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Article

***SORL1* as a Putative Candidate Gene for a Novel Recessive form of Complicated Hereditary Spastic Paraplegia: Insights from a Deep Functional Study**

Ananthapadmanabha Kotambail ^{1,†}, Yogananda Shamamandri Markandeya ^{2,†}, R Mahima ¹, Ramya Sukrutha ¹, Madhura Milind Nimonkar ², Suravi Sasmita Dash ², Chandrajit Prasad ³, Ghati Kasturirangan Chetan ¹, Pooja Mailankody ⁴ and Gautham Arunachal ^{1,*}

¹ Department of Human Genetics

³ Department of Neuro Imaging and Interventional Radiology

⁴ Department of Neurology

⁵ National Institute of Mental Health and Neuro Sciences (NIMHANS), Bengaluru, Karnataka, India

[†] These authors equally contributed to the work.

* Correspondence: gautham.arunachal@gmail.com

Abstract

Genes regulating the endolysosome and autophagy pathway are the major aetiological contributors to hereditary spastic paraplegia (HSP). A pathogenetic link involving macroautophagy between HSP and Alzheimer disease (AD) has been well-established. *SORLA*, an endosomal trafficking protein, has a pivotal role in maintaining the homeostasis of glutamatergic neurons and white matter tract microarchitecture. So far, *SORL1* is only associated with a dominant form of AD and amyloid angiopathy. Here, we investigated a case with adult-onset spastic paraparesis and cerebromicroangiopathy (CRV). Whole-genome sequencing revealed a biallelic NC_000011.10(NM_003105.6):c.1211+1G>A consensus splice donor variant in *SORL1*. Transcriptomics confirmed aberrant splicing, predicting a derailment of the reading frame and significant nonsense-mediated decay. The demonstration of reduced levels of *SORLA* and, consequently, a significant enlargement of endosomes and lysosomes in patient-derived fibroblasts, further cemented the pathogenicity of the variant. The probability that *SORL1* is recessive and aetiologically related, gathers support from the constraint score metrics (pRec-1.0), meiotic recombination studies, *SORL1*^{-/-} mouse models with the phenotype reminiscent of HSP with CRV, and in vitro studies demonstrating endolysosomal enlargement in *SORL1*^{-/-} glutamatergic neurons. Collating all the evidence from phenotype analysis, functional studies, and literature suggests, *SORL1* is probably a new candidate for a recessive form of complicated HSP.

Keywords: *SORL1*; complicated HSP; cerebral amyloid angiopathy; lysosomal enlargement; NMD

1. Introduction

Hereditary Spastic Paraplegia (HSP) is a designation given to a constellation of symptoms characterised predominantly by weakness and spasticity of the lower limbs. HSP is a heterogeneous group of Mendelian disorders that is pan-ethnic, and has a collective prevalence of 2–5 per 100,000 [1]. It is clinically categorised into pure/uncomplicated and complex/complicated types with variable ages of onset. In all forms of HSPs, there is axonal degeneration of the corticospinal tract and its cell bodies. There can be a variable involvement of Fasciculus Gracilis. Substantial intra- and inter-familial variation in the phenotype is well known, even among the carriers of the same pathogenic variant [2]. Hence, accurate genotype-phenotype correlation is challenging. Moreover, there is a growing recognition of a substantial overlap between HSP and categories otherwise considered

distinct traditionally, such as inherited ataxias (spastic-ataxias), extrapyramidal disorders (Parkinsonian-pyramidal syndromes), dementias, and others.

Of the 150 HSP-linked genes, 70 have been definitively confirmed; while the others remain candidates [3]. Causative genes are involved in any one of the diverse cellular pathways, such as axonal transport, endosomal trafficking, etc. One such biological process, responsible for at least 24 subtypes of HSP, is the endolysosome and autophagy pathway [3]. Its role in several neurodegenerative disorders involving the motor neuron, such as HSP, ALS, and CMT, is beyond doubt. The classical examples are *SPG11*, *KIF1A*, *KIF5A*, *VCP*, and spastacin-related disorders. Since post mitotic differentiated neurons cannot divide, to preserve viability during stress, they rely heavily on the endolysosome and autophagy pathway [4]. It does so through precise cargo delivery through membrane trafficking and degradation, thus ensuring the removal of cytotoxic macromolecules or undesirable organelles. Hence, any malfunctioning of the endolysosome and autophagy pathway may promote neuronal dysfunction and death, thereby precipitating neurodegenerative disorders.

The protein SORLA (Sorting-related receptor with A-type repeats), a member of the VPS10 domain receptor family, is a key player in the endolysosome and autophagy pathways. It is involved in the sorting and recycling of Amyloid Precursor Protein (APP). SORLA is encoded by the gene Sortilin-related receptor 1 (*SORL1*). In conjunction with other retromer complexes, SORLA transports APP to the trans-Golgi network (TGN) or recycles APP to the plasma membrane from early endosomes, preventing the detrimental amyloidogenic pathway [4]. *SORL1* has a well-established association with the dominant form of familial Alzheimer disease (AD) and cerebral amyloid angiopathy (CAA) [5–7]. Glutamatergic neurons derived from human induced pluripotent stem cells (hiPSCs) of AD patients with heterozygous loss-of-function variants (LOF) have enlarged endosomes, a pathological hallmark of AD [8]. Enlarged endosomes are a result of the clogging of endolysosome and autophagy pathways by the amyloidogenic products [9]. However, recent in vitro studies have suggested a more severe molecular phenotype in the homozygous knockout (KO) of *SORL1* [8]. Parting AD and CAA, no other clinical phenotypes have been described so far with *SORL1*, especially in a recessive fashion.

Recently, there has been mounting evidence for the phenotypic overlap between HSP and AD, along with an established pathogenetic link [10]. Several genes aetiologically associated with both phenotypes are part of the endolysosome and autophagy pathway. The most prominent among them is *PSEN1*. In this light, we thoroughly investigated a case of progressive spastic paraparesis with cerebroretinal vasculopathy (CRV). Post comprehensive sequencing of the whole genome and an exhaustive search for a causative locus only yielded a biallelic consensus splice donor variant in *SORL1*. Here, we provide evidence in the form of aberrant RNA phenotypes, aberrant protein expression, as well as endosomal and lysosomal enlargement to support our claim that the biallelic splice donor variant is indeed a LOF variant and is leading to clogging of the endolysosome and autophagy pathway. The evidence from our data as well as the available molecular data on *SORL1* and clinical correlates supports our hypothesis that *SORL1* is a potential candidate for an as-yet-undescribed recessive type of complicated HSP.

2. Materials and Methods

2.1. Clinical Evaluation

Proband, a 22-year-old male, is the first born of second-degree consanguineous parentage (Figure 1). He presented with a history of lower limb weakness, gait difficulty (Supplementary Video), and frequent falls since the last three years. The onset was insidious, with a gradually progressive course. There was no history of bladder/bowel involvement, sensory disturbance, upper limb weakness, fasciculations, seizures, cognitive impairment, or constitutional symptoms. Antenatal history was uneventful. He had a smooth neonatal transition period with a birth weight of 2.6 kg. He had mild talipes of the left foot at birth, which, upon correction, did not pose any gait impediment. There was no developmental delay or regression. His scholastic performance up to the 9th standard

was just average. However, he was completely independent in all the activities of daily living, including the handling of monetary affairs. His parents in their early 5th decade have no symptoms of cognitive deterioration. No significant family history of neurological illness/dementia in the second and third degree relatives.

On examination, his head circumference was 53.5 cm, within the normal range; his height was 146.5 cm (<3 SD); and his weight was 36 kg (<3 SD). Tests for cognitive functions such as frontal aphasia battery, visuospatial memory, calculation, and attention were all normal. He had spasticity of the lower limbs with exaggerated deep tendon reflexes and bilateral extensor plantar responses. Reflexes in the upper limbs were brisk; however, power and tone were normal. He demonstrated a spastic gait. Sensory and cerebellar examinations were normal. Fundus examination revealed dot haemorrhages and a single cotton wool spot bilaterally. The nerve conduction studies and tibial somatosensory evoked potentials were normal.

Asymmetric T2/FLAIR hyperintensities were seen on the MRI in the pons, both centrum semiovale, and corona radiata (Figure 2). The same locations revealed punctate mineralization on susceptibility-weighted imaging (SWI) and curvilinear enhancement on contrast imaging. MRA revealed the left vertebral artery terminating in PICA, along with evidence of a persistent left hypoglossal artery. The MRI spine screening was unremarkable. The CT brain showed punctate calcifications in the locations mentioned above, as well as in the pineal gland and bilateral choroid plexus.

