Biallelic variants in *SPATA5L1* lead to intellectual disability, spastic-dystonic cerebral palsy, epilepsy and hearing loss

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Abstract

Spermatogenesis associated 5 like 1 (SPATA5L1) represents an orphan gene encoding a protein of unknown function. We report 28 biallelic variants in *SPATA5L1* associated with sensorineural hearing loss in 47 individuals from 28 (26 unrelated) families. In addition, 25/47 affected individuals (53%) presented with microcephaly, developmental delay/intellectual disability, cerebral palsy, and/or epilepsy. Modeling indicated damaging effect of variants on the protein, largely via destabilizing effects on protein domains. Brain imaging revealed diminished cerebral volume, thin corpus callosum, and periventricular leukomalacia, and quantitative volumetry demonstrated significantly diminished white matter volumes in several individuals. Immunofluorescent imaging in rat hippocampal neurons revealed localization of Spata5l1 in neuronal and glial cell nuclei, with more prominent expression in neurons. In the rodent inner ear, Spata5l1 is expressed in the neurosensory hair cells and inner ear supporting cells. Transcriptomic analysis performed using fibroblasts from affected individuals was able to distinguish affected from controls by principal components. Analysis of differentially expressed genes and networks suggested a role for SPATA5L1 in cell surface adhesion receptor function, intracellular focal adhesions, and DNA replication and mitosis. Collectively, our results indicate that biallelic *SPATA5L1* variants lead to a human disease characterized by SNHL with or without a nonprogressive mixed neurodevelopmental phenotype.

Keywords

Neurodevelopmental disorder, movement disorder, cerebral palsy, epilepsy, SPATA5L1, intellectual disability, AAA+ superfamily, ATPase, sensorineural hearing loss.

REPORT

Neurodevelopmental disorders (NDDs) frequently co-occur, as disruption of early brain morphogenesis and connectivity can affect multiple intersecting domains of development. These disorders represent a wide spectrum of clinical manifestations, ranging from single organ (e.g., brain) pathology to embryonic lethality due to failure of vital organs, yet common, recognizable NDD phenotypes include intellectual disability, hearing loss, cerebral palsy, autism, and epilepsy. Prior work has revealed that homozygous or compound heterozygous variants in the spermatogenesis associated 5 gene (*SPATA5*, MIM: 613940) cause an NDD syndrome[1] that features microcephaly, cortical visual impairment, intellectual disability, spastic cerebral palsy, epilepsy, and sensorineural hearing loss (SNHL) (MIM: 616577). Neuroimaging features included hypomyelination in some individuals and a thin corpus callosum. Based on the domain structure, SPATA5 has been grouped into the ATPase associated with diverse activities (AAA+) protein family[2]. Knockdown of *Spata5* in rat cortical neurons led to abnormal mitochondrial morphology and fission/fusion ratios[3], suggesting a role in energy metabolism. In humans, SPATA5 has a paralog, spermatogenesis associated 5 like 1 (SPATA5L1), that is 35% identical and 52% similar by Drosophila RNAi Research Center Integrative Ortholog Prediction Tool (DIOPT) alignment. Previous genome-wide association studies have found that *SPATA5L1* resides within a locus associated with chronic kidney disease in a combined North American and Dutch cohort[4], which was replicated in Japanese[5] and Mongolian[6] cohorts. However, no Mendelian disease-associated variants have been previously reported in *SPATA5L1*.

Here, as part of large-scale sequencing screens of individuals with SNHL and cerebral palsy, respectively, we detected biallelic, predicted deleterious variants in *SPATA5L1* (HGNC:28762, NM_024063.3). Using GeneMatcher[7] services, we subsequently connected with colleagues worldwide. Together, we report 28 unique *SPATA5L1* variants in 47 affected individuals from 28 (26 unrelated) families. All human subjects' studies were performed in accordance with the ethical standards of the responsible committee on human experimentation according to institutional and national standards. Proper informed consent was obtained for all participants. Sequencing was performed at numerous centers, but all used Illumina systems and institutional pipelines based on current GATK best practices[8; 9]. Details regarding sequencing metrics and variant prioritization can be found in **Supplemental Methods**. Among the identified variants, 25 were present in the cohort in compound heterozygous form and three were found as homozygous variants (**Figure S1**). Out of these three, one missense variant, c.1199C>T (p.(Thr400Ile)), also segregates in a compound heterozygous fashion in two other families. Most putatively damaging variants were private, with the exception of five that were detected in multiple families: c.527G>T (p.(Gly176Val)), c.1398T>G (p.(Ile466Met)), c.606_619dup14 (p.(Glu207Glyfs*25)), c.1199C>T (p.(Thr400Ile)) and c.2066G>T (p.(Gly689Val)), with the former two found in both the neurologic presentation and isolated hearing loss cases and the latter two found only in individuals with neurologic presentation. The pathogenicity of missense variants was predicted by ≥3 algorithms (**Table S1**). None of the identified variants were found in homozygous form in gnomAD.

