Delineation of the early-onset retinal dystrophy associated with steroid 5α-reductase type 3-congenital disorder of glycosylation (SRD5A3-CDG)

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Abstract word count: 345

Text word count: 2,998

Revision 1: 02-January-2017

Abstract

Importance: Steroid 5α-reductase type 3 congenital disorder of glycosylation (SRD5A3-CDG) is a rare disorder of N-linked glycosylation. The retinal phenotype is not well described and could be important for disease recognition since it appears to be a consistent primary presenting feature.

Objective: To investigate a series of patients with the same steroid 5α-reductase type 3 (*SRD5A3*) mutation thereby characterising the retinal manifestations and other associated features.

Design, setting and participants: Seven affected individuals from four unrelated families presenting with early-onset retinal dystrophy (EORD) as a primary manifestation underwent comprehensive ophthalmic assessment, including retinal imaging and electrodiagnostic (EDT) testing. Developmental and systemic findings were also recorded. Molecular genetic approaches including target-enrichment NGS, autozygosity mapping and apex microarray, were used to try and reach a diagnosis; all were mutation negative. Whole exome (WES) or whole genome sequencing (WGS) was used to identify the causative variant. Biochemical profiling was conducted to confirm a CDG Type I defect.

Main outcome measures: Detailed clinical phenotypes, genetic and biochemical results.

Results: The mean age of participants at their most recent exam was 17.1 years (SD 3.9), all were of South Asian ethnicity and 71.4% of the cohort was female. WES and WGS identified the same homozygous *SRD5A3* c.57G>A, p.(Trp19Ter) variant as the underlying cause of EORD in each family. Detailed ocular phenotyping identified early-onset (≤3 years of age) visual loss (mean BCVA = +0.95 LogMar (SD: 0.34)), childhood-onset nyctalopia, myopia (mean refractive error -6.71 (SD-4.22)) and nystagmus. Six of seven patients had learning difficulties and psychomotor delay. Fundus autofluorescence imaging and optical coherence tomography scans were abnormal in all patients, and EDT revealed rod and cone dysfunction in the five patients tested.

Conclusions and relevance: These data suggest mutations in *SRD5A3* cause EORD, a previously under-described feature of SRD5A3-CDG that is progressive and may lead to serious visual impairment. *SRD5A3* and other glycosylation disorder genes should be considered as a cause of

retinal dystrophy even where systemic features are mild. Further delineation of *SRD5A3* associated eye phenotypes can help inform genetic counselling for prognostic estimation of visual loss and disease progression.

Text

Congenital disorders of glycosylation (CDG) are a large group of neurometabolic diseases caused by impaired glycoconjugate synthesis. Type I CDGs (CDG-I), result from disruptions in the early N-linked glycosylation pathway[1]. Numerous CDG-I sub-types exist that are characterised by neurological, developmental, hepatic and coagulation abnormalities, alongside ocular, muscular, skeletal, dermatological, cardiovascular, or genitourinary involvement in some forms.[1,2] Approximately 23 different genes have been associated with this group of disorders.[1] Steroid 5α-reductase type 3 (*SRD5A3*, MIM 611715) encodes a polyprenol reductase enzyme required for the synthesis of dolichol, the end product of the mevalonate pathway.[3] Dolichol undergoes phosphorylation to produce dolichol phosphate that serves as the lipid-anchor for N-glycan biosynthesis in the endoplasmic reticulum.[3]

Biallelic mutations in *SRD5A3* cause SRD5A3-CDG (formerly known as CDG-Iq; MIM 612379), a phenotypically variable form of CDG-I that features nystagmus, optic atrophy, visual loss, muscle hypotonia, intellectual disability and cerebellar ataxia. [3,4] Biochemically, SRD5A3-CDG is characterised by a transferrin isoelectric focusing (TIEF) pattern that is typical of CDG-I.[5] Defective glycan synthesis results in altered sialotransferrin forms, detectable by charge differences and characterized by increased di- and/or asialotransferrin in cases of CDG-I.[5] Kahrizi syndrome, featuring iris coloboma, juvenile cataract, contractures, kyphosis, mental retardation, motor delay and lack of speech (MIM 612713), has also been reported in association with biallelic variants in *SRD5A3*.[6] Patients described thus far, have considerable phenotypic overlap with SRD5A3-CDG, though demonstrate a normal TIEF profile.[6,7] Unlike other CDG-I subtypes, all patients with SRD5A3-CDG develop abnormal ocular phenotypes and almost always experience early-onset visual loss, such that the ocular presentation can be an early and obvious disease-delineating feature.

Previous studies of this disorder focus on genetic findings in relation to the neurometabolic and developmental manifestations of the condition, with only one study having acknowledged a retinal abnormality.[8] Hence, the appearance, onset and progression of the SRD5A3-CDG-related retinal phenotype is poorly understood. We report detailed ocular and developmental phenotypes in seven individuals with early-onset retinal dystrophy (EORD), from four unrelated families who were found to harbour the same *SRD5A3* mutation via whole exome (WES) or whole genome sequencing (WGS).

Methods

Clinical Assessment

Study participants were ascertained from Manchester Centre for Genomic Medicine (Manchester, England), Moorfields Eye Hospital (London, England) and St James's University Hospital (Leeds, England). The Northwest Research Ethics Committee granted approval for all aspects of this study (11/NW/0421 and 15/YH/0365) and the protocol observed the tenets of the Declaration of Helsinki. Written informed consent was obtained from each study participant, or parental consent was obtained on behalf children, as an essential pre-requisite for study inclusion.

