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Dual diagnosis of *UQCRFS1*-related mitochondrial complex III deficiency and recessive *GJA8*-related cataracts

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Ethics statement

This study was approved by the National Institutes of Health Institutional Review Board (IRB) (IRB# 15HG0130) and written informed consent and parental permission were obtained from all participants. Research biochemical studies of de-identified skin fibroblasts were performed at University of Colorado Anschutz Medical Campus under an IRB-approved protocol (COMIRB# 18–1828).

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Supplemental Methods

The University of Washington Center for Mendelian Genomics protocol for genome sequence realignment and variant calling methods and the method for mitochondrial-focused proteomics analysis are provided in greater detail.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rare.2024.100040.

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Abstract

Biallelic pathogenic variants in *UQCRFS1* underlie a rare form of isolated mitochondrial complex III deficiency associated with lactic acidosis and a distinctive scalp alopecia previously described in two unrelated probands. Here, we describe a participant in the Undiagnosed Diseases Network (UDN) with a dual diagnosis of two autosomal recessive disorders revealed by genome sequencing: *UQCRFS1*-related mitochondrial complex III deficiency and *GJA8*-related cataracts. Both pathogenic variants have been reported before: *UQCRFS1* (NM_006003.3:c.215–1 G>C, p.Val72_Thr81del10) in a case with mitochondrial complex III deficiency and *GJA8* (NM 005267.5:c.736 G>T, p.Glu246*) as a somatic change in aged cornea leading to decreased junctional coupling. A multi-modal approach combining enzyme assays and cellular proteomics analysis provided clear evidence of complex III respiratory chain dysfunction and low abundance of the Rieske iron-sulfur protein, validating the pathogenic effect of the *UQCRFS1* variant. This report extends the genotypic and phenotypic spectrum for these two rare disorders and highlights the utility of deep phenotyping and genomics data to achieve diagnosis and insights into rare disease.

Keywords

UQCRFS1 ; *GJA8* ; mitochondrial complex III; alopecia; cataracts; rare disease; phenotypic spectrum

1. Introduction

Retrospective and observational studies of clinical exome sequencing results estimate the prevalence of multiple genetic diagnoses in a single individual to be between 1.4 % and 7.2 % [1–3]. Patients with multiple genetic diagnoses or potentially relevant findings after clinical exome sequencing are more likely to come from consanguineous unions and have multi-system disease than patients with fewer clinical exome findings [1–4]. When more than one disorder is present in a single individual, it becomes increasingly difficult to attribute specific clinical features to rare disorders with limited phenotypic descriptions. Here, we report a male proband of consanguineous Afghan descent whose clinical and molecular investigations revealed a dual diagnosis of two homozygous recessive disorders: *UQCRFS1*-related mitochondrial complex III deficiency and *GJA8*-related cataracts. Beyond extending the phenotypic spectrum of both disorders, we highlight the value of leveraging clinical and multi-modal phenotyping to conclude complex diagnostic odysseys.

2. Materials and Methods

2.1. Patient enrollment and phenotyping

This study was approved by the National Institutes of Health Institutional Review Board (IRB) (IRB# 15HG0130) and written informed consent and parental permission were obtained from all participants. Records review and remote telemedicine assessment at age 3 years were followed by an in-person assessment at 3.8 years. Research biochemical studies of de-identified skin fibroblasts were performed at University of Colorado Anschutz Medical Campus under an IRB-approved protocol (COMIRB# 18-1828).

2.2. Genome sequencing

Blood-derived DNA from the proband, parents, and unaffected sister underwent genome sequencing. PCR-free genome sequence (GS) libraries with 450 bp average size and 150 bp paired-end reads were sequenced on the NovaSeq 6000 using the NovaSeq 5000/6000 S4 Reagent Kit (300 cycle; 20012866). FASTQs were generated following Illumina's recommended protocol and aligned to the GRCh38 human reference genome to generate sample-level BAM files. BAMs were evaluated using several quality control (QC) metrics, including average coverage > 40X, > 97.5 % coverage at > 20X, and >95 % concordance with genotype array. After QC, sample-level VCF files were generated using the Illumina DRAGEN platform's haplotype-based variant caller for single nucleotide variants (SNVs) and small insertions/deletions (indels), whereas structural variants (SVs) were called by Manta[5]. Baylor Genetics analyzed these files to provide a clinical GS report.

BAM files were reprocessed following the University of Washington Center for Mendelian Genomics (UW-CMG) GS protocol (Supplemental Methods). BAMs were re-aligned to GRCh37 to capture alignment-specific variants. SNV/indel variant calling was performed using GATK HaplotypeCaller (v3.7)[6,7] to generate a multi-sample VCF. SNV/indels were flagged using the GATK filtration walker to mark those with low quality scores, allelic imbalance, long homopolymer runs, or low quality by depth. SVs were identified using LUMPY [8], genotyped using svtyper, and a multi-sample VCF was generated using smoove (https://github.com/brentp/smoove).

2.3. Research GS analysis

The multi-sample VCFs generated by the UW-CMG were annotated with allele frequencies in reference data sets, predicted consequences, and evidence of conservation and/or pathogenicity as previously described [9]. Sample-level QC was performed using peddy (v0.4.7) [10], confirming sample identity through genetically derived sex, ancestry, and pedigree relationships and 36–42X median sequencing depth for each participant. Pedigree and genotype data were combined using GEMINI (v30.1) [11]. SNV/indels co-segregating with the phenotype were restricted to those with moderate or high impact consequences, GATK filters of "PASS" or "SBFilter", maximum alternate allele frequency (maxAAF) ≤ 0.005 across reference populations [12–15], a lower bound of the 95 % maxAAF confidence interval within UW-CMG data ≤ 0.05, depth ≥ 6, genotype quality ≥ 20, and no missing genotypes. Similar filters were applied to SVs co-segregating with the phenotype, including depth ≥ 6, genotype quality ≥ 20, and maxAAF ≤ 0.05 across reference panels [16,17] given these resources have small samples and include individuals affected by pediatric and/or Mendelian disorders. Custom queries identified all rare variants with maxAAF < 0.005 observed in the proband where: 1) the proband was homozygous for the alternative allele and the unaffected relatives were not, 2) ClinVar reported evidence of pathogenicity as of April 2021 [18], or 3) fell within or near genes underlying mitochondrial disorders or glycogen storage disorders. All variants meeting any of these criteria were evaluated by the Pacific Northwest (PNW) Undiagnosed Diseases Network (UDN) at a series of case review meetings. Variant plots were generated using Adobe Illustrator.

2.4. Functional studies

Functional studies in fibroblasts included respiratory chain enzyme activities measured spectrophotometrically (Supplemental Methods) [19,20]. The results were described as initial velocity rates for complexes I, II, II-III combined and citrate synthase, and as rate constants for complexes III and IV. Ratios of each complex activity over citrate synthase activity and over complex II activity are provided. Proband results are also described as Z-scores relative to the log-normal control distribution. The respiratory chain complexes were separated by non-denaturing blue native polyacrylamide gel electrophoresis (BN-PAGE) and activities of complexes I, II, IV and V assayed by in-gel activity staining [21,22]. Complex I assembly was assessed by a non-denaturing polyacrylamide gel electrophoresis followed by western blotting [23]. Proteomics analysis was performed with the proband sample analyzed in triplicate along with five individual in-assay controls as described [23,24] with modifications listed in Supplemental Methods. Mass spectrometry quantity (MS2) from protein groups were imported into Perseus (v2.0.5.0) [25], mitochondrial proteins were identified by MitoCarta3.0 [26], and volcano plots were generated using normalization via subtract row cluster means prior to a two-sample t-test with significance set to +/− 2 fold-change and p-value <0.05. From the mitochondrial abundance-corrected values, the Relative Complex Abundance (RCA) was calculated in R (v4.3.0) and RStudio (v2023.03.1+446) and patient values compared to those derived from a similar analysis in 14 normal controls and a patient affected by *UQCR-C2*-related mitochondrial complex III deficiency as a positive control [23,24].

3. Results

3.1. Medical history

The proband is the second child of consanguineous Afghan parents, first cousins through their maternal lines (Fig. 1). He has a healthy older sister, while two other pregnancies resulted in a 32-week stillborn male and an ectopic pregnancy. Following an unremarkable pregnancy, the proband was born at 39-weeks' gestation by cesarean section after failure to progress. Birth weight was at the 39th percentile (3210 g), length at 72nd percentile (51 cm), and occipitofrontal circumference at 22nd percentile (OFC; 33.5 cm) (US for-age-percentiles for boys from birth to 36 months). Tachypnea at age 17 hours prompted intensive care unit admission where a severe lactic acidosis was identified (lactate >20 mmol/L; reference range [ref. range] <2.4). Plasma ammonia was elevated (157 mmol/L; ref. range 30–90), while plasma amino acids revealed a markedly elevated alanine (1213 μmol/L; ref. range 205–540 μmol/L), elevated proline (596 μmol/L; ref. range 89–273), and elevated tyrosine (239 μmol/L; ref. range 27–104 μmol/L). Urine organic acids confirmed massive excretion of lactate with lesser amounts of pyruvate and 3-hydroxybutyric acid. A normal echocardiogram was also recorded.

He was discharged home at 45 days of life, feeding independently on fortified formula. Postnatal growth was restricted, in part related to a labile clinical course with repeatedly elevated plasma lactate levels at age 10 months (as high as 11.1 mmol/L; ref. range 0.7 – 2.1). Persistent feeding problems, emesis, oral aversion, and faltering growth resulted in a gastrostomy tube placement at age one year. Growth improved although emesis persisted,

frequently associated with episodic lactic acidosis and viral respiratory tract infections, requiring multiple hospitalizations. Normal levels of plasma copper, ceruloplasmin, 7-dehydrocholesterol, growth differentiation factor 15 (GDF15 = 739 pg/ml; normal <750), thyroid stimulating hormone and biotinidase activity were recorded within the first year of life. At age 13 months, his weight was at the 21st percentile (9 kg, Z-score = −0.82 SD), height was 33rd percentile (75.7 cm, Z-score = −0.45 SD), and head circumference was 4th percentile (44 cm, Z-score = −1.79 SD) (WHO growth standards for boys aged 0–2 years) Fig. 2.

Bilateral cataracts were detected at age 2 months, described as symmetric pinpoint nuclear opacities in each eye with more subtle lamellar changes (~3 mm). The lamellar changes were stable, visible on retinoscopy but were not completely opaque on slit lamp. He did not have impaired vision. An echocardiogram at age 13 months demonstrated mild left ventricular posterior wall and septal hypertrophy. An auditory brainstem response study at 20 months demonstrated bilateral mixed conductive and sensorineural hearing loss (SNHL), prompting ear tube placement and hearing aid fitting. Head MRI at age 20 months demonstrated normal brain structure.

