GGPS1-associated muscular dystrophy with and without hearing loss

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Abstract
Ultra-rare biallelic pathogenic variants in geranylgeranyl diphosphate synthase 1 (GGPS1) have recently been associated with muscular dystrophy/hearing loss/ovarian insufficiency syndrome. Here, we describe 11 affected individuals from four unpublished families with ultra-rare missense variants in GGPS1 and provide follow-up details from a previously reported family. Our cohort replicated most of the previously described clinical features of GGPS1 deficiency; however, hearing loss was present in only 46% of the individuals. This report consolidates the disease-causing role of biallelic variants in GGPS1 and demonstrates that hearing loss and ovarian insufficiency might be a variable feature of the GGPS1-associated muscular dystrophy.
GGPS1-Associated Muscular Dystrophy

R. Kaiyrzhanov et al.

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Introduction

Geranylgeranyl diphosphate synthase 1 (GGPS1) is a member of the prenyltransferase family and encodes a protein with geranylgeranyl diphosphate (GGPP) synthase activity. The enzyme catalyzes the synthesis of GGPP from farnesyl diphosphate and isopentenyl diphosphate. GGPP is an important molecule responsible for the C20-prenylation of proteins. Prenylation provides proteins with a hydrophobic C terminus, the consequence of which is a greatly increased capacity to interact with cellular membranes, which have a high concentration of signaling molecules.1,2 Prenylation is particularly important for small guanosine triphosphatases (GTPases) such as the Rab family, which have crucial roles in vesicular trafficking in mammalian cells.2 The roles of prenylated proteins in cells are well conserved across species.1

While monoallelic variants in GGPS1 have been identified as a risk factor for atypical femoral bone fractures in females exposed to bisphosphonates, ultra-rare biallelic pathogenic missense variants in GGPS1 have recently been associated with muscular dystrophy/hearing loss/ovarian insufficiency syndrome.2,3 Here, we describe 11 affected individuals from four previously unreported British, Iranian, Egyptian, and Brazilian families harboring ultra-rare missense variants in GGPS1 and provide follow-up clinical data from the previously reported two Pakistani cases by Tucker et al. (2020).3
Subjects and Methods

Two of the novel families reported here were recruited from Iran and Egypt as part of the SYNaPS project at the Institute of Neurology, University College London (UCL). The third and fourth novel families were identified through a collaboration between Great Ormond Street Institute of Child Health (UCL) and the Center for Mendelian Genomics, Broad Institute, USA and School of Medicine of Universidade de Sao Paulo, respectively. The corresponding author was contacted from Tucker et al. (2020)³ to obtain follow-up details from the reported 2 persons with homozygous GGPS1 variants. To identify the genetic cause of the disease in the affected individuals, exome sequencing on DNA extracted from probands’ leukocytes and variant filtering were performed as described (Table 1 for genetic methods). To assess Ggps1 RNA expression in the mouse cochlear epithelium at various stages (embryonic (E) 14, and postnatal (P) 1 and P7), publicly available single cell RNA-seq data were visualized using the gene expression analysis resource (gEAR).⁷ The publicly available Mouse Cell Atlas, generated as previously described,⁸ includes single-cell RNA sequencing data used to visualize global expression of Ggps1 in the female E14.5 gonad (version 2.0 with >520,000 cells from over 10 mouse tissues grouped into 95 major clusters) and neonatal skeletal muscle excised from the leg (version 1.0 with >400,000 cells from over 10 mouse tissues grouped into 104 major clusters). Clusters showing expression (red) are marked in black numbers representing the cluster code defined on the Mouse Cell Atlas.⁸ The study was approved by the ethics Institutional Review Board of UCL and additional local ethics committees of the participating centres. Informed consent was obtained from all families.

