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*PRPS1* loss-of-function variants, from isolated hearing loss to severe congenital encephalopathy:  
new cases and literature review.

Oriane Mercati<sup>1</sup>, Marie-Thérèse Abi Warde<sup>2</sup>, Geneviève Lina-Granade<sup>3</sup>, Marlène Rio<sup>4</sup>, Solveig Heide<sup>5</sup>, Pascale de Lonlay<sup>6</sup>, Irène Ceballos-Picot<sup>7</sup>, Matthieu P. Robert<sup>8</sup>, Vincent Couloigner<sup>9</sup>, Jacques Beltrand<sup>10</sup>, Nathalie Boddaert<sup>11</sup>, Diana Rodriguez<sup>12</sup>, Elisa Rubinato<sup>1,4</sup>, Jean-Michel Lapierre<sup>13</sup>, Christophe Merlette<sup>14</sup>, Sylvia Sanquer<sup>14</sup>, Agnès Rötig<sup>15</sup>, Holger Prokisch<sup>16</sup>, Stanislas Lyonnet<sup>4,17</sup>, Natalie Loundon<sup>9</sup>, Josseline Kaplan<sup>18</sup>, Jean-Paul Bonnefont<sup>4,18</sup>, Arnold Munnich<sup>4,19</sup>, Claude Besmond<sup>19</sup>, Laurence Jonard<sup>1,4</sup>, Sandrine Marlin<sup>1,4,17</sup>.

We declare no conflict of interest

1. Centre de Référence des Surdités Génétiques, Institut Imagine, Hôpital Necker-Enfants Malades, AP-HP.Centre, Paris, France.
2. Département de NeuroPédiatrie, CHU, Strasbourg, France.
3. Service d'Otorhinolaryngologie et chirurgie cervico-faciale Pédiatrique, CHU, Hospices civils de Lyon, France.
4. Fédération de Génétique, Hôpital Necker-Enfants Malades, AP-HP.Centre, Paris, France.
5. Département de Génétique, Groupe Hospitalier Pitié-Salpêtrière, AP-HP, Paris, France.
6. Centre Référence des Maladies héréditaires du Métabolisme, Hôpital Necker-Enfants Malades, AP-HP.Centre, Paris, France.
7. Laboratoire de Biochimie Métabolomique et Protéomique, Hôpital Necker-Enfants Malades, AP-HP.Centre, Paris, France.
8. Service d'Ophthalmologie pédiatrique, Hôpital Necker-Enfants Malades, AP-HP.Centre, Paris, France.
9. Service d'Otorhinolaryngologie et chirurgie cervico-faciale Pédiatrique, Hôpital Necker-Enfants Malades, AP-HP.Centre, Paris, France.
10. Service Endocrinologie, Gynécologie et Diabétologie Pédiatrique, Hôpital Necker-Enfants Malades, AP-HP.Centre, Paris, France.
11. Département de Radiologie pédiatrique, Hôpital Necker-Enfants-Malades, AP-HP.Centre; UMR 1163, Institut Imagine, Université Paris Descartes, Paris, France.
12. Service de NeuroPédiatrie, Hôpital Trousseau, AP-HP, Paris, France.
13. Service de Biochimie Métabolomique et Protéomique, AP-HP.Centre, Hôpital Universitaire Necker-Enfants malades, Paris, France
14. Plateforme de spectrométrie de masse, AP-HP.Centre, Hôpital Universitaire Necker-Enfants malades, Paris, France
15. Laboratoire de Génétique des maladies Mitochondriales, INSERM UMR1163, Institute Imagine, Université Paris Descartes, Paris, France.
16. Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany.
17. Laboratoire d'Embryologie et de Génétique des Malformations Congénitales, INSERM UMR 1163, Institut Imagine, Université Paris Descartes, Paris, France.

18. Laboratoire de Génétique Ophthalmologique INSERM UMR1163, Institut Imagine, Université Paris Descartes, Paris, France.

19. Université Paris Descartes, Institut Imagine, Paris, France.

## **Abstract**

We describe two sporadic and two familial cases with loss-of-function variants in *PRPS1*, which is located on the X chromosome and encodes phosphoribosyl pyrophosphate synthetase 1 (PRS-1). We illustrate the clinical variability associated with decreased PRS-1 activity, ranging from mild isolated hearing loss to severe encephalopathy. One of the variants we identified has already been reported with a phenotype similar to our patient's, whereas the other three were unknown. The clinical and biochemical information we provide will hopefully contribute to gain insight into the correlation between genotype and phenotype of this rare condition, both in females and in males. Moreover, our observation of a new family in which hemizygous males display hearing loss without any neurological or ophthalmological symptoms prompts us to suggest analysing *PRPS1* in cases of isolated hearing loss. Eventually, *PRPS1* variants should be considered as a differential diagnosis of mitochondrial disorders.

## Keywords

*PRPS1*, hearing loss, deafness, Arts syndrome, intellectual deficiency

## Introduction

*PRPS1* (MIM\*311850) is located in Xq22.3 and encodes the enzyme phosphoribosyl pyrophosphate synthetase (PRS-1) which catalyses the synthesis of phosphoribosyl pyrophosphate (PRPP), an essential precursor of nucleotide synthesis, from ribose-5-phosphate (R5P) and ATP. PRS-1 is active as a hexamer composed of three homodimers arranged in a propeller-like shape. Each homodimer has an active site (with ATP- and R5P-binding sites) and two allosteric sites, involved in feedback inhibition of the enzyme by nucleotide products that result from PRPP utilization <sup>1</sup>.