Clinical genetic testing was non-diagnostic, including both duo BCM-MitomeNGS^{SM} mitochondrial disease panel testing of 200 nuclear and mitochondrial genes in the proband and mother and trio exome sequencing of the proband and parents conducted within the first week of life. A maternally inherited heterozygous pathogenic variant in *BTD* (c.968 A>G; p.His323Arg) associated with autosomal recessive biotinidase deficiency [MIM: 253260] and a paternally inherited heterozygous variant of uncertain significance (VUS) in *SDHB* (c.607 G>A; p. Gly203Arg) associated with autosomal recessive mitochondrial complex II deficiency [MIM: 619224] were reported in the proband. A SNP microarray study at 5 months of age identified 12 regions of homozygosity > 5 Mb, consistent with parental consanguinity (~248.86 Mb) without detection of pathological structural variants. Reanalysis of the exome data one year later was non-diagnostic.

3.2. Deep Phenotyping by the UDN

A video-telehealth examination at age 3 years showed a non-dysmorphic globally delayed male with mild limb and axial hypotonia, absent scalp hair, and sparse body hair with retained eyelashes and eyebrows. Clinical evaluation aged 3.5 years revealed expressive and receptive language levels at 6 months with guttural verbalizations and an inability to follow commands. Fine motor skills were estimated at a 10- to 12-month level and gross motor at 15-months level.

Aged 3.8 years, a head MRI was normal and magnetic resonance spectroscopy imaging demonstrated a slight deficit of N-acetyl aspartate in the midline parietal gray matter and left centrum semiovale with elevation of creatine in the superior cerebellar vermis and pons. An electromyogram and nerve conduction velocity study were normal. A sedated auditory brainstem response test confirmed bilateral SNHL. An abdominal ultrasound detected a mildly enlarged liver and right kidney. Biochemical testing of blood showed a normal acylcarnitine profile and carnitine levels, plasma amino acids, urine amino acids, and GDF15 (512 pg/ml; ref. range <750). Urine organic acids demonstrated excretion of Krebs

cycle intermediates in the presence of mild ketonuria and minimal lactic aciduria, consistent with a mitochondrial respiratory chain disorder. Parental ophthalmological evaluations were unremarkable.

3.3. Quad GS analysis

Baylor Genetics confirmed the regions of homozygosity previously detected by microarray (~253 Mb) and reported VUS co-segregating with disease in genes underlying disorders sharing phenotypic features with the proband: a homozygous likely pathogenic variant in *GJA8* (c.736 G>T, p.Glu249*), a *de novo* heterozygous VUS in *POU3F3* (c.370 G>T, p.Gly124Cys), a homozygous VUS in *ARMC9* (c.1148 C>T, p.Thr383Met), and a homozygous VUS in *GLDC* (c.334+1115 A>T). Baylor Genetics provided a separate research-tier variant table to UDN investigators noting 5 rare homozygous variants co-segregating with the phenotype which did not meet clinical reporting criteria, including variants in *ADARB1* (c.–219–14589 G>T and c.664 C>T, p.Leu222Phe), *DHPS* (c.379 G>A, p.Val127Ile), *INTS1* (c.6248 T>G, p.Phe2083Cys), and *UQCRFS1* (c.215–1 G>C). PNW UDN analysis identified no additional candidate variants. The clinical team evaluated the phenotypic overlap between the proband and each Mendelian condition associated with these strong candidates (Supplemental Table 1) and determined the homozygous variants in *UQCRFS1* (NM_006003.3:c.215–1 G>C, p. Val72_Thr81del10) and *GJA8* (NM_005267.5:c.736 G>T, p.Glu246*) were diagnostic, as described below.

3.4. Autosomal recessive UQCRSF1-related complex III deficiency

The proband is homozygous for a splice acceptor site variant in *UQCRFS1* (encoding ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1), while his unaffected relatives were heterozygous. This nucleotide is highly conserved (GERP score = 4.38 [27]) and the variant allele has evidence for pathogenicity (phred-scaled CADD v1.6 score = 32 [28]), is predicted to cause acceptor loss (SpliceAI delta score = 0.99), and was not observed in reference population databases. This variant was previously reported as pathogenic for mitochondrial complex III deficiency [MIM: 618775], characterized by lactic acidosis, hypertrophic cardiomyopathy and alopecia [29]. Clinical testing over-looked this variant because *UQCRFS1* was not included in the BCM-MitomeNGS^{SM} panel while clinical GS analysis did not recognize its effect on splicing and prior publication.

Complex III deficiency is characterized by decreased complex III enzyme activity and lactic acidosis and is frequently observed with developmental delay, encephalo-myopathy, growth deficiency, hypoglycemia, hypotonia, and ataxia (Table 1) [30,31]. SNHL is a well-established feature of complex III disorders while cataracts are much less prevalent. Gusic *et al.* (2020) demonstrated complex III deficiency in patient fibroblasts with the identical *UQCRFS1* pathogenic variant in our proband, with similar borderline low activity of complex III [29]. Direct comparison revealed our proband closely matched the two cases, except the original cases lacked cataracts or SNHL [29].

Functional studies in our proband's fibroblasts showed decreased activity of isolated complex III to 41 % of average controls, but not of the combined complex II+III activity (Table 2). The activity of citrate synthase was relatively increased, suggesting compensatory

increase in mitochondrial biogenesis, and the ratio of complex III activity to citrate synthase was decreased to 24 % of control mean. BN-PAGE with in-gel activity staining revealed no abnormalities for complexes I, II IV and V including normal assembly of complex V (Fig. 3). Tracing the assembly of complex I showed normal amounts of the holocomplex and 230 kDa intermediate but an unusual trace amount at 950 kDa of lesser intensity than for a primary complex I disorder (Fig. 3A). Label-free quantitative proteomics analyses showed a decrease in all subunits of complex III including UQCRFS1, a decrease in complex I subunits, and in SQOR abundance (Fig. 4A). When all the subunits of a complex were analyzed in RCA analysis, complex III was significantly decreased to 76 % relative to in-assay controls, complex I was borderline decreased to 85 % relative to in-assay controls, and the proband exhibited a significant increase in the mitoribosomal subunits (Fig. 4b, Table 3).

3.5. Autosomal recessive GJA8-related cataracts

It was initially unclear whether our proband's cataracts were due to the homozygous stop-gain variant in *GJA8* (c.736 G>T, p.Glu246*) or from the *UQCRSF1*-related complex III deficiency. *GJA8* encodes gap junction protein alpha 8 also known as connexin50 (Cx50) which forms gap junctions critical for lens development, supporting circulation and maintaining lens transparency [32,33]. While pathogenic missense variants in *GJA8* are most frequently associated with autosomal dominant congenital cataracts, autosomal recessive frameshifts have been reported [34–37] (Fig. 2). Biallellic *GJA8* congenital cataract patients may also manifest microcornea and/or microphthalmia [34–37], absent in our proband. While heterozygous carriers of recessive pathogenic variants may manifest slight lens opacities, these were absent in the parents. *GJA8* p.Glu246* occurs at a conserved nucleotide (GERP score = 3.48), the variant has compelling evidence of pathogenicity (phred-scaled CADD score = 35), and has been reported once in the ExAC Asian sample [38].

Like *GJA8* p.Glu246*, most recessive pathogenic variants in *GJA8* result in truncated Cx50 and functional studies indicate these can lead to mislocalization to the endoplasmic reticulum and increased intracellular degradation, decreasing the level of Cx50 localizing to the plasma membrane and active gap junctions [39,40]. *GJA8* p.Glu246* deletes most of the last intracellular domain of this multi-pass membrane protein and occurs in the second of two exons, suggesting the mRNA would be stable while the fate of the protein is uncertain. Extensive terminal deletions of Cx50, including *GJA8* p.Glu246*, are observed as somatic changes in aged cornea, decrease junctional coupling, and are consistent with the proposed mechanisms of cataract formation [41,42].

4. Discussion

The UDN is a research study that combines emerging genomic technologies and team science with the goal of identifying the cause of disease in individuals and families affected by previously undiagnosed conditions. In this case, detailed GS and molecular analyses determined our proband‘s complex phenotype was the outcome of two separate autosomal recessive conditions: mitochondrial complex III deficiency and *GJA8*-related cataracts.

Coding changes in 6 of 11 complex III components and 5 of 8 assembly factors underlie nuclear mitochondrial complex III deficiency while mutations in *MT-CYB* can also cause isolated complex III deficiency [31]. Most of these conditions are rare, described in < 3 unrelated families and many are consanguineous. Cataracts are rarely observed in mitochondrial disease [43–47] but may be under-recognized [48–50]. However, *BCS1L*-related mitochondrial complex III deficiency [MIM: 262000] includes the SNHL and scalp alopecia present in our proband [29,30,51–53], suggesting they are distinctive features for these sub-types of complex III deficiency.

Complex III catalyzes the transfer of electrons from reduced coenzyme Q to cytochrome c, thereby contributing to the proton gradient across the inner mitochondrial membrane required for ATP synthesis [54–56]. It forms a homodimeric complex and has three subunits containing the catalytically active core that involves hemes and an iron-sulfur cluster: cytochrome b, cytochrome c1, and the Rieske iron-sulfur protein (UQCRFS1). The functional characterization of complex III disorders in fibroblasts is challenging, particularly for partial deficiencies. Deficiencies are usually more pronounced in the isolated complex III enzyme activity assay when assayed as the optimized decylubiquinol:cytochrome c oxidoreductase [57] than in the combined complex II-III or the combined complex I-III assays. These combined assays cannot reliably detect pathogenic complex III defects as they yielded normal results for our proband and the previously reported patients [29], perhaps due to the low rate control that complex III exerts over them [58–60]. The substantial variability in the isolated complex III assay still only resulted in a borderline value when the proband had 41 % the control mean activity (Z-score = −2.4 SD) and 24 % of control mean for the complex III activity/citrate synthase ratio. Proteomics analysis showed a clear decrease in the abundance of complex III, further validated in the quantitative RCA analysis, and revealed secondary changes related to the decrease in complex III. Complex I physically interacts with complex III in the respiratory chain supercomplex [61], and complex III is required for the assembly of complex I by reduced maturation of the N-module [62], resulting in decreased complex I in complex III deficiency states [63]. The c.215–1 G>C variant has been shown to result in strongly reduced steady state UQCRFS1 protein level and on blue native PAGE to result in strongly reduced $CIII_2$ homodimer of complex III, which is needed for complex I assembly [27]. Indeed, a faint band at 950 kDa was present in our complex I assembly assay, compatible with accumulation of a subcomplex without the addition of the N-module, and a mildly reduced complex I abundance on RCA. This secondary reduction in complex I was not sufficient to affect the amount of the holocomplex and did not affect activity as measured by enzymatic assay or BN-PAGE. The reduction of SQOR protein was interesting, as SQOR donates electrons to the coenzyme Q pool although a physical interaction with complex III has not been described. Together, these results clearly indicate a defect in complex III stability and enzymatic function, with mild secondary effects at least on complex I formation, attributable to this *UQCRFS1* pathogenic variant. Our restricted access to patient fibroblasts limited our analyses: *GJA8* is not expressed in fibroblasts and could not be evaluated with proteomics, while muscle and liver tissue is preferred for electron microscopy assessment of mitochondrial disorders.