All affected individuals with biallelic variants in *SPATA5L1* presented with mild, moderate, or severe hearing loss, with about half (25/47, 53%) also exhibiting neurologic features, particularly global developmental delay/intellectual disability (seen in all cases with neurologic involvement). Other prominent neurological findings included spastic-dystonic cerebral palsy in approximately two-thirds, epilepsy (16/25, 64%) and cortical visual impairment

(15/25, 60%). Clinical features of our cohort are summarized in **Table 1** and **Figure 1A** (and detailed in **Table S2** and **S3**). Operational definitions for presence/absence of NDDs can be found in **Supplementary Methods**. Case video review indicated visual impairment, impaired expressive language, intellectual disability, and mixed movement disorders with resultant orthopedic complications (**Videos S1-6**).

Most individuals exhibited a movement disorder, typically spasticity (17/25, 68%), dystonia (15/25, 60%), or a combination of these two forms of hypertonia (13/25, 52%). This usually occurred in a quadriplegic or generalized distribution. More than half (17/25, 68%) of the individuals had isolated hypotonia, although these cases tended to be younger and may not have manifested their full motor phenotype. Some individuals were reported to exhibit ataxia, while non-epileptic myoclonus was identified in one. Stereotypies were seen in several individuals as well. The degree of cognitive impairment seen in affected individuals varied from severe to profound. For severely affected individuals, hyporesponsiveness to environmental stimuli was seen. Autistic features were absent except for two individuals. Additional neuropsychiatric features were not reported, and behavior problems were not prominent. A combination of focal and generalized seizure types was reported. Few individuals (4/25, 16%) exhibited infantile spasms, often associated with a clinical diagnosis of West syndrome. Other forms of generalized seizures included myoclonic (7/25, 28%), absence (3/25, 12%), and generalized tonic-clonic (11/25, 44%) events. Focal epilepsy (4/25, 16%), sometimes with secondary generalization, was evident in several individuals as well, and a subset demonstrated mixed focal and generalized semiologies. One individual was reported to have evidence of electrical status epilepticus in slow wave sleep (ESES), and seizures in some were intractable or described in the context of a developmental or epileptic encephalopathy.

Microcephaly was present in about half of the cases (13/25, 52%). Facial dysmorphism, assessed locally and confirmed by a trained dysmorphologist (MCF) whenever possible, was noted in one-third (9/25) of affected individuals. Facial features included downslanting palpebral fissures, widow's peak, low frontal hairline, large ears, tooth malformation, high palate, bitemporal narrowing, sparse eyebrows, depressed nasal bridge, large ears and micrognathia as well as prominent upper lip, small chin and mild telecanthus, evident in individual facial photographs (**Figure 1B**). A gestalt representation of 'SPATA5L1 facies' was also constructed using the Face2Gene RESEARCH application (FDNA Inc., Boston, MA, USA). However, this facial gestalt did not highlight any consistent dysmorphism, evidenced by the lack of a significant difference between the case and the age, sex, and ethnicity matched control cohort (p=0.223, **Figure S2**).

Neuroimaging findings were assessed by a board-certified neuroradiologist (PC). Some individuals' brains were morphologically normal, but relatively consistent features included diminished cortical volume, periventricular leukomalacia, widened Sylvian fissures, anterior temporal hypoplasia, and hypoplastic corpus callosum (**Figure 2A**). More variable features included delayed myelination in toddlers, an "ears of the lynx"-like appearance, incomplete hippocampal rotation, optic nerve hypoplasia, and small pons. To further characterize the structural neuroanatomic findings, a quantitative volumetric analysis was performed using DICOM data from clinical magnetic resonance images using a previously described method[10]. This analysis revealed a significance decrease in white matter volume in *SPATA5L1* cases compared to controls (**Figure 2B**).

