

Missense variants in the X-linked gene *PRPS1* cause retinal degeneration in females

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Abstract

Retinal dystrophies are a heterogeneous group of disorders of visual function leading to partial or complete blindness. We report the genetic basis of an unusual retinal dystrophy in five families with affected females and no affected males. Heterozygous missense variants were identified in the X-linked *PRPS1* gene: c.47C>T, p.(Ser16Phe); c.586C>T, p.(Arg196Trp); c.641G>C, p.(Arg214Pro) and c.640C>T, p.(Arg214Trp). Missense variants in *PRPS1* are usually associated with disease in male patients, including Arts Syndrome, Charcot-Marie-Tooth and non-syndromic sensorineural deafness. In our study families, affected females manifested a retinal dystrophy with inter-ocular asymmetry. Three unrelated females from these families had hearing loss leading to a diagnosis of Usher Syndrome. Other neurological manifestations were also observed in three individuals. Our data highlight the unexpected X-linked inheritance of retinal degeneration in females caused by variants in *PRPS1*, and suggest that tissue specific skewed X-inactivation or variable levels of PRS-I deficiency are the underlying mechanism(s). We speculate that the absence of affected males in the study families suggests that some variants may be male embryonic lethal when inherited in the hemizygous state. The unbiased nature of next generation sequencing enables all possible modes of inheritance to be considered for association of gene variants with novel phenotypic presentation.

Key Words Retinal Dystrophy, *PRPS1*, PRS-I, Next generation sequencing

1. Introduction

Inherited retinal dystrophies (IRD) are a heterogeneous group of visual function disorders that lead to partial or complete blindness, affecting approximately 1 in 2500-3000 individuals, and represent the commonest cause of blindness in the working age population and second commonest in childhood in England and Wales (Holt, et al., 2015; Liew, et al., 2014). IRD may be classified according to whether there is a primary defect of cone or rod systems or both, and according to whether retinal dysfunction is generalised or confined to the central retina (macular dystrophy). These disorders can occur in isolation (non-syndromic IRD) or with additional systemic manifestations (syndromic IRD). The genetic aetiology of IRD is remarkably heterogeneous, with over 300 disease-causing genes identified (The Retinal Information Network <http://www.sph.uth.tmc.edu/RetNet/>; accessed April 1st, 2017). The implicated genes are involved in the function of numerous pathways in the retina, including phototransduction, retinal metabolism, ciliogenesis and RNA splicing (Nash, et al., 2015); however, the genetic cause of IRD in approximately 40% of cases remains unsolved (Ellingford, et al., 2016; Khan, et al., 2017).

The identification of disease-causing variants in IRD has been accelerated by the implementation of next generation sequencing (NGS) technologies. Targeted NGS sequencing of known genes can identify disease-causing variants in an estimated 60% of non-syndromic cases and 80% of syndromic cases (Bravo-Gil, et al., 2016; Ellingford, et al., 2016; Glockle, et al., 2014). In patients that lack a molecular diagnosis, whole exome sequencing (WES) and whole genome sequencing (WGS) are effective tools to identify new potential causative genes and variants (Arno, et al., 2016; Audo, et al., 2014; Chaki, et al., 2012).

In our collaborative study of unsolved IRD cases, through the UK Inherited Retinal Disease Consortium, the Japan Eye Genetic Consortium and the 100,000 Genomes Project, approximately 1,614 unrelated individuals were analysed by WES or WGS.

Here we describe retinal dystrophy in 9 affected females from 5 unrelated families with no affected males. Whole exome or whole genome sequencing (WES; WGS) revealed heterozygous missense variants in the Phosphoribosyl Pyrophosphate Synthetase 1 (*PRPS1*) gene, which are more usually implicated in rare syndromic disease in male patients including Arts Syndrome (MIM# 301835), X-linked Charcot-Marie-Tooth (CMTX5, MIM# 311070) and X-linked non-syndromic sensorineural deafness (DFNX1, MIM# 304500).

2. Material and methods

2.1 Clinical assessment of study families

The study protocols adhered to the tenets of the Declaration of Helsinki and received approval from the local Ethics Committee of all participant institutes; Moorfields Eye Hospital, National Institute of Sensory Organs, and the Jikei University School of Medicine. Written, informed consent was obtained from all participants or parents of children prior to their inclusion in this study. Families 1 to 4 were recruited in the UK (Figure 1).

Four probands, the affected mother of the proband of family 1, and the affected daughter of the proband of family 2 underwent detailed clinical examination, including best-corrected decimal visual acuity (BCVA), 35-degree color fundus photography (TRC-NW8, Topcon (Great Britain) Ltd., UK), ultra-widefield confocal scanning laser imaging (California, Optos plc, UK), fundus autofluorescence (FAF) imaging (Spectralis, Heidelberg Engineering Inc., CA), spectral-domain optical coherence tomography (SD-OCT; Spectralis, Heidelberg Engineering Inc., CA), kinetic and static visual field testing (Goldmann perimetry and Humphrey Field Analyzer, HFA3, Carl Zeiss Meditec AG, Germany), and color vision

testing with both Ishihara and Hardy Rand and Rittler Pseudoisochromatic plate testing.