The first variants of *PRPS1* were identified in male patients who presented superactivity of PRS-1 (MIM #300661), gout and/or uric acid lithiasis either isolated or associated with sensorineural hearing loss (HL) or neurological involvement <sup>2, 3</sup>. Variants responsible for a decrease in PRS-1 activity were then described in several syndromes associating HL and neurological symptoms: Arts syndrome (MIM#301835), a severe condition combining congenital profound bilateral sensorineural HL with delayed motor development, intellectual deficiency, peripheral neuropathy, optic atrophy and recurrent infections, frequently fatal during childhood <sup>1, 4, 5</sup>; Charcot-Marie-Tooth type 5 disease (CMTX5, MIM#311070) which associates prelingual sensorineural HL and peripheral neuropathy, with or without optic atrophy <sup>6, 7, 8, 9</sup>; more recently, a previously unreported phenotype was described and considered as a new syndrome associating prenatal growth retardation, intellectual deficiency, spastic tetraparesis, conductive and sensorineural HL, retinal dystrophy and diabetes insipidus <sup>10</sup>. Eventually, *PRPS1* was the second gene to be identified as a cause of isolated X-linked sensorineural HL, after *POU3F4* <sup>11</sup>.

However, given the atypical presentations and the significant overlap between phenotypes associated with *PRPS1* loss-of-function, it has been suggested to consider these conditions as a clinical continuum rather than separate entities<sup>1,9,12</sup>. HL is the most frequent symptom: in twenty-two families out of the twenty-seven described so far, at least one subject had childhood-onset sensorineural HL, in three families it was reported with later onset, and in the last two it was not reported at all.

We identified *PRPS1* variants in four families presenting with phenotypes ranging from a non-syndromic HL to severe congenital encephalopathy. A mitochondrial disease had first been suspected in the three probands who had syndromic HL, two of which showed respiratory chain complex I deficiency.

Here we discuss genotype-phenotype correlation, in our cases and those previously described, with particular focus on auditory symptoms. In addition we suggest to extend the *PRPS1* analysis to all cases associating HL and neuropathy in males, HL and retinopathy or optic atrophy in females, and also to all non-syndromic sensorineural HL.

## **Case reports, biochemical and molecular analyses**

### **Family 1**

Proband 1 is the first child of unrelated parents of French origin, who previously had an early miscarriage (10 weeks of gestation (GW)). The mother was born from a triplet pregnancy (one of her sisters has Little's disease and epilepsy without cognitive disability). She has a short stature (149 cm) and suffers from mild to moderate sensorineural HL diagnosed at 8. The father reports having had epilepsy in infancy (Fig.1).

Intrauterine growth restriction (IUGR) was diagnosed at 17 GW, with mainly short long bones. Etiological investigations found normal 46,XY male karyotype, 4p and *SHOX* hybridizations, normal electrophoresis of cholinesterase. A bone scan at 29 GW showed delayed mineralization. Skeletal radiographies of the mother revealed short femoral necks and a narrow spinal canal.

The proband was born at 33 GW by emergency C-section because of stagnation of intra-uterine growth and abnormal foetal cardiac rhythm. All birth parameters were below the 1<sup>st</sup> percentile: weight 860 g, length 33.5 cm, head circumference (HC) 25 cm. Apgar scores were 6, 7 and 8 at 1, 5 and 10 min, respectively. Pathology examination revealed an immature and hypotrophic placenta with numerous vascular lesions that was in favour of a vascular origin of the IUGR.

The patient suffered from respiratory distress syndrome and required endotracheal intubation for 38 days. At 10 days, he had ulceronecrotic enterocolitis, followed by coagulase negative staphylococcal septicaemia. He was fed by nasogastric tube for one year.

Several transfontanellar ultrasonographies and electroencephalographies (EEG) performed in the first 2 months were normal. Brain MRI at 3 months was normal. Fundoscopic examination was initially normal, but at 4 months patches of retinal atrophy were found. Neurological presentation included profound developmental delay since birth, with truncal hypotonia and spastic tetraparesis. The patient presented a developmental delay and a significant failure to thrive. At the age of one, he was diagnosed with bilateral moderate sensorineural HL. He has had no visual contact since he was born. Ophthalmological examination at 22 months showed no light perception, frequent digito-ocular sign and no pupillary light reflex. Fundoscopic examination revealed severe macular atrophy, peripheral clumping, pale optic discs and attenuated vessels; ISCEV (International Society for Clinical Electrophysiology of Vision) full-

field electroretinogram (ERG) with scleral electrodes showed no detectable cone and rod responses, confirming the diagnosis of a syndromic Leber congenital amaurosis (severe congenital retinal dystrophy).

At the age of 21 months, he presented with polyuria and polydipsia and he was diagnosed with diabetes insipidus that responded well to desmopressin. There was no history of recurrent infections or seizures.

Upon clinical examination at 4 years 9 months, his weight was at -4 SD, his height at -5 SD and his head circumference at -5 SD. He had a pyramidal syndrome, predominantly involving upper limbs. He still had no head control.

Brain MRI at 7 showed enlarged fourth ventricle, *cisterna magna* and *cisterna pontis*, thin optic nerves, and normal spectroscopic profile (Table 1).

### *Etiological investigations*

Extensive metabolic analyses were normal, notably mitochondrial respiratory chain investigations were normal in muscle and no mitochondrial DNA anomaly was identified in blood sample. Wolfram syndrome was suspected, but no mutation could be found in *WFS1* gene. Subsequently, exome capture was performed and resulted in the identification of the hemizygous variant c.586C>T, p.Arg196Trp in *PRPS1* (NM\_002764.3). Sanger sequencing confirmed this variant in the patient and his mother. It was not found in the father nor in the younger brother (Fig.1). This class-V variant (according to the international classification) was reported in a similar phenotype in two male siblings and was predicted to affect the interaction between pyrophosphate and PRS-I, thereby decreasing the enzyme activity<sup>10</sup>; it has also been found recently in a family with only affected females, who suffered from retinal dystrophy, either isolated or associated with HL or neurological involvement<sup>13</sup>.

