frequency less < 0.01) we used the SKAT-O method implemented in Rvtest package.¹²

Results

Here we describe five affected individuals from a large Greek family presenting either with PD or dementia (Figure 1). The average age at onset was 56.5 years (Table 1). Three of the patients (Figure 1: R1F6, R1F9, R1F16_2) presented with cardinal symptoms of PD including tremor, bradykinesia, and rigidity with good response to levodopa treatment. Parkinsonism was asymmetrical without any other atypical features. Dopamine agonists were initiated and levodopa treatment was added after a couple of years. Two of them developed severe dyskinesias after 5 years of levodopa treatment despite reasonable doses (Figure 1: R1F6, R1F9). Later symptoms included Parkinson's disease dementia (PDD) such as impaired attention, memory and executive dysfunction presented in all three individuals with PD. Moreover, depression and apathy were also detected. The diagnosis of PDD was made based on the Movement Disorders Society (MDS)-proposed diagnostic criteria.¹³ Further laboratory testing and structural imaging with brain MRI scans excluded other possible causes of parkinsonism and AD CSF biomarker analysis was normal. (Figure 2).

Two additional members of the pedigree (Figure 1: R1F1, R1F10) presented initially with cognitive impairment without parkinsonism. Detailed clinical history revealed that early symptoms in these cases were memory impairment, concentration difficulties, disorientation, visuospatial deficits, and depression. Clinical examination and, assisted by imaging studies such as brain MRI SCAN, SPECT and DAT scan, as well as CSF biomarkers, including CSF A β and tau measures, classified these patients as Alzheimer's disease. The patients fulfilled the National Institute on Aging and the Alzheimer's Association revised criteria.¹⁴ Structural neuroimaging with MRI scans showed mild to moderate hippocampal atrophy, mild cortical atrophy, and moderate vascular ischemic changes (Figure 2). Differential diagnosis between Lewy body disease (LBD) and Alzheimer's disease might be difficult because of similarities of clinical symptoms in both neurodegenerative diseases; however, DAT scan was

normal in all three patients and diagnostic criteria for LBD were not fulfilled.¹⁵

Disease-causing variant identification

Screening for variants in genes associated with PD using the data generated with WES from all three individuals identified a total of 110 variants from 43 PD-associated genes. After searching the Human Molecular Genomic Database (HGMD), seven of these variants from four genes were considered PD-associated polymorphisms and none was considered disease causing or able to explain the disease in this population.

We subsequently screened for segregating rare coding variants in all protein coding genes. In total, 72 variants were identified with only one being present in the linked region described below (Supplementary Data S2). The heterozygous variant c.G1135T (p.G379W) in *SORL1*, a gene known to cause familial AD, segregated with the disease in the extended family. The variant was absent from major variant databases, including GnomAD, and our in-house 5000 controls. It was located inside a constrained element at a highly conserved nucleotide position (GERP = 4.15). The p.G379W change was predicted to be damaging by Mutation Taster with a confidence probability of 0.99 (SIFT = 0.00, Polyphen-2 = 1.00) and had a CADD PHRED score of 31. Glycine 379 is located within SORLA vacuolar protein sorting 10 (VPS10p) domain (Figure 3) and change to threonine is predicted to affect protein structure. No other rare variants in the *SORL1* gene or in all the other genes contained in this peak on chromosome 11 segregated with disease. The WGS analysis confirmed this finding and failed to identify any other variants in the linked region.

Additionally, burden tests were performed for all rare variants, all functional variants (including nonsynonymous, splice-site, and loss of function variants), and separately on rare nonsynonymous variants. We did not find an association between rare *SORL1* variants and PD in any of the burden analyses (Supplementary Data S3).

Linkage and Haplotype analysis

We performed a parametric linkage analysis assuming an autosomal dominant model using all available individuals. This analysis identified a single peak suggesting linkage on chromosome 11 (LOD score of 2.2). The 12.8Mb region (rs10750111 - rs12146531) contained 153 protein coding genes and included the *SORL1* gene. Haplotype analysis confirmed co-segregation between the affected status and the *SORL1* region in the family (Figure 1). Frequency estimation of c.G1135T (p.G379W) *SORL1* variant in controls and sporadic PD.