Intriguingly, a subset of individuals (DY1-DY11) with biallelic *SPATA5L1* variants presented with isolated, non-syndromic hearing loss without neurological features (22/47, 47%, **Figure S1**, **Table S2**). These individuals were all of Ashkenazi Jewish decent, and in all the missense variant c.1398T>G was identified in compound-heterozygosity with various other pathogenic alleles suggesting a hypomorphic founder allele, resulting in a partial rather than complete loss of protein function. According to gnomAD, the allele frequency of this variant is 0.0029 in individuals of Ashkenazi Jewish background, indicating a frequency of homozygotes of 0.8/100,000. The fact that no cases with SNHL hearing loss and homozygosity for the c.1398T>G allele was identified further raises the question if the variant would not lead to a clinical phenotype in homozygous form. Like the cases with neurologic involvement, the bilateral SNHL associated with isolated cases was mild to profound. There appeared to be some benefit to cochlear implants among individuals who received this intervention.

SPATA5L1 belongs to the AAA+ ATPases protein superfamily, a functionally diverse group of enzymes that hydrolyze ATP to induce changes in target substrates. Variants identified in our cohort are spread throughout the gene and protein (**Figure 3A**, visualized using Geneious Prime 2021.0.1). Structural effects of a subset of missense variants detected in our cohort were assessed by VIPUR[11] and Missense3D[12] based on a three-dimensional model of SPATA5L1. Of the 13 variants investigated, 11 variants were predicted to have a deleterious effect on protein structure (**Figure 3B**) via a combination of destabilizing effects including steric clashes, loss of hydrophobic packing, loss of polar interactions, and the emergence of buried charged residues (**Figure S3**). All variants predicted to be deleterious are expected to destabilize domains within the SPATA5L1 protein, except for the p.(Gly689Val) variant, which is predicted to create steric clashes with the ATP ligand, affecting ATP binding properties.

We next sought to define the typical protein localization of SPATA5L1. *SPATA5L1* mRNA has been detected (albeit at low levels) in both neurons and glia, both during embryonic and adult stages of human brain development (**Figure 4A**). We confirmed this at the protein level in rat dissociated hippocampal cultures, identifying Spata5l1 immunoreactivity within neurons, astrocytes, oligodendrocytes, and microglia, with the most prominent staining in neurons (**Figure 4B**). *Spata5l1* localized primarily to the nucleus. Within the ear, both inner and outer hair cells exhibit detectable levels of *Spata5l1* transcript (**Figure 4C**). Rat whole mounts stained with a commercial antibody against Spata5l1 demonstrated that Spata5l1 is present in hair cells and pillar cells of the organ of Corti (**Figure 4D**), suggesting that loss of wild-type protein may lead to sensorineural hearing loss by disrupting normal function within these structures.

The precise function of SPATA5L1 in brain, inner ear and other tissues is currently unknown. However, the sequence similarity and overlapping clinical manifestations in individuals harboring putatively loss of function or protein-damaging variants in SPATA5 and SPATA5L1 led us to speculate as to a potential redundancy between the two proteins. Given the proposed role of SPATA5 in mitochondrial function[1], we assessed oxidative phosphorylation (OXPHOS) in primary fibroblasts from affected individuals 2-3, 2-4, 7-9 (i.e., individuals 3 and 4, family 2 and individual 9, family 7) in comparison to passage and age-matched controls using the Seahorse XF96 assay. These assays indicated no impairment of OXPHOS in affected fibroblasts (**Figure S4**), suggesting that despite the degree of clinical overlap seen in affected individuals, the two proteins may have divergent functions. Indeed, a mitochondrial localization for SPATA5L1 is not predicted using MitoMiner[13] and it is absent from

the MitoCarta3.0 human and mouse inventory[14]. However, alterations to mitochondrial biogenesis remain possible, and we cannot exclude disruption to mitochondrial morphology and dynamics (as observed in SPATA5 deficient neurons)[3].

Next, we turned to an unbiased transcriptome approach to try to distinguish SPATA5L1 fibroblast cell lines from controls. The analysis was performed in the same affected (plus 1-1 and 4-6 i.e. individual 1, family 1 and individual 6, family 4) cell lines utilized for the Seahorse study and in passage and age-matched controls. Principal component analysis from RNA-Seq data indicated that individuals harboring biallelic SPATA5L1 variants could be distinguished from controls based on their differentially expressed genes (**Figure 5A**). This provided proof of principle data supporting the p.(Ala41Pro), p.(Val245Glu), p.(Ile435fs), p.(Ile466Met), and p.(Ala519Asn) variants as *bona fide* disease-associated variants. These findings allowed us to pool these data for subsequent analyses. Significantly upregulated networks were not identified. However, several significantly down-regulated genes were identified (**Figure 5B** and **5C**). These genes converged on several hubs (**Figure 5D-F**), pointing to a role for SPATA5L1 in mitosis (mitotic nuclear division, sister chromatid segregation, mitotic spindle organization, kinetochores) and DNA replication (DNA conformation change, single stranded DNA binding, DNA helicase activity). Adhesion receptors, which connect cell-substrate junctions (**Figure 5E**) and include fibronectin-binding (**Figure 5F**) integrins (i.e., *ITGA8*; **Figure 5B**), cadherins (**Figure 5F**), and immunoglobulin superfamily members (i.e., *L1CAM*; **Figure 5B**) were significantly downregulated. Members of the AAA+ ATPases protein superfamily have known roles in mitosis, DNA replication, metabolism, and repair processes[15]. For example, cytoplasmic dynein plays a role in mammalian mitotic spindle formation[16], while WRNIP1 protects stalled replication forks from degradation[17]. Our transcriptome analyses posit a potential role for SPATA5L1 in mitosis and DNA replication, however additional studies are required to assess this possibility.