Results

The affected individuals are six males and seven females, all of whom were born at full term, with all but two (P10 and P11) conceived from consanguineous unions (Fig. 1A). Eleven affected individuals are alive with a mean current age of 10 ± 6 years (range 4–24), and two individuals died at the ages of 11 months and 30 years old due to respiratory insufficiency and choking, respectively. Antenatal history was unremarkable in all but 3/13 individuals accompanied with stiffness (6/12), calf hypertrophy (4/12), hyporeflexia/areflexia (12/12), and myalgia (6/10). Gait was waddling in 4/11 and broad-based in 2/11 persons (Tables 1 and 2). Electromyography findings were compatible with myopathy in 8/13 and elevated creatinine kinase (CK) (1594-27000 U/L) was registered in 10/10 individuals. Lower limb muscle magnetic resonance imaging in Family 3 (P10), performed at the age of 8 months old, showed generalized muscle atrophy without fatty infiltration (Fig. 1E). Muscle biopsy from the quadriceps at age 8 months old in P10 showed type 1 fiber predominance and central nucleation with Z line streaming and minicores evident on electron microscopy (Fig. 1D, IV). Muscle biopsy from the biceps in Family 4 (P11) showed fatty infiltration with mitochondrial changes (Fig. 1D, V–VII), whereas in Family 5 it showed a dystrophic pattern.

On exome sequencing, an ultra-rare homozygous missense variant in exon 4 of GGPS1 c.269A > G, p.(Asn90Ser) (NM_004837.4) residing within a 9.77 Mb region of homozygosity was identified in Family 1 (Fig. 1A–C, Figure S1). The p.(Asn90Ser) variant is located in a highly
Table 1. Clinical features of the affected individuals with biallelic GGPS1 variants.

| Person | Family 1 (P1–P3) | Family 2 (P4–P9) | Family 3 (P10) | Family 4 (P11) | Family 5 (from Tucker et al., 2020) P12, P13 | Foley et al. 2020 (11 persons/6 families) | Tucker et al. 2020 (4 persons/2 families) |
|--------|------------------|------------------|----------------|----------------|---------------------------------------------|--------------------------------------------|-------------------------------------------|
| Variant details | Variant type | Homozygous | Homozygous | Compound heterozygous | Homozygous | Homozygous | Homozygous | Homozygous |
| | Variants | c.269A > G | c.439A > G | c.196A > C | c.770 T > G | c.269A > G | c.269A > G | c.782G > A, c.269A > G |
| | at the cDNA level | (NM_004837.4) | | and | and | | | |
| | Variant at the protein level | p.(Asn90Ser) | p.(Met147Val) | p.(Ile66Leu) | p.(Phi257Cys) | p.(Asn90Ser) | p.(Asn90Ser) | |
| Methods | Makranyanasis et al. | Makranyanasis et al. | Pais et al. 2022, Natera-de Benito et al. | Foley et al. 2020 | Tucker et al. 2020 | Foley et al. 2020 | Tucker et al. 2020 |
| Maximum allele frequency in variant databases | | | | | | | |
| | ACMG | <0.000001 | Absent | Absent | Absent | <0.000001 | <0.0001 to <0.000001 | <0.00001 |
| | Epidemiology | Sex | M, F | F | M, F | M, F | M - F | M - 1 |
| | Consanguinity | 3+ | 6+ | – | 8 y.o. | 12 y | 2 y.o., 1.2 y.o. | 1.5 y, 1.5 y, 3 y.o. |
| | Current age | 11 y.o. (P1), 11 m.o. (P2) | 23.5 y.o., 4 y. 8 m.o. | 5 y 7 m.o., 4 y.o. | 5 y.o., 30 y.o. | 11 y.o., 18 y.o. | 9 y.o., 11 y.o. | 11 m.o. (P1) |
| | Medical history | Age of death | 30 y.o. (P9) | 6 y.o. | 4 y.o. | 4 y.o. | 13 y.o. (P12), 13 y.o. | 11 y.o. (P13) |
| | Type of progression | Slow | Slow | Moderate** (P12), Slow** (P13) | NA | E (P12), E (P13) | 8+ | 3+, 1- |
| | Failure to thrive | 2+, 1NA(P2) | 6- | – | – | – | – | – |
| | Sensoryneural hearing loss | 3+ | 6- | + | + | + | + | + |
| | Progressive muscle weakness, onset age | 15 y.o (P3) | 4 y.o., 2 y.o., 1.2 y.o. | 15 y.o., 2 y.o., 3 y.o. | 18 m | 11 y.o.** (P12), ** (P13) | 10+, 1- | Mild (3), Severe (1) |
| | Joint contractures | 2+, 1NA (P2) | 2+, 4- | – | + | + | + | 4+ |
| | Respiratory insufficiency, age of onset | 13 m.o. (P1) | 20 y.o., 20 y.o., 20 y.o. | 20 y.o., 20 y.o., 20 y.o. | 10 y.o. | 13 y.o. (P12), – (P13) | 8+, 1NA | 1+ |
| | Non-invasive ventilation | 3+ | 1+, 5- | + | – | + | + | 2+ |
| | POI | 2 y.o. | 2 y.o. | 11 y.o. (P12), – (P13) | 4+ | 3+, 3 uncertain to age | 2+ |
| | Cardiac involvement | 2+, 1NA(P2) | 30 y.o. | 11 y.o. | 11 y.o. (P12), – (P13) | 4+ | 3+, 3 uncertain to age | 2+ |
| | Loss of ambulation (age) | 7 y.o. (P1, P3), 11 y.o. | 2+, 18 y.o. and 17 y.o. | 9 y.o. | 9 y.o. | 11 y.o. (P12), – (P13) | 5+, 11 y.o, 13 y.o, 15,12,11 | NA |