Despite the fact that his clinical profile did not match, we assessed him for intracranial calcifications, including interferonopathy, based on the imaging findings. The calcium studies were normal. ESR, CRP, and extensive autoimmune workups including RA, ANA, and ANCA were negative. CSF studies, including workups for infections, were unremarkable. VDRL, HIV, HTLV I, and II were non-reactive. HBsAg and anti-HCV antibodies were negative. Vitamin B12, homocysteine, tandem mass spectrometry, venous blood lactate, and ammonia were normal. Echocardiography, ultrasound abdomen and pelvis were normal. Audiological evaluation revealed bilateral mild conductive hearing loss, which was secondary to CSOM. However, brain stem auditory evoked potentials were normal bilaterally. In view of the clinical phenotype, course, and negative laboratory studies, a provisional diagnosis of complicated HSP (progressive spastic paraparesis with CRV) was considered. We performed whole-exome sequencing (WES) to determine the genetic causality.

2.2. Whole-Exome and Whole Genome Sequencing

WES and mitochondrial genome libraries were prepared using the Twist comprehensive exome kit (36.8 Mb, Twist Bioscience, USA). Paired-end 150 bp (PE150) sequencing was performed on NextSeq 550. Later, both the proband and asymptomatic mother (MOP) were also sequenced on a larger 43 Mb WES panel (KAPA HyperExome), as per the manufacturer's protocol (Roche Sequencing Solutions, USA). The libraries were sequenced on DNBSEQ-G400 (MGI Tech Co., Latvia) with PE 100 bp reads. We generated more than 10 Gb of data for the proband and MOP. Over 95% of bases had Phred Q scores >30.

Short read whole genome sequencing (SRWGS) library was constructed using KAPA HyperPrep kit. The library was sequenced in was performed DNBSEQ-G400 with PE 150 bp reads. A total of 92.41 Gb of sequencing raw data was generated for SRWGS.

2.3. Long-Read Whole-Genome Sequencing

Long-read whole-genome sequencing (LRWGS) library was constructed from 1 µg of the proband's DNA using the ligation sequencing kit (SQK-LSK114, Oxford Nanopore Technologies Ltd., UK). The libraries were sequenced on the Promethion 2i sequencer using R10.4.1 flowcells as per the manufacturer's protocol. The sequencing and simultaneous base calling were performed using Dorado v0.6.2. FASTQ reads with a mean Q score above 10 (Q10+) were filtered and considered for further analysis.

2.4. Variant Calling and Interpretation

We used FastQC v0.12.1 to evaluate FASTQ read quality. We aligned WES and SRWGS reads to the GRCh38 reference genome and performed variant calling using the Sentieon DNaseq pipeline, adhering to GATK best practices. LRWGS reads were aligned to GRCh38 using Minimap2. Variant calling was performed using wf-human-variation workflow v2.2.0 on EPI2ME v5.1.10 including calling of SNVs, small indels, structural variation (SV) and repeat expansions. We used VarSeq v2.5 (Golden Helix, Inc., USA) for variant annotation, filtering, and interpretation as per the ACMG/AMP guidelines [11]. Variants were annotated against various public databases of allele frequencies and phenotype associations, sequence conservation, functional effect, and splicing effect predictions.

Copy number variations (CNVs) were called using VarSeq CNV pipeline, and CNVs with a p value of <0.0005 and a z score value greater than ± 2 were considered for further evaluation. We annotated and interpreted CNVs in accordance with the ACMG guidelines. We used the VarSeq pipeline and AutoMap [12] to call copy-neutral loss of heterozygosity (LOH) regions as well as SV analysis for both the SRWGS and LRWGS datasets. Sanger sequencing was performed using the BDT v3.1 kit and SeqStudio system to validate the candidate variants.

2.5. RNA Sequencing

High-quality RNA from peripheral blood mononuclear cells (PBMCs) of proband and MOP was extracted and sequenced in duplicate. We also sequenced three healthy male and female control samples (HCON) matched for age. Strand-specific total RNA-seq libraries were prepared using 700 ng of total RNA utilising KAPA Hyper RNA kit (Roche Sequencing Solutions, USA). The libraries were sequenced on the NextSeq 550 with PE150 reads, which generated ~60 million reads per sample.

Reads were aligned to the GRCh38 reference using STAR v2.7.11b [13]. Gene-level quantification was performed using HTSeq v0.11 and Salmon v1.10 [14,15]. Differential gene expression analysis was performed using DESeq2 [15]. Differentially expressed genes (DEGs) showing log2 fold change (log2FC) of ± 1 and above with a padj value of <0.05 were considered significant. Significant DEGs were further analysed for gene ontology, pathways, and gene set enrichment analysis (GSEA) using WebGestalt [17]. Enriched gene ontology terms or molecular pathways identified were tested using FDR correction, and a p value of <0.05 was considered significant.

2.6. Detection of Splicing Aberration

As the pathogenic variant identified in the proband was predicted to result in splicing aberrations, we pursued detecting, analysing, and quantifying alternative splicing events. We utilised MAJIQ to detect and quantify local splicing variations (LSVs) and VOILA for visualisation [18]. Aligned reads from proband, MOP, six healthy age-matched controls, and three neurotypical brain tissues from the primary motor cortex (M1) and middle frontal gyrus (MC-MFG) were utilised. In-house MC-MFG data was utilised to show that the canonical transcript (ENST00000260197.12) is predominantly expressed in the human frontal cortex and that LSVs between HCON and MC-MFG are similar. Another reason is that, the M1 cortex is the likely pathological site in the proband; therefore, any outcome of our analysis of the clinically accessible tissue should mirror that of M1. Percent spliced in (PSI, Ψ) for each of the LSVs was calculated along with relative LSV differences (delta PSI, $\Delta\Psi$) between the groups using the MAJIQ quantifier.

2.7. Western Blot

Proteins from PBMC lysates were resolved by 7.5% SDS-PAGE and transferred to a PVDF membrane. Membrane was blocked and probed overnight with primary anti-SORL1 and anti-GAPDH antibodies (Abcam Inc., USA). Membranes were labelled with secondary antibodies and visualised using chemiluminescence detection.

2.8. Fibroblast Culture and Confocal Immunofluorescence Imaging

Previous studies have shown that the loss of *SORL1* in hiPSC-derived neurons leads to enlarged early endosomes and lysosomes [8,19]. Hence, we planned to perform confocal immunofluorescence imaging of endosomes and lysosomes from patient derived fibroblasts and controls. The tissue from the skin biopsy was minced and cultured in DMEM with 10% foetal bovine serum (FBS) as per the standard protocols [20]. Fibroblasts were then plated on coverslips, fixed with 4% paraformaldehyde, and permeabilized with 0.1% TritonX-100. Cells were blocked with 5% normal goat serum and labelled with anti-EEA1 and anti-LAMP1 (Cell Signalling Technology, USA). This was followed by staining with fluorochrome-conjugated secondary antibodies (Alexa fluor 488, Invitrogen), followed by counterstaining with DAPI. Cells were visualised using Zeiss LSM 980 confocal microscope with a suitable filter. Images were acquired for 15–20 fields and 20–25 cells were analysed. Maximum intensity projections of confocal stacks were generated and exported to ImageJ. Individual puncta were separated by segmentation, and 'Analyze Particle' command was used to measure the vesicle size [8,19]. Unpaired, two-tailed t-test was used for statistical analysis.

3. Results

3.1. Clinical Outcome at Follow-Up

The proband was prescribed antispastic medications and a regular physiotherapy regimen. The follow-up, 20 months after the first visit, revealed no new complaints. Only the frequency of falls had marginally increased. However, the neurological findings were comparable to the initial examination. Follow-up MRI/MRA indicated no interval changes. The clinical phenomenology, indolent course, and follow-up imaging data suggested a diagnosis of complicated HSP rather than monogenic interferonopathy, vasculitis, primary angiitis of the CNS, or a disorder of calcium homeostasis.

3.2. Results of WES and WGS

We analysed comprehensively annotated variants in a hierarchical filtering process using the latest versions of gene panels from PanelApp Australia or PanelApp UK [21]. We used a panel of 163 unique HSP-associated genes, which revealed no overt pathogenic or likely pathogenic variants. Scouring through the data for rare and ultra-rare variants in the panel yielded no significant variants. In the second line of evaluation, a panel of 55 genes, including *TREX1*, *RNASEH2A/2B,2C*, *LSM11*, *ARAR*, *IFIH1*, *SAMHD1*, and *RNU7-1*, were comprehensively screened for significant variants to rule out monogenic interferonopathy. Further, brain small vessel disease with calcification syndromes due to *COL4A1*, *COL4A2*, and *COLGALT1* were screened. This line of investigation did not yield any positive results. Finally, a comprehensive evaluation of over 1000 genes predisposing to either intracranial calcification, ALS, spastic ataxia, vasculitis, mitochondrial disorders, Fabry's disease, or *CSF1R* was performed, which yielded no disease-causing variants. Further, no large or small SVs encompassing genes or contiguous genes relevant to the above-mentioned panels were detected.