5. Conclusions

The proband described in this case report ultimately received a dual diagnosis of nuclear encoded complex III deficiency and *GJA8*-related cataracts only after the deep clinical and molecular phenotyping offered by the UDN. He demonstrates the underlying metabolic derangement in complex III deficiency due to *UQCRFS1* loss-of-function. Our proband shares metabolic instability with episodic lactic acidosis resulting from respiratory chain disruption, cardiomyopathy, marked developmental and growth delays, and scalp alopecia with the two previously reported cases. Alopecia in the presence of a primary mitochondrial disorder is suggestive of CIII deficiency [29,52]. Unlike the patient previously described with the same *UQCRSF1* genotype who ultimately died from progressive hypertrophic cardiomyopathy in infancy, our proband had a transitory cardiomyopathy, expanding the clinical trajectories for this disorder. Both patients bearing this pathogenic variant in *UQCRFS1* share Afghani ancestry, suggesting a founder allele [29] which physicians should consider when caring for patients with similar backgrounds presenting acutely with mitochondrial disease, especially when paired with alopecia. A multi-modal investigation of mitochondrial function combining enzyme activity assays and proteomics analysis proved an effective way to confirm the pathogenicity of this variant and its functional consequences on mitochondrial biochemistry. With respect to the discovery of recessive *GJA8*-related cataracts, our patient represents an example of a germline variant observed among somatic-acquired terminal deletions in aged cornea. Finally, these results highlight the importance of searching for multiple pathogenic variants in patients with complex phenotypes, especially for those born to consanguineous parents and with atypical features for the disease designation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marisa Friederich reports financial support was provided by CureARS. Johan Van Hove reports financial support was provided by CureARS. Elizabeth Blue reports a relationship with International Genetic Epidemiology Society that includes: board membership. Gail Jarvik reports a relationship with American Society of Human Genetics that includes: board membership and travel reimbursement. If there are other authors, they

declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The UDN shares phenotype and sequence data through dbGaP while phenotypes and candidate genes are shared through PhenomeCentral and phenotypes and candidate variants present in the ClinVar database.

Abbreviations:

BCM	Baylor College of Medicine
GS	genome sequence
Indel	insertion/deletion ≤50 bp
MaxAAF	maximum alternate allele frequency
MIM	Mendelian Inheritance in Man
MRI	magnetic resonance imaging
NGS	next-generation sequencing
PCR	polymerase chain reaction
QC	quality control
Ref	reference
SNHL	sensorineural hearing loss
SNV	single nucleotide variant
SV	structural variant
UDN	Undiagnosed Diseases Network
VCF	variant call format
VUS	variant of unknown significance

References

[1]. Posey JE, Harel T, Liu P, Rosenfeld JA, James RA, Coban Akdemir ZH, Walkiewicz M, Bi W, Xiao R, Ding Y, Xia F, Beaudet AL, Muzny DM, Gibbs RA, Boerwinkle E, Eng CM, Sutton VR, Shaw CA, Plon SE, Yang Y, Lupski JR, Resolution of Disease Phenotypes Resulting from Multilocus Genomic Variation, N. Engl. J. Med 376 (2017) 21–31, 10.1056/NEJMoa1516767. [PubMed: 27959697]

[2]. Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, Ward P, Braxton A, Wang M, Buhay C, Veeraraghavan N, Hawes A, Chiang T, Leduc M, Beuten J, Zhang J, He W, Scull J, Willis A, Landsverk M, Craigen WJ, Bekheirnia MR, Stray-Pedersen A, Liu P, Wen S, Alcaraz W, Cui H, Walkiewicz M, Reid J, Bainbridge M, Patel A, Boerwinkle E, Beaudet AL, Lupski JR, Plon

SE, Gibbs RA, Eng CM, Molecular findings among patients referred for clinical whole-exome sequencing, JAMA 312 (2014) 1870–1879, 10.1001/jama.2014.14601. [PubMed: 25326635]

[3]. Balci TB, Hartley T, Xi Y, Dyment DA, Beaulieu CL, Bernier FP, Dupuis L, Horvath GA, Mendoza-Londono R, Prasad C, Richer J, Yang XR, Armour CM, Bareke E, Fernandez BA, McMillan HJ, Lamont RE, Majewski J, Parboosingh JS, Prasad AN, Rupar CA, Schwartzentruber J, Smith AC, Tetreault M, FORGE Canada Consortium, Care4Rare Canada Consortium, Innes AM, Boycott KM, Debunking Occam's razor: Diagnosing multiple genetic diseases in families by whole-exome sequencing, Clin. Genet 92 (2017) 281–289, 10.1111/cge.12987. [PubMed: 28170084]

[4]. Smith ED, Blanco K, Sajan SA, Hunter JM, Shinde DN, Wayburn B, Rossi M, Huang J, Stevens CA, Muss C, Alcaraz W, Hagman KDF, Tang S, Radtke K, A retrospective review of multiple findings in diagnostic exome sequencing, half are Distinct. half are overlapping Diagn.. Genet Med 21 (2019) 2199–2207, 10.1038/s41436-019-0477-2. [PubMed: 30894705]

[5]. Chen X, Schulz-Trieglaff O, Shaw R, Barnes B, Schlesinger F, Kallberg M, Cox AJ, Kruglyak S, Saunders CT, Manta: rapid detection of structural variants and indels for germline and cancer sequencing applications, Bioinformatics 32 (2016) 1220–1222, 10.1093/bioinformatics/btv710. [PubMed: 26647377]

[6]. Poplin R, Ruano-Rubio V, DePristo MA, Fennell TJ, Carneiro MO, Van der Auwera GA, Kling DE, Gauthier LD, Levy-Moonshine A, Roazen D, Shakir K, Thibault J, Chandran S, Whelan C, Lek M, Gabriel S, Daly MJ, Neale B, MacArthur DG, Banks E, Scaling accurate genetic variant discovery to tens of thousands of samples, bioRxiv (2017) 201178, 10.1101/201178.

[7]. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, del Angel G, Rivas MA, Hanna M, McKenna A, Fennell TJ, Kernytsky AM, Sivachenko AY, Cibulskis K, Gabriel SB, Altshuler D, Daly MJ, A framework for variation discovery and genotyping using next-generation DNA sequencing data, Nat. Genet 43 (2011) 491–498, 10.1038/ng.806. [PubMed: 21478889]

[8]. Layer RM, Chiang C, Quinlan AR, Hall IM, LUMPY: a probabilistic framework for structural variant discovery, Genome Biol. 15 (2014) R84, 10.1186/gb-2014-15-6-r84. [PubMed: 24970577]

[9]. Kobren SN, Baldridge D, Velinder M, Krier JB, LeBlanc K, Esteves C, Pusey BN, Zuchner S, Blue E, Lee H, Huang A, Bastarache L, Bican A, Cogan J, Marwaha S, Alkelai A, Murdock DR, Liu P, Wegner DJ, Paul AJ, Undiagnosed Diseases Network, Sunyaev SR, Kohane IS, Commonalities across computational workflows for uncovering explanatory variants in undiagnosed cases, Genet Med 23 (2021) 1075–1085, 10.1038/s41436-020-01084-8. [PubMed: 33580225]

[10]. Pedersen BS, Quinlan AR, Who's Who? Detecting and Resolving Sample Anomalies in Human DNA Sequencing Studies with Peddy. Am. J. Hum. Genet 100 (2017) 406–413, 10.1016/j.ajhg.2017.01.017. [PubMed: 28190455]

[11]. Paila U, Chapman BA, Kirchner R, Quinlan AR, GEMINI: integrative exploration of genetic variation and genome annotations, PLoS Comput. Biol 9 (2013) e1003153, 10.1371/journal.pcbi.1003153. [PubMed: 23874191]

[12]. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR, A global reference for human genetic variation, Nature 526 (2015) 68–74, 10.1038/nature15393. [PubMed: 26432245]

[13]. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alfoldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, Birnbaum DP, Gauthier LD, Brand H, Solomonson M, Watts NA, Rhodes D, Singer-Berk M, England EM, Seaby EG, Kosmicki JA, Walters RK, Tashman K, Farjoun Y, Banks E, Poterba T, Wang A, Seed C, Whiffin N, Chong JX, Samocha KE, Pierce-Hoffman E, Zappala Z, O'Donnell-Luria AH, Minikel EV, Weisburd B, Lek M, Ware JS, Vittal C, Armean IM, Bergelson L, Cibulskis K, Connolly KM, Covarrubias M, Donnelly KM, Ferriera S, Gabriel S, Gentry J, Gupta N, Jeandet T, Kaplan D, Llanwarne C, Munshi R, Novod S, Petrillo N, Roazen D, Ruano-Rubio V, Saltzman A, Schleicher M, Soto J, Tibbetts D, Tolonen C, Wade G, Talkowski ME, Genome Aggregation Database Consortium, Neale BM, Daly DP, MacArthur DG, The mutational constraint spectrum quantified from variation in 141,456 humans, Nature 581 (2020) 434–443, 10.1038/s41586-020-2308-7. [PubMed: 32461654]