Our clinical, radiologic, genomic, and transcriptomic evidence support the existence of a mixed neurodevelopmental syndrome with hearing loss due to biallelic variants in *SPATA5L1*. The pathogenicity of the variants we identified is supported by their rarity (many private variants), predicted deleteriousness by multiple algorithms, consistent phenotype in affected individuals, and RNA-Seq validation of several variants. Although we did identify individual loss of function (premature stop, frameshift, start-loss, stop-loss, or canonical splice site) alleles, we did not identify biallelic loss of function variants, suggesting that perhaps human knockout genotypes might show reduced viability. We were not able to clearly identify any firm genotype-phenotype correlations. Our morphologic neuroimaging analyses revealed thin corpus callosum, diminished cortical volume, open opercula, and anterior temporal hypoplasia in several individuals, while quantitative morphometry indicated that white matter volume was significantly diminished in multiple members of the cohort. When observed, microcephaly correlated with reduced white matter volume (as observed in cases 10-2, 12-1, 13-1).

Although *SPATA5L1* is an orphan gene, our transcriptomic studies provide some clues as to its function. Adhesion receptors collectively play a major role in the control of cell-extracellular matrix (fibronectin-integrin and immunoglobulin superfamily members) and cell-cell interactions (cadherin family members). These interactions in turn integrate cell growth/migration and proliferation based on environmental cues such as contact inhibition. Fibronectin-integrin binding is known to be mediated through focal adhesions, intracellular cytoskeleton/signaling hubs that transmit extracellular cues through phosphorylation events (i.e., protein serine-threonine kinase activator activity) and ultimately control DNA replication

and mitosis. Although neuroimaging in our cases did not indicate malformations of cortical development that would suggest abnormalities of neuronal migration, the diminished cortical volumes and microcephaly seen may reflect impairment of neuronal cell division during brain development.

In conclusion, we present evidence that rare coding variants and loss of function alleles in *SPATA5L1* lead to hearing loss and a mixed neurodevelopmental disorder that features microcephaly, global developmental delay/intellectual disability, spastic-dystonic cerebral palsy (with some children presenting with hypotonia), and focal or generalized epilepsy. Although our studies support a role for SPATA5L1 in DNA replication, further experimental studies will be required to support or refute this hypothesis.

Supplemental Data

Supplemental Data include 4 Figures and 3 Tables.

Declaration of Interests

The authors declare no competing interests.

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Table 1. Biallelic variants in *SPATA5L1* cause a neurodevelopmental disorder, featuring intellectual disability, cerebral palsy, epilepsy, and hearing loss.