In order to explore the possibility of hidden causative variants in the known genes not captured by WES, we performed both SRWGS well as LRWGS. A concordance rate calculation for QC passed variants between SRWGS and LRWGS was performed by intersecting both VCF files using BCFTOOLS [22]. The analysis showed >95% concordance, suggesting high-quality LRWGS data. We then focused our attention on the rare/ultra-rare variants in the non-coding regions of the aforementioned genes with bioinformatic variant effect predictions on aberrant splicing/expression. It yielded a few variants of uncertain significance, which had insufficient evidence for disease causation, based on either in silico predictions or the experimental evidence of aberrant expression/splicing from the RNA-seq data. Furthermore, no pathogenic CNVs, repeat expansions, or structural variations were found for the genes/genomic regions relevant to the phenotype. Also, no copy neutral LOHs spanning the genes relevant to the phenotype were found.

Upon screening for all the pathogenic or likely pathogenic variants in any gene, an ultra-rare consensus splice donor variant in *SORL1* was found. The homozygous variant,

NC_000011.10(NM_003105.6):c.1211+1G>A in intron 8 of *SORL1* was observed (WES depth 57x; SRWGS depth 22x; LRWGS depth 18x) (Figure 3). The variant has been previously reported only once in a heterozygous state in gnomAD v4.1 (1/1612006 alleles, allele frequency=0.0000006203). This variant has not been reported in TOPMed, 1000 genomes, or in our in-house database consisting of over 12,000 alleles of Indian ancestry. The variant is in a clinically relevant transcript (MANE Select: ENST00000260197.12/NM_003105.6), potentially resulting in an aberration of normal splicing. As it disrupts the donor splice site for an exon, 6577 base pairs upstream of the penultimate exon junction complex, it was therefore predicted to cause nonsense-mediated decay (NMD). LOF is a known pathogenetic mechanism for *SORL1*. Hence, as per the ACMG/AMP guidelines, the variant was considered to be likely pathogenic (PM2, PVS1) [10]. MOP was found to be a heterozygous carrier of the variant. The proband's asymptomatic father declined to provide his sample for testing. However, phased LRWGS data demonstrated two different haplotype patterns flanking the pathogenic variant, suggesting the inheritance of the variant, one each from both parents. Further, the proband was found to harbour *APOE* $\epsilon 3/\epsilon 4$ genotype.

SORL1 has a well-established association with not only sporadic AD but also with monogenic forms of AD (dominant inheritance with age-dependent penetrance) [23]. However, the constraint score for *SORL1* resembles that of a recessive gene with a pRec score of 1 (pLI=0, LOEUF=0.55) [23]. Yet another clue towards the possibility of a recessive inheritance, comes from the homozygous KO mouse models exhibiting gait difficulties and vasculopathy [25]. Further, the variant was observed in a large copy neutral LOH, which was 4.3 Mb in size by WES and 0.9 Mb by LRWGS. This large copy neutral LOH, along with other large LOHs genome-wide, confirmed parental relatedness. This also indicates that the homologous chromosomal segments in the LOH regions are identical by descent rather than by chance. All these pointers strongly suggested a recessive pattern of inheritance for the phenotype, prompting further investigation.

3.3. Aberrant RNA Phenotype

As the variant predicted aberrant splicing and possible NMD, deep RNA-seq was performed. RNA-seq detected ~8894 protein-coding transcripts, each with >5 TPM (transcripts per million count). All the samples had comparable expression for the housekeeping genes. Of the SNVs called from RNA-seq, >95% were concordant with those from WES. We performed an integrated analysis with variants from WES, SRWGS, LRWGS and RNA-seq. However, no hidden pathogenic/likely pathogenic variants were identified from the RNA-seq data relevant to the phenotype.

We then focused our analysis on the *SORL1* transcript. The overall depth of the entire transcript in the proband as compared to controls was remarkably low. DEG analysis revealed a downregulation of *SORL1* transcripts with a log₂FC of -1.218 from the HTseq-DESeq2 workflow (padj=0.0046) and -1.66 (padj=0.000000043) from the Salmon-DESeq2 workflow (Supplementary Table 1). This translates to a striking 2.5-fold underexpression of *SORL1* as compared to controls. These results undisputedly confirmed NMD, which is a known pathogenetic mechanism in *SORL1* [26,27]. Based on the observed TPM values, we predicted NMD in 61.32% of transcripts, while 38.67% of the remaining, probably escaped. This is in line with the evidence that NMD is never complete and the proportion of escape varies by gene as well as the location of premature termination codons (PTCs) [28]. While MOP showed a partial downregulation of *SORL1*, it was not statistically significant. As the NMD was incomplete, further analysis of splicing aberrations was possible in the remainder of the transcripts.

Sashimi plots in IGV displayed at least 5 alternative splicing events (Supplementary Figure S1), which were further investigated by generating splice graphs and junctional read counts in MAJIQ/VOILA (Figure 4). The pattern of splicing and LSVs were identical between HCON and MC-MFG, suggesting only the canonical isoform is expressed in both tissues. Therefore, any aberration in splicing detected in PBMCs should mirror that of MC-MFG. Further, no other *SORL1* isoform but the canonical one was detected. MAJIQ revealed six distinct LSVs for the proband and MOP as compared to HCON/MC-MFG. LSV1, utilising the canonical splice sites of intron 8, had no junctional

reads in the proband with Ψ values (Table 1) strongly suggesting a complete abrogation of the 5' canonical splice site. Whereas, about 62% of transcripts in MOP were observed to have LSV1, as compared to >90% in HCON/MC-MFG. Because of this complete abrogation, a single target LSV with two 5' cryptic splice sites were activated (LSV2 and LSV3), with ~30% junctional reads for each of the LSVs in the proband. Another noteworthy finding was that, both the proband and MOP had a higher retention of intron 8 as compared to HCON/MC-MFG (LSV6). Since we employed a total RNA sequencing protocol, many of the reads spanning the entirety of the introns are probably originating from the pre-mRNA molecules. Even if a proportion of such reads are discounted, the extent of the intron retention event is at least 2-4 times higher in the proband as compared to HCON/MC-MFG.

We also observed single-source LSV (SS-LSV) events at the edges of exon 6 and exon 7, leading to exon skipping. The first SS-LSV at the junction of exon 6 skips exons 7 and 8 (LSV4), while the second at exon 7 skips exon 8 alone (LSV5). Since exons 8 and 9 are asymmetric alternative exons, any skipping event that results in splicing of exon 9 with any symmetrical exon would derail the open reading frame [29]. All six LSVs (Figure 5) described above result in the shifting of the reading frame and the incorporation of a PTC and many more PTCs theoretically downstream. These transcripts with PTCs, if translated, would result in highly truncated polypeptides of size less than 410 amino acids, roughly 1/5th of the full-length protein. Even though some transcripts have escaped NMD and proceeded to translation, we predict that the ribosome quality control complex would prevent the formation of truncated polypeptides cotranslationally [30].

3.4. In Silico Functional Analysis of DEGs

Overrepresentation analysis in WebGestalt for gene ontology relevant to biological processes utilising 16 statistically significant DEGs predicted a potential alteration in the regulation of tau-protein kinase activity (GO:1902947) with a p value of 0.000007 (FDR - 0.03) (Supplementary Table 2). This was predicted to be a consequence of *SORL1* downregulation. Homozygous *SORL1* KO mouse models have clearly established the detrimental effect on the subcellular distribution of phosphorylated tau, especially in the somatodendritic regions [31]. *SORL1* has been strongly linked with tau trafficking, as it aids tau internalisation within the cell. It has been shown that, abnormal tau aggregates in a cascading manner, termed tau seeding, when *SORL1* is dysfunctional [32]. It is plausible that altered tau levels may contribute to dysregulation of tau-protein kinase activity. This result was further validated by network topology-based analysis using a larger list of DEGs with log2FC greater than ± 1 and $p < 0.05$, regardless of padj (Supplementary Figure S6). Further, DEG analysis did not show overexpression of interferon-stimulated genes or aberrant expression of any of the genes related to monogenic interferonopathy or intracranial calcifications (Supplementary Table 3).