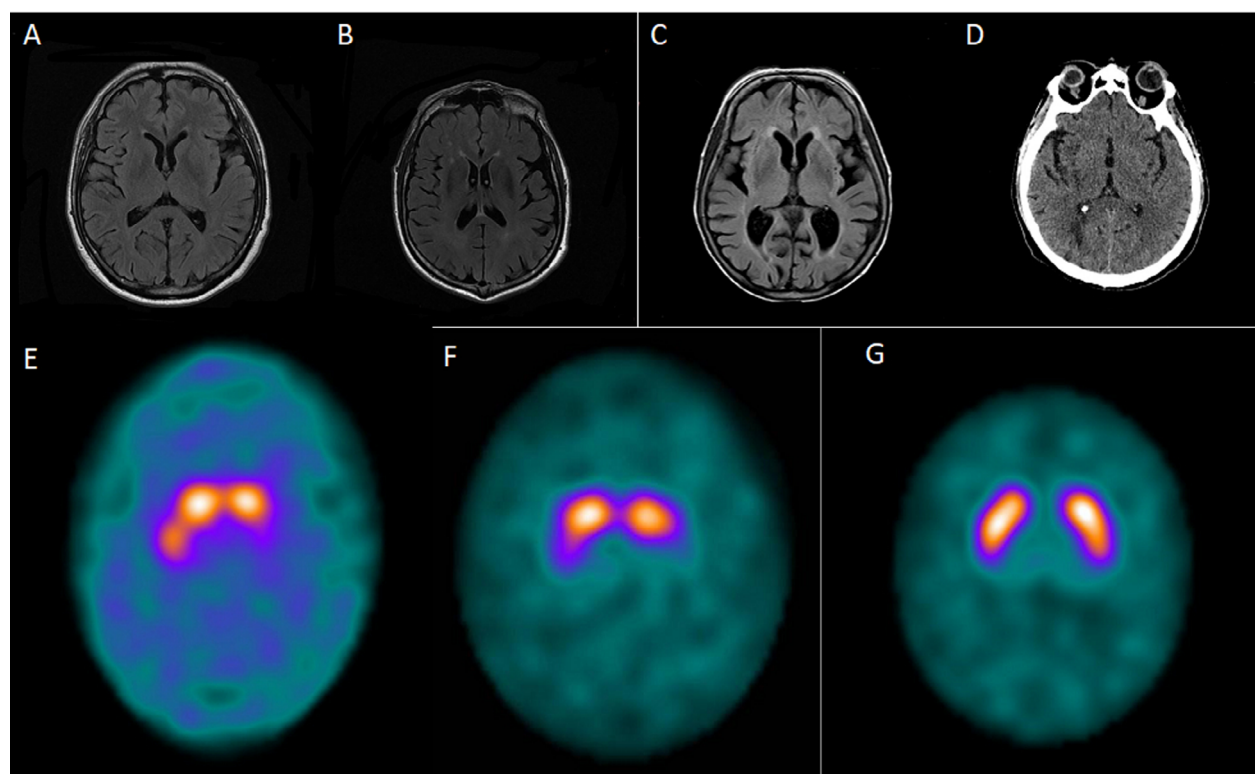


FIGURE 2. (A–C) MRI scans of members of the pedigree, showing no signs of atrophy (A:RI9, B:RIF16), and generalized cerebral atrophy and periventricular leukoencephalopathy (C:RIF10). (D) Brain CT scan of individual RIF1 showing mild atrophy and periventricular leukoencephalopathy. (E–G) DaT scan images showing asymmetrical radiotracer uptake in individual RIF16 (E) and in individual RIF9 (F) and normal symmetrical uptake in individual RIF10 (G)

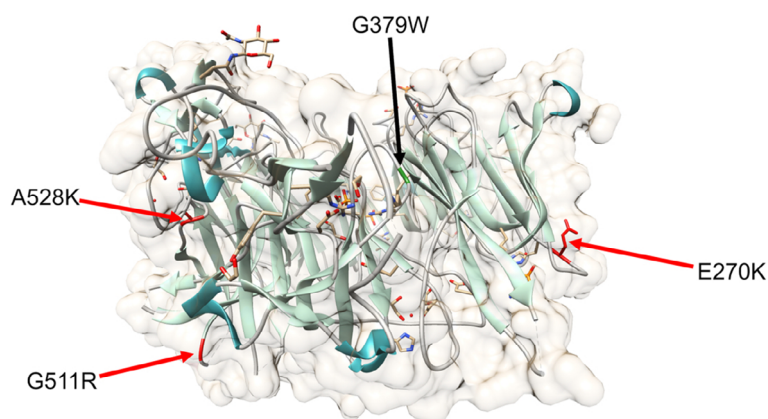


FIGURE 3. A crystal structure of SorLA Vps10p domain in ligand-free form, with the location of the G379 residue indicated by a black arrow and a selection of AD associated variants indicated in red (structural coordinates from PDB 3WSX, Ref. [13])

Mutation screening of sporadic PD cases identified one additional case with the c.G1135T(p.G379W) *SORL1* variant and haplotype analysis revealed a shared 5.2Mb haplotype surrounding the *SORL1* region. This PD patient was of Greek origin, from the same village as the described family but kinship analysis excluded close relatedness. No other sporadic patients from the village or from the larger Greek cohort carried the

variant. All controls were negative for the presence of the variant.