After *PRPS1* mutation identification, additional analyses were performed in the proband: levels of uric acid were subnormal in serum (229 to 368  $\mu\text{mol/L}$ ; N: 120 - 320  $\mu\text{mol/L}$ ) as well as in urine (0.72 mmol/L; N: 1 – 5 mmol/L). PRS-1 activity was nil in erythrocytes (control: 0.65 nmol/min/mg hemoglobin) and fibroblasts (control: 0.45 nmol/min/mg prot) as well. HGPRT (hypoxanthine-guanine phosphoribosyl transferase) and APRT (adenine phosphoribosyl transferase) activities were normal in the same samples, attesting the specific PRS-1 deficiency for this patient (in erythrocytes: patient HGPRT: 2.2 nmol/min/mg Hb, control: 2.0-2.2; patient APRT: 0.52 nmol/min/mg Hb, control: 0.4-0.6. In fibroblasts: patient HGPRT: 1.19 nmol/min/mg prot., control: 0.46. Patient APRT: 1.68 nmol/min/mg prot., control: 1.33).

Retrospective examination of the mother at 28 years of age showed, in association with her known hearing impairment, moderate cone-rod retinal dystrophy with a nystagmus. She had complained about visual difficulties since the age of 24.

Her uric acid levels in serum and urine were normal, but PRS-1 activity was decreased in erythrocytes with a 30% residual activity (0.23 nmol/min/mg Hb, control: 0.65).

The proband's younger sister, who carries the *PRPS1* variant, presented at six months with convulsions with ocular revulsions and flexion spasms. Echocardiography and *fundus oculi* were normal. Neurological examination found axial hypotonia. Morphological peculiarities were noted: palpebral fissures asymmetry with a small right fissure, unilateral agenesis of the fifth digit, clinodactyly of the left second digit. She was able to sit at 10 months; at 27 months she could walk unsteadily and say a few words (weight: -3.5 SD, height -1.5 SD, HC -1.5 SD). She suffered from mastoiditis; the following immunity blood tests were normal. At 18 months, she had normal free-field audiometry.

The unusually early neurological presentation in this female patient prompted us to perform array-CGH (400kb resolution), which was considered normal, and to test X-chromosome inactivation, which was not informative in the mother and partially skewed in the sister, the paternal allele being more frequently expressed in blood cells (80%). PRS-1 activity was decreased in the sister's erythrocytes (1.0 nmol/min/mg Hb, control: 1.6) while HGPRT activity was normal (patient HGPRT: 4.5 nmol/min/mg Hb, control: 2.9).

## **Family 2**

Proband 2 is the first child of an unrelated couple. A maternal uncle and a male cousin on the paternal side have intellectual deficiency, without known etiology (Fig.1). The proband was born after uneventful pregnancy at 40 GW with a weight of 3180 g (10<sup>th</sup>-25<sup>th</sup> percentiles), a length of 49 cm (25<sup>th</sup>-50<sup>th</sup> p) and a head circumference of 33 cm (5<sup>th</sup>-10<sup>th</sup> p). Apgar scores were 8/10.

When he was six months-old, his parents noticed a strabismus associated with bilateral nystagmus. Decreased amplitudes of both cones and rod responses on reproducible ISCEV full-field ERG allowed to make the diagnosis of early-onset severe retinal dystrophy.

Ophthalmological outcome was then marked by ERG extinction at the age of 2. Motor retardation was diagnosed at 9 months; he had generalized hypotonia associated with absent tendon reflexes. Electromyogram (EMG) revealed axonal and demyelinating sensorimotor neuropathy. The patient firstly sat at 14 months and was able to make a few steps with gait ataxia at 2 years 9 months. At the age of 18 months, a delayed language development conducted to auditory testing and diagnosis of bilateral severe progressive sensorineural HL. Several brain MRIs until 4 years 4 months were normal apart from bilateral thin optic nerves. At last clinical evaluation, he was 17 years old. He used a wheelchair but was able to walk a few steps with support. His gait was ataxic. He had good comprehension but speech was dysarthric (Table 1).

### *Etiological investigations*

First metabolic studies including peroxysomal and lysosomal analysis were normal.

Mitochondrial respiratory chain analysis after muscular biopsy revealed a marked complex I deficiency (27 nmol/min/mg prot, control values: 42-107), leading to suspect a mitochondrial disease during several years. However, this deficiency was not found on fibroblasts, and advanced genetic studies including mitochondrial DNA analysis (research of : the main mutations responsible for NARP and MELAS syndromes ; mutations m.10158T>C and m.10191T>C in *ND3* ; m.12706T>C, m.13513G>A and m.13514A>G in *ND5* ; m.14487T>C in *ND6*) and nuclear genes analysis (*NDUFS1*, *NDUFS4*, *NDUFS7*, *NDUFS8*, *NDUFVI*) did not identify any abnormality. *PRPS1* targeted sequence analysis by Sanger sequencing was performed according to standard protocols and revealed a mosaicism in blood cells with a rate of about 70% of a hemizygous missense variant (c.383A>T, p.Asp128Val), that was not found in the mother. It has never been described in the literature but affects a highly-conserved residue corresponding to a Mg<sup>2+</sup> binding site and is predicted to be deleterious (SIFT/MutationTaster, class IV variant according to the international classification) (Table 1). Additional whole exome sequencing (WES) testing was undergone, revealing no candidate mutation except *PRPS1* already identified variant.