Family 5 was recruited in Japan. The proband and the two affected older daughters underwent clinical evaluation including measurements of the BCVA, visual field testing, 35-degree color fundus photography (VISUCAM NM/FA; Carl Zeiss Meditec AG, Dublin, CA, USA), Spectralis HRA (Heidelberg Engineering, Heidelberg, Germany) for fundus autofluorescence imaging (5-III:2), and SD-OCT (Cirrus, Carl Zeiss Meditec AG, Germany), static visual field testing (Humphrey Field Analyzer, HFA3, Carl Zeiss Meditec AG, Germany), and color vision testing with Farnsworth D-15 test.

Full-field ERG assessment of generalised retinal function (Families 2, 4 and 5) and pattern ERG assessment of macular function (families 2 and 4) were performed with corneal electrodes and incorporated the standards of the International Society for Clinical Electrophysiology of Vision (Bach, et al., 2013; McCulloch, et al., 2015), with the exception of the flash ERGs in one child (2-IV:5), recorded using lower eyelid skin electrodes according to a paediatric protocol.

2.2 Next Generation Sequencing

DNA samples for the five probands were analysed by NGS: family 1, 2, 3 and 5 were investigated using WES and family 4 using WGS. For more details see Supp. material and methods.

2.3 Sanger sequencing

In order to confirm the *PRPS1* variants identified by NGS (position according to reference transcript NM_002764 and X-chromosome co-ordinates in hg19), polymerase chain reaction (PCR) amplification and bi-directional Sanger sequencing was performed using standard reagents and conditions, and oligonucleotide primers flanking the variant (primer sequences

available on request). Segregation analysis was performed where familial samples were available.

2.4 *In silico* analysis of *PRPS1* variants

The UCSC genome browser (<http://genome.ucsc.edu/>) and Phenopolis (<https://Phenopolis.github.io>; (Pontikos, et al., 2016)) were used for visualisation and analysis of existing variation. Genetic variant deleteriousness was scored with PolyPhen v2.2.2 (<http://genetics.bwh.harvard.edu/pph2/>; accessed on 11/01/2017; (Adzhubei, et al., 2010)) and SIFT (<http://sift.jcvi.org/>; accessed on 11/01/2017; (Ng and Henikoff, 2001)). The evolutionary conservation of variants was assessed by multiple sequence alignment across species using Homologene (<https://www.ncbi.nlm.nih.gov/homologene>; accessed on 11/01/2017). The impact of pathogenic variants on the three-dimensional structure of the protein was assessed with a model of phosphoribosyl pyrophosphate synthetase-1 (PRS-I) based on the crystal structure of the human PRS-I protein (Protein Database, PDB: 2H06, <http://www.rcsb.org/pdb/home/home.do>) using Swiss Model (Arnold, et al., 2006), <https://swissmodel.expasy.org/>, accessed on the April 1st, 2017) with standard parameters and visualizing it with YASARA v16.9.23 (<http://www.yasara.org/>; (Krieger and Vriend, 2014)). *PRPS1* variants associated with disease have been submitted to ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>).

3. Results

3.1 Phenotypic Characteristics

The phenotypic characteristics of nine affected individuals in the study families (Figure 1) are summarised in Table 1. The mean age in 2017 was 38 years with a range of 13 to 94 years. Photoaversion was reported in 3 patients (1-II:1, 3-II:1, 5-II:2) and 4 subjects had night blindness (2-IV:5, 5-II:2, 5-III:1, 5-III:2). Visual acuity reduction was described in 3 patients

(2-III:4, 4-II:1, 5-II:2), with one subject having blurred vision in the left eye due to intraocular lens dislocation (1-I:2).

Visual acuity varied; two patients had severe visual loss (less than 6/36 in the better eye), and six had relatively preserved BCVA (better than 6/9 in the better eye). The mean age of onset of visual symptoms was 19 years with a range of 5 to 29 years.

Fundus examination, color fundus photography, and FAF imaging identified a variable degree of patchy mid-peripheral retinal atrophy and/or retinal pigment epithelial disturbance in all patients, with a variable degree of inter-ocular asymmetry in all nine patients (Figures 2-3). Retinal pigment epithelial (RPE) mottling and/or a tapetal-like reflex on FAF was observed in 6 out of 8 subjects with available images (1-II:1, 2-III:4, 2-IV:5, 3-II:1; 4-II:1, 5-III:1; Figures 2-3). Macular involvement was apparent in four patients and optic disc atrophy was detected in seven (Table 1).