[14]. Taliun D, Harris DN, Kessler MD, Carlson J, Szpiech ZA, Torres R, Taliun SAG, Corvelo A, Gogarten SM, Kang HM, Pitsillides AN, LeFaive J, Lee SB, Tian X, Browning BL, Das S, Emde AK, Clarke WE, Loesch DP, Shetty AC, Blackwell TW, Smith AV, Wong Q, Liu X, Conomos MP, Bobo DM, Aguet F, Albert C, Alonso A, Ardlie KG, Arking DE, Aslibekyan S, Auer PL, Barnard J, Barr RG, Barwick L, Becker LC, Beer RL, Benjamin EJ, Bielak LF, Blangero J, Boehnke M, Bowden DW, Brody JA, Burchard EG, Cade BE, Casella JF, Chalazan B, Chasman DI, Chen YI, Cho MH, Choi SH, Chung MK, Clish CB, Correa A, Curran JE, Custer B, Darbar D, Daya M, de Andrade M, DeMeo DL, Dutcher SK, Ellinor PT, Emery LS, Eng C, Fatkin D, Fingerlin T, Forer L, Fornage M, Franceschini N, Fuchsberger C, Fullerton SM, Germer S,Gladwin MT, Gottlieb DJ, Guo X, Hall ME, He J, Heard-Costa NL, Heckbert SR, Irvin MR, Johnsen JM, Johnson AD, Kaplan R, Kardia SLR, Kelly T, Kelly S, Kenny EE, Kiel DP, Klemmer R, Konkle BA, Kooperberg C, Kottgen A, Lange LA, Lasky-Su J, Levy D, Lin X, Lin KH, Liu C, Loos RJF, Garman L, Gerszten R, Lubitz SA, Lunetta KL, Mak ACY, Manichaikul A, Manning AK, Mathias RA, McManus DD, McGarvey ST, Meigs JB, Meyers DA, Mikulla JL, Minear MA, Mitchell BD, Mohanty S, Montasser ME, Montgomery C, Morrison AC, Murabito JM, Natale A, Natarajan P, Nelson SC, North KE, O'Connell JR, Palmer ND, Pankratz N, Peloso KG, Peyser PA, Pleiness J, Post WS, Psaty BM, Rao DC, Redline S, Reiner AP, Roden D, Rotter JI, Ruczinski I, Sarnowski C, Schoenherr S, Schwartz DA, Seo JS, Seshadri S, Sheehan VA, Sheu WH, Shoemaker MB, Smith NL, Smith JA, Sotoodehnia N, Stilp AM, Tang W, Taylor KD, Telen M, Thornton TA, Tracy RP, Van Den Berg DJ, Vasan RS, Viaud-Martinez KA, Vrieze S, Weeks DE, Weir BS, Weiss ST, Weng LC, Wilier CJ, Zhang Y, Zhao X, Arnett DK, Ashley-Koch AE, Barnes KC, Boerwinkle E, Gabriel S, Gibbs R, Rice KM, Rich SS, Silverman EK, Qasba P, Gan W, NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium, Papanicolaou GJ, Nickerson DA, Browning SR, Zody MC, Zollner S, Wilson JG, Cupples LA, Laurie CC, Jaquish CE, Hernandez RD, O'Connor TD, Abecasis GR, Sequencing of 53,831 diverse genomes from the NHLBI TOPMed Program, Nature 590 (2021) 290–299, 10.1038/s41586-021-03205-y. [PubMed: 33568819]

[15]. UK10K Consortium, Walter K, Min JL, Huang J, Crooks L, Memari Y, McCarthy S, Perry JR, Xu C, Futema M, Lawson D, Iotchkova V, Schiffels S, Hendricks AE, Danecek P, Li R, Floyd J, Wain LV, Barroso I, Humphries SE, Hurles ME, Zeggini E, Barrett JC, Plagnol V, Richards JB, Greenwood CM, Timpson NG, Durbin R, Soranzo N, The UK10K project identifies rare variants in health and disease, Nature 526 (2015) 82–90, 10.1038/nature14962. [PubMed: 26367797]

[16]. Geoffroy V, Lamouche JB, Guignard T, Nicaise S, Kress A, Scheidecker S, Le Bechec A, Muller J, The AnnotSV Webserver in 2023: updated visualization and ranking, Nucleic Acids Res 51 (2023) W39–W45, 10.1093/nar/gkad426. [PubMed: 37216590]

[17]. Geoffroy V, Herenger Y, Kress A, Stoetzel C, Piton A, Dollfus H, Muller J, AnnotSV: an integrated tool for structural variations annotation, Bioinformatics 34 (2018) 3572–3574, 10.1093/bioinformatics/bty304. [PubMed: 29669011]

[18]. Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, Chitipiralla S, Gu B, Hart J, Hoffman D, Jang W, Karapetyan K, Katz K, Liu C, Maddipatla Z, Malheiro A, McDaniel K, Ovetsky M, Riley G, Zhou G, Holmes JB, Kattman BL, Maglott DR, ClinVar: improving access to variant interpretations and supporting evidence, Nucleic Acids Res 46 (2018) D1062–D1067, 10.1093/nar/gkx1153. [PubMed: 29165669]

[19]. Frazier AE, Vincent AE, Turnbull DM, Thorburn DR, Taylor RW, Assessment of mitochondrial respiratory chain enzymes in cells and tissues, Methods Cell Biol. 155 (2020) 121–156, 10.1016/bs.mcb.2019.11.007. [PubMed: 32183956]

[20]. Chatfield KC, Coughlin CR 2nd, Friederich MW, Gallagher RC, Hesselberth JR, Lovell MA, Ofman R, Swanson MA, Thomas JA, Wanders RJ, Wartchow EP, Van Hove JL, Mitochondrial energy failure in HSD10 disease is due to defective mtDNA transcript processing, Mitochondrion 21 (2015) 1–10, 10.1016/j.mito.2014.12.005. [PubMed: 25575635]

[21]. Coughlin CR 2nd, Scharer GH, Friederich MW, Yu HC, Geiger EA, Creadon-Swindell G, Collins AE, Vanlander AV, Coster RV, Powell CA, Swanson MA, Minczuk M, Van Hove JL, Shaikh TH, Mutations in the mitochondrial cysteinyl-tRNA synthase gene, CARS2, lead to a severe epileptic encephalopathy and complex movement disorder, J. Med Genet 52 (2015) 532–540, 10.1136/jmedgenet-2015-103049. [PubMed: 25787132]

[22]. Van Coster R, Smet J, George E, De Meirleir L, Seneca S, Van Hove J, Sebire G, Verhelst H, De Bleecker J, Van Vlem B, Verloo P, Leroy J, Blue native polyacrylamide gel electrophoresis: a powerful tool in diagnosis of oxidative phosphorylation defects, Pedia Res 50 (2001) 658–665, 10.1203/00006450-200111000-00020.

[23]. Friederich MW, Erdogan AJ, Coughlin CR 2nd, Elos MT, Jiang H, O'Rourke CP, Lovell MA, Wartchow E, Gowan K, Chatfield KC, Chick WS, Spector EB, Van Hove JLK, Riemer J, Mutations in the accessory subunit NDUFB10 result in isolated complex I deficiency and illustrate the critical role of intermembrane space import for complex I holoenzyme assembly, Hum. Mol. Genet 26 (2017) 702–716, 10.1093/hmg/ddw431. [PubMed: 28040730]

[24]. Tucker EJ, Baker MJ, Hock DH, Warren JT, Jaillard S, Bell KM, Sreenivasan R, Bakhshalizadeh S, Hanna CA, Caruana NJ, Wortmann SB, Rahman S, Pitceathly RDS, Donadieu J, Alimi A, Launay V, Coppo P, Christin-Maitre S, Robevska G, van den Bergen J, Kline BL, Ayers KL, Stewart PN, Stroud DA, Stojanovski D, Sinclair AH, Premature Ovarian Insufficiency in CLPB Deficiency: Transcriptomic, Proteomic and Phenotypic Insights, J. Clin. Endocrinol. Metab 107 (2022) 3328–3340, 10.1210/clinem/dgac528. [PubMed: 36074910]

[25]. Tyanova S, Cox J, Perseus: A Bioinformatics Platform for Integrative Analysis of Proteomics Data in Cancer Research, Methods Mol. Biol 1711 (2018) 133–148, 10.1007/978-1-4939-7493-1_7. [PubMed: 29344888]

[26]. Rath S, Sharma R, Gupta R, Ast T, Chan C, Durham TJ, Goodman RP, Grabarek Z, Haas ME, Hung WHW, Joshi PR, Jourdain AA, Kim SH, Kotrys AV, Lam SS, McCoy JG, Meisel JD, Miranda M, Panda A, Patgiri A, Rogers R, Sadre S, Shah H, Skinner OS, To TL, Walker MA, Wang H, Ward PS, Wengrod J, Yuan CC, Calvo SE, Mootha VK, MitoCarta3.0: an updated mitochondrial proteome now with sub-organelle localization and pathway annotations, Nucleic Acids Res 49 (2021) D1541–D1547, 10.1093/nar/gkaa1011. [PubMed: 33174596]

[27]. Davydov EV, Goode DL, Sirota M, Cooper GM, Sidow A, Batzoglou S, Identifying a high fraction of the human genome to be under selective constraint using GERP++, PLoS Comput. Biol 6 (2010) e1001025 10.1371/journal.pcbi.1001025. [PubMed: 21152010]

[28]. Rentzsch P, Schubach M, Shendure J, Kircher M, CADD-Splice-improving genome-wide variant effect prediction using deep learning-derived splice scores, Genome Med 13 (2021) 31, 10.1186/s13073-021-00835-9. [PubMed: 33618777]

[29]. Gusic M, Schottmann G, Feichtinger RG, Du C, Scholz C, Wagner M, Mayr JA,Lee CY, Yepez VA, Lorenz N, Morales-Gonzalez S, Panneman DM, Rotig A, Rodenburg RJT, Wortmann SB, Prokisch H, Schuelke M, Bi-Allelic UQCRFS1 Variants Are Associated with Mitochondrial Complex III Deficiency, Cardiomyopathy, and Alopecia Totalis, Am. J. Hum. Genet 106 (2020) 102–111, 10.1016/j.ajhg.2019.12.005. [PubMed: 31883641]

[30]. Benit P, Lebon S, Rustin P, Respiratory-chain diseases related to complex III deficiency, Biochim Biophys. Acta 1793 (2009) 181–185, 10.1016/j.bbamcr.2008.06.004. [PubMed: 18601960]

[31]. Banerjee R, Purhonen J, Kallijarvi J, The mitochondrial coenzyme Q junction and complex III: biochemistry and pathophysiology, FEBS J. 289 (2022) 6936–6958, 10.1111/febs.16164. [PubMed: 34428349]

[32]. Berthoud VM, Gao J, Minogue PJ, Jara O, Mathias RT, Beyer EC, Connexin Mutants Compromise the Lens Circulation and Cause Cataracts through Biomineralization, Int J. Mol. Sci 21 (2020), 10.3390/ijms21165822.

[33]. Sellitto C, Li L, White TW, Connexin50 is essential for normal postnatal lens cell proliferation, Invest Ophthalmol. Vis. Sci 45 (2004) 3196–3202, 10.1167/iovs.04-0194. [PubMed: 15326140]

[34]. Ma AS, Grigg JR, Ho G, Prokudin I, Farnsworth E, Holman K, Cheng A, Billson FA, Martin F, Fraser C, Mowat D, Smith J, Christodoulou J, Flaherty M, Bennetts B, Jamieson RV, Sporadic and Familial Congenital Cataracts: Mutational Spectrum and New Diagnoses Using Next-Generation Sequencing, Hum. Mutat 37 (2016) 371–384, 10.1002/humu.22948. [PubMed: 26694549]

[35]. Ponnam SP, Ramesha K, Matalia J, Tejwani S, Ramamurthy B, Kannabiran C, Mutational screening of Indian families with hereditary congenital cataract, Mol. Vis 19 (2013) 1141–1148 (https://doi.org/). [PubMed: 23734083]

[36]. Ponnam SP, Ramesha K, Tejwani S, Ramamurthy B, Kannabiran C, Mutation of the gap junction protein alpha 8 (GJA8) gene causes autosomal recessive cataract, J. Med Genet 44 (2007) e85, 10.1136/jmg.2007.050138. [PubMed: 17601931]

[37]. Schmidt W, Klopp N, Illig T, Graw J, A novel GJA8 mutation causing a recessive triangular cataract, Mol. Vis 14 (2008) 851'856 (https://doi.org/). [PubMed: 18483562]

[38]. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG, Exome Aggregation Consortium, Analysis of protein-coding genetic variation in 60,706 humans, Nature 536 (2016) 285–291, 10.1038/nature19057. [PubMed: 27535533]

[39]. Minogue PJ, Beyer EC, Berthoud VM, A connexin50 mutant, CX50fs, that causes cataracts is unstable, but is rescued by a proteasomal inhibitor, J. Biol. Chem 288 (2013) 20427–20434, 10.1074/jbc.M113.452847. [PubMed: 23720739]

[40]. Somaraju Chalasani ML, Muppirala M, G.P SP, Kannabiran C, Swarup G, A cataract-causing connexin 50 mutant is mislocalized to the ER due to loss of the fourth transmembrane domain and cytoplasmic domain, FEBS Open Bio 3 (2013) 22–29, 10.1016/j.fob.2012.11.005.