### **Family 3**

Proband 3 is the first child of unrelated parents of Turkish origin, apparently healthy and without any familial medical history (Fig.1). He was born after provoked delivery for abnormal fetal cardiac rhythm at 35 GW + 4 days. He had severe growth retardation: weight 1760 g (3<sup>rd</sup> percentile), length 43 cm (3<sup>rd</sup> -10<sup>th</sup> p) and head circumference 29.5cm (10<sup>th</sup> p). He initially

presented a good psychomotor development, sitting at 6 months, walking at 14 months. Bilateral profound sensorineural HL was diagnosed at 6 months. Between the ages of 8 months and 2 years, he had five episodes of neck contractions; his clinical examination and brain MRI were normal. At 4 years of age, absence epilepsy was diagnosed and confirmed by EEG showing bilateral diffuse spikes and sharp waves. Absences disappeared under valproic acid treatment. At 6 years old, an episode of ataxia occurred eight days after an otitis. Subsequent neurological examination found increased polygon of support and a head circumference of 48 cm (-2 SD). EEG was normal; in contrast, NCV (Nerve Conduction Velocity) test and EMG showed a diffuse peripheral motor and sensitive neuropathy. ISCEV full-field ERG found a decrease both cone and rod responses. Redox points were normal in blood but lactate was increased (2.4 mmol/L) in the cerebrospinal fluid. Cerebral MRI was normal. At 13 years, his neuropathy had not evolved and he had no mental disability (Table 1). As the redox points were slightly abnormal in blood, mitochondrial studies were performed: muscle biopsy showed a chronic neurogenic remodelling of fibers but mitochondrial respiratory chain was normal; mitochondrial biochemistry analyses in fibroblasts demonstrated a complex I deficiency (6 nmol/min/mg prot, control values: 9-17) but no mtDNA mutation, deletion or depletion were detected. MLPA of 17p11.2 region as well as sequencing of genes *PMP22*, *P0*, *GDAP1* and *SH3TC2*, involved in Charcot-Marie-Tooth disease, were normal. Eventually, WES identified the new c.206A>C, p.Glu69Ala variant in *PRPS1*, which was then confirmed by Sanger sequencing and has occurred *de novo*. This variant was considered of unknown pathogenicity (class III variant) (Table 1).

#### **Family 4**

At the first genetic consultation, the index case (V.1) was 23 years old. He had started walking at 11 months. A sensorineural HL had been diagnosed at 8 and had then evolved, ended up being

profound for the right ear and severe for the left ear, with a bilateral U-shape curve (Fig.1 and Table S1). His neurological and ophthalmological examinations were normal. CT-scan of temporal bones did not show any cochleovestibular malformation. His sister (V.2) did not complain about hearing troubles at the age of 25 but tonal audiograms revealed a right hearing defect of 35 dB for the 8000 Hz frequency. Otoacoustic emissions (500 to 3000 Hz) were present in both ears. She did not complain about visual problems. Their mother (IV.2), 53 years old, had normal hearing and otoacoustic emissions were present in both ears. The maternal grandfather (III.1) was reported to have had progressive isolated HL like his own maternal uncle (II.3). Two cousins of the index case (V.4 and V.5) also had hearing impairment: V.4 was 31 years old at first examination and presented with a moderate bilateral sensorineural HL that remained quite stable over time, with a flat or ascending audiological curve; his brother (V.5) had a U-shaped bilateral HL that evolved from moderate to severe between 33 and 49 years of age. Both men had normal neurological examination and *fundus oculi*.

#### *Etiological investigations*

Because of an X-linked transmission hypothesis and in the absence of cochleovestibular malformation, targeted *SMPX* and *PRPS1* analyses were performed by Sanger sequencing. A new *PRPS1* hemizygous variant, (c.136G>C, p.Glu46Gln), was found in subject V.4. Sanger analyses subsequently confirmed familial co-segregation of the variant with the phenotype. This variant was considered of unknown pathogenicity (class III variant) according to the international classification (Table 1). Consistently, PRS-1 activity was decreased in erythrocytes (0.1 nmol/min/mg Hb, control: 1.6) while HGPRT activity was normal (patient HGPRT: 3.5 nmol/min/mg Hb, control: 2.9).

The four variants were absent from dbSNP and GnomAD databases. The p.Arg196Trp variant had been reported in LOVD (#0000642641) ; we added p.Asp128Val, p.Glu69Ala and p.Glu46Gln (#0000598418, #0000598419 and #0000598420 respectively).

## Discussion

*PRPS1* is the most ubiquitously expressed gene of a three-member family which also comprises *PRPS2* and *PRPS1L1* (or *PRPS3*). Alterations of *PRPS1* open reading frame that have been reported so far are all missense variants and can be divided into two groups according to their functional consequence on the enzyme activity: gain-of-function variants, responsible for phosphoribosyl pyrophosphate synthetase superactivity, and loss-of-function variants. Nine different variants have been characterized in PRS-1 superactivity, all disrupting at least one of the allosteric sites of the protein, thereby decreasing feedback inhibition by nucleotides<sup>14, 15</sup>.