SDOCT demonstrated outer retinal disruption in the atrophic regions of seven patients with available images (Figure 2, 1-II:1, 1-I:2, 2-III:4, 4-II:1, 5-II:2, 5-III:1, 5-III:2). Foveal structure was relatively preserved in five patients (1-II:1, 1-I:2, 4-II:1, 5-III:1, 5-III:2) and choroidal thickness was maintained in all seven subjects.

Electrophysiological assessment was performed in five patients and showed generalized retinal dysfunction with a similar degree of cone and rod photoreceptor involvement in two subjects (2-III:4, 4-II:1), with mild worsening of rod system function in 4-II:1 between the ages of 6 and 12 years (Figure S1). In a 12-year-old girl there was ERG evidence of a cone-rod dystrophy (2-IV:5) and in one other subject a cone dystrophy (5-III:1). The ERGs were normal in one patient (5-III:2). There was significant inter-ocular ERG asymmetry in cases 2-III:4 (Figure S1) and 2-IV:5. Subjects that underwent additional PERG testing showed P50

reduction in keeping with severe macular involvement with evidence of progression in 4-II:1 (Figure S1).

Visual field testing was performed in six patients. Visual field defects corresponding to the amount of RPE atrophy was detected in these six patients, with a variable degree of interocular asymmetry. Constricted peripheral visual fields with relatively preserved central field were found in four patients (1-II:1, 4-II:1, 5-III:1, 5-III:2), and large central field defects with areas of residual peripheral field were observed in two subjects (2-III:4, 5-II:2).

Generalised dyschromatopsia was detected in all four patients with available data (1-II:1, 2-III:4, 2-IV:5, 5-II:2).

Sensorineural hearing loss was reported in three patients (Table 1). Hearing loss was first reported in childhood and diagnosed in two patients (3-II:1, 4-II:1) and one subject showed severely reduced responses at the pure tone test in her 40s (5-II:2). Asymmetrical hearing loss was reported in one patient (4-II:1), while there were multiple infection histories in the middle ear.

Additional neurological abnormality was reported in three patients (1-II:1, 5-II:2, 5-III:2). Gait deficit was detected in one proband of family 1 (1-II:1), who was previously diagnosed with possible post-polio syndrome. The mother of this proband of family 1 (1-I:2) also had gait difficulty; however, detailed information is unavailable. Ataxia of gait was also detected in the proband and older daughter of the proband of family 5 (5-II:2, 5-III:1), while no ataxia was reported by the younger daughter of the proband (5-III:2).

3.2 Identification of rare *PRPS1* variants in affected females

The pedigrees of the study families are shown in Figure 1. A female in Family 1 (GC20175, individual 1-II:1), was diagnosed with rod-cone dystrophy with inter-ocular asymmetry, inherited from her mother. Given the asymmetry of retinal degeneration and RPE mottling/

tapetal-like retinal reflex in this individual, an X-linked condition was considered, which led to screening of Retinitis Pigmentosa 2 gene (*RP2*) and Retinitis Pigmentosa GTPase Regulator gene (*RPGR*) (including the Open Reading Frame 15 (ORF15)). As no pathogenic variant was identified, WES was performed and variants were filtered and prioritised for potential autosomal dominant or X-linked inheritance. Heterozygous rare variants with a maximum of 2 in 8000 alleles in University College London Exome (UCLEx N=4,000) and a maximum of 5 in 121412 alleles in the Exome Aggregation Consortium (ExAC, including WES data of 60,706 unrelated individuals without paediatric onset severe Mendelian disease) databases were identified in 88 autosomal genes and 3 genes on the X-chromosome (Supp. Table S1). A single heterozygous variant (GRCh37 Chr6:65301316, NM_001142800: c.4444G>A p.E1482K) was identified in Eyes Shut Homolog (*EYS*), a gene previously implicated in autosomal recessive Retinitis Pigmentosa (RP). In the absence of a second allele, this was deemed unlikely to be causative of the phenotype. Interestingly, a rare heterozygous missense variant was identified in the X-linked gene *PRPS1* (Supp. Table S1, GRCh37 ChrX:106871905, NM_002764: c.47C>T (p.(Ser16Phe), RNA not analysed)) which was absent from the ExAC and Genome Aggregation Database (gnomAD) datasets and was predicted to be probably pathogenic by *in silico* prediction algorithms (Table 2).

Variants in *PRPS1* encoding the protein PRS-I cause rare forms of DFNX1, CMTX5, Arts Syndrome and PRS-I superactivity (MIM# 300661) in affected males (Gandia, et al., 2015; Mittal, et al., 2015). However, recently a family with only affected females with optic atrophy, RP and neurological features overlapping CMTX5 and Arts syndrome was reported with a *PRPS1* variant affecting the same codon as we report in Family 1, c.46T>C, p.(Ser16Pro) (Almoguera, et al., 2014), implicating the *PRPS1* variant as the top candidate in Family 1. Sanger sequencing confirmed the *PRPS1* variant in the affected female proband, and its absence in unaffected siblings (1-II:3 and 1-II:5, Figure 1 and Supp. Figure S2).