Each patient underwent full ophthalmic assessment including visual acuity and dilated fundus examination. Fundus photographs were obtained using conventional 35^{o} colour fundus photography (Topcon Great Britain, Ltd., Berkshire, UK) or Wide-field Optos™ colour fundus imaging (Optos plc, Dunfermlin, UK). Fundus autofluorescence (FAF) imaging was conducted using either the 55^{o} Spectralis (Heidelberg Engineering Ltd., Heidelberg, Germany) or ultra-widefield confocal scanning laser imaging (Optos™ plc, Dunfermlin, UK). Optical coherence tomography (OCT) was performed using the Spectralis OCT platform (Heidelberg Engineering). Five patients underwent electroretinography (ERG), three using gold foil electrodes and performed to standards specified by the International Society for Clinical Electrophysiology of Vision (ISCEV) and two using surface

electrodes.[9,10] Developmental and dysmorphology assessments were conducted by a clinical geneticist or inherited metabolic disease specialist.

Molecular Investigations

Genetic Analysis

Target-next generation sequencing (105 gene inherited retinal dystrophy panel testing and whole exome sequencing (WES)) was conducted as previously detailed by Arno *et al.* (2016).[11]

Briefly: the proband of families I and III underwent screening for a panel of 105 known inherited retinal dystrophy (IRD) genes (described in O'Sullivan et al., 2012)[12] at the Manchester Genomic Diagnostic Laboratory. Family II (GC15567) underwent SNP analysis using an Affymetrix 50k Xba SNP chip (Affymetrix Inc., Santa Clara, CA, USA) on DNA samples from the parents, one affected and two unaffected children to identify regions of homozygosity in the affected child for the prioritization of candidate genes. The proband from family IV was screened using a commercially available apex microarray for 344 published disease-causing variants in eight genes associated with Lebers congenital amaurosis (LCA) and EORD (Asper Ophthalmics, Tartu, Estonia). The proband from family I-III underwent WES as part of an ongoing study of inherited retinal disease in families without a molecular diagnosis following targeted gene panel screening (UK Inherited Retinal Disease Consortium, UKIRDC).

The affected individual and unaffected parents of family IV underwent whole genome sequencing (WGS) as part of the 100,000 Genomes Project. Briefly, genomic DNA was processed using the Illumina TruSeq DNA PCR-Free Sample Preparation kit (Illumina Inc) and sequenced using an Illumina HiSeq X Ten, generating minimum coverage of 15X for >97% of the callable autosomal genome. Reads were aligned to build GRCh37 of the human genome using the Isaac aligner (Illumina Inc). SNVs and indels were identified using Platypus v0.8.1 and annotated using Cellbase (https://github.com/opencb/cellbase). Variant filtering was performed using MAF in publicly available and in-house datasets, predicted protein impact and familial segregation. Surviving variants

were prioritized using two prespecified virtual gene panels from PanelApp (https://bioinfo.extge.co.uk/crowdsourcing/PanelApp/): Intellectual disability v1.2, which includes *SRD5A3*, and Posterior segment abnormalities v1.7. Allelic state was required to match the curated mode of inheritance for variants in panel genes.

The *SRD5A3* c.57G>A p.Trp19Ter homozygous variant (GenBank accession NM_024592) was confirmed by Sanger sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Corporation, Foster City, Ca, USA).

Biochemical Studies

Where samples were made available, Type I N-glycosylation defect was confirmed using isoelectric focussing of serum transferrin and blood coagulation studies.[5]

Results

Patient Phenotypes

The mean age of participants at their most recent exam was 17.1 years (SD 3.9), all were of South Asian ethnicity and 71.4% of the cohort was female. Phenotypes are summarized in Table 1.

Family I

Family I, originally from India, had a history of consanguinity and no prior family history of health problems.

The proband, **patient I.I**, from family I (G40001, Figure 1) was born slightly under-weight at 6lbs and was mildly jaundiced after birth. A developmental and dysmorphology assessment by a clinical geneticist found only mild developmental delay. She walked at 18 months and developed speech at the normal time. She attended mainstream school where she received assistance because of her visual problems, but was able to complete the same level of work as her peers.

At five weeks of age she was not fixing and following but was otherwise well. At the age of 5 years, ophthalmic review identified a decline in visual acuity; fundus imaging and electrophysiological testing led to a preliminary diagnosis of CSNB (Table 1 and Figure 2). At her latest visit at 20 years of age, right and left best-corrected visual acuity (BCVA) measured 1.5 Logmar (20/800 Snellen equivalent) with a mild myopic refractive error (Table 1). Fundus autofluoresence (FAF) imaging was also abnormal (Figure 2).

Patient I.II was born at term following an uneventful pregnancy and was otherwise fit and well. At five years of age, she was described as being hyperactive with an attention deficit and suffering from frequent sleep disturbances. At age 7 years, examination by a clinical geneticist diagnosed a social communication disorder, behavioural problems and learning difficulties. Dysmorphology assessment identified thick hair, thick gums, coarse facies and slender, tapered fingers.

Aged 2 months, I.II presented with multi-planar nystagmus. On examination, she was found to be mildly myopic, while fundoscopy revealed only attenuated retinal blood vessels and ERG demonstrated no recordable response in the dark (Figure 2 and Table 1). FAF at 3 years was also abnormal (Figure 2). At age 7 years, she began to report symptoms of nyctalopia.

Family II

The proband (II.I) and her affected sister (II.I) from family II (GC15567, Figure 1) were born to first cousin parents of a family originating from Gujarat, India.