Twenty-four loss-of-function variants, the vast majority of which affect only one family, have been described and associated with a great clinical heterogeneity, encompassing four distinct phenotypes: X-linked isolated hearing-loss (DFN2 or, formerly, DFNX1), CMTX5, Arts syndrome and another syndrome combining prenatal growth retardation, severe neurological symptoms and diabetes insipidus<sup>10</sup>. Nevertheless, some authors questioned these distinctions and suggested to consider PRS-1 loss-of-function as an unique condition associated with a phenotypic continuum, the clinical presentation being highly dependent on the level of residual enzymatic activity<sup>1</sup>. This idea was then strengthened by several reports of atypical profiles and intermediate phenotypes: a patient presented with symptoms of Arts syndrome and CMTX5, while his elder sister, 42, had clinically isolated congenital HL and discrete parietal and cerebellar atrophy on the MRI, and their carrier mother, 66, did not show any symptom<sup>12</sup>. Two patients carrying different *PRPS1* variants associated with different phenotypes (one has CMTX5 and the other has Arts

syndrome) both presented with transient proximal muscle weakness after infections<sup>5,9</sup>. Robusto and colleagues described three families with apparently isolated sensorineural HL, but after careful neurological evaluation, clinical or subclinical peripheral neuropathy signs were actually found in some affected males and carrier females of two families<sup>16</sup>. Gandia and colleagues reported a family with strong phenotypic variability in males, going from isolated HL to CMTX5, regardless of the patients' ages; the variant involved, p.Gly306Glu, presumably affects interactions at the trimer interface of the enzyme, as had been shown for other variants responsible for DFN2, including p.Gly306Arg which affects the same amino-acid. The authors did not find any variation in different candidate modifier genes<sup>1, 17</sup>. Unfortunately, they did not measure PRS-1 activity in their patients; it could have been informative to compare the level of residual activity in the more and less severely affected patients. Last, the variant p.Val142Pro was surprisingly reported to cause a complex phenotype with symptoms of both PRS-1 superactivity and decreased activity in a single individual, questioning the relevance of separating gain- and loss-of-function anomalies of *PRPS1*. This variant was predicted by molecular modelling to affect both allosteric sites, involved in inhibition of the enzyme, and ATP-binding site, suggesting that it could have antagonist effects on PRS-1 activity. Biochemical assays showed that activity was absent in the erythrocytes of the patient and that in contrast, it was in the normal range in his fibroblasts, although it did not increase with higher concentrations of the activator phosphate as it did in control cells, which suggests that PRS-1 was constitutively active in patient's fibroblasts. The authors hypothesize that the variant may have opposite effects in different cell types, causing a gain of function in dividing cells and a loss of function in postmitotic cells<sup>18</sup>.

In spite of the overall heterogeneity and possible intrafamilial variability (even between males), which render genetic counselling particularly complex, some observations are in favour of the

existence of a strong genotype-phenotype correlation. Indeed, in the literature, *PRPS1* loss-of-function variants related to CMTX5 and Arts syndrome are usually predicted to destabilize the overall structure of the protein, while the impact of those associated with DFN2 is predicted to be restricted to the ATP-binding site <sup>14</sup>. This generally correlates with the level of residual activity. The most severe phenotypes are associated with a very low PRS-I activity in erythrocytes or fibroblasts, whereas on the other side of the spectrum, the patients presenting with isolated HL and female carriers have less dramatically decreased enzyme activity <sup>11, 16, 19</sup>.

Moreover, each variant that has been identified in two different families (p.Ser16Pro, p.Arg196Trp and p.Arg214Trp) is associated with close phenotypes (Fig. 2). Notably, our male patient carrying the p.Arg196Trp variant (family 1) and the two siblings previously described with the same variant show great phenotypic similarity, with relatively rare signs as intrauterine and postnatal growth retardation, congenital retinal dysfunction, diabetes insipidus and white matter dedifferentiation <sup>10</sup>. This suggests a tight correlation between protein structure and activity at this particular site; the variant seems to be responsible for a dramatic destabilization of the protein that affects the greatest variety of tissues. In accordance with previous findings, activity was absent in the erythrocytes and fibroblasts of our patient (while his mother has a 30% decrease in erythrocytes compared to controls), and extremely low –yet detectable– in erythrocytes of the previously described siblings. In the erythrocytes of the proband’s sister, however, PRS-1 activity was only moderately decreased; this is consistent with predominant expression of the wild-type paternal allele found in blood cells. Interestingly, this variant has recently been identified in another family with two females suffering from retinal dystrophy, both being part of sibships composed of females only. This particular profile prompts to evoke embryonic lethality of the variant in this family <sup>13</sup>. The mother of the two affected siblings reported by Al-Maawali and colleagues had also had two intrauterine foetal deaths of males at 23

and 24 GW; growth retardation and very high maternal  $\alpha$ -fetoprotein had been found in both cases. The authors did not provide any information on the mother's phenotype<sup>10</sup>. Regarding other female carriers, the proband's mother in family 1 of this study had short stature and a sensory phenotype reminiscent of Usher syndrome, type 2 or 3. Her daughter had normal *fundus oculi* and audiogram at respectively six and eighteen months. Quite surprisingly, she presented with very precocious neurological involvement with flexion spasms. The two affected females of the lastly described family, aged 40 and 13, have retinal dystrophy without hearing impairment. The variants p.Ser16Pro and p.Arg214Thr were also reported in families with affected females only. For p.Ser16Pro, in the first family, all four affected women showed optic atrophy followed by typical or sectorial *retinitis pigmentosa* and various degree of neurological involvement. Ages of onset of the different symptoms were greatly variable. The most severe phenotype comprised peripheral neuropathy, mild developmental delay, hypotonia, ataxia, essential tremor and HL, while the less affected individual only had essential tremor in addition to visual symptoms<sup>20</sup>. In the second family, both affected females had optic atrophy, retinal dystrophy and gait deficit but no HL<sup>13</sup>. The variant p.Arg214Thr has been described in two sporadic female cases; both patients displayed optic atrophy, retinal dystrophy and HL, and had been initially clinically diagnosed with Usher syndrome (type 2 or 3).