A simplex affected female (GC19753, individual 2-III:4) was diagnosed with an asymmetrical photoreceptor dystrophy with severe macular involvement. Given the interocular asymmetry of retinal degeneration in this individual, an X-linked condition was considered, and variants in *RP2* and *RPGR* (including ORF15) were excluded. In addition, targeted capture sequencing of 176 genes (Manchester Centre for Genomic Medicine) known to be associated with IRD did not reveal a causative variant, so WES was performed. Variants were filtered and prioritised considering potential *de novo*, X-linked or recessive inheritance (see Supp. material and methods), revealing a *PRPS1* variant as the most compelling candidate (GRCh37 ChrX:106888462; c.586C>T (p.(Arg196Trp), RNA not analysed); NM_002764). This variant was absent in the ExAC and gnomAD databases and was predicted to be potentially pathogenic by both Polyphen and SIFT (Table 2). Sanger sequencing confirmed the variant in the affected female proband. Subsequently, one of the proband's daughters presented with vision problems (2-IV:5), and genotyping confirmed that she had inherited the *PRPS1* variant c.586C>T, p.(Arg196Trp) (Supp. Figure S2).

The proband (GC19557, 3-II:1) of family 3 was diagnosed with RP and hearing loss, initially described as atypical Usher syndrome type II or III, with no family history of the condition. Known genes associated with Usher syndrome (*MYO7A*, *USH1C*, *CDH23*, *PCDH15*, *USH1G*, *USH2A*, *GPR98*, *WHRN*, *CLRN1*, *SLC4A7*) were screened and were variant negative (National Collaborative Usher Study (Stabej, et al., 2012)). WES was performed and the variants were filtered and prioritised for all possible modes of inheritance (see Supp. material and methods). A unique variant in *PRPS1*, (GRCh37 ChrX:106888516; c.640C>T (p.(Arg214Trp), RNA not analysed; NM_002764), was considered the most probable cause of disease. The variant was not found in the ExAC or gnomAD databases and was predicted to be potentially pathogenic by both Polyphen and SIFT (Table 2). Interestingly, the same variant was identified in the proband of family 4 (GC19559, 4-II:1) who was also diagnosed

with atypical Usher syndrome type II, with no family history. WGS was performed for the proband and her parents as part of the 100,000 genomes project (4-II:1, 4-I:1, 4-I:2) and variants were filtered and prioritised for potential *de novo* or autosomal recessive inheritance. No potential compound heterozygous or homozygous variants survived filtering (see Supp. material and methods). A *PRPS1* variant c.640C>T (p.(Arg214Trp), RNA not analysed) was considered the most compelling candidate, and was not found by WGS in the unaffected parents (4-I:1 and 4-II:2). Sanger sequencing confirmed that the variant has occurred as a *de novo* variant in the affected female (Figure 1 and Supp. Figure S2).

An affected female in Family 5 (5-II:2) was diagnosed with cone-rod dystrophy at the age of 26. No family history was reported and there was a history of miscarriage before having three daughters. Fundus examination of her three daughters was normal (5-III:1, 5-III:2, 5-III:3) at that time. The latest clinical investigations were performed 16 years after the baseline diagnosis. The female proband had developed hearing loss and ataxia of gait was also noted. All three daughters reported visual impairment. Two had subtle fundus abnormalities with disc pallor (5-III:1, 5-III:2); and fundus abnormality, optic nerve hypoplasia and a gait deficit were detected in the youngest daughter (not included in this study). One, had ataxia of gait with evidence of cone dystrophy (5-III:2) and two individuals had inferior visual field defects (5-III:1, 5-III:2). WES was performed for the proband, and variants were filtered and prioritised for potential *de novo*, X-linked or recessive inheritance. A variant, c.641G>C (p.(Arg214Pro), RNA not analysed) (GRCh37 ChrX:106888517; NM_002764), in *PRPS1* was identified as the most likely cause. This variant was absent in the ExAC or gnomAD databases and was predicted to be possibly pathogenic by SIFT (Table 2). Sanger sequencing confirmed the variant in the proband and absence in her unaffected mother and sister (5-I:2 and 5-II:3). Two symptomatic daughters available for genetic testing were found to carry the *PRPS1* variant (5-III:1 and 5-III:2, Figure 1 and Supp. Figure S2).