(Continued)
| Person | Family 1 | Family 2 | Family 3 | Family 4 | Family 5 (from Tucker et al., 2020) | Foye et al. 2020 (11 persons/6 families) |
|--------|----------|----------|----------|----------|-----------------------------------|------------------------------------------|
| Development | Age of sitting | 1 y.o. (P1), NA (P1, P3) | 1 y.o. (P4, P5) | 11 m.o. (P6-P8, 9 m.o. (P9) | 8 m.o. (P12), 9 m.o. (P13) | NA |
| Age of walking | 2.5 y.o. (P1, P3), (NA) | 2.2 y.o. 1.7 y.o. 2 y.o. | 1.4 y.o. 2.1 y.o. | 18 m | 24 m.o. **, 18 m.o. ** | 18 m.o. |
| Age of first words | 8 m.o. (P1, P3), 9 m.o. (P2) | 18 m | Normal (P12), Few words* (P13) | NA | NA |
| Physical examination | Age of sitting | 11 y.o. (P1), 9 y.o. (P3), NA (P2) | 23.5 y.o., 4 y.o., 5.5 y.o, 5 y.o. | 11 m.o 8 m NA (P12), 9 m.o. (P13) | 7 y.o | 12 y \( \text{NA} \) |
| Neurological examination | Progressive scoliosis | —(P1), NA(P2), —(P3) | 3, 3, — | + | +** | NA (P13) |
| Short stature | 2+ | NA(P2) | 4, 2— | (25th centile for age) | — | — | — | — | +** | (2) |
| Hypotonia | 3− | NA(P2) | 6+ | — | + | + | + | (2) | + | 2+ |
| Muscle weakness | Generalized (2), Proximal (4) | 4 limb Proximal/Axial | Axial and proximal | LL > UL** | (2) | NA |
| Muscle hypertrophy | 2−, NA(P2) | 4−, 2− | 6− | — | — | NA | —** | NA (P13) | —** | (2) |
| Peripheral neuropathy | 001, P3, NA(P2) | 0 (2), (4) | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ |
| Muscle atrophy | 2+ | NA(P2) | 6+ | + | + | + | + | (2) | + | 2+ |
| Myalgia | 2−, NA(P2) | 6+ | — | — | NA | NA | NA | NA | NA | NA |
| Pattern of muscular weakness | Broad-based (P1, P3), NA(P2) | Non-ambulant (2), Waddling gait (3), | Normal gait (1) | Unsteady | Non-ambulant | NA (P12), Waddling gait* | Non-ambulant (5), NA (6) | NA |
| Gait | Broad-based (P1, P3), NA(P2) | Non-ambulant (2), Waddling gait (3), | Normal gait (1) | Unsteady | Non-ambulant | NA (P12), Waddling gait* | Non-ambulant (5), NA (6) | NA |
| Details on Hearing loss | The age of onset | 11 m.o.(P1), 6 m.o. (P2, P3) | 6 Intact | Normal newborn hearing screen. Hearing loss detected at 4 years | Childhood | Intact (P12), From birth (P13) | From neonatal to childhood | 3+, Childhood |
| The type of hearing loss | SNHL | 6− | SNHL | SNHL | —(P12), SNHL (P13) | SNHL | SNHL (3) |
| Laterality | Bilateral (P1, P3), NA(P2) | 6− | Bilateral | Bilateral | Bilateral (P13) | Bilateral | Bilateral (3) |
| Degree | Severe (P1, P3), NA(P2) | 6− | Severe | Severe | 80 dB **(P13) | NA | 9+ | NA |
| Elevated CK (age) | 3 NA | 6− | 6+ | <7 m.o. 1594 U/L, 4 y.o 5400 U/L | 19 m | 80 dB **(P13) | NA | 9+ | NA |
| FSH | 3 NA | na (5), NA (1) | NA | NA | 2 NA | 88.2 IU/L, 50.3 IU/L, 53.2 IU/L | 60 IU/L, 35.8 IU/L |
| EMG | 3 NA | Normal | Myopathic changes (6) | High | 2 NA | 88.2 IU/L, 50.3 IU/L, 53.2 IU/L | 60 IU/L, 35.8 IU/L |
| Muscle biopsy/ histochemistry | 3 NA | 6 NA | (8 m.o) Type 1 fiber predominance, central nuclei, Z line streaming, mini-cores. | Fatty infiltration and mitochondrial changes | 2 NA | Normal** | 2 Dystrophic pattern ** | 9+, dystrophic, with evidence of degeneration and regeneration and internalized nuclei. | 3+, fatty infiltration consistent with an underlying muscular dystrophy. |
| Muscle MRI | 3 NA | 6 NA | (8 m.o) generalized muscle atrophy without fatty infiltration. | NA | 2 NA | NA | NA |