Clinical presentations associated with *PRPS1* variants are thus generally less severe and/or of later onset in females than in males, but are still quite variable, ranging from the absence of symptoms to rather pronounced sensory and neurological involvement. Several studies investigated X-inactivation patterns in carrier females; although no skewing was observed in two families with severely affected boys<sup>1, 18</sup>, two other families showed a skewed X-inactivation pattern correlating to a certain extent with the levels of residual PRS-1 activity and with the

severity of symptoms in females<sup>12, 20</sup>. Robusto and colleagues reported unbalanced expression of wild-type and mutant alleles which did not strictly reflect X-inactivation pattern, suggesting that correlation between inactivation degree and enzyme activity may not be straightforward in all cases<sup>16</sup>. X-inactivation pattern analysis in carrier females of family 1 was not informative in the mother but revealed skewed inactivation in her daughter, with 80% inactivation of the mutated maternal allele. Accordingly, we cannot conclude as for the pathophysiology of her neurological features. As she was quite young at the time of last examination, the actual severity of her phenotype is uncertain.

The most widespread symptom of *PRPS1* impairment is HL. In mice, Liu and colleagues showed strong expression of *PRPS1* mRNA in cochlear hair cells and greater epithelial ridge during embryonic development and early postnatal life, suggesting a role of the protein in the development and function of the inner ear. At P6, additional expression in spiral ganglia was found<sup>11</sup>, but no evidence of auditory nerve impairment has been reported in affected patients so far.

Seven families of isolated X-linked sensorineural HL due to a variant in *PRPS1* have already been reported<sup>11, 16, 17, 19</sup>. There does not seem to be any recognizable pattern emerging from these observations. The age of onset in males is variable, from birth to early adulthood, but tends to be more often postlingual. The deficit is frequently symmetrical, progressive and its severity ranges from moderate to profound, with a great diversity in audiogram shapes: flat, downsloping, upsloping or U-shaped. Carrier females have later-onset HL, which is usually mild to moderate (but may progress to profound, as it has been described once by Liu and colleagues<sup>11</sup>) and, when detailed, more often asymmetrical (Table S1). Of note, a few of them in their fifth or sixth decade still have normal hearing. In our family 4, subjects V.2 and IV.2, aged 25 and 53 respectively,

only have subclinical signs of hearing impairment.

Although the prevalence of *PRPS1* anomalies in isolated HL is unknown, Kim and colleagues identified a variant in a multiplex family out of 42 Korean sporadic cases or families compatible with recessive inheritance in which the genes most frequently involved had been excluded<sup>19</sup>. We therefore recommend testing *PRPS1* in all familial cases of isolated deafness compatible with X-linked inheritance with normal CT scan of temporal bones (hence not suggestive of *POU3F4* impairment) and normal *GJB2* sequencing. A few studies performed whole-exome or whole-genome sequencing on small cohorts of sporadic isolated deafness cases of various ethnic origins (each study was performed on 11 to 22 cases in which at least mutations in *GJB2* had been excluded). They did not find any candidate variant in *PRPS1*<sup>21, 22, 23, 24</sup>.

As mentioned above, *PRPS1* loss of function may be reminiscent of Usher syndrome type 2 or 3 in females; it should therefore be tested in cases associating optic atrophy or retinal dystrophy and HL in females. It is also one of the main differential diagnoses for mitochondriopathies, which had been suspected in three families out of the four described in the present study.

Pei and colleagues created a model of *PRPS1* inactivation in zebrafish, in which there are two paralogues of the gene, *prps1a* and *prps1b*. Animals mutated for both genes (“double mutants”) displayed dysmorphic craniofacial features, severely reduced body size, eye defects, and had a reduced lifespan, with only 5% of individuals reaching adulthood. In wild-type animals, *prps1a* and *prps1b* were strongly expressed in the inner ear; double mutants showed a decrease in the number of inner ear hair cells. They also presented with an early reduction in the number of leukocytes, which can be reminiscent of the recurrent infections described in Arts syndrome<sup>25</sup>.

The only therapeutic option envisaged to date is S-adenosylmethionine (SAM), which could

allow replenishment of purine nucleotide pools, with limited side effects. A few years ago, de Brouwer and colleagues reported that two Australian brothers with Arts syndrome were being given SAM supplementation diet and that preliminary data suggested improvement of their condition, with a dramatic decrease in hospitalisation time, and stabilisation of ataxia and HL <sup>1</sup>. More recently, Kim and colleagues considered DFNX1 would be a good model to test for SAM supplementation; indeed, the difference of PRS-1 activity between affected males and asymptomatic carrier females is smaller than in other *PRPS1*-related disorders, so treatment would require minimal doses, and the evolution of hearing impairment could easily be followed and compared to the evolution prior to any supplementation. However, Pei and colleagues did not observe any improvement of the phenotype of double mutant zebrafish after SAM supplementation; consistently, they could not find any difference in SAM levels between mutant and WT animals <sup>25</sup>.

We present four families with *PRPS1* loss-of-function variants that, taken together, recapitulate almost all the different phenotypes described so far, with overlaps of conditions initially considered as distinct. Our data are therefore consistent with the notion of clinical continuum associated with PRS-1 deficiency and are also in favour of a strong genotype-phenotype correlation, *via* reproducible effects of a given variant on the level of PRS-1 activity, which seems to be quite precisely determined by structural characteristics of the enzyme.