[41]. Chai Z, Goodenough DA, Paul DL, Cx50 requires an intact PDZ-binding motif and ZO-1 for the formation of functional intercellular channels, Mol. Biol. Cell 22 (2011) 4503–4512, 10.1091/mbc.Ell-05-0438. [PubMed: 21965293]

[42]. Slavi N, Wang Z, Harvey L, Schey KL, Srinivas M, Identification and Functional Assessment of Age-Dependent Truncations to Cx46 and Cx50 in the Human Lens, Invest Ophthalmol. Vis. Sci 57 (2016) 5714–5722, 10.1167/iovs.16-19698. [PubMed: 27787559]

[43]. Fnon NF, Hassan HH, Ali HM, Sobh ZK, Sengers syndrome: a rare case of cardiomyopathy combined with congenital cataracts in an infant: post-mortem case report, Cardiovasc Pathol. 54 (2021) 107371, 10.1016/j.carpath.2021.107371. [PubMed: 34274418]

[44]. Zhao T, Goedhart CM, Sam PN, Sabouny R, Lingrell S, Cornish AJ, Lamont RE, Bernier FP, Sinasac D, Parboosingh JS, Care4Rare Canada Consortium0, J.E. Vance, Claypool SM, Innes AM, Shutt TE, PISD is a mitochondrial disease gene causing skeletal dysplasia, cataracts, and white matter changes, Life Sci. Alliance 2 (2019), 10.26508/lsa.201900353.

[45]. Wortmann SB, Zietkiewicz S, Kousi M, Szklarczyk R, Haack TB, Gersting SW, Muntau AC, Rakovic A, Renkema GH, Rodenburg RJ, Strom TM, Meitinger T, Rubio-Gozalbo ME, Chrusciel E, Distelmaier F, Golzio C, Jansen JH, van Karnebeek C, Lillquist Y, Lucke T, Ounap K, Zordania R, Yaplito-Lee J, van Bokhoven H, Spelbrink JN, Vaz FM, Pras-Raves M, Ploski R, Pronicka E, Klein C, Willemsen MA, de Brouwer AP, Prokisch H, Katsanis N, Wevers RA, CLPB mutations cause 3-methylglutaconic aciduria, progressive brain atrophy, intellectual disability, congenital neutropenia, cataracts, movement disorder, Am. J. Hum. Genet 96 (2015) 245–257, 10.1016/j.ajhg.2014.12.013. [PubMed: 25597510]

[46]. Schwartzentruber J, Buhas D, Majewski J, Sasarman F, Papillon-Cavanagh S, Thiffault I, Sheldon KM, Massicotte C, Patry L, Simon M, Zare AS, McKernan KJ, Consortium FC, Michaud J, Boles RG, Deal CL, Desilets V, Shoubridge EA, Samuels ME, Mutation in the nuclear-encoded mitochondrial isoleucyl-tRNA synthetase IARS2 in patients with cataracts, growth hormone deficiency with short stature, partial sensorineural deafness, and peripheral neuropathy or with Leigh syndrome, Hum. Mutat 35 (2014) 1285–1289, 10.1002/humu.22629. [PubMed: 25130867]

[47]. Di Fonzo A, Ronchi D, Lodi T, Fassone E, Tigano M, Lamperti C, Corti S, Bordoni A, Fortunato F, Nizzardo M, Napoli L, Donadoni C, Salani S, Saladino F, Moggio M, Bresolin N, Ferrero I, Comi GP, The mitochondrial disulfide relay system protein GFER is mutated in

autosomal-recessive myopathy with cataract and combined respiratory-chain deficiency, Am. J. Hum. Genet 84 (2009) 594–604, 10.1016/j.ajhg.2009.04.004. [PubMed: 19409522]

[48]. McElnea EM, Pick ZS, Smyth AC, Stevenson LJ, McKelvie PA, Loughnan MS, McNab AA, Ptosis, ophthalmoplegia and corneal endothelial disease - ocular manifestations of mitochondrial disease, Am. J. Ophthalmol. Case Rep 22 (2021) 101073, 10.1016/j.ajoc.2021.101073. [PubMed: 33869891]

[49]. Shurman DL, Glazewski L, Gumpert A, Zieske JD, Richard G, In vivo and in vitro expression of connexins in the human corneal epithelium, Invest Ophthalmol. Vis. Sci 46 (2005) 1957–1965, 10.1167/iovs.04-1364. [PubMed: 15914609]

[50]. Ma AS, Grigg JR, Prokudin I, Flaherty M, Bennetts B, Jamieson RV, New mutations in GJA8 expand the phenotype to include total sclerocornea, Clin. Genet 93 (2018) 155–159, 10.1111/cge.13045. [PubMed: 28455998]

[51]. Suthammarak W, Morgan PG, Sedensky MM, Mutations in mitochondrial complex III uniquely affect complex I in Caenorhabditis elegans, J. Biol. Chem 285 (2010) 40724–40731, 10.1074/jbc.M110.159608. [PubMed: 20971856]

[52]. Hinson JT, Fantin VR, Schonberger J, Breivik N, Siem G, McDonough B, Sharma P, Keogh I, Godinho R, Santos F, Esparza A, Nicolau Y, Selvaag E, Cohen BH, Hoppel CL, Tranebjaerg L, Eavey RD, Seidman JG, Seidman CE, Missense mutations in the BCS1L gene as a cause of the Bjornstad syndrome, N. Engl. J. Med 356 (2007) 809–819, 10.1056/NEJMoa055262. [PubMed: 17314340]

[53]. Chinnery PF, Elliott C, Green GR, Rees A, Coulthard A, Turnbull DM, Griffiths TD, The spectrum of hearing loss due to mitochondrial DNA defects, Brain 123 (Pt 1) (2000) 82–92, 10.1093/brain/123.l.82. [PubMed: 10611123]

[54]. Crofts AR, Holland JT, Victoria D, Kolling DR, Dikanov SA, Gilbreth R, Lhee S, Kuras R, Kuras MG, The Q-cycle reviewed: How well does a monomeric mechanism of the bc(1) complex account for the function of a dimeric complex? Biochim Biophys Acta 1777 (2008) 1001–1019, 10.1016/j.bbabio.2008.04.037. [PubMed: 18501698]

[55]. Xia D, Esser L, Tang WK, Zhou F, Zhou Y, Yu L, Yu CA, Structural analysis of cytochrome bc1 complexes: implications to the mechanism of function, Biochim Biophys. Acta 1827 (2013) 1278–1294, 10.1016/j.bbabio.2012.11.008. [PubMed: 23201476]

[56]. Vercellino I, Sazanov LA, The assembly, regulation and function of the mitochondrial respiratory chain, Nat. Rev. Mol. Cell Biol 23 (2022) 141–161, 10.1038/s41580-021-00415-0. [PubMed: 34621061]

[57]. Chretien D, Slama A, Briere JJ, Munnich A, Rotig A, Rustin P, Revisiting pitfalls, problems and tentative solutions for assaying mitochondrial respiratory chain complex III in human samples, Curr. Med Chem 11 (2004) 233–239, 10.2174/0929867043456151. [PubMed: 14754419]

[58]. Taylor RW, Birch-Machin MA, Bartlett K, Lowerson SA, Turnbull DM, The control of mitochondrial oxidations by complex III in rat muscle and liver mitochondria. Implications for our understanding of mitochondrial cytopathies in man, J. Biol. Chem 269 (1994) 3523–3528 (https://doi.org/). [PubMed: 8106394]

[59]. Taylor RW, Birch-Machin MA, Bartlett K, Turnbull DM, Succinate-cytochrome c reductase: assessment of its value in the investigation of defects of the respiratory chain, Biochim Biophys. Acta 1181 (1993) 261–265, 10.1016/0925-4439(93)90030-5. [PubMed: 8391327]

[60]. Birch-Machin MA, Turnbull DM, Assaying mitochondrial respiratory complex activity in mitochondria isolated from human cells and tissues, Methods Cell Biol. 65 (2001) 97–117, 10.1016/s0091-679x(01)65006-4. [PubMed: 11381612]

[61]. Letts JA, Fiedorczuk K, Degliesposti G, Skehel M, Sazanov LA, Structures of Respiratory Supercomplex I+III(2) Reveal Functional and Conformational Crosstalk., e6, Mol. Cell 75 (2019) 1131–1146, 10.1016/j.molcel.2019.07.022. [PubMed: 31492636]

[62]. Protasoni M, Perez-Perez R, Lobo-Jarne T, Harbour ME, Ding S, Penas A, Diaz F, Moraes CT, Fearnley IM, Zeviani M, Ugalde C, Fernandez-Vizarra E, Respiratory supercomplexes act as a platform for complex III-mediated maturation of human mitochondrial complexes I and IV, EMBO J. 39 (2020) e102817, 10.15252/embj.2019102817. [PubMed: 31912925]

[63]. Acin-Perez R, Bayona-Bafaluy MP, Fernandez-Silva P, Moreno-Loshuertos R, Perez-Martos A, Bruno C, Moraes CT, Enriquez JA, Respiratory complex III is required to maintain complex I in mammalian mitochondria, Mol. Cell 13 (2004) 805–815, 10.1016/sl097-2765(04)00124-8. [PubMed: 15053874]

[64]. Hikmat O, Isohanni P, Keshavan N, Ferla MP, Fassone E, Abbott MA, Bellusci M, Darin N, Dimmock D, Ghezzi D, Houlden H, Invernizzi F, Kamarus Jaman NB, Kurian MA, Morava E, Naess K, Ortigoza-Escobar JD, Parikh S, Pennisi A, Barcia G, Tylleskar KB, Brackman D, Wortmann SB, Taylor JC, Bindoff LA, Fellman V, Rahman S, Expanding the phenotypic spectrum of BCS1L-related mitochondrial disease, Ann. Clin. Transl. Neurol 8 (2021) 2155–2165, 10.1002/acn3.51470. [PubMed: 34662929]