Family	Family 1	Family 2	Family 3	Family 4	Family 5	Family 6	Family 7	Family 8	Family 9
Patient	**1 (proband)**	**3 (proband)**	**5**	**6**	**7**	**8**	**9**	**10 (proband)**	**13**
cDNA (NM_024063.3)	c.1304_1305del (pat); c.121G>C (mat)	c.734T>A (pat); c.1398T>G (mat)	c.1A>T (pat); c.2066G>T (mat)	c.515C>A (pat); c.196G>T (mat)	c.1973G>A (pat); c.2176_2177del (mat)	c.76A>G (pat); c.1079T>C (mat)	c.1556C>A (hom)	c.1199C>T (hom)	c.1676delC (pat); c.1682T>C (mat)
Protein (NP_076968.2)	p.(Ile435Argfs*4) (pat); p.(Ala41Pro) (mat)	p.(Val245Glu) (pat); p.(Ile466Met) (mat)	p.(M1?) (pat); p.(Gly689Val) (mat)	p.(Pro172His) (pat); p.(Asp66Tyr) (mat)	p.(Arg658Lys) (pat); p.(Val726Lysfs*13) (mat)	p.(Thr26Ala) (pat); p.(Phe360Ser) (mat)	p.(Ala519Asp) (hom)	p.(Thr400Ile) (hom)	p.(Ala559Glufs*33) (pat); p.(Leu561Ser) (mat)
Ancestry	Iraqi	European, with Ashkenazi Jewish ancestry	American; mat. ethnicity: Italian, pat. ethnicity: Italian & Afro-American	Mixed European	Spanish	African American	Turkish	Kazakh	Mixed, Eastern European, and Scandinavian
Sex	Male	Male	Female	Male	Male	Female	Female	Female	Male
Age at diagnosis	First year of life	32 months	27 months	23 years	4 months	9-12 months	5 years	7 months	1 month
Hearing impairment	+	+	+	+	+	+	+	+	+
Spasticity	+	-	+	-	-	-	+	+	+
Dystonia / hypotonia	+ / -	+ / +	- / +	- / N/A	- / +	N/A / -	+ / -	+ / +	- / +
Pattern	Spastic quadriplegia	N/A	Spastic quadriplegia	N/A	N/A	N/A	Spastic-dystonic tetraparesis	Spastic quadriplegia	Spastic quadriplegia
Microcephaly (HC)	+ (acq., 47cm @ 6 years)	-	+ (acq., 46cm @ 3 years)	-	+ (acq., 45cm @ 1 year)	-	+ (27cm @ birth)	+ (45cm @ 6 years)	-
DD / ID	+ Profound DD	+ Global DD	+ Global DD	+ Severe DD	+ Profound DD	+	+ Severe DD	+ Profound DD / ID	+ Profound DD
Epilepsy	+	-	-	+	-	+	+	+	+
Dysmorphic features	-	+	-	-	-	-	-	+	+
Visual impairment	Cortical visual blindness	-	Cortical visual impairment	-	-	N/A	Severe cortical visual impairment	Probable cortical blindness	Severe impairment
MRI findings	Progressive CO and CB volume loss; thin CC; periventricular T2 hyperintensities; subtle GP hyperintensity bilaterally	Concern for delayed myelination	Delayed myelination; thin CC	CC lipoma, otherwise normal	Normal	Thin CC; enlarged 4th ventricle; mega cisterna magna; possible brainstem hypoplasia; possible delayed myelination	Profound cortical atrophy with predominant reduction of the white matter. Cerebellum and brainstem normal	Paucity of periventricular WM bilaterally with patchy confluent T2 hyperintensity; thin CC; generalized CO atrophy	Normal (age 14 months); repeat MRI showed diminished cortical volume

Family	Family 10	Family 11	Family 12	Family 13	Family 14	Family 15	Family 16	Family 17	Family 18
Patient	**14**	**15**	**16 (proband)**	**18**	**19**	**20**	**21**	**22 (proband)**	**25**
cDNA (NM_024063.3)	c.190C>T; c.1826C>G	c.85T>G (hom)	c.1199C>T (pat); c.1090-2A>G (mat)	c.2066G>T (pat); c.527G>T (mat)	c.527G>T (pat); c.2006T>G (mat)	c.527G>T; c.1199C>T	(c.213T>G; c.1313T>C) (hom)	c.1091T>A (pat); c.1918C>T (mat)	c.1648_1649insC; c.2066G>T
Protein (NP_076968.2)	p.(Arg64Trp); p.(Ser609*)	p.(Cys29Gly) (hom)	p.(Thr400Ile) (pat); p.(?) (mat)	p.(Gly689Val) (pat); p.(Gly176Val) (mat)	p.(Gly176Val) (pat); p.(Met669Arg) (mat)	p.(Gly176Val); p.(Thr400Ile)	p.(Phe71Leu) (hom); p.(Leu438Pro) (hom)	p.(Val364Glu) (pat); p.(Arg640*) (mat)	p.(Phe550Serfs*16); p.(Gly689Val)
Ancestry	African American	Turkish	European (German)	Italian	European (German)	German	Arabian	German	Italian
Sex	Male	Male	Male	Male	Female	Male	Male	Male	Male
Age at diagnosis	3 months	14 months	5 years	5 years, 6 months	2-3 months	15 years, 6 months	8 weeks	14 years, 9 months	5 months
Hearing impairment	+	+	+	+	+	+	+	+	+
Spasticity	+	-	+	+	+	+	+	+	+
Dystonia/hypotonia	+ / +	+ / -	+ / +	+ / +	+ / +	+ / +	+ / +	+ / +	+ / +
Pattern	Spastic quadriplegia	Quadriparesis	Spastic quadriplegia	Spastic quadriplegia	Spastic quadriplegia	Hypotonic dystonic spastic quadriplegia	Hypotonic dystonic spastic quadriplegia	Hypotonic dystonic spastic quadriplegia	Hypotonic dystonic spastic quadriplegia
Microcephaly (HC)	+	+ (34cm @ birth)	-	+ (acq.)	+ (cong.)	+ (-2 SD @ 1 year)	+ (32cm @ birth)	-	+ (48cm @ 6 years)
DD / ID	+ Profound global DD	+	+ Profound global DD	+ Severe ID	+ Severe ID	+ Profound global DD	+ Profound global DD	+ Profound global DD	+ Profound global DD
Epilepsy	+	+	-	+	-	+	+	+	+
Dysmorphic features	-	-	-	+	-	N/A	+	-	+
Visual impairment	-	-	Mild myopia	N/A	Severe impairment	-	Severe impairment	Severe impairment	Central visual impairment
MRI findings	Diffusely diminished CO volumes; *ex vacuo* dilatation LV; delayed myelination; thin CC	Bilateral peritrigonal hyperintensity	Diminished CO volume; periventricular WM hyperintensity	Ventricle enlargement (slight); WM hyperintensity	Diffuse slightly diminished CO volume; thin CC; lactate peak visualized on MRS (2 years)	Mildly diminished CO volume; mildly atrophic BG, delayed myelination of the CC	Mega cisterna magna; embryonic variant posterior cerebral artery	Normal (11 months); slightly enlarged ventricles (19 months); no progression of ventricular enlargement (25 months); delayed myelination, thin CC (4.5 years)	Brain hypomyelination, diffuse slight brain atrophy