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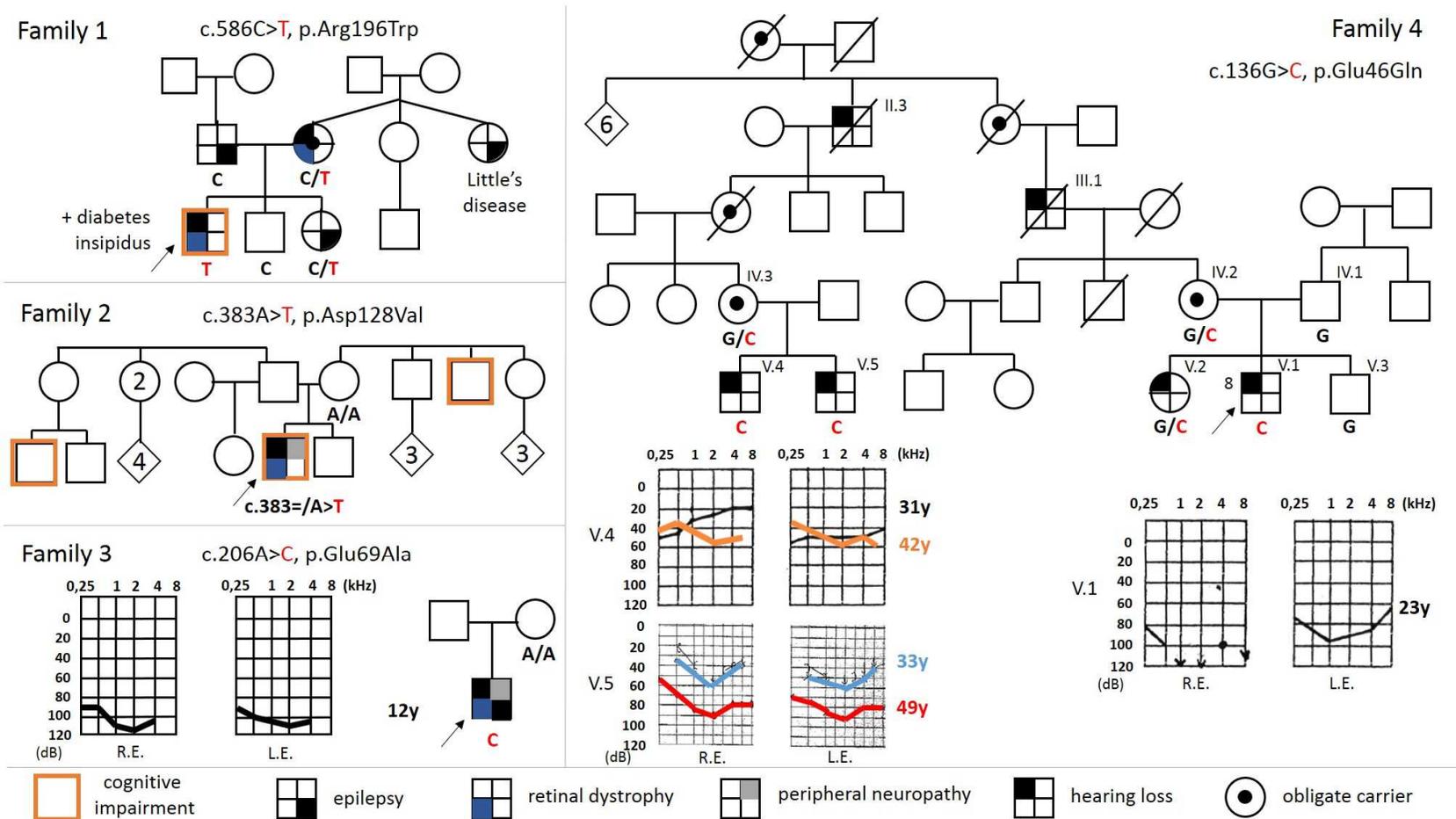
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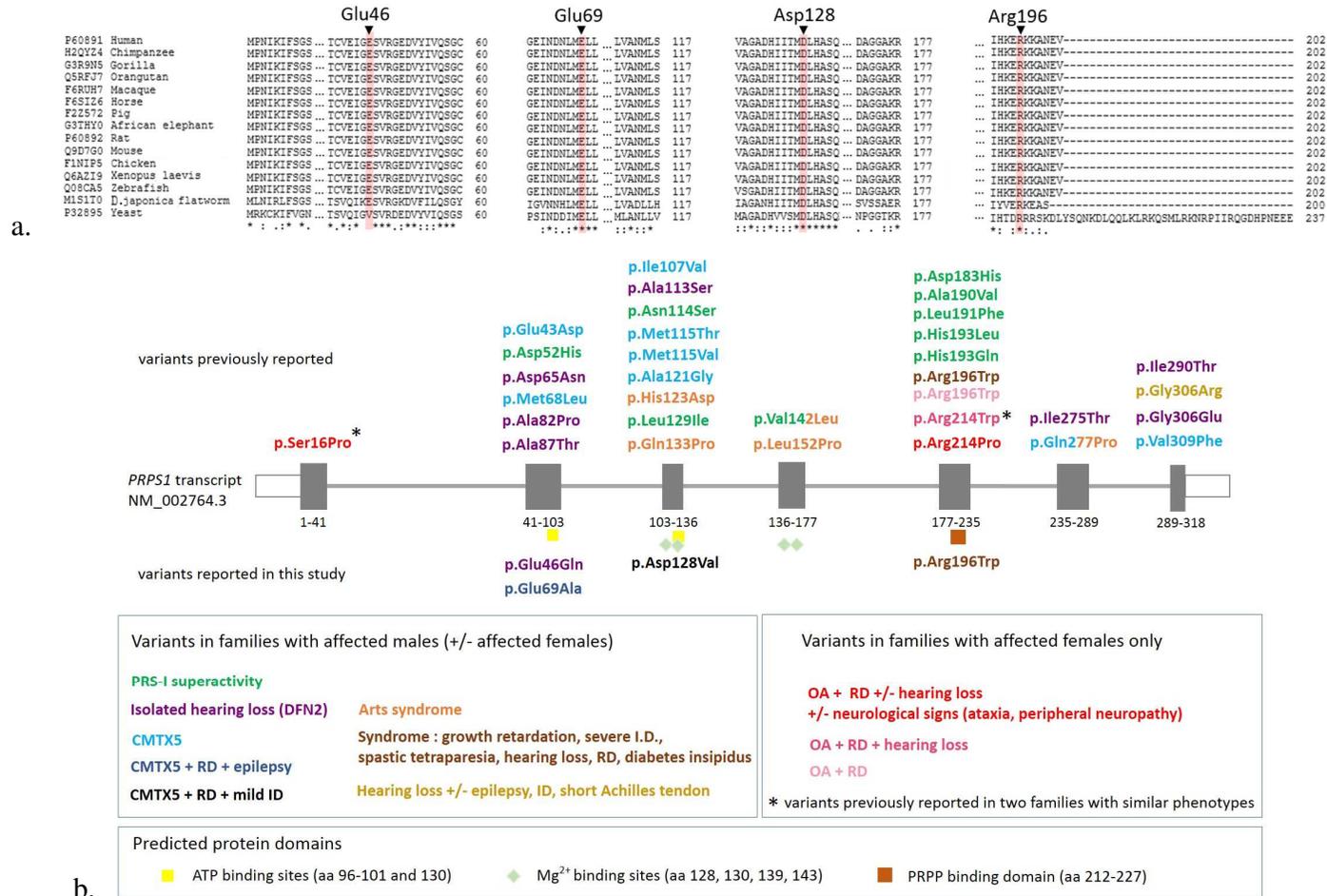
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**Fig.1. Pedigrees of four new families with *PRPS1* variants, and clinical information**