[65]. Kotarsky H, Karikoski R, Morgelin M, Marjavaara S, Bergman P, Zhang DL, Smet J, van Coster R, Fellman V, Characterization of complex III deficiency and liver dysfunction in GRACILE syndrome caused by a BCS1L mutation, Mitochondrion 10 (2010) 497–509, 10.1016/j.mito.2010.05.009. [PubMed: 20580947]

[66]. Baker RA, Priestley JRC, Wilstermann AM, Reese KJ, Mark PR, Clinical spectrum of BCS1L Mitopathies and their underlying structural relationships, Am. J. Med Genet A 179 (2019) 373–380, 10.1002/ajmg.a.61019. [PubMed: 30582773]

[67]. Kanako KI, Sakakibara N, Murayama K, Nagatani K, Murata S, Otake A, Koga Y, Suzuki H, Uehara T, Kosaki K, Yoshiura KI, Mishima H, Ichimiya Y, Mushimoto Y, Horinouchi T, Nagano C, Yamamura T, Iijima K, Nozu K, BCS1L mutations produce Fanconi syndrome with developmental disability, J. Hum. Genet 67 (2022) 143–148, 10.1038/sl0038-021-00984-0. [PubMed: 34650211]

[68]. Gaignard P, Menezes M, Schiff M, Bayot A, Rak M, Ogier de Baulny H, Su CH, Gilleron M, Lombes A, Abida H, Tzagoloff A, Riley L, Cooper ST, Mina K, Sivadorai P, Davis MR, Allcock RJ, Kresoje N, Laing NG, Thorburn DR, Slama A, Christodoulou J, Rustin P, Mutations in CYC1, encoding cytochrome c1 subunit of respiratory chain complex III, cause insulin-responsive hyperglycemia, Am. J. Hum. Genet 93 (2013) 384–389, 10.1016/j.ajhg.2013.06.015. [PubMed: 23910460]

[69]. Anastasio N, Tarailo-Graovac M, Al-Khalifah R, Legault L, Drogemoller B, Ross CJ, Wasserman WW, van Karnebeek C, Buhas D, Mitochondrial Complex III Deficiency with Ketoacidosis and Hyperglycemia Mimicking Neonatal Diabetes, JIMD Rep. 31 (2017) 57–62, 10.1007/8904_2016_557. [PubMed: 27074787]

[70]. Alfattal R, Alfarhan M, Algaith AM, Albash B, Elshafie RM, Alshammari A, Alahmad A, Dashti F, Alsafi R, Alsharhan H, LYRM7-associated mitochondrial complex III deficiency with non-cavitating leukoencephalopathy and stroke-like episodes, Am. J. Med Genet A 191 (2023) 1401–1411, 10.1002/ajmg.a.63143. [PubMed: 36757047]

[71]. Koch J, Freisinger P, Feichtinger RG, Zimmermann FA, Rauscher C, Wagentristl HP, Konstantopoulou V, Seidl R, Haack TB, Prokisch H, Ahting U, Sperl W, Mayr JA, Maier EM, Mutations in TTC19: expanding the molecular, clinical and biochemical phenotype, Orphanet J. Rare Dis 10 (2015) 40, 10.1186/S13023-015-0254-5. [PubMed: 25887401]

[72]. Ardissone A, Granata T, Legati A, Diodato D, Melchionda L, Lamantea E, Garavaglia B, Ghezzi D, Moroni I, Mitochondrial Complex III Deficiency Caused by TTC19 Defects: Report of a Novel Mutation and Review of Literature, JIMD Rep. 22 (2015) 115–120, 10.1007/8904_2015_419. [PubMed: 25772319]

[73]. Wang Y, Fukuda T, Isaji T, Lu J, Gu W, Lee HH, Ohkubo Y, Kamada Y, Taniguchi N, Miyoshi E, Gu J, Loss of alpha1,6-fucosyltransferase suppressed liver regeneration: implication of core fucose in the regulation of growth factor receptor-mediated cellular signaling, Sci. Rep 5 (2015) 8264, 10.1038/srep08264. [PubMed: 25652335]

[74]. Mordaunt DA, Jolley A, Balasubramaniam S, Thorburn DR, Mountford HS, Compton AG, Nicholl J, Manton N, Clark D, Bratkovic D, Friend K, Yu S, Phenotypic variation of TTC19-deficient mitochondrial complex III deficiency: a case report and literature review, Am. J. Med Genet A 167 (6) (2015) 1330, 10.1002/ajmg.a.36968. [PubMed: 25899669]

[75]. Conboy E, Selcen D, Brodsky M, Gavrilova R, Ho ML, Novel Homozygous Variant in TTC19 Causing Mitochondrial Complex III Deficiency with Recurrent Stroke-Like Episodes: Expanding the Phenotype, Semin Pedia Neurol. 26 (2018) 16–20, 10.1016/j.spen.2018.04.003.

[76]. Habibzadeh P, Inaloo S, Silawi M, Dastsooz H, Farazi Fard MA, Sadeghipour F, Faghihi Z, Rezaeian M, Yavarian M, Bohm J, Faghihi MA, A Novel TTC19 Mutation in a Patient With Neurological, Psychological, and Gastrointestinal Impairment, Front Neurol. 10 (2019) 944, 10.3389/fneu.2019.00944. [PubMed: 31551910]

[77]. Mahale RR, Arunachal G, Gautam J, Dutta D, Kovoor J, Mailankody P, Padmanabha H, Mathuranath PS, Parkinsonism, Olivary Hypertrophy and Cerebellar Atrophy with TTC19 Gene Mutation, Ann. Indian Acad. Neurol 24 (2021) 991–993, 10.4103/aian.AIAN_625_20. [PubMed: 35359541]

[78]. Xuan X, Ruan J, Wu C, Gao Y, Li L, Lei X, A TTC19 mutation associated with progressive movement disorders and peripheral neuropathy: Case report and systematic review, CNS Neurosci. Ther. 30 (2024) e14425, 10.1111/cns.l4425. [PubMed: 37927170]

[79]. Khan H, Harripaul R, Mikhailov A, Herzi S, Bowers S, Ayub M, Shabbir MI, Vincent JB, Biallelic variants identified in 36 Pakistani families and trios with autism spectrum disorder, Sci. Rep 14 (2024) 9230, 10.1038/s41598-024-57942-x. [PubMed: 38649688]

[80]. Tucker EJ, Wanschers BF, Szklarczyk R, Mountford HS, Wijeyeratne XW, van den Brand MA, Leenders AM, Rodenburg RJ, Reljic B, Compton AG, Frazier AE, Bruno DL, Christodoulou J, Endo H, Ryan MT, Nijtmans LG, Huynen MA, Thorburn DR, Mutations in the UQCC1-interacting protein, UQCC2, cause human complex III deficiency associated with perturbed cytochrome b protein expression, PLoS Genet 9 (2013) e1004034, 10.1371/journal.pgen.1004034. [PubMed: 24385928]

[81]. Feichtinger RG, Brunner-Krainz M, Alhaddad B, Wortmann SB, Kovacs-Nagy R, Stojakovic T, Erwa W, Resch B, Windischhofer W, Verheyen S, Uhrig S, Windpassinger C, Locker F, Makowski C, Strom TM, Meitinger T, Prokisch H, Sperl W, Haack TB, Mayr JA, Combined Respiratory Chain Deficiency and UQCC2 Mutations in Neonatal Encephalomyopathy: Defective Supercomplex Assembly in Complex III Deficiencies, Oxid. Med Cell Longev 2017 (2017) 7202589, 10.1155/2017/7202589. [PubMed: 28804536]

[82]. Wanschers BF, Szklarczyk R, van den Brand MA, Jonckheere A, Suijskens J, Smeets R, Rodenburg RJ, Stephan K, Helland IB, Elkamil A, Rootwelt T, Ott M, van den Heuvel L, Nijtmans LG, Huynen MA, A mutation in the human CBP4 ortholog UQCC3 impairs complex III assembly, activity and cytochrome b stability, Hum. Mol. Genet 23 (2014) 6356–6365, 10.1093/hmg/ddu357. [PubMed: 25008109]

[83]. Haut S, Brivet M, Touati G, Rustin P, Lebon S, Garcia-Cazorla A, Saudubray JM, Boutron A, Legrand A, Slama A, A deletion in the human QP-C gene causes a complex III deficiency resulting in hypoglycaemia and lactic acidosis, Hum. Genet 113 (2003) 118–122, 10.1007/s00439-003-0946-0. [PubMed: 12709789]

[84]. Zhang T, Hong F, Qian G, Tong F, Zhou X, Huang X, Yang R, Huang X, Analysis of UQCRB gene mutation in a child with mitochondrial complex III deficiency], Zhonghua Yi Xue Yi Chuan Xue Za Zhi 34 (2017) 382–386, 10.3760/cma.j.issn.l003-9406.2017.03.015. [PubMed: 28604960]

[85]. Koc Yekeduz M, Oncul U, Rose E, Ezgu F, Eminoglu FT, A Patient with Recurrent Severe Hypoglycemic Attacks and Mitochondrial Complex III Deficiency, Nuclear Type 3: a Novel UQCRB Variant, Mol. Syndr 13 (2022) 64–68, 10.1159/000517761.

[86]. Burska D, Stiburek L, Krizova J, Vanisova M, Martinek V, Sladkova J, Zamecnik J, Honzik T, Zeman J, Hansikova H, Tesarova M, Homozygous missense mutation in UQCRC2 associated with severe encephalomyopathy, mitochondrial complex III assembly defect and activation of mitochondrial protein quality control, Biochim Biophys. Acta Mol. Basis Dis 1867 (2021) 166147, 10.1016/j.bbadis.2021.166147. [PubMed: 33865955]

[87]. Miyake N, Yano S, Sakai C, Hatakeyama H, Matsushima Y, Shiina M, Watanabe Y, Bartley J, Abdenur JE, Wang RY, Chang R, Tsurusaki Y, Doi H, Nakashima M, Saitsu H, Ogata K, Goto Y, Matsumoto N, Mitochondrial complex III deficiency caused by a homozygous UQCRC2 mutation presenting with neonatal-onset recurrent metabolic decompensation, Hum. Mutat 34 (2013) 446–452, 10.1002/humu.22257. [PubMed: 23281071]

[88]. Bansept C, Gaignard P, Lebigot E, Eyer D, Delplancq G, Hoebeke C, Mazodier K, Ledoyen A, Rouzier C, Fragaki K, Ait-El-Mkadem Saadi S, Philippe C, Bruel AL, Faivre L, Feillet

F, Abi Warde MT, UQCRC2-related mitochondrial complex III deficiency, about 7 patients, Mitochondrion 68 (2023) 138–144, 10.1016/j.mito.2022.12.001. [PubMed: 36509339]