Abbreviations: CO = cortical, CB = cerebellar, CC = corpus callosum, DD = developmental delay, ID = intellectual disability, GP = globus pallidus, WM = white matter, T2 is an MRI signal acquisition parameter, BG = basal ganglia, MRS = magnetic resonance spectroscopy, mat. = maternal, pat. = paternal, + = clinical feature detected, - = clinical feature not observed, N/A = no information provided for clinical feature, SD = standard deviation, P = percentile, acq. = acquired postnatally, cong. = congenital. HC = head circumference

Legends

Figure 1 - Prevalent clinical features of individuals with biallelic variants in *SPATA5L1*.
(A) Bar graph illustrating the prevalence of the most relevant clinical features from the 25 individuals for whom full datasets were available from 18 families with the neurodevelopmental phenotype. Blue: individuals with the clinical feature. Gray: individuals without the clinical feature.
(B) Representative clinical features of individuals carrying biallelic *SPATA5L1* variants with the severe neurodevelopmental phenotype, showing subtle and non-specific dysmorphic features, including downslanting palpebral fissures, bitemporal narrowing and depressed nasal bridge.

Figure 2 - Neuroimaging features in individuals harboring biallelic predicted deleterious variants in *SPATA5L1*.
(A) T1 and T2/FLAIR MRI images were assessed for the presence of periventricular leukomalacia (defined as T2 hyperintensity and/or diminished white matter volume/*ex vacuo* ventriculomegaly evident adjacent to the ventricles); anterior temporal lobe hypoplasia; widened Sylvian fissures (characterized as diminished coverage of the insular cortex), diminished cortical volume; and thin/dysplastic corpus callosum. Ages were: 4 years-old (1-1, 1-2), 21 years-old (7-9), 10 years-old (8-10), 7 years-old (8-11), 6 years-old (8-12), 1 year-old (12-16).
(B) Box-plot of six structural measures quantified from brain MRI volumes, represented as z-scores, in comparison to an age-matched control cohort of typically developing children.

Figure 3 - Distribution and predicted structural effects of *SPATA5L1* variants.
(A) Alternative splicing leads to two distinct *SPATA5L1* transcripts (top), resulting in a full-length and short isoform (bottom). The variant numbering is based on the full-length transcript and isoform, NM_024063.3 and NP_076968.2. Non-coding and coding regions of exons are denoted by flat-edged and pointed-edged rectangles, respectively.
(B) Structural effects of a subset of variants identified in this study were evaluated using a three-dimensional model of SPATA5L1, based on the structure of the homologous ATPase p97 (PDB: 5FTN). The SPATA5L1 structure is shown in ribbon presentation, depicting the N-terminal domain (green) and two conserved ATPase domains (AAA, blue and cyan). ATP ligands are shown in stick presentation. Altered residues are highlighted as balls and labelled. Red and orange balls indicate variants that were classified as deleterious by two or one methods, respectively. Yellow balls indicate variants that were predicted to have little effect on protein structure. The variants depicted in more detail in **Figure S3** are labelled in bold letters.