Proband is indicated by arrows. When known, the genotype is mentioned under the symbol of the individual. Audiograms of five affected males are represented (R.E.: right ear, L.E.: left ear) ; age at testing is indicated on the right.

Correspondence between cDNA and gDNA nomenclatures of variants : c.586C>T : g.106888462C>T, c.383A>T : g.106884208A>T, c.206=/A>C : g.106882608=/A>C, c.136G>C : g.106882538G>C (nomenclature follows HGVS recommendations)



**Fig. 2: PRPS1 variants and associated phenotypes** Adapted from Almuquera *et al.*, 2014 and Fiorentino *et al.*, 2017.

a. Multiple sequence alignment of *PRPS1* orthologues using ClustalOmega (Sievers *et al.*, 2011). Corresponding UniProt references are given on the left; the four amino-acids affected by the variants we identified are indicated.

b. *PRPS1* transcript (NM\_002764.3) : coding parts are represented by grey-filled boxes (with corresponding amino-acids written below), 5' and 3'-UTRs by empty boxes, introns by a grey line. Above the transcript are indicated variants reported in the literature so far; below are the variants we report. Two-coloured variants are associated with combined phenotypes in the same individual, while a variant written twice in different colours is associated with different phenotypes in two unrelated families.

OA: optic atrophy, RD: retinal dystrophy, CMTX : Charcot-Marie-Tooth disease type 5, X-linked (peripheral neuropathy + hearing loss +/- OA).

Protein domains are based on UniProtKB entry P60891.

	<b>Proband 1</b>	<b>Proband 2</b>	<b>Proband 3</b>	<b>Proband 4</b>
<b>cDNA change</b>	c.586C>T	c.383A>T	c.206A>C	c.136G>C
<b>genomic DNA change (GRCh37)</b>	g.106888462C>T *	g.106884208A>T *	g.106882608A>C *	g.106882538G>C *
<b>Proteic change</b>	p.Arg196Trp	p.Asp128Val	p.Glu69Ala	p.Glu46Gln
<b>SIFT prediction (score)</b>	deleterious (0)	deleterious (0)	deleterious (0)	deleterious (0)
<b>Mutation Taster (score)</b>	disease-causing (101)	disease-causing (152)	disease-causing (107)	disease-causing (29)
<b>ACMG classification</b>	Likely pathogenic	Likely pathogenic	Pathogenic	Likely pathogenic
<b>Phenotype</b>	Syndrome of IUGR, severe DD, spastic tetraparesis, congenital RD and diabetes insipidus	CMTX5	CMTX5	DFN2
<b>Age at last examination</b>	4 y 9 m	17 y	13 y	23 y
<b>Speech development</b>	absent	delayed	normal	normal
<b>Hearing loss (HL)</b>	bilateral, moderate, SN	bilateral, severe, SN	bilateral, profound, SN	bilateral, severe/profound, SN
<b>Age at HL diagnosis</b>	1 year	18 months	6 months	8 years
<b>Walking age</b>	NA	33 months	14 months	11 months
<b>MRI (age)</b>	enlarged 4 <sup>th</sup> ventricle, <i>cisterna magna</i> and <i>cisterna pontis</i> ; optic nerve hypoplasia (7y)	bilateral optic nerve atrophy (4 y 4 m)	normal (6 y)	-
<b>Additional info</b>	severe IUGR	ataxic gait and dysarthric speech	- severe IUGR - good psychomotor development - epilepsy - peripheral neuropathy	-

**Table 1: PRPS1 variants and main clinical features of probands**

IUGR: intra-uterine growth restriction, DD: developmental delay, RD: retinal dystrophy, NA: not applicable; a dash means that the information is missing. \* not reported in dbSNP / GnomAD databases. SIFT and Mutation Taster predictions were made using protein ID ENSP00000361512 and transcript ID ENST00000372435, respectively.