3.3 Pathogenicity and potential consequences of missense variants in *PRPS1*

All four rare *PRPS1* missense variants (p.Ser16Phe, p.Arg196Trp, p.Arg214Pro and p.Arg214Trp) identified in this study are absent from control datasets and are predicted to be damaging by both SIFT and Polyphen, (with the exception of p.Arg214Pro, Table 2). Where samples were available, the variants segregated with the phenotype in families (Figure 1 and Supp. Figure S2). Multiple sequence alignment of PRS-I orthologues confirmed that the serine residue at position 16 and the arginine residues at positions 196 and 214 are highly conserved in orthologues at least to zebrafish (Figure 4A). PRS-I is highly conserved across different species, with 100% conservation in primates, wolf, cow, mouse and rat, and over 88% conservation in insects. Variants in *PRPS1* have been previously associated with different phenotypes in males: DFNX1, CMTX5, Arts Syndrome, PRS-I superactivity and some overlapping phenotypes. Causative variants for these different hemizygous phenotypes have been identified in coding exons 2 to 7. In *PRPS1* exon 1, only one variant (p.Ser16Pro) has been identified in a single family with only affected females (Figure 4B). The phenotypes associated with previously reported variants are summarised in Figure 4B.

PRPS1 codes for the enzyme PRS-I, a 318 amino acid protein that catalyses the synthesis of phosphoribosyl pyrophosphate (PRPP) from adenosine triphosphate (ATP) and ribose-5-phosphate (R5P) (Becker, 2001; Kornberg, et al., 1955). PRPP is essential for the *de novo* synthesis of purine (Hartman and Buchanan, 1958), pyrimidine (Lieberman, et al., 1955), and pyridine nucleotides (Preiss and Handler, 1958). PRS-I acts as a hexamer, which consists of three homodimers arranged in a propeller-like shape (Figure 5A) (de Brouwer, et al., 2010; Li, et al., 2007). Each of the homodimers has an active site, comprising the binding site for ATP (including the flexible loop, the PPi binding loop and the flag region) and R5P (Figure 4B), and two regulatory allosteric sites, I and II (de Brouwer, et al., 2010; Li, et al., 2007).

Three-dimensional modelling of PRS-I indicates that serine 16 is located in the first α -helix of the protein in the N-terminal domain, while the two affected arginine residues at position 196 and 214 are located in random coils (Figure 5 B, C, D). Serine 16 has been previously predicted to create a hydrogen bond with Glycine 13 using Swiss Model based on the PDB 2H06 model (Almoguera, et al., 2014). In the crystallography model, the side chain of Arginine 196 is in close proximity to the bound ATP, with a distance between the guanidinium group of the Arginine and the α -phosphate of ATP of about 7.4 Å (Li, et al., 2007). This close proximity suggests that Arginine 196 is involved in the interaction of PRS-I with the PPI, and in the stabilization of the transition state (Li, et al., 2007). The substitution of this conserved arginine with tryptophan has been previously predicted to abolish this interaction, resulting in decreased enzyme activity (Mittal, et al., 2015). This has been noted in the three-dimensional model created using Swiss Model (Arnold, et al., 2006) in which the substitution, Arg196Trp, alters the conformation of the connection loop between two β -sheets (Figure 5B). Arginine 214 forms part of the binding site of the R5P (Figure 4B) and in the three-dimensional model the substitution of arginine 214 with tryptophan leads to the loss of a hydrogen bond with cysteine 165, possibly altering the binding site for R5P (Figure 5C). The same hydrogen bond is lost with the substitution of arginine 214 to proline (Figure 5D). Therefore, all of the variants identified are predicted to either disrupt the function, or stability, of PRS-1.

4. Discussion

Here we characterise an inherited retinal dystrophy in five families in which only female members were affected, including cases with markedly asymmetrical disease on fundus examination, retinal imaging and/or functional phenotyping. Variable clinical presentation was evident in the nine affected subjects, consistent with previous reports (Al-Maawali, et al., 2015; Almoguera, et al., 2014), including variable disease onset and visual acuity, fundus

appearance, and evidence of hearing loss and/or systemic abnormality. Almoguera and colleagues reported variable onset in six affected patients with *PRPS1*-associated disorders, including initial presentation of optic atrophy, night blindness and reduced visual acuity, followed by presentation of retinitis pigmentosa, and later onset of hearing impairment and neurological abnormalities. In our 5 youngest cases (younger than 30 years at the latest examination), optic disc atrophy was observed in 4, one had night blindness and one had reduced visual acuity. Retinal atrophy was present at a wide range of ages in our cohort. The absence of macular involvement was common, with maintained foveal structure and function observed even in a patient at the age of 71 (1-II:1). Two patients with RPE disturbance at the macula maintained BCVA (2-IV:5, 3-II:1). However, one subject (4-II:1) had severely reduced macular function (as detected with pattern ERG; a 15-degree stimulus field), with maintained foveal structure and function (as was detected, and can be monitored over time, with multifocal ERG) (Almoguera, et al., 2014; Fujinami, et al., 2013). Full-field ERGs showed a similar degree of generalised cone and rod photoreceptor dysfunction or predominant or selective cone system dysfunction, and were normal in one young case (5-III:2). Overall, these observations demonstrate the importance of comprehensive assessment of both generalised retinal and macular structure and function in the diagnosis and monitoring of *PRPS1* retinopathy.