[89]. Vidali S, Gerlini R, Thompson K, Urquhart JE, Meisterknecht J, Aguilar-Pimentel JA, Amarie OV, Becker L, Breen C, Calzada-Wack J, Chhabra NF, Cho YL, da Silva-Buttkus P, Feichtinger RG, Gampe K, Garrett L, Hoefig KP, Holter SM, Jameson E, Klein-Rodewald T, Leuchtenberger S, Marschall S, Mayer-Kuckuk P, Miller G, Oestereicher MA, Pfannes K, Rathkolb B, Rozman J, Sanders C, Spielmann N, Stoeger C, Szibor M, Treise I, Walter JH, Wurst W, Mayr JA, Fuchs H, Gartner U, Wittig I, Taylor RW, Newman WG, Prokisch H, Gailus-Durner V, Hrabe de Angelis M, Characterising a homozygous two-exon deletion in UQCRH: comparing human and mouse phenotypes, EMBO Mol. Med 13 (2021) e14397, 10.15252/emmm.202114397. [PubMed: 34750991]

[90]. Barel O, Shorer Z, Flusser H, Ofir R, Narkis G, Finer G, Shalev H, Nasasra A, Saada A, Birk OS, Mitochondrial complex III deficiency associated with a homozygous mutation in UQCRQ, Am. J. Hum. Genet 82 (2008) 1211–1216, 10.1016/j.ajhg.2008.03.020. [PubMed: 18439546]

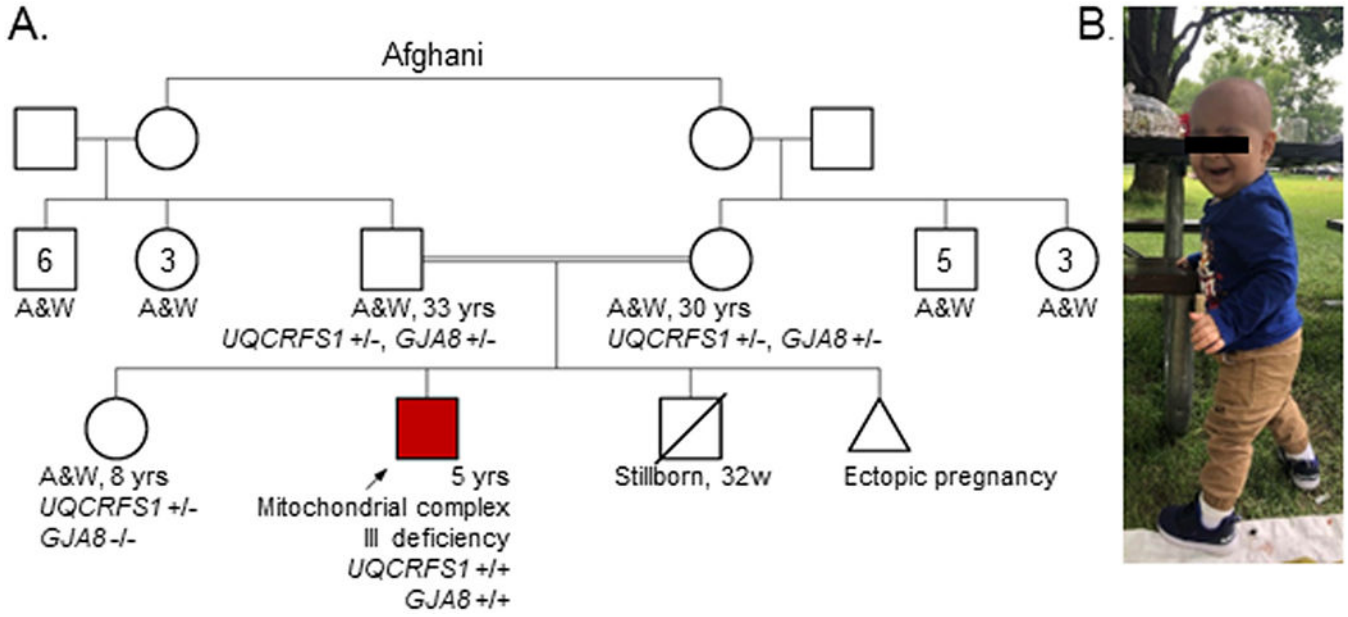

Fig. 1. Proband pedigree and clinical images.

Panel A: Pedigree depicting family history and *UQCRFS1* and *GJA8* genotypes. The proband's parents are first cousins through their maternal lines. Panel B: Photo of the proband at nearly 2 years of age illustrating non-dysmorphic craniofacial features, normal proportionate stature, and scalp alopecia.

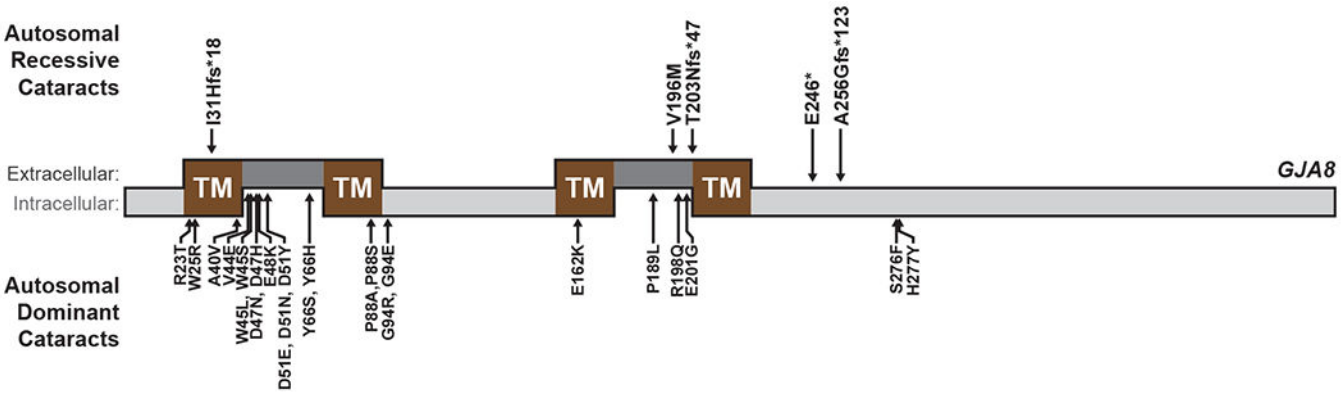

Fig. 2. Diagram of the proband's *GJA8* variant in context of known pathogenic variants.

The proband's Q246Ter variant is shown in relation to variants underlying either recessive (top) or dominant (bottom) cataracts. Transmembrane domains (TM) are shown in brown.

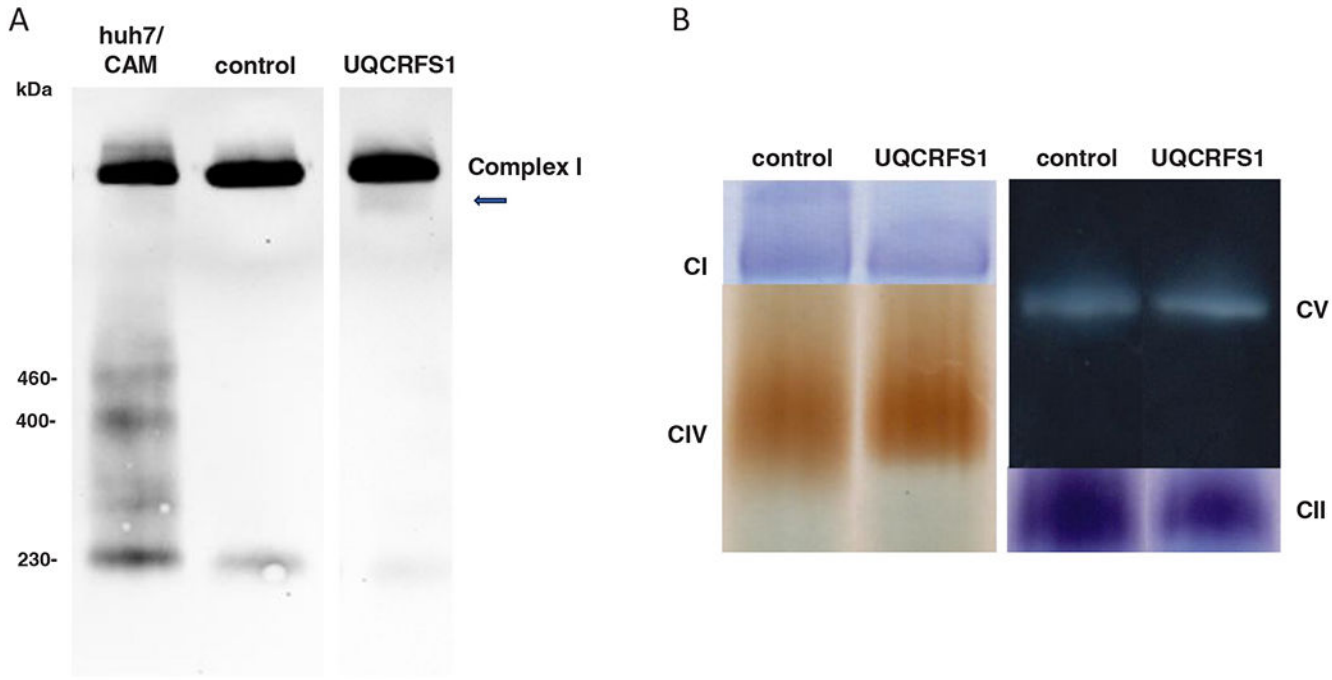

Fig. 3. : Analysis of mitochondrial function in fibroblasts.

Panel A: Mitochondrial complex I (CI) assembly is followed on non-denaturing gel after western blotting and identification with an antibody against NDUFS2. Normal fibroblasts show a large amount of fully assembled complex I at 1000 kDa with a small band at 230 kDa. The proband's cells show the normal amount of the holocomplex and the 230 kDa band. There is a faint band at 950 kDa (arrow) which represents an intermediate without the incorporation of the N-module. Huh-7cells treated with chloramphenicol are shown as a positive control. Panel B: The activity and assembly of the respiratory chain enzyme complexes were analyzed by BN-PAGE followed by in-gel activity staining. The activities of all enzyme complexes were normal, including assembly of complex V. Abbreviations: CI = complex I, CII = complex II, CIII = complex III, CIV = complex IV, CV = complex V.