Examination of **patient II.I** by a clinical geneticist found delayed motor and speech development with associated learning difficulties at young age. She was found to have variable manifest nystagmus and myopia at age 18 months. At five years of age, her BCVA measured 3/12 single Kays (0.60 logMAR equivalent; 20/80 Snellen equivalent) in the right and left (Table1). Electrodiagnostic testing at the age of 11 years identified both rod and cone system dysfunction. In her second decade she became symptomatic with nyctalopia and photophobia. Funduscopic, FAF and OCT

examinations identified multiple abnormalities indicative of retinitis pigmentosa (RP) (Table 1, Figure 2 and Figure 3).

Patient II.II was reviewed by a paediatrician aged 18 months and found to have normal muscle tone but increased, brisk reflexes and mild hyperkeratosis on the right leg. She also had developmental delay and learning difficulties with normal growth parameters, with a normal skeletal survey. She is particularly anxious and has a very short memory.

II.II was found to have pendular nystagmus and roving eye movements at 3 months of age. Electrodiagnostic testing at the age of 7 years suggested rod and cone dysfunction. By the age of 15 years her myopia increased and she was experiencing poor night vision and photophobia. Fundus, FAF and OCT examinations were abnormal and indicative of RP in the absence of pigmentary changes (Table 1, Figure 2 and Figure 3).

Family III

The affected sibling pair from family III (LDS3659, Figure 1) were born to apparently non-consanguineous parents originating from India.

Patient III.I experienced learning difficulties from a young age and was described as having a slightly 'clumsy' walking style. She was noticed to have poor visual behaviour, by her family within the first year of life. A myopic refractive error was detected at 18 months, which progressed to high myopia by the age of 16 years (Table 1). Examination of the fundus, by colour and FAF imaging, revealed abnormalities suggestive of retinal pigment epithelium (RPE) malfunction (Table 1 and Figure 2). OCT scans were corroborative of this and indicated loss of outer segment structures with complete loss of photoreceptor layer (Figure 3).

Patient III.II, when examined aged 14 years, was found to have an ataxic gait and reduced upper limb co-ordination- both signs of mild cerebellar disease. He also demonstrated global developmental delay and experienced recurrent respiratory tract infections.

He experienced loss of vision with associated roving eye movements between two and three years of age. He also presented with early-onset nyctalopia and high myopia (Table 1). Ophthalmic examination revealed abnormalities similar to those of his brother apart from a small region of photoreceptor preservation within the central macular (Table 1 and Figure 3).

Family IV

The proband (IV.I) from family IV, a male, was born to apparently non-consanguineous parents originating from India (GC15063, Figure 1). Examination of patient IV.I at 4.5 years of age identified developmental delay, learning difficulties and abnormal curvature of the spine (Table 1). Ophthalmic history revealed infantile-onset nystagmus and reduced vision. At 4.5 years, he was found to have reduced visual acuity (0.60 logMAR RE and LE) and myopia (Table 1). At his most recent examination at 24 years of age, his vision had deteriorated (Table 1) and fundus exam revealed retinal vessel attenuation and pale optic discs (Figure 1). ERG indicated both rod and cone dysfunction (Table 1) and OCT scan revealed loss of outer segments structures with relative preservation of the central macular, bilaterally (Figure 3).

Molecular Analysis

Clinically available genetic testing did not identify any potentially pathogenic variants in 105 known retinal dystrophy genes in the proband of families I and III. Autozygosity mapping and candidate gene sequencing did not identify any pathogenic variants in the proband of family II. Apex array analysis in patient IV.I was also mutation negative. Subsequent WES or WGS led to the identification of *SRD5A3* c.57G>A, p.(Trp19Ter) homozygous variant in each proband. Sanger sequencing confirmed the presence and zygosity of this variant in every affected member of each family. The

SRD5A3 p.(Trp19Ter) variant has an allele frequency of 0.001174 in 4684 controls of South Asian ethnicity, according to the ExAC dataset. In homozygous state, this same variant has been described as the cause of SRD5A3-CDG in four unrelated families.[4,8,13]

Both siblings from family III underwent screening for biochemical abnormalities that may be associated with congenital disorders of glycosylation.[5] Mild abnormalities of blood clotting (activated partial thromboplastin time (APTT) 43.6s, APTT ratio 1.4) and a microcytic hypochromic blood profile were observed in both. Liver function tests were normal, however, a CDG type I pattern of transferrin glycoforms was observed.

Discussion

Biallelic mutations in *SRD5A3* cause SRD5A3-CDG (CDG-Iq; MIM612379) a phenotypically variable disorder of N-linked glycosylation that is normally characterised by neuro-developmental abnormalities and ophthalmic manifestations.[3,4] We report seven patients from four families with a retinopathy consequent upon the SRD5A3 p.(Trp19Ter) mutation. This mutation has been reported to cause SRD5A3-CDG previously, in four unrelated families.[4,8,13] Our case series provides an in-depth description of the ocular symptomology and appearance over the course of ophthalmic follow-up. The retinopathy, unlike the extra-ocular features of this disease, appears to be slowly progressive. On fundal view, signs of retinal disease may be very subtle and bone spicules absent in young patients. Likewise, syndromic manifestations associated with mutation of *SRD5A3* may also be very mild. This detailed description of retinal phenotype could be important for early disease recognition since it appears to be a consistent primary presenting feature.Early-onset visual loss (≤ 3 years of age, mean BCVA = +0.95 LogMar (SD= 0.34)) and nystagmus are consistent manifestations associated with the SRD5A3 p.(Trp19Ter) variant in this cohort of seven patients. Other shared ocular findings were: retinal arteriolar attenuation in the absence of bone spicule formation (n=7), childhood-onset nyctalopia (n=5) and optic disc pallor (n=5). Each of the patients reported in this