*P, person; NA, not available; na, not applicable; y.o., years old; m.o., months old; SNHL, sensorineural hearing loss; LL, lower limbs; UL, upper limbs; POI, primary ovarian insufficiency; FSG, follicle-stimulating hormone; EMG, electromyography; MRI, magnetic resonance tomography; m, male; f, female; CK, creatine kinase; DTRs, deep tendon reflexes; dB, decibel.

**Database checked include: GnomAD v3, gnomAD v2.1.1, TopMED Bravo, UKBiobank, Iranome, GME Variome, In-house Database. The total number of alleles considered was ~1,314,000.

**New information that was not previously reported by Tucker et al. (2020).
conserved GGPS1 protein region. The variant is absent in gnomAD and a number of large publicly available databases apart from 2 heterozygous alleles from the Centogene and TOPMed databases, and is predicted to be damaging by multiple in silico tools. (Table S2). The variant segregated with the phenotype within the family (Fig. 1A). Both Family 1 and Family 5 from Tucker et al. (2020) carried the same recurrent homozygous GGPS1 c.269A > G, p.(Asn90Ser) variant. An ultra-rare homozygous missense variant in exon 4 of GGPS1 c.439A > G, p.(Met147Val) (NM_004837.4) residing within a 9 Mb region of homozygosity was identified in Family 2 (Fig S1). This variant is located in a highly conserved GGPS1 protein region with predicted-damaging scores on various in silico tools (Table S2). The variant segregated with the phenotype within the family (Fig. 1A).

Based on the extremely rare frequency of the identified GGPS1 variants (PS4, PM2), co-segregation in two unrelated families with multiple affected individuals for the GGPS1 c.269A > G, p.(Asn90Ser) variant (PP1 moderate) and segregation in multiple affected family members from different branches for the GGPS1 c.439A > G, p.(Met147-Val) variant (PP1 moderate), as well as predicted-deleterious effect from multiple in silico tools, we classified both variants from Family 1 and 2 as likely pathogenic according to the American College of Medical Genetics and Genomics (ACMG) guidelines for the interpretation of sequence variants.9

Two ultra-rare missense variants in exon 4 of GGPS1 were identified, in trans, in the proband of Family 3 (P10), c.196A > C p.(Ile66Leu) and c.545T > C p.(Leu182Pro) (Fig. 1). Both variants are absent from publicly available databases and are conserved across species (Table S2). The maternally inherited variant, c.196A > C p.(Ile66Leu), affects the alpha 3 helix of GGPPS whilst the paternally inherited variant affects the alpha 7 helix of the protein. Both implicated residues lie within the enzymes central catalytic barrel. The c.196. A > C p.(Ile66Leu) variant lies between two magnesium ion cofactor ligand bindings sites and the c.545T > C p.(Leu182Pro) variant lies near to the dimethylallyl diphosphate substrate binding sites within the GGPPS protein (Fig. S2). The individual’s phenotype was felt to be highly specific for GGPS1 related muscular dystrophy (Table 1). Owing to the rarity of the variants, and lack of further segregation and functional study data, these variants are classified as of uncertain significance according to ACMG guidelines. However, given the affected person’s phenotype and proximity of the affected residues to key domains within GGPPS we feel they can be considered disease causing in P10.