Hearing impairment was reported in three patients (20 to 71 years at the latest examination) and systemic abnormalities were found in three (17 to 45 years). Our study also suggests earlier presentation of optic atrophy and later presentation of hearing impairment and systemic abnormalities; although there was still a degree of variability and further investigation with longitudinal analysis would be needed to confirm these observations. Overall, these findings illustrate that *PRPS1*-associated disorders can be progressive and affect multiple organs, such that careful observation with systemic survey is encouraged.

NGS analysis of families with only affected females revealed an X-linked mode of inheritance and causative variants in *PRPS1*, with four missense variants (c.47C>T, p.(Ser16Phe); c.586C>T, p.(Arg196Trp); c.640C>T, p.(Arg214Trp); c.641G>C, p.(Arg214Pro)) identified in five unrelated families. The common phenotype in all cases was patchy bilateral retinal degeneration, frequently asymmetrical with RPE mottling and/or a tapetal-like reflex in most FAF images and with evidence of cone or cone and rod dysfunction in most. It is noted that in the absence of a family history, similar asymmetrical features may be confused with some acquired or inflammatory retinal disorders. A high proportion of adult obligate carriers of X-linked RP manifest fundus abnormalities, such as a tapetal reflex and full-field ERG abnormalities which are frequently asymmetrical. This may be attributed to skewed inactivation of X-chromosomes in females, whereby a higher proportion of cells express the variant, rather than wild-type allele.

In a previous report (Almoguera, et al., 2014), skewed X chromosome inactivation was experimentally tested in blood leukocytes from three patients, whose symptoms included IRD. Only one of the affected individuals showed significant asymmetric inactivation, that correlated with the severity of the phenotype. The lack of a skewed pattern of X-inactivation in two other subjects does not exclude this mechanism, as it may be tissue specific (Van den Veyver, 2001) and restricted to tissues primarily affected in this condition, i.e. the retina or the nervous system.

Interestingly, three affected individuals from families 3, 4, and 5 also presented with hearing loss, caused by different missense variants affecting the same amino acid residue, p.Arg214Trp and p. Arg214Pro. These variants occurred *de novo* in families 4 (4-II:1) and 5 (5-II:2) and are the only variants reported in this study that are associated with hearing impairment.

In our study, disease progression was confirmed in the three affected females in family 5. Hearing loss and ataxia developed in the proband in her forties and ocular manifestations of retinal changes and disc pallor were first confirmed in the two affected daughters in their twenties. A progressive phenotype has previously been reported for affected females diagnosed with CMTX5 (Almoguera, et al., 2014; Park, et al., 2013; Synofzik, et al., 2014).

The majority of reported *PRPS1* missense variants cause a reduction in PRS-I enzymatic activity (Almoguera, et al., 2014; Gandia, et al., 2015). There is considerable variation in the resulting phenotype in hemizygous males, including DFNX1, CMTX5 disease and Arts Syndrome, and the phenotypic severity is considered to be related to the degree of reduced PRS-I activity. Modest reductions in activity are associated with X-linked non-syndromic sensorineural deafness where patients have post-lingual progressive hearing loss. A moderate reduction of enzymatic activity is associated with X-linked CMTX5 where affected individuals develop hearing impairment, optic atrophy and peripheral neuropathy. More severe reductions of PRS-I activity are associated with Arts Syndrome, characterised by hearing impairment, optic atrophy, peripheral neuropathy, central neuropathy and a deficient immune response.

Severe PRS-I deficiency associated with the p.Arg196Trp variant has been reported in two male siblings with central neuropathy, including severe developmental delay and spastic quadraparesis, in addition to prenatal growth retardation and dysmorphic facial features (Al-Maawali, et al., 2015). Additional reported phenotypic features include macular coloboma-like lesions with retinal dystrophy, short stature and diabetes insipidus. Here we report two additional affected females with this variant (family 2).

PRS-I superactivity has also been implicated as the cause of disease resulting from missense variants, characterised by hyperuricemia and hyperuricosuria resulting in childhood gout.

Similar to the reduction in PRS-I enzymatic activity, PRS-I superactivity results in a wide range of phenotypes: patients with a milder phenotype have uric acid crystalluria or urinary stones as the first clinical sign, followed by gout arthritis if the uric acid level is not controlled. More severe phenotypes are characterized by early childhood gout arthritis that is also accompanied with a combination of neurodevelopment abnormalities, hypotonia, ataxia and sensorineural hearing loss (Mittal, et al., 2015).