3.5. Western Blot and Immunofluorescence Analysis

Western blot revealed a near absence of full-length SORLA signal in the proband (Figure 5A) compared to the control, while GAPDH showed normal expression. This was in line with our prediction from the RNA-seq results. Previous studies have shown that hiPSC-derived neurons from an individual carrying a heterozygous truncating variant in *SORL1* invariably lead to enlarged early endosomes, whereas homozygous KO of *SORL1* neurons not only demonstrated enlarged early endosomes but also enlarged lysosomes [8,19]. To study the impact of homozygous LOF in *SORL1* on the cellular consequences, the proband's fibroblasts were interrogated with anti-EEA1 and anti-LAMP1 markers by immunofluorescence. Early endosome size was substantially larger ($p = 2.0308 \times 10^{-5}$) in proband-derived fibroblasts ($0.44 \pm 0.03 \mu\text{m}^2$, $n=16$) compared to controls ($0.27 \pm 0.01 \mu\text{m}^2$, $n=28$) (Figure 5B). The fibroblasts from the proband also had considerably larger lysosomes ($0.53 \pm 0.01 \mu\text{m}^2$, $n=25$) compared to controls ($0.24 \pm 0.01 \mu\text{m}^2$, $n=23$) (Figure 5C, $p = 8.5471 \times 10^{-20}$). Our findings not only corroborate the in vitro studies on homozygous KO of *SORL1* hiPSC-derived excitatory neurons, but they are also the first of their kind, to the best of our knowledge, from patient-derived fibroblasts. Enlarged endosomes have also been observed in the neuronal progenitor cells

derived from *SORL1* KO hiPSCs [19]. This indicates that early in the neuronal lineage, *SORL1* dysfunction can lead to endolysosomal traffic jams. These results provide irrefutable and conclusive functional evidence for the pathogenic effects of the LOF discovered in our proband. Hence, the variant c.1211+1G>A in *SORL1* was reclassified as pathogenic using PS3 functional study criteria (PM2, PVS1, PS3).

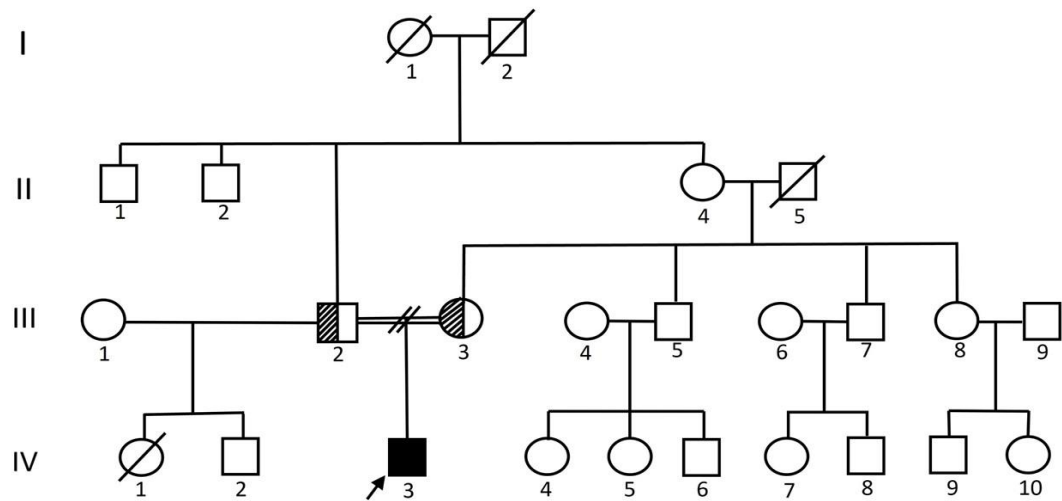


Figure 1. Family pedigree representing four generations. Proband (generation IV, individual 3) is denoted with an arrow mark who is homozygous for NC_000011.10(NM_003105.6):c.1211+1G>A. Parents (generation III, individual 2, 3) are unaffected carriers. .

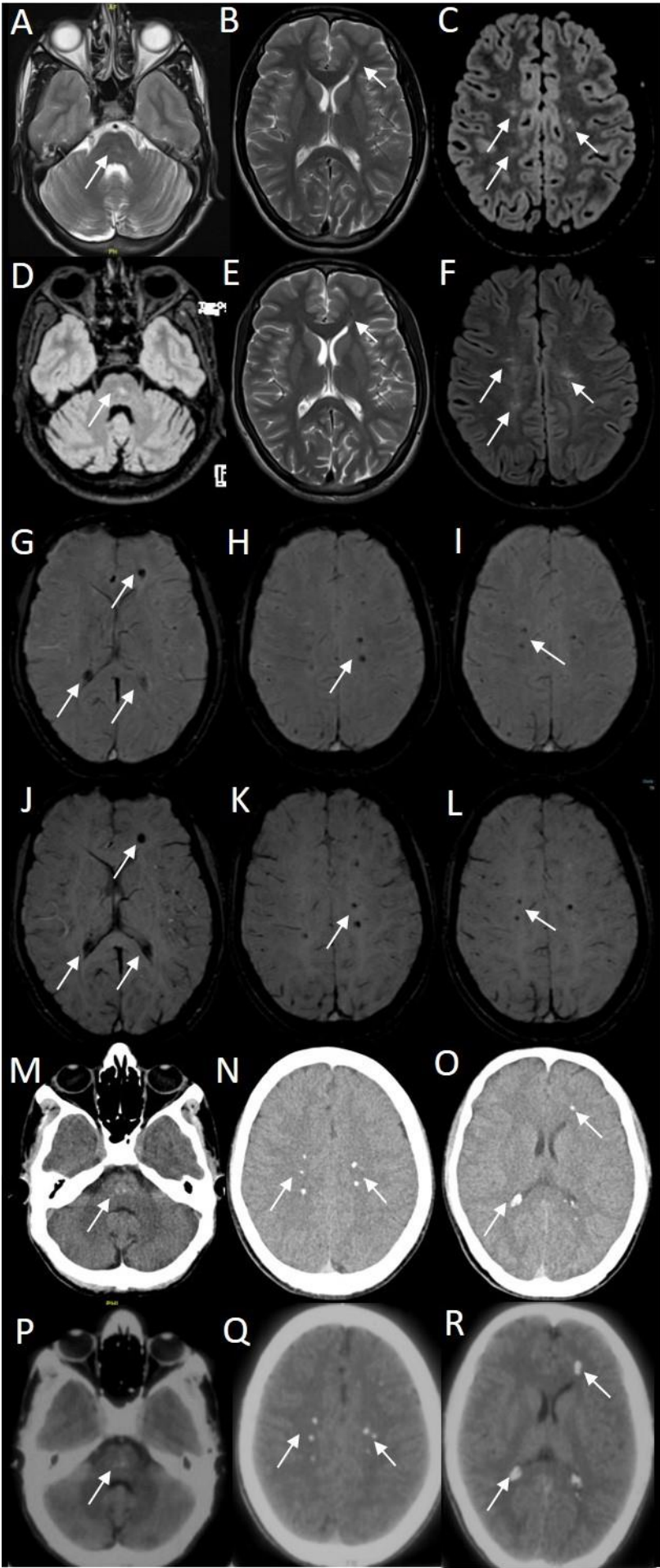


Figure 2. Magnetic resonance imaging of the proband showed asymmetric hyperintensities in pons, bilateral centrum semiovale and deep white matter of the left frontal cortex (white arrows on T2/FLAIR axial images A, B, C, D, E, F). Note that the MR images A-C and G-I were performed in April 2024 and images D-F and J-K were performed in June 2022. Susceptibility weighted imaging (SWI) showed punctate mineralization (white arrows on SWI axial images G-L). CT brain showed punctate calcifications in the pons, bilateral centrum semiovale and deep white matter of the left frontal cortex (white arrows on CT images M-R). Note that the CT images M-O were performed in June 2022 and images P-R were performed in September 2021.



Figure 3. Visualization of the homozygous splice donor variant NC_000011.10(NM_003105.6):c.1211+1G>A in *SORL1* (highlighted in the box) using Integrated Genomic Viewer (IGV) from the data generated by (A) WES, (B) LRWGS and (C) RNA-seq.