An ultra-rare missense variant c.770T > G; p.(Phe257Cys) was identified in Family 4. The variant is absent from all publicly available databases, is conserved across species, and is predicted damaging from a variety of in silico tools (Table S2). The variant segregated with the phenotype within the family (Fig. 1A).

Expression patterns of Ggps1 in the mouse inner ear, skeletal muscle, and female gonad at different embryonic and postnatal stages were studied using single-cell RNA sequencing. Single-cell RNA sequencing of the cochlear epithelium in the E14, P1, and P7 mouse shows Ggps1 with a sustained, diffuse expression at these timepoints (Fig. S3). Global view of Ggps1 expression in the E14.5 female gonad showed clusters most densely in the germ cells and embryonic gonad cells (Fig. S4), whereas neonatal skeletal muscle showed several clusters reflecting comparatively lower expression, for example, in clusters representing stromal cells and chondrocytes (Fig. S5).
Discussion

Protein prenylation is an important downstream part of the mevalonate pathway, which is a pivotal process for the synthesis of specialized lipids that play a crucial role in cellular processes at all levels. The mevalonate pathway has recently been suggested to be a novel pathway for muscular dystrophy, hearing loss, and infertility. Evidence for this comes from the description of 11 individuals from 6 independent families presenting with early...
Table 2. Frequency of the clinical features in the GGPS1 cohorts.

| Features                        | Current report | All reported cases |
|---------------------------------|----------------|--------------------|
| Decreased foetal movements      | 3/13           | 4/14               |
| Birth OFC ≤25 percentile        | 6/10           | 6/10               |
| Manifestation with delayed milestones | 10/13         | 12/15              |
| Slow progression                | 12/13          | 25/26              |
| Failure to thrive               | 3/12           | 11/20              |
| Hearing loss                    | 6/13           | 18/26              |
| Generalized or proximal         | 12/13          | 25/26              |
| Muscle weakness                 |                |                    |
| Respiratory insufficiency       | 8/13           | 16/23              |
| Non-invasive ventilation        | 6/13           | 10/17              |
| Muscular atrophy                | 12/12          | 12/12              |
| Myalgia                         | 6/10           | 6/12               |
| Muscle stiffness                | 6/12           | 6/12               |
| Reduced or absent tendon reflexes| 12/12          | 12/12              |
| Calf hypertrophy                | 4/12           | 4/12               |
| Joint contractures              | 7/12           | 11/23              |
| Progressive scoliosis           | 7/11           | 15/21              |
| Waddling gait                   | 4/11           | 4/11               |
| Broad-based gait                | 2/11           | 2/11               |
| Loss of ambulation              | 6/12           | 11/25              |
| Elevated creatine kinase        | 10/10          | 19/19              |
| Mortality                       | 2/13           | 2/26               |

onset muscular dystrophy combined with congenital sensorineural hearing loss and primary ovarian insufficiency in females. All but one affected individual in the report by Foley et al. (2020)² had congenital sensorineural hearing loss, and all 3 postpubertal females had a laboratory-confirmed primary ovarian failure. Andrological examinations on the postpubertal male individuals in the Foley et al. (2020)² report were not available, but none of the 5 male adult individuals has had children. Although a combination of sensorineural hearing loss and muscular dystrophy or myopathy has been described in early-onset facioscapulohumeral dystrophy and Vici syndrome, and an association of early-onset sensorineural hearing loss and primary ovarian insufficiency without muscular dystrophy have been recognized in Perrault syndrome, a constellation of all these three features had not been previously recognized prior to the Foley et al. (2020)² study.² Disease course, in particular proximal muscle weakness, was progressive in the series by Foley et al. (2020)² with some interfamilial variability in the rates of deterioration. When comparing the description and course of muscle clinical signs in these previously described affected individuals with expression in the neonatal mouse muscle, there are hints of relatively low Ggps1 expression at this stage compared to the female gonad from E14.5 and we cannot exclude that expression in the muscle increases throughout later development. Muscle histopathology findings in the cohort described by Foley et al. (2020)² were dystrophic with variable evidence of regenerating fibers, internal nucleation, and core-like regions.