Although most female carriers in families with *PRPS1* variants were noted to be asymptomatic (Kim, et al., 2007), some studies report that female carriers in families with Arts syndrome or CMTX5 occasionally exhibit delayed onset hearing impairment, sometimes combined with ataxia and neuropathy (Almoguera, et al., 2014; Arts, et al., 1993; de Brouwer, et al., 2010; Kim, et al., 2016; Liu, et al., 2010; Synofzik, et al., 2014). In addition, a missense variant, p.Ser16Pro, has been reported in affected females in one family as the cause of optic atrophy, RP and neurological features overlapping CMTX5 and Arts syndrome, with no affected males (Almoguera, et al., 2014). The same amino acid residue is substituted in Family 1 (p.Ser16Phe) with a similar phenotypic presentation.

While it has not been possible to experimentally confirm the PRS-I activity in our patients, the missense variant, p.Arg196Trp, in family 2 has been shown to reduce PRS-I enzymatic activity (Al-Maawali, et al., 2015). A similar decrease of enzymatic activity in females was reported for the missense variant p.Ser16Pro (Almoguera, et al., 2014), a different substitution at the same amino acid position affected in Family 1 (p.Ser16Phe). It is likely that substitution at the same amino acid position will have a similar effect on PRS-I activity. The phenotype of families 3 (p.Arg214Trp), 4 (p.Arg214Trp) and 5 (p.Arg214Pro) is consistent a decrease of PRS-I enzymatic activity.

5. Conclusions

Skewed X-inactivation and/or variable levels of PRS-I deficiency or hyperactivity most likely contribute to the phenotypic variability of disease in males and females with missense variants in *PRPS1*. We speculate that the absence of affected males in the study families, and rarity of the variants in the population, suggests that some of these variants may not be tolerated when inherited in the hemizygous state in males and could be male embryonic lethal. Indeed, of the three affected amino acid positions 16, 196 and 214 that give rise to a phenotype in females, only one has been previously reported in affected males with the most severe phenotype caused by a *PRPS1* variant; of four male children, only two survived the pregnancy and one died during childhood (Al-Maawali, et al., 2015). The phenotype included severe intellectual disability and spastic quadraparesis, macular coloboma-like lesions with retinal dystrophy, short stature and diabetes insipidus. Alternatively selective involvement of females could be indicative of male sparing rather than male lethality (Ryan, et al., 1997).

The high conservation of the protein sequence is reflected in the lack of nucleotide variability across human genomes. In the ExAC database, there are no loss of function variants and only 8 missense variants were identified in the major isoform, whereas a gene of similar size to *PRPS1* would be expected to harbour on average 6.4 loss-of-function and 76.4 missense variants. The discrepancy between observed and expected variant rate, leads to this gene having a high probability of loss-of-function intolerance (pLI= 0.87) (Lek, et al., 2016). If population variability is only considered for hemizygous variants, the number of variants drops even further with only one missense variant identified in *PRPS1* in the general population.

To date, only variants at amino acid position 16, 196 and 214 have been associated with a retinopathy. More patients need to be investigated both genetically and phenotypically before it will be possible to reach any conclusion on the specific role of these residues, or the relationship between specific domains of PRS-I and retinal function.

Patients with Arts syndrome have shown an encouraging response to a trial intervention with S-adenosylmethionine (SAM), as a substrate for the alternative purine synthesis pathway (de Brouwer, et al., 2010; Liu, et al., 2013). SAM can replenish ATP and GTP independently of PRPP by direct conversion into adenine via the polyamine pathway. In addition, methyltransferases convert SAM into S-adenosylhomocysteine, which can be transformed into adenosine and adenine by S-adenosylhomocysteine hydrolase (de Brouwer, et al., 2010). Treatment with SAM could therefore be considered for patients with *PRPS1* retinopathy to potentially slow the progression of retinal degeneration.

This report highlights the unexpected finding of retinal degeneration in females caused by missense variants in the X-linked gene *PRPS1* and expands our understanding of the phenotypic outcome of specific variants. The features of retinal degeneration include RPE mottling described as a tapetal-like reflex and intraocular asymmetry, and are similar to those seen in some female carriers of *RPGR* and *RP2* (Ebenezer, et al., 2005; Kousal, et al., 2014; Pelletier, et al., 2007; Tee, et al., 2016), and *PRPS1* should be considered in females with such generalised retinal dystrophy after exclusion of the more common causes, especially in the absence of affected males in an extended family.

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Disclosure Statement

The authors declare no conflict of interest.

Author Contributions

Conception and design: Hardcastle, Michaelides, Webster.

Analysis and interpretation: Fiorentino, Fujinami, Arno, Robson, Pontikos, Plagnol, Hayashi, Iwata, Henderson, Cheetham, Webster, Michaelides, Hardcastle.

Data collection: Armengol, Parker, Fowler, Rendon, Gardner, Fiorentino, Fujinami, Arno, Robson, Pontikos, Hayashi, Iwata, Henderson.

Obtained funding: Hardcastle, Michaelides, Webster, Cheetham, Fujinami, Hayashi, Iwata.