This patient had typical PD with an age of onset at 46. He initially developed asymmetrical bradykinesia and tremor on the right hand side and then bilaterally. Approximately after 10 years on levodopa treatment, he developed dyskinesias and he was then treated with levodopa/carbidopa intestinal gel with good response and

without complications. Cognitive functions were normal and Mini Mental and Addenbrooke's Cognitive examination scores were within the normal limits.

Discussion

The genetic association of *SORL1* with AD is well established.^{16,17} *SORL1* is responsible for encoding SORLA, a sorting receptor that is associated with retromer complex. SORLA is a large protein with an estimated size of 250 KDa. It contains a VPS10p domain, which is important for binding with cargo molecules, such as the amyloid precursor protein (APP) and other interactors.¹⁸ The VPS10p domain forms a ten bladed beta propeller which interacts with ABeta complex. The p.G379W mutation is located inside the sixth blade, adjacent to loop one which is crucial for binding affinity with ABeta.¹⁸

While most families with *SORL1* mutations present with a pure AD phenotype,¹⁶ it is noteworthy that the clinical spectrum in *SORL1* families is broad and extends beyond straightforward AD features.¹⁷ Cucarro *et al.* described two families with AD and parkinsonian features or AD and DLB.¹⁹ Additionally, Maple-Grodem and colleagues²⁰ report that variability in *SORL1* is associated with dementia in cases with parkinsonism. Other variants in *SORL1* have been found in pathologically confirmed DLB cases with rapid progression, clinically resembling Creutzfeldt-Jacob disease.²¹ These observations highlight the phenotypic pleiotropy where mutations in the same gene give rise to diverse phenotypes thus increasing the complexity of genotype–phenotype correlations.

Our study describes a *SORL1* mutation in a family presenting with features of AD and PD. These findings support the presence of broader phenotypic variability in patients with *SORL1*, highlighting the need for mutation screening of *SORL1* in patients with monogenic forms of neurodegenerative diseases to further establish a full phenotypic spectrum. As we did not find enrichment of rare variants in *SORL1*, the association of this gene with PD may be limited to this mutation, or it may act as a modifier gene.

Many familiar cases associated with PD genes so far were phenotypically heterogenous but within the spectrum of PD/PDD/DLB. However, in our family the clinical picture as well as all imaging and CSF biomarker studies are implicating PD/PDD/AD. All affected males have PD while females have AD, however, more studies are needed in order to determine whether *SORL1* may affect phenotypic expression through a sex-specific mechanism.²² Further investigation of SORLA in the context of PD is needed to elucidate the molecular mechanisms that underlie the possible association of the *SORL1* gene with this disorder, and any potential overlap with other PD

genes, such as *VPS35*—a key component of the retromer complex.

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have not participated in reviewing the data analysis or content of the manuscript. Genome Sequencing in Lewy Body Dementia and Neurologically Healthy Controls: A Resource for the Research Community.” was generated by the International LBD Genomics Consortium (iLBDGC), under the co-directorship by Dr. Bryan J. Traynor and Dr. Sonja W. Scholz from the Intramural Research Program of the U.S. National Institutes of Health. The iLBDGC Investigators have not participated in reviewing the data analysis or content of the manuscript. For a complete list of contributors, please see: Chia *et al.*, 2021 (*Nat. Genetics*).²³ STEADY-PD III is a 36-month, Phase 3, parallel group, placebo-controlled study of the efficacy of isradipine 10 mg daily in 336 participants with early Parkinson’s Disease that was funded by the National Institute of Neurological Disorders and Stroke (NINDS) and supported by The Michael J Fox Foundation for Parkinson’s Research and the Parkinson’s Study Group. The STEADY-PD III Investigators have not participated in reviewing the data analysis or content of the manuscript. The full list of STEADY PD III investigators can be found at: <https://clinicaltrials.gov/ct2/show/NCT02168842>.

Author Contributions

G.X.: conception and design of the study, acquisition and analysis of data, drafting the manuscript. J.V.: acquisition and analysis of data, drafting the manuscript. K.S.: acquisition and analysis of data, drafting the manuscript. A.E., Z.GO.: acquisition and analysis of data. H.H., J.H., A.S.: conception and design of the study, acquisition and analysis of data. T.B., M.F., M.H., P.A.L., G.M.H., C.S., J.V., S.B., L.F. A.K.: acquisition and analysis of data.

Conflict of Interest

The authors report no conflicts of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Whole genome sequencing variant filtering pipeline analysis

Data S2. Segregating rare coding variants in all protein coding genes identified by WES

Data S3. Gene burden analysis results