series also experienced varying degrees of progressive myopia (mean refractive error -6.71 (SD= 4.22)), ranging from relatively mild to high (Table 1). None of our patients were either microphthalmic, nor did they have ocular colobomata as has been described in association with other *SRD5A3* mutations.[4] Mutual systemic associations included learning difficulties and developmental delay. One patient was found to have only mild developmental delay as a young child (<5 years of age), which may have been attributable to her severe visual impairment since she went on to meet normal developmental and intellectual milestones with increasing age.

Despite the absence of a pigmentary retinopathy, widespread loss of outer retinal structures was evidenced by OCT, with relative preservation of foveal photoreceptors, and only mild epiretinal membrane formation (Figure 3). Electroretinography, where performed (n=5), identified dysfunction in both rod and cone pathways at the level of the photoreceptor allowing discrimination from disorders involving the photoreceptor-bipolar cell synapse, such as CSNB, as three out of seven patients here initially received a clinical diagnosis of CSNB. Previous reports of patients with *SRD5A3* mutations have not described OCT findings. There has been a single description of retinal bone spicule pigmentation in an adult sibling pair with the *SRD5A3* p.(Trp19Ter) variant. Due to lack of previous descriptions of RP as a feature of SRD5A3-CDG, Kara et al., 2014 hypothesized that it may be a late onset feature of the condition.[8] Our findings suggest that the onset of retinal degeneration is likely to occur in childhood in at least a proportion of cases and indeed, ocular imaging and FAF do suggest early dysfunction of the RPE.

Rhodopsin is a pigment containing, G protein-coupled receptor that is expressed in rod photoreceptors cells where it specifically localises to the rod outer segments (ROS)[14]. Studies have shown that the N-terminus of rhodopsin contains two N-linked glycosylation sequences.[15] Mutations at glycosylated amino acid residues or surrounding glycosylation consensus sequences of rhodopsin cause autosomal dominant and sectoral RP in humans.[16,17] Studies in animal models expressing non-glycosylated rhodopsin have shown that although the mutant proteins undergo

normal biosynthesis, folding and trafficking, they confer toxicity, causing rod cell death, leading to light-sensitive retinal degeneration.[18] Evidence on whether non-glycosylated rhodopsin incorporates into and initiates disk morphogenesis in ROS is conflicting.[19,20] It is possible that the SRD5A3 p.(Trp19Ter) variant prevents normal glycosylation of rhodopsin in the retina and subsequently impairs its normal incorporation and/or function in the ROS, thereby leading to defective phototransduction and loss of vision, before eventual photoreceptor death and the presentation of RP. Similarly, non-glycosylation of other retinal proteins such as ABCA4, known to have seven N-glycosylation sites, could also lead to defective phototransduction, and eventual cell death.[21] This is an area that warrants further research.

The SRD5A3 p.(Trp19Ter) variant has a frequency of 0.0012 in the South Asian population according to the ExAC dataset- a frequency that is 30 times higher than other ethnic groups, suggesting that this is an ancestral variant within this specific population. Further, findings from our cohort suggest that phenotypic subtleties mean this condition goes unrecognised or unsuspected. Alongside recent evidence for a role of other glycosylation disorder genes in non-syndromic retinal dystrophy (POMGNT1[22] and DHDDS[23]), we suggest that CDG genes should be considered in clinical diagnostic gene panels for retinal disease.

Conclusions

This case series is the first to provide a detailed account of the retinal dystrophy consequent upon the p.(Trp19Ter) mutation in *SRD5A3*, delineating the complex phenotype associated with SRD5A3-CDG. Furthermore, we illustrate the wide variability in onset and progression of the disorder in patients with the same null mutation. We report EORD as a novel feature of SRD5A3-CDG and suggest that retinal degeneration without pigmentary change may be an early manifestation of CDG that may progress to RP over time. Crucially, our findings also suggest that *SRD5A3* may cause these ocular manifestations alongside only mild learning difficulties, in some instances, in contrast to the

neurodevelopmental delay and other systemic features usually associated with SRD5A3-CDG[3,4]. Our work adds to cumulative evidence that NGS offers a proficient means of diagnosis for this genetically heterogeneous and phenotypically variable group of conditions.[6,24,25] For CDG, precise diagnosis enables the provision of more accurate prognostic information regarding loss of vision and risk of later onset manifestations. Better understanding of the pathogenesis of *SRD5A3* mediated retinal disease could lead to the development of novel therapeutic strategies. Findings in our cohort show that the macular, although non-functional, remains structurally intact making this condition a good target for gene therapy.