Biallelic pathogenic missense GGPS1 variants identified in Foley et al. (2020)² have been shown to moderately impair the enzymatic activity of geranylgeranyl diphosphate synthase (GGPPS) (only for 50%) in vitro, and the possible effects of these missense variants on GGPPS function have been speculated to be subtle, putatively including the impairment of the dynamic subcellular localization of the enzyme for cell-type-specific processes, particularly in muscle, ovary, and the inner ear. It had been suggested that the localization of the missense GGPS1 variants residing outside of the catalytic core of the enzyme could account for the very specific and recognizable clinical phenotype. However, the variants reported in P10 (Family 3) co-locate within the catalytic core of GGPPS and implicate impaired substrate and co-factor binding as potential pathogenic mechanisms, resulting in a phenotype consistent with other reported individuals.

Family 2 in our cohort presents hitherto the largest family (6 affected) with biallelic GGPS1 variants reported. Affected individuals from our report replicated most of the previously described clinical features associated with GGPS1 defects. This includes muscular dystrophy with progressive proximal muscle weakness and episodes of variably elevated CK, scoliosis, and respiratory insufficiency. Muscle histopathology findings were also variable but largely consistent with those previously reported by Foley et al. (2020)² including dystrophic changes, regenerating fibers, core-like lesions, internal nucleation, and mitochondrial changes. The lack of dystrophic histopathology and fat infiltration observed in the muscle biopsy and lower limb MRI of Family 3 (P10) may reflect the early age (8 months) at which these investigations were performed, compared to other reported persons. The families described in our report display inter- and intra-familial variability with respect to hearing loss, which was detected only in all affected siblings from Family 1, the probands from Families 3 and 4, and one affected sibling from Family 5. This could suggest that hearing loss might be a variable feature of GGPS1-associated muscular dystrophy and is an interesting observation in light of its diffuse expression in the embryonic and postnatal mouse inner ear. Since most of the subjects with unimpaired hearing in the current report were from Family 2, carrying the GGPS1 c.439A > G, p.(Met147Val) variant, we cannot exclude the possibility of a variant-specific effect. Additionally, our data shows that primary ovarian insufficiency might also be a variable feature of GGPS1 deficiency, despite strong Ggps1 expression in relevant cells in the embryonic mouse female gonads. Although it is difficult to determine the exact molecular cause of the observed clinical variability, we can
hypothesize a possible link with localisation of the GGPS1 variants within the gene.

One possible limitation of the present study is the absence of in vitro enzyme activity measurements. However, taking into account the findings from the study by Foley et al. (2020),2 one could expect that the impact of the missense variants from our study on GGPPS function could be subtle as well, with an insignificant reduction of the enzymatic activity of GGPPS. All missense GGPS1 variants c.269A > G, p.(Asn90Ser), c.439A > G, p.(Met147Val), c.196A > C p.(Ile66Leu), c.545 T > C p.(Leu182Pro) and c.770 T > G p(Phe257Cys) reported in the current study are ultra-rare across several large genetic databases including 1,314,000 alleles (Table S2) and uniformly predicted to be damaging in various in silico prediction tools supporting their disease-causing roles.

Collectively, this report consolidates the disease-causing role of biallelic variants in GGPS1, demonstrates that hearing loss and ovarian insufficiency might be variable features of the GGPS1-associated muscular dystrophy and implicates impaired substrate and co-factor binding as potential pathogenic mechanisms in this novel form of congenital muscular dystrophy.

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Conflict of Interest

None of the authors has any conflict of interest to disclose.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Supporting Information

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

Figure S1 Region of homozygosity around the variant of interest indicated by a red box.11
Figure S2 3D protein modeling of the GGPS1 variants in Family 3 (P10).
**Figure S3** Expression of *Ggps1* in the mouse cochlea through single-cell RNA-sequencing data.

**Figure S4** Expression of *Ggps1* in the mouse embryonic (E14.5) gonad through single-cell RNA-sequencing data.

**Figure S5** Expression of *Ggps1* in mouse neonatal leg muscle through single-cell RNA-sequencing data.

**Table S1** Extended clinical features of the cases with biallelic *GGPS1* variants.

**Table S2** Population frequencies and in silico pathogenicity predictions for *GGPS1* variants reported in this study.

**Video S1** Shows P1 with a waddling gait, lower limb weakness, and inverted feet.

**Video S2** Shows P4 who is unable to stand unassisted and walk due to the lower limb weakness.

**Video S3** Shows P5 with a suggestion of Gower’s sign and waddling gait.

**Video S4** shows P6 with proximal lower limb weakness.

**Video S5** shows P7 with positive Gower’s sign.

**Video S6** shows P8 with positive Gower’s sign.