Figure 4 - *Spata5l1* is expressed in the inner ear and the brain of rodents/humans.
(A) *SPATA5L1* is expressed in all cellular subtypes of the human brain (Brain RNA-Seq database).
(B) Representative immunofluorescent labeling of endogenous SPATA5L1 (green) in rat hippocampal neurons in culture highlight the co-localization of the protein within the nuclei of neurons (MAP2), as well as the nuclei of glial cells (red): microglia (IBA1), astrocytes (GFAP) and oligodendrocytes (Olig2). Signal intensity of SPATA5L1 immunolabeling suggests protein expression is higher in neurons compared to glial cells. Arrows indicate the localization of SPATA5L1 in the nuclei of glial cells. All images are projections of confocal optical section stacks. Scale bar: 25µm.
(C) *Spata5l1* is expressed at low levels in hair cells (inner and outer) as well as supporting cells (Pillar and Deiter cells) in adult mice (adapted from gEAR portal). HC, hair cells.

(D) Representative immunofluorescent labeling of endogenous SPATA5L1 (green) in Sprague Dawley rat organ of Corti at E20. Immunolabeling shows SPATA5L1 is present in the hair and pillar cells of the organ of Corti. DAPI (blue) and Rhodamin Phalloidin (red) were used to counterstain the nuclei and the cytoskeleton, respectively. All images are projections of confocal optical section stacks. Scale bar: 10µm.

Figure 5 - Analysis of gene expression patterns in *SPATA5L1* fibroblasts by RNA-Seq reveals differential expression of DNA replication and mitosis-related genes.

(A) Principal components analysis plot shows a differential clustering of SPATA5L1 samples (n=4) from control samples (n=4).

(B) Transcriptomic heatmap of the top 20 differentially expressed genes (top ten with fold change >1.5, $p<0.05$ and bottom 10 with fold change <0.5, $p<0.05$). Red/yellow colors represent highly expressed genes and blue colors represent under-expressed genes for these 20 genes in the respective case and control samples. The legend corresponds to expression values.

(C) Volcano plot highlighting genes with large fold changes that are either significantly upregulated or downregulated between SPATA5L1 and control samples. Log_2 fold change of normalized counts (red dots indicate genes with $p < 10^{-16}$).

(D) Over-representation analysis (ORA) for down-regulated genes (log_2 fold change <-1, $p < 0.05$) for GO-BP, Gene Ontology Biological Processes.

(E) Over-representation analysis (ORA) for down-regulated genes (log_2 fold change <-1, $p < 0.05$) for GO-CC, Gene Ontology Cellular Components.

(F) Over-representation analysis (ORA) for down-regulated genes (log_2 fold change <-1, $p < 0.05$) for GO-CC, GO-MF, Gene Ontology Molecular Functions.

Supplemental Figures

Figure S1 - Segregation of biallelic *SPATA5L1* variants with disease in 47 affected individuals.
Twenty-eight (26 unrelated) families of diverse origins show segregation of a severe, autosomal recessive neurodevelopmental disorder or non-syndromic hearing loss with homozygous or compound heterozygote *SPATA5L1* variants. Affected individuals in families DY1 to DY11 presented with isolated, non-syndromic mild to severe hearing loss. Affected individuals shaded in black, unaffected individuals shaded in white. Double lines indicate consanguineous unions, triangles indicate miscarriages.

Figure S2 – Facial gestalt representation of individuals harboring biallelic, predicted deleterious variants in *SPATA5L1*.
(A) Using the Face2Gene research application, a composite image based on the frontal facial images of cases with *SPATA5L1*-associated disorder was created (left image). The mask of the healthy controls was generated by an age-, sex- and ethnicity-matched control group (right image). The composite image of individuals with *SPATA5L1* variants did not reveal any common dysmorphic findings. (B) The aggregated binary comparison (left image) demonstrates no significant difference between the two cohorts ($p = 0.194$). Area Under the Curve (AUC) was 0.633 indicating that a facial analysis cannot reliably distinguish between cases and controls.