Acknowledgements

Financial Sources and Role of Sponsor: This work was funded by RP Fighting Blindness and Fight for Sight (RP Genome Project GR586) and Rosetrees Trust, Fight for Sight (family II), Moorfields Eye Hospital (MEH) Special Trustees, National Institute for Health Research Biomedical Research Centre at Moorfields Eye Hospital National Health Service Foundation Trust and UCL Institute of Ophthalmology (KNK, ARW, AJH). KNK is supported by a National Institute for Health Research Rare Diseases Translational Research Collaboration (NIHR RD-TRC) fellowship award. The authors would also like to acknowledge the support of the Manchester Academic Health Science Centre and the Manchester National Institute for Health Research Biomedical Research Centre. The views expressed are those of the authors, and not necessarily those of the NHS, the NIHR or the Department of Health. Funding bodies did not have any specific role in design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

This research was made possible through access to the data and findings generated by the 100,000 Genomes Project. The 100,000 Genomes Project is managed by Genomics England Limited (a wholly owned company of the Department of Health). The 100,000 Genomes Project is funded by the

National Institute for Health Research and NHS England. The Wellcome Trust, Cancer Research UK and the Medical Research Council have also funded the research infrastructure. The authors also wish to acknowledge Genomics England and the Ophthalmology Genomics England Clinical Interpretation Partnership (GeCIP) for enabling this research. The authors would also like to thank the families for agreeing to participate in this study.

Conflict of interest: No conflicting relationship exists for any author

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Table 1: Ophthalmic and phenotypic presentations of patients with SRD5A3 p.(Trp19X) mutation

Family (gender)/I.D number	I.I (F)/G40001.1)	I.II (F)/G40001.2	II.I (F)/GC15567.1	II.II (F)/GC15567.2	III.I (F)/LDS3659.1	III.II (M)/LDS3659.2	IV.I (M)
Ethnicity	South Asian	South Asian	Indian	Indian	Pakistani	Pakistani	Indian
Age at onset	5w	2m	18m	3m	<1y	2-3y	<1y
Age at last exam	20y	13y	18.5y	14.5y	16y	14y	24y
Consanguinity	+		+			-	-
Ophthalmic findings							
Ophthalmic history	Failure to fix and follow, multi-planar nystagmus, mild myopia from 2m, nyctalopia from 6y, initial diagnosis of CSNB made at 6y	Multi-planar nystagmus, strabismus, progressive myopia from 2m, nyctalopia from 7y	Variable manifest nystagmus, squint, myopia from 18m	Nystagmus and roving eye movements from 3m, myopia, poor night vision and photophobia	Roving eye movements and nyctalopia from <1y, high myopia, exophoria decompensating into an exotropia from 16y, central scotomata	Roving eye movements from 2-3y, nyctalopia, high myopia, exophoria	Early-onset nystagmus and myopia
BCVA (Snellen equivalent) [age]	1.5 LogMar (20/640) RE and LE [20y]	1.3 LogMar (20/400) RE and LE [7y] 1.04 LogMar (20/250) RE; 1.20 LogMar (20/320) LE [13y]	0.900 crowded LogMar (20/160) RE; 0.800 (20/125) crowded LogMar LE [6y] 0.72 LogMar (20/100) RE; 0.36 LogMar (20/50) LE [18.5y]	1.0 LogMar (20/200) RE; 0.8 LogMar (20/125) LE [15y]	Data not available		0.6 LogMAR (20/80) RE and LE [4.5y] 1.0 LogMAR (20/200) RE and LE [24y]
Refractive error [age]	RE:-1.00/+0.25x90; LE: -1.25/+0.25x80 [20y]	-2.00/+1.00x100 RE; -3.00/+1.00 x 80 LE [2m] -6.00/+1.75X90 RE and -6.50/+1.00 x90 LE [3y]	-2.5/-2.5 x 180 RE; -1.5/-3.0 x 170 LE [6y] -3.00/-3.5 x 180 RE; -3.50/-4.0 x 160 LE [18.5y]	-1.5/-1.25 x 180 RE; -2.00/-2.00 x 180 LE [18m] -5.5/-3.75x155 RE; -5.5/-3.75x100 LE [15y]	-15.50/+0.25x109 RE; -14.00/+1.00x92 LE [16y]	-9.50/+1.50x103 RE, -8.25/+2.5x106 LE [14y]	RE: -7.00/-0.75 x 180; LE: -7.5DS [24y]
Fundus imaging	Optic disc pallor, foveal hypoplasia, granular appearance of peripheral retina, attenuated retinal vasculature.	Subtle temporal optic disc pallor, mildly attenuated retinal arterioles, prominent nerve fibre layer visible radiating around the superior and inferior vascular arcades. Patchy (RE) and stippled (LE) macular reflex.	Tilted optic disc with temporal pallor, peri-papillary atrophy temporally, absence of foveal reflex (LE only), attenuated retinal vasculature.	Myopic tilted discs, attenuated retinal vasculature, subtle mottling in the retinal periphery (data not shown)	Myopic tilted discs, attenuated retinal vasculature, subtle mottling in the retinal periphery (data not shown)		Optic disc pallor, attenuated retinal vasculature
FAF	Well defined ring of hyper-autofluorescence around the macula			Diffuse ring of hyper-fluorescence at the periphery of the macular with normal autofluorescence centrally apart from a hyper-autofluorescent dot at the fovea	Diffuse ring of hyper-autofluorescence around the macula	Well defined ring of hyper-autofluorescence around the macula (data not shown)	Diffuse ring of hyper-autofluorescence around the macula
OCT	Data not available		Widespread loss of outer retinal structures with relative preservation of foveal structures including photoreceptors.	Widespread loss of outer retinal structures with relative preservation of foveal structures including photoreceptors.	Widespread loss of outer retinal structures and complete absence of the photoreceptor layer	Widespread loss of outer retinal structures with relative preservation of foveal structures including photoreceptors.	Widespread loss of outer retinal structures with relative preservation of foveal structures including photoreceptors.
ERG (age at testing)	Indicative of rod-cone dystrophy (no details available) (5y)	Low amplitude light-adapted response, extinguished dark-adapted response (2m)	Undetectable rod-specific responses and delayed and subnormal cone-specific responses (11y)	Limited compliance with test but reduced and delayed cone-specific responses found with rod involvement	Data not available	Data not available	Profoundly electronegative ERG, and grossly delayed cone-specific responses
Developmental/Neurological findings	Mild developmental delay up to 5 years of age	Dysmorphic, communication and behavioural problems, learning difficulties, recurrent respiratory infections, gait ataxia.	Psychomotor delay, learning difficulties	Increased, brisk reflexes, psychomotor delay and learning difficulties	Learning difficulties, gait ataxia, normal reflexes, mild upper limb co-ordination difficulties on finger-nose test. Normal height, weight and head circumference.	Learning difficulties, developmental delay, gait ataxia, normal reflexes, mild upper limb co-ordination difficulties on finger-nose test. Recurrent respiratory infections. Normal height, weight and head circumference.	Developmental delay, learning difficulties, scoliosis
Other Investigations	Urine organic acids (normal) Plasma phytanic acid levels (normal)	Hearing assessment (normal); uMPS (normal); Oligosaccharides (normal); Lysosomal enzymes (normal); X-ray (normal); aCGH (normal)	VLCFAs (normal) Lysosomal enzymes (normal) White cell and plasma enzymes (normal)	VLCFAs (normal) Lysosomal enzymes (normal) White cell and plasma enzymes (normal)			