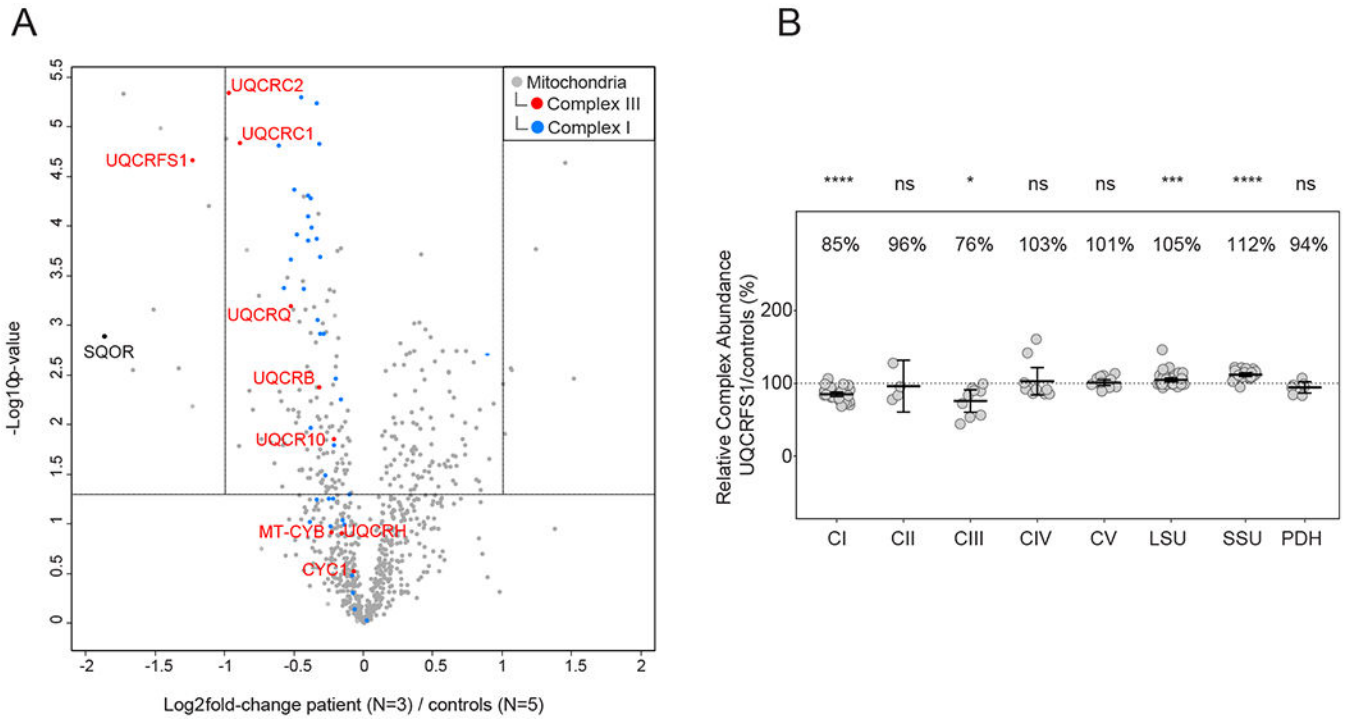

Fig. 4. : Proteomics analysis of the fibroblasts of the patient with UQCRS1 variants compared to controls.

Panel A: Volcano plot of differential mitochondrial protein abundance results after correction of mitochondrial abundance. The x-axis represents the difference between the patient and control mean values. This shows a decrease in the abundance of the subunits of complex III (red), complex I (blue), and the SQOR protein (black). Proteins encoding subunits of complex I or III are shifted to the left, indicating reduced abundance in the proband. The horizontal line shows a cutoff p-value <0.05. Panel B: Quantification of mitochondrial complex abundance shows a decrease in complex III, and a smaller decrease in complex I, and an increase in the mitoribosomal subunits. These data represent an aggregation of the data presented in Fig. 4A. The p-value and the relative % of the complex in the proband compared to the controls are provided in sequential rows above the figure. Abbreviations: * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, ns = non-significant. CI = complex I, CII = complex II, CIII = complex III, CIV = complex IV, CV = complex V, mtLSU = large subunit of the mitoribosome, mtSSU = small subunit of the mitoribosome, PDH = pyruvate dehydrogenase complex.

Table 1

Phenotypic spectrum of recessive mitochondrial complex III disorders, including our proband. Where possible, we provide the number of cases with a feature divided by the number of cases with data available. The proband in our case report is included in the *UQCRFS1* column.

Gene	*BCS1L*	*CYC1*	*LYRM7*	*TTC19*	*UQCC2*	*UQCC3*	*UQCRB*	*UQCRC2*	*UQCRFS1*	*UQCRH*	*UQCRQ*
MIM	124000 *	615453	615838	615157	615824	616111	615158	615160	618775	620137	615159
# patients	56	3	17	23	2	1	3	12	3	2	20
Age of onset	N; I; C	N; C	I; C; A	I; A 9/22	N	N	N 3/3	N 4/12; I; C	N 3/3	C	I
Lactic acidosis	E; P 44/53	E	E; P 11/17	8/14	E; P 2/2	E	E	11/12	E; P	E	M
Hyperammonemia	0/2	3/3	2/9	0/5	0/2	+	−	7/10	1/3	2/2	NR
Hypoglycemia	18/30	0/3	6/17	0/6	0/2	+	1	11/12	1/3	2/2	NR
Hyperglycemia	0/30	IR 2/3, KA 2/3	1/17	0/4	0/2	+	−	0/12	0/3	0/2	NR
Development	23/28	1/3	12/15	18/20	1/1	GDD	GDD 1/3	6/11	3/3	0/2	ID 20/20
Encephalo-myopathy	17/27	3/3	6/14	16/16	2/2	+	3/3	7/12	3/3	0/2	20/20
Neurological complications	+ 9/20; ND 6/10	0/3	+ 7/13; ND 11/15	ATX 20/20; S 16/20; ND 15/19; PN 6/9; PD 1/20; CV 1/20	0/2	+	0/3	ATX,T 6/11; ND 1/2	0/3	0/2	ATX,D,ND 20/20
Psychiatric features	7/9	0/3	NR	6/10	1/1	+	−	−	NR	NR	20/20
Seizures/Abnormal EEG	12/26	0/3		2/5	2/2	+	−	3/11	0/3	0/2	slowing in some
MRI	WM 8/10	PE 1/3	CY,WM 17/17	CA,CY,LS,OD 19/19	NR	NR	WM 1/3	ABN 5/7; BG,GA 2/5	0	NR	ABN, BG 5/5
Hearing loss	21/35	0/3	0/1	NR	1/1	−	−	1/6	2/3	1/2	−
Ophthamologic	12/32; CAT 3/32	PT 1/3	AS,NYS,OA 11/15	RC 1/13	0/2	−	−	3/5	CAT 1/3	−	−
Growth deficiency	34/45	IUGR, PNG 2/3	3/8	2/13	IUGR 2/2	IUGR	−	+ 6/9; IUGR 5/6	IUGR 1/3; PNG 2/3	2/2	−
Hepatopathy	24/43	0/3	3/6	0/2	0/2	−	−	6/10	2/3	0/2	−
Renal tubulopathy	31/35	0/3	1/2	NR	1/2	−	−	1/4	−	0/2	20/20
Alopecia/pili torti	7/15	0/3	3/3	NR	NR	−	−	−	3/3	0/2	−
Other	IO 15/40	NR	NR	NR	DM,PP,UDT 1/2	−	NR	MC 3/12	HCM 2/3	MO 1/2	NR
References	[64,66,67	[68,69]	[70]	[71–79]	[64,80,81]	[82]	[83–85]	[86–88]	[29]	[89]	[90]

KEY: + = reported; - = absent; A = adulthood; ABN = abnormal; AS = abnormal saccades; ATX = ataxia; BG = basal ganglia; C = childhood; CA = cerebellar atrophy; CAT = cataracts; CV = cerebrovascular accident; CY = cysts; D = dystonia; DM = dysmorphic; E = episodic; GA = global atrophy; GDD = global developmental delay; HCM hypertrophic cardiomyopathy; I = infancy; ID =

intellectual disability; IO = iron overload; IR = insulin responsive; IUGR = intrauterine growth retardation; KA = ketoacidosis; LS = Leigh syndrome like; M = mild; MC = microcephaly; MO = microtia; N = neonatal; ND = neurodegeneration; NR = not reported in original publications; NY = nystagmus; OA = optic atrophy; OD = olivary degeneration; P = persistent; PD = parkinsonism; PE = enlarged pituitary gland; PN = neuropathy; PNG = postnatal growth retardation; PP = postaxial polydactyly; PT = congenital ptosis; RC = retinal cherry red spots; S = spasticity; T = tremor; UDT = unilateral undescended testis; WM = white matter disease.

*Variants in *BCSL1* also cause Bjornstad [MIM: 262000] and GRACILE [MIM: 603358] syndromes (reviewed here [64,65]), but those disorders are excluded from this table as they are well characterized elsewhere.

Table 2

Respiratory chain enzyme activities in fibroblasts.

The activities in fibroblasts of the respiratory chain enzymes are provided as activities in nmol.min^{-1}.mg protein^{-1} or as a rate constant over protein for complexes III and IV. The ratio of the activities is also provided over citrate synthase and over complex II. The activities are also expressed as a Z-score of the log normal distribution of control fibroblasts. Activities significantly outside the control range are shown in bold. Abbreviations: CI = complex I, CII = complex II, CIII = complex III, CIV = complex IV, CV = complex V, CS = citrate synthase.

FIBROBLAST:	Activity (controls)	Z-score	Ratio/CS	Z-score	Ratio/CII	Z-score
CI	90.7 (49.3–131.1)	0.2	135 (145–396)	−1.2	571 (237–754)	1.0
CII	158.8 (130.9–364.4)	−1.0	**236** (297–863)	−2.4	NA	NA
CIII	**6.3** (8–29.2)	−1.9	**9** (19–65)	−2.4	39 (36–114)	−1.8
CII-CIII	158.2 (61.8–158.8)	1.3	235 (131–376)	0.3	996 (263–1100)	1.4
CIV	5.1 (2.2–7.1)	0.4	8 (6–23)	0.1	32 (12–35)	0.8
CS	673.6 (253.5–554.1)	1.9	NA	NA	NA	NA

Table 3

Respiratory chain complex abundances in fibroblasts of patients with complex III deficiency and controls.

	CI	CII	CIII	CIV	CV	mtSSU	mtLSU	PDH
UQCRFS1	**85**	96	**76**	103	101	105	112	94
UQCRC2	**70**	109	**58**	116	109	90	93	106
Negative Controls								
Mean±SD	98.3±5.4	97.3±10.3	97.1±5.1	97.9±13.6	99.2±4.3	100.7±9.1	107.4±13.2	95.8±7.5
Range	87–105	77–110	88–103	83–118	93–108	92–121	89–128	83–106

The relative complex abundances of the respiratory chain complexes, the mitoribosome subunits, and the pyruvate dehydrogenase complex as determine by proteomics analysis are listed as percent of in-assay controls (n = 5) for our proband, shown in comparison to a range of 14 normal controls and another patient with pathogenic variants in *UQCRC2* underlying a different nuclear complex III deficiency. The mean, the standard deviation (SD) and the observed range among controls are provided. This control data did not deviate significantly from a normal distribution. Patient results significantly outside the range observed in control subjects are shown in bold. Abbreviations: CI = complex I, CII = complex II, CIII = complex III, CIV = complex IV, CV = complex V, mtLSU = large subunit of the mitoribosome, mtSSU = small subunit of the mitoribosome, PDH = pyruvate dehydrogenase complex.