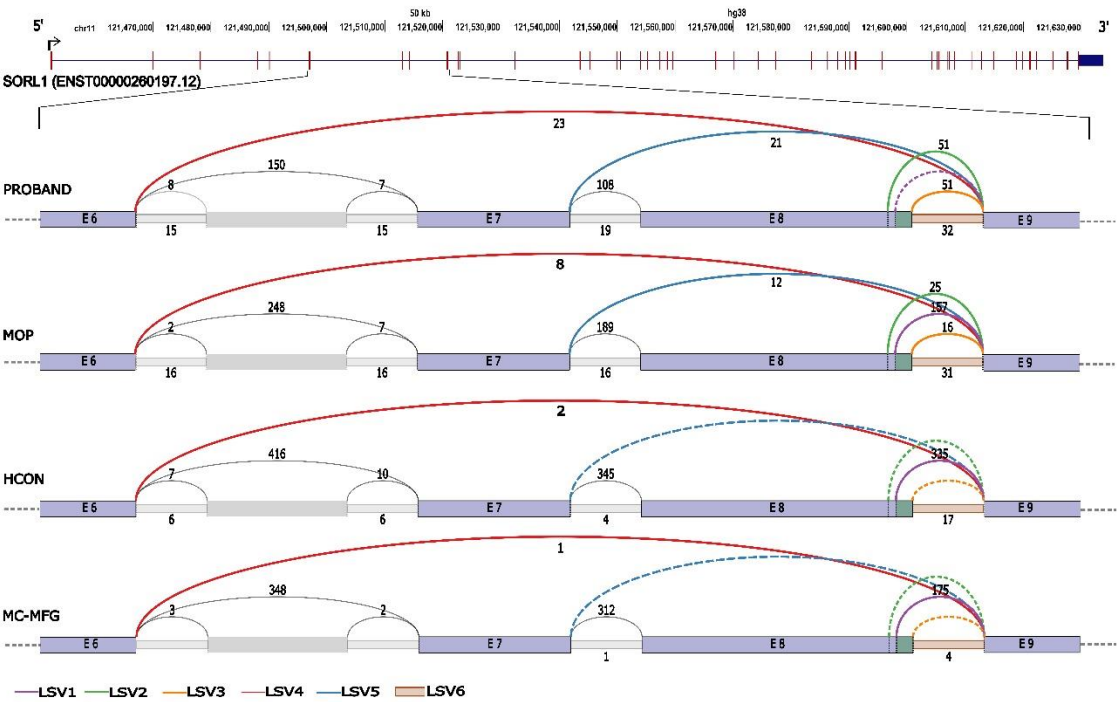


Figure 4. LSVs identified in *SORL1* transcript using MAJIQ pipeline. Significant LSVs (numbered LSV1-6) are represented as coloured arcs spanning the splice junctions. LSVs spanning only exon 6 to 9 are represented. Exons of the canonical transcript are represented as light lavender coloured rectangles with the respective exon

numbers. De novo exons detected from RNA-Seq data are shown by a lighter tint and have not been assigned with numbers. The number of junctional reads supporting each splicing event in the RNA-seq data is indicated near each arc. The dashed lines indicate junctions that lack any supporting reads for the splice event. Details of each LSVs are explained in the main text.

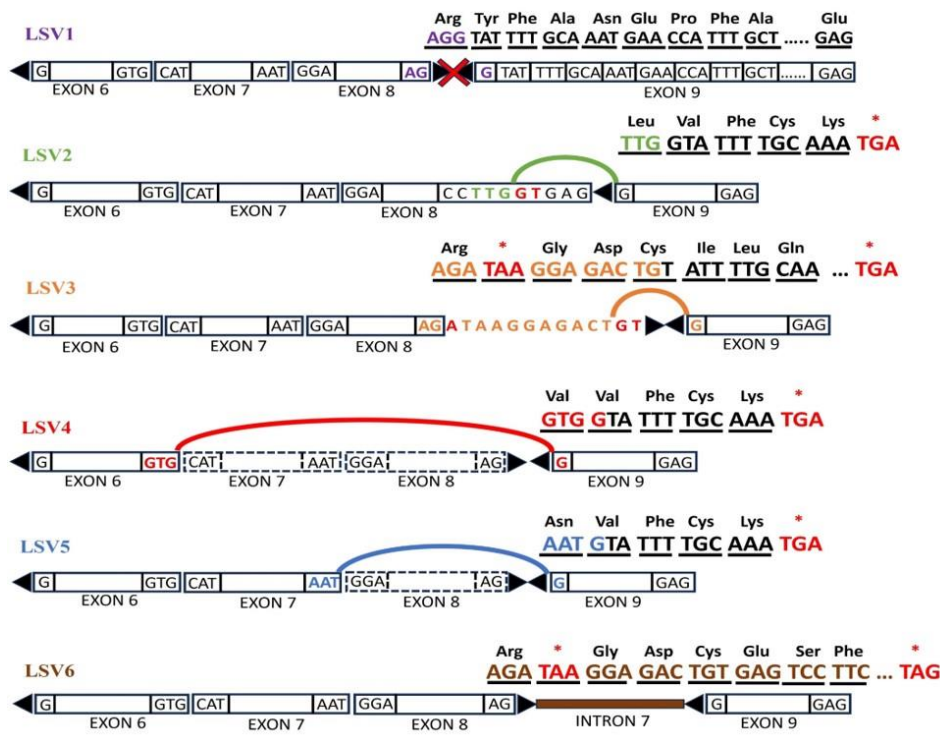


Figure 5. Illustration shows asymmetric alternative exons 8 and 9 depicted with sharp ends as well as symmetric exons with blunt ends. The respective nucleotides at the exon-intron junctions are also depicted. Any splicing of asymmetric and symmetric exons leads to shifting of reading frame with incorporation of PTCs.

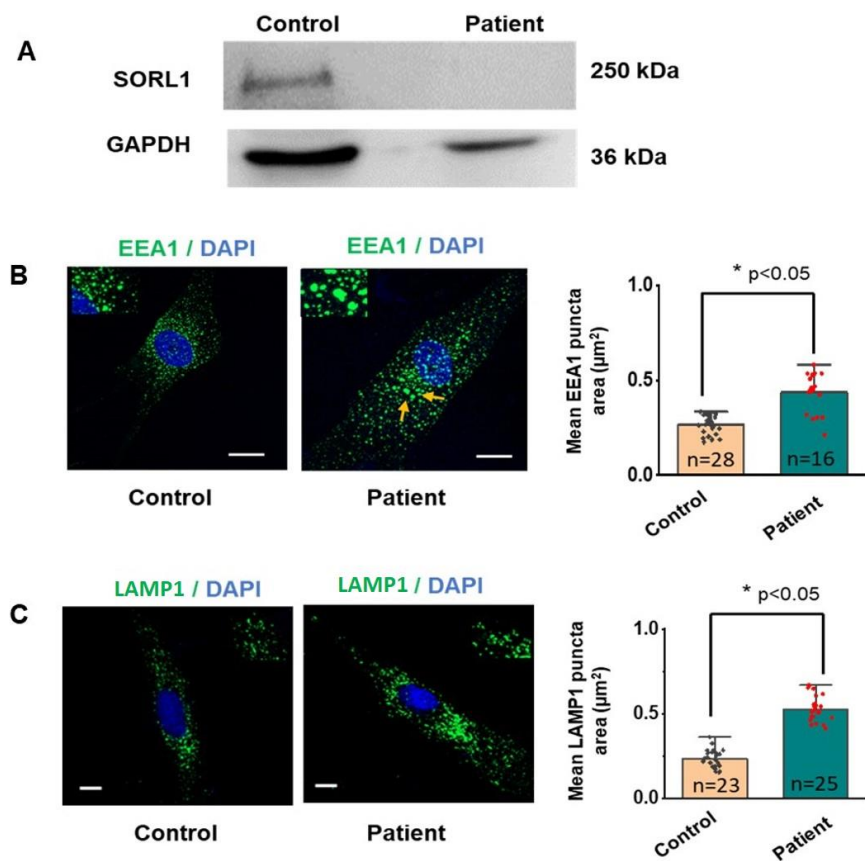


Figure 6. Enlarged endosomes and lysosomes in patient-derived fibroblasts. (A) Representative qualitative western blot image showing loss of SORLA protein expression in PBMC of proband compared to control. (B) Representative confocal images of control and patient fibroblasts immunolabelled for EEA1 (green)/DAPI (blue). Magnified view of the images are provided in the dashed squares (figure inset). Dot plot represents mean EEA1 puncta area in control and patient cells. (C) Representative confocal images of control and patient fibroblast expressing late endosomes and lysosomes (green) / DAPI (blue). Magnified view of the images are provided in the dashed squares (figure inset). Scale bar = 20 μm . Data was analysed using t-test, * $p < 0.05$.

Table 1. Details of LSVs identified from RNA-seq as per MAJIQ/VOILA. Significant LSVs depicted in Figure 4 are provided with detailed description, chromosomal location and corresponding Ψ values for each sample group.