n.d.: not disclosed; w: weeks; m: months; y: years; RE: right eye; LE: left eye; ERG: electroretinography; OCT: optical coherence tomography; FAF: fundus autofluoresence; uMPS: urine mucoplysaccharides; + present; - absent; DS: dioptre sphere; VLCFAs: very long chain fatty acids; aCGH: array comparative genomic hybridization

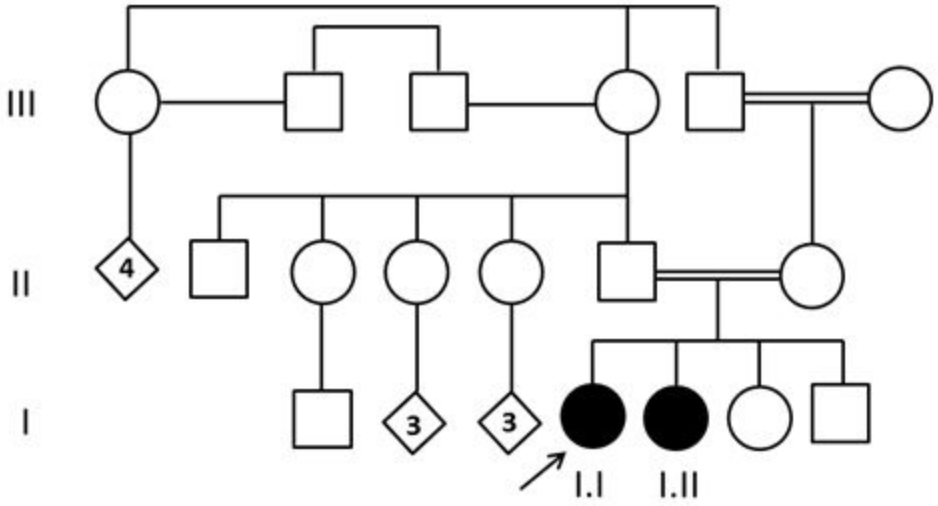
Figure Legends

Figure 1: Pedigrees of families (I-IV) included in this study. Arrows indicate proband.

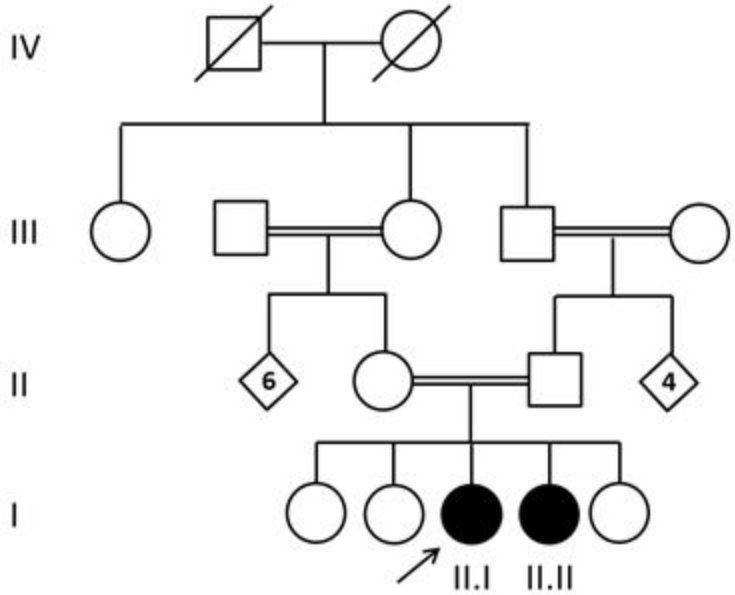
Figure 2: Colour fundus and fundus autofluorescence (FAF) images of patients with *SRD5A3* p.(Trp19Ter) variant. a,c,e,g: Wide-field Optos™ colour fundus imaging; i,k,m,o,q,s: 35° colour fundus photography; **b,d,f,h,j,l,n,p,r:** FAF imaging RE: right eye; LE: left eye; Y: years of age; RE: right eye; LE: Left eye, FAF: Fundus autofluoresence; AF: autofluorescence.