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Figure Legends

Figure 1. Retinal dystrophy pedigree structure and segregation of *PRPS1* variants

Pedigrees of five study families (families 1 to 5) with affected females and no affected males. An arrow indicates the proband in each family. The *PRPS1* variant identified in each family is indicated (position according to reference transcript NM_002764). Mut = *PRPS1* causative variant present, WT = wild type allele, ? indicates that the disease status of the individual is unknown.

Figure 2. Retinal imaging of an affected individual (1-II:1) of Family 1 with *PRPS1* pathogenic variant (c.47C>T p.(Ser16Phe)) and two affected individuals of Family 2 (2-III:4; 2-IV:5) with *PRPS1* pathogenic variant (c.586C>T, p.(Arg196Trp))

A fundus photograph of Subject 1-II:1 diagnosed with rod-cone dystrophy and possible neurological abnormality shows localized multiple atrophic regions with retinal pigment epithelial (RPE) disturbance, intraretinal bone spicule pigmentary deposition, attenuated retinal vessels, and pale disc in the right eye (A). Fundus autofluorescence (FAF) imaging identified multiple areas of reduced autofluorescence corresponding to areas of atrophy seen clinically and additional areas of increased signal with marked inter-ocular asymmetry (B. right eye; C. left eye). Spectral domain optical coherence tomography (SDOCT) demonstrated relative preservation of foveal lamination with loss of peripheral macular outer retinal structure (D). Fundus photographs of Subject 2-III:4 diagnosed with non-syndromic cone-rod dystrophy show asymmetrical diffuse RPE atrophic changes at the posterior pole with macular involvement (E. right eye; F. left eye). FAF imaging identified multiple areas of low signal surrounded by areas of high signal, corresponding to the retinal changes, with marked inter-ocular asymmetry (G. right eye; H. left eye). Fundus photographs of Subject 2-

IV:5 diagnosed with non-syndromic cone-rod dystrophy show asymmetrical mild disturbance of the RPE at the posterior pole with macular involvement, and mottled RPE and/or tapetal-like reflex. (I. right eye; J. left eye). FAF imaging identified areas of high signal at the posterior pole, corresponding to the retinal disturbance (K. right eye; L. left eye).

Figure 3. Retinal imaging of an affected individual of Families 3 with *PRPS1* pathogenic variant (c.640C>T, p.(Arg214Trp)) and two affected individuals of Family 5 with *PRPS1* pathogenic variant (c.641G>C, p.(Arg214Pro))

Fundus photographs of Subject 4-II:1 diagnosed with atypical Usher syndrome type II shows mild diffuse RPE disturbance temporally and pale optic discs, with mottled RPE and/or tapetal-like reflex (A. right eye; B. left eye). FAF imaging identified multiple areas of high signal, corresponding to the retinal changes (C. right eye; D. left eye). Fundus photographs of Subject 5-II:2 diagnosed with cone-rod dystrophy, hearing loss, and ataxia revealed severe atrophic changes mainly at the posterior pole (E. right eye; F. left eye). FAF imaging of Subject 5-III:2 diagnosed with cone-rod dystrophy and ataxia identified multiple areas of high signal, with inter-ocular asymmetry (G. right eye; H. left eye).

Figure 4. Schematic of *PRPS1* gene and PRS-I protein structure

(A) Multiple sequence alignment orthologues shows that the serine 16, arginine 196 and arginine 214 are highly conserved across different species. (B) Schematic of the genomic and protein structure. Representation of the positions of PRS-I missense variants identified in this study (in black below the gene structure), and previously known missense variants causing Deafness X-linked 1 (in purple), PRS-I superactivity (in green), Arts syndrome (in orange), Charcot-Marie-Tooth disease 5 (in blue). The variants previously associated with a retina

dystrophy phenotype are represented in red (only in females) and brown (syndromic male phenotype). Position according to reference transcript NM_002764.

Figure 5. Predicted effect of missense variants on the 3D PRS-I Protein Structure

(A) Model of PRS-I hexamer based on the crystal structure (PDB: 2H06). Highlighted in blue, light blue and red respectively are the domains containing amino acid position 16, 196 and 214. (B) Close-up model of amino acid position 196 with yellow/red as wild type and blue/light blue representing the p.Arg196Trp mutant. The substitution alters the conformation of the connection loop between two β -sheets as pointed out by the arrows. (C) Close-up structure model of the amino acid position 214. In yellow/red the wild type and in red/ blue the p.Arg214Trp variant. Arrows show the loss of a hydrogen bond between amino acids 214 and cysteine 165 due to the p.Arg214Trp substitution. (D) Close-up structure model of the amino acid position 196. In yellow/red the wild type and in blue/light blue the p.Arg214Pro variant. Arrows show the loss of a hydrogen bond between amino acids 214 and cysteine 165 due to the p.Arg214Pro substitution.