Figure S3 - Predicted effects of individual SPATA5L1 variants on protein structure.
(A) The wild-type (WT) amino acid A41 sidechain is in close spatial proximity to L39/G40. The variant P41 sidechain causes steric clashes with L39/G40 (red dotted circle) resulting in a destabilization of the N-domain.
(B) WT F360 forms hydrophobic interactions to L329/V340 while variant S360 cannot (magenta dotted circle) due to the shorter and polar sidechain compared to F360.
(C) The sidechain of WT T400 forms a hydrogen bond (green dotted line) to A397. In the I400 variant, no hydrogen bond to A397 can be formed by the nonpolar I400 sidechain.
(D) WT A519 forms hydrophobic interactions with V515, F526, and I560. In the D519 variant, a charged sidechain (see red oxygen atoms within the magenta circle) is placed in a hydrophobic environment, causing domain destabilization.

Figure S4 - Biallelic *SPATA5L1* variants cause no OXPHOS impairment in SPATA5L1 case-derived fibroblasts.
Mitochondrial oxygen consumption rate (OCR) and derived respiratory parameters of control and SPATA5L1 case-derived fibroblasts. (A) Combined traces of OCR (pmol/min/protein) and OCR after addition of specific mitochondrial pathway inhibitors: 1µM oligomycin (oligo), 1.5 µM carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP), and 1µM rotenone (Rot) with 1µM antimycin A (Ant), allowing assessment of basal respiration, ATP-linked respiration, proton leak, maximal respiration, spare respiratory capacity, and non-mitochondrial respiration (B-G). All data are means ± standard deviation of two control and three SPATA5L1 fibroblasts from affected individuals (2-1, 2-2, 7-4), with one, two or three independent experiments, each with three technical replicates. Data in B-G displayed using SuperPlots (PMID: 32346721).

Supplementary Tables

Table S1 - Summary of *SPATA5L1* variants identified in this study in association with a severe neurodevelopmental disorder or an isolated sensorineural hearing loss phenotype.

Table S2 - Biallelic variants in *SPATA5L1* cause a complex neurodevelopmental disorder or isolated, sensorineural hearing loss.

Table S3 - Biallelic *SPATA5L1* variants are associated with progressive, sensorineural hearing loss of varying severity.

Supplementary Videos

Video S1 - Family 5, Individual 7
Case 7 exhibits mild bilateral chorea of the upper limbs as a toddler.

Video S2 - **Family 7, Individual 9**
Case 9 at age 2 years demonstrates global growth failure and hypertonia of the limbs.

Video S3 - Family 8, Individuals 10, 11, 12
Proband (case 10): An examination of case 10 at age 9 years reveals visual impairment and spastic quadriplegia with diminished head control and scissoring of the lower limbs without clear hyperreflexia. Amyotrophy of the extremities is evident, as is pes cavus.
Sibling (case 11): Evaluation at age 7 years shows visual impairment with lateral-beating nystagmus. Spastic quadriplegia is noted with poor head control. Persistent glabellar tap reflex without habituation is noted as a frontal release sign. Amyotrophy of the limbs is evident and marked scoliosis and pes planus are present.
Sibling (case 12): Assessment at age 5 years reveals visual impairment, spastic quadriplegia with poor head control, diffuse hyperreflexia, and equivocally positive Babinski signs. Pes planus of the feet is again seen.

Video S4 - Family 17, Individual 24
Evaluation of this 10 year-old boy revealed dystonic posturing of the upper and lower limbs.

Video S5 - Family 18, Individual 25
Dystonic posturing of the hand and mouth is evident while the individual is sitting.

Video S6 - Family 18, Individual 25
One of the affected individual's seizures in captured, beginning as a gelastic seizure with stereotyped laughter, followed by behavioral arrest with stereotyped blinking evolving into rhythmic saccadic eye movements, and concluding with post-ictal hyporesponsiveness.

Data and Code Availability

Variants identified in this study have been submitted to ClinVar (accession numbers pending).

Original data is available from the authors upon reasonable request.

Web Resources

Brain RNA-Seq, http://www.brainrnaseq.org/

BrainSpan Atlas of the Developing Human Brain, https://www.brainspan.org/

CADD, https://cadd.gs.washington.edu/

DIOPT https://www.flyrnai.org/cgi-bin/DRSC_prot_align.pl

gEAR Portal, https://umgear.org/

gnomAD, https://gnomad.broadinstitute.org/

Gene expression in spiral ganglion neuron subtypes, https://lallemendlabcochlea.shinyapps.io/shinyapp-sgns_diversity/

MARRVEL, http://marrvel.org/

MutationTaster, http://mutationtaster.org/

OMIM, http://omim.org/

PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/

Provean, http://provean.jcvi.org

SIFT, http://sift.jcvi.org/

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