LSV ID	LSV description	Junction coordinates (GRCh38)	Median Ψ Values			
			Proband	MOP	HCON	MC-MFG
LSV1	Canonical 5' splice junction (exon 8)	chr11:121514321-121520657	0	0.6249	0.9083	0.9565
LSV2	Alternative 5' splice junction – single target LSV (exon 8)	chr11:121514316-121520657	0.2908	0.1006	0	0
LSV3	Alternative 5' splice junction – single target LSV (exon 8)	chr11:121514332-121520657	0.2829	0.0652	0	0

LSV4	Exon skipping – single source LSV (exon 6 to exon 9)	chr11:121497049- 121520657	0.1236	0.0295	0.006	0.0012
LSV5	Exon skipping – single source LSV (exon 7 to exon 9)	chr11:121513104- 121520657	0.1184	0.0469	0	0
LSV6	Intron retention (intron 8)	chr11:121514322- 121520656	0.1868	0.1245	0.084	0.0388

4. Discussion

Our very first objective was to make sense of the complex clinicoradiological findings. Proband clearly had a new-onset spastic paraparesis with an indolent course, mild involvement of the upper limbs, retinal dot haemorrhages, and growth failure. The MR imaging findings, which showed multispot white matter hyperintensities and involvement of perivascular spaces in the centrum semiovale, along with fundoscopic findings, collectively indicate CRV. We had ruled out all other causes of retinal abnormalities, such as diabetes, HIV, etc. Having ruled out infectious and autoimmune aetiologies, along with static imaging findings, a diagnosis of complicated HSP was entertained. Further, intracranial calcifications have been reported with HSP subtypes such as Spastic paraplegia type 56, Autosomal Recessive, due to CYP2U1. Pedigree analysis supported a recessive pattern of inheritance. However, even after LRWGS , transcriptome analysis, and homozygosity mapping for the known loci for HSP as well as intracranial calcifications including monogenic interferonopathy, calcium homeostasis disorders, and brain small vessel diseases, no significant aetiology was found. Congenital TORCH was another group of disorders that required consideration. However, a new-onset progressive spastic paraparesis in adulthood due to congenital infections without other pathognomonic signs and symptoms of TORCH sequelae such as sensorineural hearing loss (infancy or late-onset), retinal scars, cataracts, and developmental delay is unheard of. The case had remained undiagnosed genetically until a homozygous pathogenic variant was identified in *SORL1*. Only once, in the literature has there been a passing mention of *SORL1* with a heterozygous variant as the cause of spasticity [33].

The second objective was to find an explanation for the possible scientific link between biallelic *SORL1* LOF and progressive spastic paraparesis with CRV. Of late, there has been mounting evidence for the phenotypic overlap between HSP and AD, along with an established pathogenetic link [10]. Over 13 genes are currently known to be associated with both phenotypes. To name a few, *ATP13A2*, *ALS2*, *ATP13A2*, *C19orf12*, *CYP27A1*, *PSEN1*, *SPART*, *SPG11*, *SPG21*, and *TTR* have been strongly linked with both. *PSEN1*, which regulates the fate of APP, is strongly associated with HSP [10]. At least 7.5–10% of *PSEN1*-related familial AD cases present with HSP years before the onset of cognitive deterioration [10]. Another 13.7% of cases develop spastic paraparesis at some point during the course. Only a rapid downhill course of *PSEN1* related HSP distinguishes it from the other forms [10,34]. This evidence provides impetus to test the hypothesis that pathogenic variants in other genes of monogenic AD can also probably be associated with the HSP phenotype. This makes the supposition of *SORL1*, which is yet another key regulator of the fate of APP along with *PSEN1*, to be associated with HSP highly feasible.

The subsequent challenge was to propose *SORL1* as a gene that exhibits recessive inheritance. Three lines of reasoning strongly suggest *SORL1* could be recessive. The first line of reasoning was based on the constraint for homozygous LOF for *SORL1*. The score of pRec 1 is the highest possible [24,35]. This score highlights that the effect of evolutionary negative selection pressure on the LOFs in a homozygous state is nearly complete. The support comes from the observation that, of the 106 *SORL1* LOFs in 1,614,324 alleles catalogued in gnomAD v4.1.0, none were homozygous [24,35]. Second line of reasoning comes from the results of homozygous knockout mouse studies. The majority of the *SORL1* null/KO mouse strains (MGI:5569945; MGI:3604352; MGI:3815203) from the

Mouse Genome Informatics (MGI) database were completely viable. However, these strains showed significant neurological deficits in the form of predominant gait difficulty in early adult life and startle reflex, as well as retinal abnormalities in late adult life, as compared to the wild-type strains. Further, a form of severe vasculopathy has also been described, reminiscent of accelerated CAA. Whereas heterozygous KO had only mild biochemical and histopathological consequences [36]. Third line of reasoning is that *SORL1* is in a region with a recombination frequency ranging from 1.4 to 1.8 cM/Mb based on different studies [37]. The absence of large LOHs encompassing *SORL1* in our in-house database of over 1000 individuals of Indian ancestry further strengthen this observation. Therefore, it is not a hotspot for LOH. The amalgamation of these three independent pieces of evidence suggests that evolutionarily, *SORL1* is shielded from homozygous pathogenic variants by frequent recombination events in the region, thereby preventing any reduction in fitness [38]. In conclusion, *SORL1* bears all the hallmarks of a recessive gene in selection terms. The final piece of evidence comes from large studies looking at the effect of certain *SORL1* risk variants associated with sporadic AD, such as rs689021 and rs668387, which increase susceptibility to late-onset AD when inherited in a recessive fashion [38].

The pathogenic variant results in aberrant splicing of exon 8, which has a mean pext score of 0.8 (proportion expressed across transcripts) and 0.9 in distinct brain regions [40]. The pext score provides information regarding the degree to which an exon is expressed across tissues in various isoforms. A high pext score of 0.8–0.9 is a suggestion in itself that the expression of exon 8 is ubiquitous and is an essential component of the polypeptide. Since exon 8 and exon 9 are asymmetric alternative exons, any mutational process that excludes exon 8 from splicing will shift the reading frame and introduce a PTC, resulting in an adverse consequence for translation or the polypeptide length [29,41]. Multiple lines of functional evidence demonstrated in our study have proven that the variant is pathogenic. First, the variant leads to loss of a significant proportion of clinically relevant transcripts through NMD and aberrant splicing in the rest. Only in a small proportion of transcripts, similar effects were observed in asymptomatic MOP. The aberrant RNA phenotype discovered in this study has a completely incongruous pattern of expression and splicing to that of wild-type transcripts of HCON/MC-MFG. Secondly, the aberrant splicing predicted a substantial protein truncation with a loss of more than 75% of the length of SORLA. Western blotting confirmed this prediction. *SORL1* is a ubiquitously expressed gene. However, so far in the literature, any aberration in the expression or functioning of *SORL1*, has been noticed to affect only the brain.

Next, our goal was to explore the literature evidence towards the role of *SORL1* in the pyramidal neurons of the M1 cortex. Given our concern for the integrity of the pyramidal neurons in M1, we examined the expression pattern of *SORL1* in this specific region. Bulk transcriptome data of neurotypical brains from the Allen Brain Atlas [42] showed a peak *SORL1* expression between the ages of 18–25 years in humans. Beyond this age window, the expression tends to decrease mildly before plateauing till the 4th decade, only to show a smaller second spike (bimodal) in the late 4th decade (Supplementary Figure S4).³ This appears to mirror the pattern of expression of APP, which is the most significant cargo for the SORLA containing sorting complex (Supplementary Figure S4) [43]. The age of onset of illness in our proband clearly coincides with the peak expression of *SORL1* and APP in the M1 cortex, suggesting a plausible causal association. This inference is further corroborated by the evidence that the homozygous ‘A’ allele of *SORL1* risk variant rs689021 leads to a considerable reduction in the expression of the *SORL1* transcript in the prefrontal cortices during childhood, late adolescence, and early adulthood compared to the wild-type allele. This reduction occurs several years before the onset of AD [39]. In addition, single-cell transcriptomics of the M1 region, as retrieved from NeMo analytics [43], showed a strikingly high expression of *SORL1* in the excitatory glutamatergic neurons as compared to inhibitory GABAergic ones. Layer 5 intratelencephalic glutamatergic neurons have the highest quantum of expression and are moderately expressed among the layer 5 extratelencephalic projecting neurons. The pattern of expression of *SORL1* among various subtypes of glutamatergic neurons mirrors that of APP (Supplementary Figure S5). This pattern is highly indicative of the crucial role *SORL1* plays in

maintaining the homeostasis of the pyramidal neurons of M1 by preventing traffic jams in the endolysosome and autophagy pathway. In addition to sorting APP, *SORL1* has been considered to be instrumental in recycling glutamate receptors (GLUA1) in the pyramidal neurons [44]. This is particularly essential for normal synaptic functioning and neurotransmission. Hence, the observed expression pattern. Further, *SORL1* has been shown to be critical for maintaining the microarchitectural integrity of frontotemporal white matter (WM) tracts [39]. The homozygous 'A' allele of *SORL1* risk variant rs689021 results in reduced fractional anisotropy as measured by diffusion tensor imaging of these tracts as compared to the wild-type allele in adolescence and early adulthood, years before the onset of AD. Hence, *SORL1* is not only essential for the cellular processes in the somatodendritic region but also for the structural integrity of multiple WM tracts containing the axonal processes.