Figure 3: Optical coherence tomography (OCT) in patients with *SRD5A3* p.(Trp19Ter) variant. OCTs are shown as horizontal (**a-e, g, h**), or vertical (**f**) scans and accompanying en face infra-red image with location at which the scan through the macular was taken (indicated by green arrow). Arrow heads demarcate the transition of absent/present photoreceptors (except in c where part of the macular is not visible, and e where the photoreceptor layer is completely absent). RE: right eye; LE: left eye; Y: years of age.

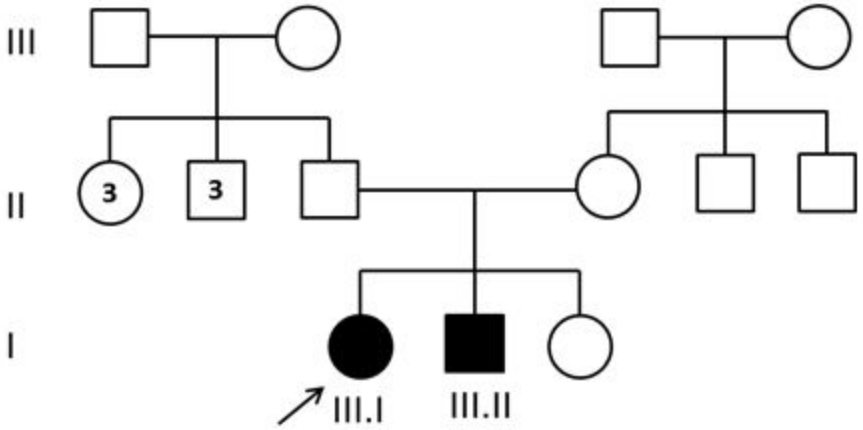
Family I G40001



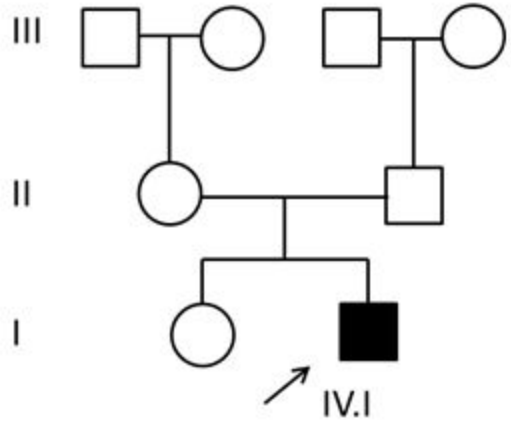
Family II G15567



Family III LDS3659



Family IV G15063



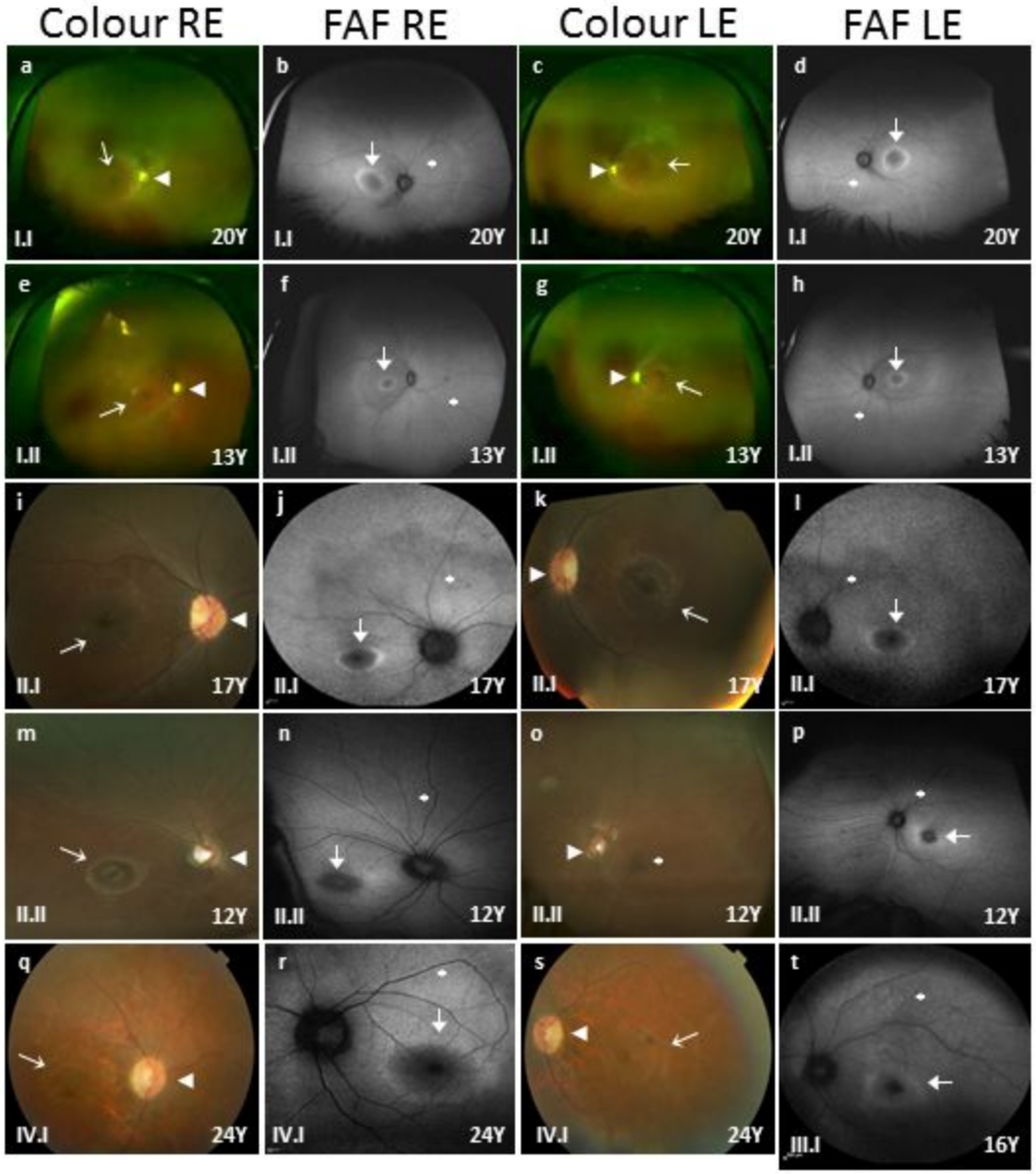

Colour RE
FAF RE
Colour LE
FAF LE
a
b
c
d
I.I
20Y
e
f
g
h
I.II
13Y
i
j
k
l
II.I
17Y
m
n
o
p
II.II
12Y
q
r
s
t
IV.I
24Y
III.I
16Y

Figure 2

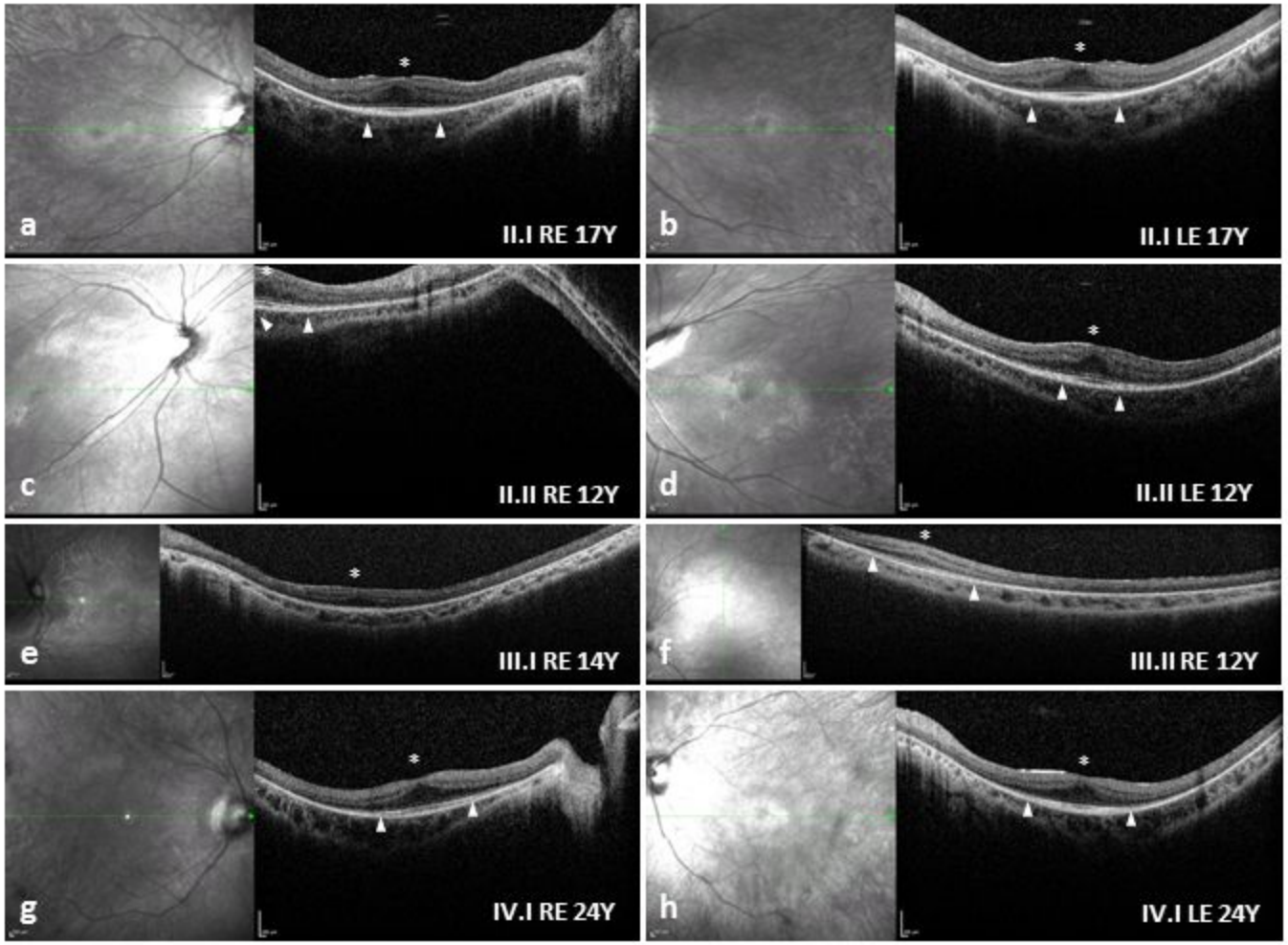

a
II.I RE 17Y
b
II.I LE 17Y
c
II.II RE 12Y
d
II.II LE 12Y
e
III.I RE 14Y
f
III.II RE 12Y
g
IV.I RE 24Y
h
IV.I LE 24Y

Figure 3