Our next objective was to establish the pathogenetic link between homozygous LOF, cellular dysfunction, and neurodegeneration. SORLA, working in conjunction with the retromer complex, is not only involved in recycling cargos back to the plasma membrane or to TGN but also sorts cargos by trafficking them into the degradation pathway in a retromer-independent manner. Hence, it is conceivable that any SORLA-mediated sorting failure can cause traffic jams in all three avenues. It has been proven time and again that SORLA dysfunction leads to a disproportionate shunting of APP into amyloidogenic degradation rather than recycling, resulting in an excess accumulation of A β 40/42 [36]. The excess accumulation of A β 40/42 fragments within the early endosomes leads to enlarged endosomes, a histopathological hallmark of AD. As can be logically predicted, the effect observed was more pronounced in the homozygous KO in vitro models [8]. Exceptional levels of A β 40/42 in these models led to not only enlarged endosomes but also lysosomal enlargement. Further, a reduction in cathepsin D levels and altered autophagy flux have been observed in these models due to an aberrant processing of APP [18]. In these homozygous KO models, it has been shown that antisense oligos against APP have rescued the observed endolysosome and autophagy pathway defects. This involvement of the entire endolysosome and autophagy pathway in the *SORL1* homozygous KO enhances accelerated neurodegeneration, as previously demonstrated. In our study, we have proven all the ill effects of the homozygous pathogenic variant on endosome and lysosome physiology through immunofluorescence studies.

Enlarged endosomes, the earliest neuropathological hallmark of AD, have been observed in pyramidal neurons of layers III and V of the neocortex, even when amyloid pathology is observed only in the entorhinal cortex (Braak stage I–III) among non-demented individuals [45]. Such changes have been observed in the prefrontal cortex, PBMCs, lymphoblastoid cell lines, and fibroblasts among individuals with Down's syndrome decades prior to the development of AD. Further, enlarged endosomes have also been observed in association with APP and PSEN1 pathogenic variants, suggesting a common pathogenetic link for APP, PSEN1, and *SORL1* involving the endolysosome and autophagy pathway [8]. All the evidence described so far from clinical, diffusion tensor imaging, neuropathological studies, and animal studies, it is clear that *SORL1* affects the M1 cortex and the WM tracts years before the development of AD. Hence, we speculate that the biallelic pathogenic variant in *SORL1* observed in our proband has significantly dysregulated the endolysosome and autophagy pathway resulting in accelerated neurodegeneration of the pyramidal neurons of the motor cortex and the WM tracts, leading to the HSP phenotype.

While considering the genotype-phenotype correlation, only two instances of biallelic LOFs in *SORL1* have been reported in individuals with early onset AD with an accelerated course previously in literature [26,27]. One family had compound heterozygous LOF variants, trans-state, and a dominant familial AD, with parents having late-onset AD. This clearly suggests accelerated neurodegeneration among individuals with biallelic LOF as compared to monoallelic. In the second family, even though the proband was homozygous for a PTC in exon 2, his illness was similar to that of his father, in terms of age of onset and course, who had the variant in a heterozygous state. Even though spasticity has not been reported in either case, *SORL1* may have pleiotropic effects like those of PSEN1. PSEN1 pathogenic variants clustering in and around exons 8–9 present with the HSP

phenotype, whereas those with variants in other exons present with pure AD.⁷ We speculate that the location of the LOF might play a role in the pleiotropic effects of *SORL1* as well. CRV changes could be a consequence of CAA. However, the pattern of calcification in CCA brains is different. Recently, though, calcification and iron deposition in the penetrating arteries have been demonstrated in CAA autopsied brains with 7T MRI [46]. We speculate that the pattern of MR imaging in our case could be secondary to an accelerated form of CAA. Further, the Genematcher platform (submission ID: 119924) we identified a match for the *SORL1* with HSP and another match with complicated recessive early-onset dementia, strengthening our view.

We provide here a consilience of evidence and a rational argument towards the possibility of *SORL1* being a candidate gene for a recessive form of complicated HSP. We recommend investigating biallelic pathogenic variants in *SORL1* among all cases of HSPs negative for the established genes. Literature similar to ours in the future can provide support for our conclusions.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Supplementary Figure 1: Visualization of RNA-Seq reads in Sashimi Plot of the proband, MOP, HCON and MC-MFG samples depicting aberrant splicing, Supplementary Figure 2: Analysis and quantification LSVs in *SORL1* by MAJIQ/VOILA, Supplementary Figure 3: Sanger electropherograms of the *SORL1* c.1211+1G>A variant, Supplementary Figure 4: *SORL1* and APP mRNA expression pattern across age groups in the human primary motor cortex, Supplementary Figure 5: *SORL1* and APP expression pattern across cell types in the human primary motor cortex (M1), Supplementary Figure 6: Network-topology based analysis of DEGs from RNA-seq data, Supplementary Table 1: List of significant DEGs with log2FC of ± 1 with a padj value of <0.05 , Supplementary Table 2: Overrepresentation analysis of gene ontology biological processes for statistically significant DEGs, Supplementary Table 3: The DEG analysis results for genes related with monogenic interferonopathy indicate that none of the genes exhibit statistically significant differential expression.

Author Contributions: AK conducted sequencing experiments, analysed data, interpreted results, and drafted the manuscript; YSM planned immunofluorescence and western blot experiments, interpreted the findings; drafted the manuscript. RS conducted bioinformatics analysis on variants obtained from WES, WGS, and RNA-seq. MMN and SSD executed immunofluorescence studies, western blots and analysed the results, respectively. CP conducted neuroimaging and interpreted the findings. CGK supported the overall study and provided expert input. PM provided complete clinical care, including investigations, diagnosis, and treatment for the proband; provided clinical expertise, including genotype-phenotype correlation. GA conceptualised the study, experimental design, interpretation of data, obtaining funding, study supervision, and drafting/revising the final manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study protocol (No.NIMHANS/43rdIEC/BS&NS.DIV/2023) was approved by the Institutional Ethics Committee of the National Institute of Mental Health and Neuro Sciences (NIMHANS), Bengaluru, India. Subjects were recruited after the appropriate consenting process as per the ICMR national ethical guidelines for biomedical and health research involving human participants, in adherence with the Helsinki principles. Written informed consent including that for photography and videography where relevant was obtained from study subjects.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The variant identified has been submitted to the ClinVar database with the accession number: VCV003238954.1.

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References

1. Shribman S, Reid E, Crosby AH, Houlden H, Warner TT. Hereditary spastic paraplegia: from diagnosis to emerging therapeutic approaches. *Lancet Neurol.* 2019;18:1136-46.
2. Fink JK. Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms. *Acta Neuropathol.* 2013;126:307-28.
3. Toupenet Marchesi L, Leblanc M, Stevanin G. Current Knowledge of Endolysosomal and Autophagy Defects in Hereditary Spastic Paraplegia. *Cells.* 2021;10.
4. Zhang H, Huang T, Hong Y, Yang W, Zhang X, Luo H, et al. The Retromer Complex and Sorting Nexins in Neurodegenerative Diseases. *Front Aging Neurosci.* 2018;10:79.
5. Verheijen J, Van den Bossche T, van der Zee J, Engelborghs S, Sanchez-Valle R, Llado A, et al. A comprehensive study of the genetic impact of rare variants in *SORL1* in European early-onset Alzheimer's disease. *Acta Neuropathol.* 2016;132:213-24.
6. Rogaeva E, Meng Y, Lee JH, Gu Y, Kawarai T, Zou F, et al. The neuronal sortilin-related receptor *SORL1* is genetically associated with Alzheimer disease. *Nat Genet.* 2007;39:168-77.
7. Du Y, Liu C, Ma C, Xu X, Zhou X, Zhou H, et al. Cerebral amyloid angiopathy-related inflammation: a case report presenting with a rare variant in *SORL1* gene. *BMC Neurol.* 2019;19:97.
8. Hung C, Tuck E, Stubbs V, van der Lee SJ, Aalfs C, van Spaendonk R, et al. *SORL1* deficiency in human excitatory neurons causes APP-dependent defects in the endolysosome-autophagy network. *Cell Rep.* 2021;35:109259.
9. Andersen, O. M., Bogh, N., Landau, A. M., Ploen, G. G., Jensen, A. M. G., Monti, G., . . . Sorensen, C. B. (2022). A genetically modified minipig model for Alzheimer's disease with *SORL1* haploinsufficiency. *Cell Rep Med*, 3(9), 100740.
10. Chelban V, Breza M, Szaruga M, Vandrovcova J, Murphy D, Lee CJ, et al. Spastic paraplegia preceding *PSEN1*-related familial Alzheimer's disease. *Alzheimers Dement (Amst).* 2021;13:e12186.
11. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17:405-24.
12. Quinodoz M, Peter VG, Bedoni N, Royer Bertrand B, Cisarova K, Salmaninejad A, et al. AutoMap is a high performance homozygosity mapping tool using next-generation sequencing data. *Nat Commun.* 2021;12:518.
13. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics.* 2013;29:15-21.
14. Anders S, Pyl PT, Huber W. HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics.* 2015;31:166-9.
15. Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. *Nat Methods.* 2017;14:417-19.
16. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 2014;15:550.
17. Liao Y, Wang J, Jaehnig EJ, Shi Z, Zhang B. WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. *Nucleic Acids Res.* 2019;47:W199-W205.
18. Vaquero-Garcia J, Aicher JK, Jewell S, Gazzara MR, Radens CM, Jha A, et al. RNA splicing analysis using heterogeneous and large RNA-seq datasets. *Nat Commun.* 2023;14:1230.
19. Knupp A, Mishra S, Martinez R, Braggins JE, Szabo M, Kinoshita C, et al. Depletion of the AD Risk Gene *SORL1* Selectively Impairs Neuronal Endosomal Traffic Independent of Amyloidogenic APP Processing. *Cell Rep.* 2020;31:107719.
20. Iannello G, Patel A, Sirabella D, Diaz AG, Hoover BN, Sarmah H, et al. Simple, Fast, and Efficient Method for Derivation of Dermal Fibroblasts From Skin Biopsies. *Curr Protoc.* 2023;3:e714.
21. Martin AR, Williams E, Foulger RE, Leigh S, Daugherty LC, Niblock O, et al. PanelApp crowdsources expert knowledge to establish consensus diagnostic gene panels. *Nat Genet.* 2019;51:1560-65.
22. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, et al. Twelve years of SAMtools and BCFtools. *Gigascience.* 2021;10.

23. Redaelli V, Ricci M, Del Sole A, Piccione M, Prioni S, Rossi G. A novel *SORL1* mutation in a pedigree affected by early-onset Alzheimer's disease. *J Alzheimers Dis Rep.* 2025;9:25424823241296017. Published 2025 Apr 2. doi:10.1177/25424823241296017
24. Chen S, Francioli LC, Goodrich JK, Collins RL, Kanai M, Wang Q, et al. A genomic mutational constraint map using variation in 76,156 human genomes. *Nature.* 2024;625:92-100.
25. Blake JA, Baldarelli R, Kadin JA, Richardson JE, Smith CL, Bult CJ, et al. Mouse Genome Database (MGD): Knowledgebase for mouse-human comparative biology. *Nucleic Acids Res.* 2021;49:D981-D87.
26. Alvarez-Mora MI, Blanco-Palmero VA, Quesada-Espinosa JF, Artech-Lopez AR, Llamas-Velasco S, Palma Milla C, et al. Heterozygous and Homozygous Variants in *SORL1* Gene in Alzheimer's Disease Patients: Clinical, Neuroimaging and Neuropathological Findings. *Int J Mol Sci.* 2022;23.
27. Le Guennec K, Tubeuf H, Hannequin D, Wallon D, Quenez O, Rousseau S, et al. Biallelic Loss of Function of *SORL1* in an Early Onset Alzheimer's Disease Patient. *J Alzheimers Dis.* 2018;62:821-31.
28. Dyle MC, Kolakada D, Cortazar MA, Jagannathan S. How to get away with nonsense: Mechanisms and consequences of escape from nonsense-mediated RNA decay. *Wiley Interdiscip Rev RNA.* 2020;11:e1560.
29. Schad E, Kalmar L, Tompa P. Exon-phase symmetry and intrinsic structural disorder promote modular evolution in the human genome. *Nucleic Acids Res.* 2013;41:4409-22.
30. Karamyshev AL, Karamysheva ZN. Lost in Translation: Ribosome-Associated mRNA and Protein Quality Controls. *Front Genet.* 2018;9:431.
31. Capsoni S, Carlo AS, Vignone D, Amato G, Criscuolo C, Willnow TE, et al. SorLA deficiency dissects amyloid pathology from tau and cholinergic neurodegeneration in a mouse model of Alzheimer's disease. *J Alzheimers Dis.* 2013;33:357-71.
32. Cooper JM, Lathuiliere A, Su EJ, Song Y, Torrente D, Jo Y, et al. *SORL1* is a receptor for tau that promotes tau seeding. *J Biol Chem.* 2024;300:107313.
33. Fazeli E, Child DD, Bucks SA, Stovarsky M, Edwards G, Rose SE, et al. A familial missense variant in the Alzheimer's disease gene *SORL1* impairs its maturation and endosomal sorting. *Acta Neuropathol.* 2024;147:20.
34. Ringman JM, Dorrani N, Fernandez SG, Signer R, Martinez-Agosto J, Lee H, et al. Characterization of spastic paraplegia in a family with a novel *PSEN1* mutation. *Brain Commun.* 2023;5:fcad030.
35. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature.* 2020;581:434-43.
36. Dodson SE, Andersen OM, Karmali V, Fritz JJ, Cheng D, Peng J, et al. Loss of LR11/SORLA enhances early pathology in a mouse model of amyloidosis: evidence for a proximal role in Alzheimer's disease. *J Neurosci.* 2008;28:12877-86.
37. Kong A, Thorleifsson G, Gudbjartsson DF, Masson G, Sigurdsson A, Jonasdottir A, et al. Fine-scale recombination rate differences between sexes, populations and individuals. *Nature.* 2010;467:1099-103.
38. Pemberton TJ, Absher D, Feldman MW, Myers RM, Rosenberg NA, Li JZ. Genomic patterns of homozygosity in worldwide human populations. *Am J Hum Genet.* 2012;91:275-92.
39. Felsky D, Szeszko P, Yu L, Honer WG, De Jager PL, Schneider JA, et al. The *SORL1* gene and convergent neural risk for Alzheimer's disease across the human lifespan. *Mol Psychiatry.* 2014;19:1125-32.
40. Cummings BB, Karczewski KJ, Kosmicki JA, Seaby EG, Watts NA, Singer-Berk M, et al. Transcript expression-aware annotation improves rare variant interpretation. *Nature.* 2020;581:452-58.
41. Kolkman JA, Stemmer WP. Directed evolution of proteins by exon shuffling. *Nat Biotechnol.* 2001;19:423-8.
42. Shen EH, Overly CC, Jones AR. The Allen Human Brain Atlas: comprehensive gene expression mapping of the human brain. *Trends Neurosci.* 2012;35:711-4.
43. Ament SA, Adkins RS, Carter R, Chrysostomou E, Colantuoni C, Crabtree J, et al. The Neuroscience Multi-Omic Archive: a BRAIN Initiative resource for single-cell transcriptomic and epigenomic data from the mammalian brain. *Nucleic Acids Res.* 2023;51:D1075-D85.
44. Small SA, Simoes-Spassov S, Mayeux R, Petsko GA. Endosomal Traffic Jams Represent a Pathogenic Hub and Therapeutic Target in Alzheimer's Disease. *Trends Neurosci.* 2017;40:592-602.

45. Cataldo AM, Mathews PM, Boiteau AB, Hassinger LC, Peterhoff CM, Jiang Y, et al. Down syndrome fibroblast model of Alzheimer-related endosome pathology: accelerated endocytosis promotes late endocytic defects. *Am J Pathol.* 2008;173:370-84.
46. Bulk M, Moursel LG, van der Graaf LM, van Veluw SJ, Greenberg SM, van Duinen SG, et al. Cerebral Amyloid Angiopathy With Vascular Iron Accumulation and Calcification. *Stroke.* 2018;49:2081-87.

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