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BRIEF REPORT

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Renal dysfunction, rod-cone dystrophy, and sensorineural hearing loss caused by a mutation in *RRM2B*

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Abstract

More than two decades ago, a recessive syndromic phenotype affecting kidneys, eyes, and ears, was first described in the endogamous Afrikaner population of South Africa. Using whole-exome sequencing of DNA from two affected siblings (and their carrier parents), we identified the novel *RRM2B* c.786G>T variant as a plausible disease-causing mutation. The *RRM2B* gene is involved in mitochondrial integrity, and the observed change was not previously reported in any genomic database. The subsequent screening revealed the variant in two newly presenting unrelated patients, as well as two patients in our registry with rod-cone dystrophy, hearing loss, and Fanconi-type renal disease. All patients with the c.786G>T variant share an identical 1.5 Mb haplotype around this gene, suggesting a founder effect in the Afrikaner population. We present ultrastructural evidence of mitochondrial impairment in one patient, to support our thesis that this *RRM2B* variant is associated with the renal, ophthalmological, and auditory phenotype.

KEYWORDS

founder effect, mitochondrial, phenotype, *RRM2B*, whole-exome sequencing

In 1993, a rare autosomal recessive syndrome was reported in 14 children from nine South African families of Afrikaner ancestry (Beighton, Bartmann, Bingham, & Sellars, 1993). Affected individuals presented with renal dysfunction, visual impairment due to rod-cone dystrophy, and sensorineural hearing loss (OMIM Entry 268315). The Fanconi-type renal involvement resulted in rickets-like skeletal changes and kidney failure was fatal in most cases. The affected families were not consanguineous; however, the Afrikaner community is genetically endogamous, originally arising from a limited number of European founders and, therefore, prone to founder

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effects, as described previously for a range of other disorders (Botha & Beighton, 1983). This study describes the identification of the genetic mutations causing this syndrome.

DNA samples were available from four members of a previously reported family (Beighton et al., 1993), that is, two affected siblings (now deceased), and their obligate carrier parents (Figure 1; Family A). Informed consent was obtained from the parents and whole exome sequencing (WES) was performed on all four samples, using the Ion™ torrent platform and the AmpliSeq™ Exome RDY Kit (Thermo Fisher Scientific, Waltham, MA). Variants were prioritized as follows: $n = 488$ displayed an appropriate autosomal recessive cosegregation pattern (including both homozygous and compound heterozygous variants); $n = 12$ of those had a minor allele frequency < 0.05 across the 1000 Genomes project (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/; Auton et al., 2015; McVean et al., 2012), Exome Variant Server, NHLBI GO Exome Sequencing Project, Seattle, WA (http://evs.gs.washington.edu/EVS/) and the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org/; Lek et al., 2016); and $n = 7$ of those were located in coding or splice regions. One of the seven variants was synonymous and was thus eliminated, leaving five missense variants and one splice variant remaining.

To prioritize the most likely disease-causing variant further, the clinical manifestations of the patients were considered. The condition resembled but was distinct from, Alström syndrome (OMIM Entry # 203800), Usher syndrome (characterized by hearing loss and retinitis pigmentosa), and Senior-Løken syndrome (OMIM Entry # 266900; Table S1). The phenotypic overlap between these disorders, together with the tissues implicated (retina, kidney, inner, and middle ear), suggested a possible cilial dysfunction. Cilia are hair-like sensory organelles that project from the surface of almost all cells in vertebrates and have diverse functions (Valente, Rosti, Gibbs, & Gleeson, 2014). Ciliopathies are a spectrum of heterogeneous, inherited disorders (including Alström, Usher, and Senior-Løken syndromes) caused by abnormalities of these ciliary structures, characteristically resulting in vision loss, hearing loss, and renal disease (Coussa et al., 2013; Nikopoulos et al., 2016; Valente et al., 2014; Waters & Beales, 2011). In the retina, the rod and cone photoreceptor cells consist of a photosensitive outer segment, which is itself a specialized primary cilium, joined by a connecting cilium to the inner segment which comprises the molecular components required for visual signal transduction of light stimuli (May-Simera, Nagel-Wolfrum, & Wolfrum, 2017). Cilia protrude from the renal tubule epithelial cells and act as sensory antennae, signaling changes in the motion and composition of urine through the renal tubule and ducts (Mitchison & Valente, 2017). Cilia are also located in the middle and inner ear, and are responsible for signal transduction of auditory stimuli, converting the mechanical energy of sound waves to electrical energy which leads to excitation of the auditory nerve (Friedman & Griffith, 2003).

Prioritization of the candidate genes was therefore performed utilizing a web-based tool, ToppGene (Chen, Bardes, Aronow, & Jegga, 2009), which calculates a similarity score between the candidate genes input and a training set of genes involved in all known ciliopathies (http://www.omim.org; accessed June 10, 2016). The attributes selected for similarity comparisons were: GO Molecular function; Biological process and Cellular component; Human and mouse phenotype; Pathways; PubMed; Co-Expression; Disease; Domain, and Gene family; and Interaction. The highest-ranked candidate variant using this approach was NM_015713.4: c.786G>T; p.(Glu262Asp) in *RRM2B* (Ensembl transcript ID ENST00000251810.7; CCDS34932.1). This homozygous

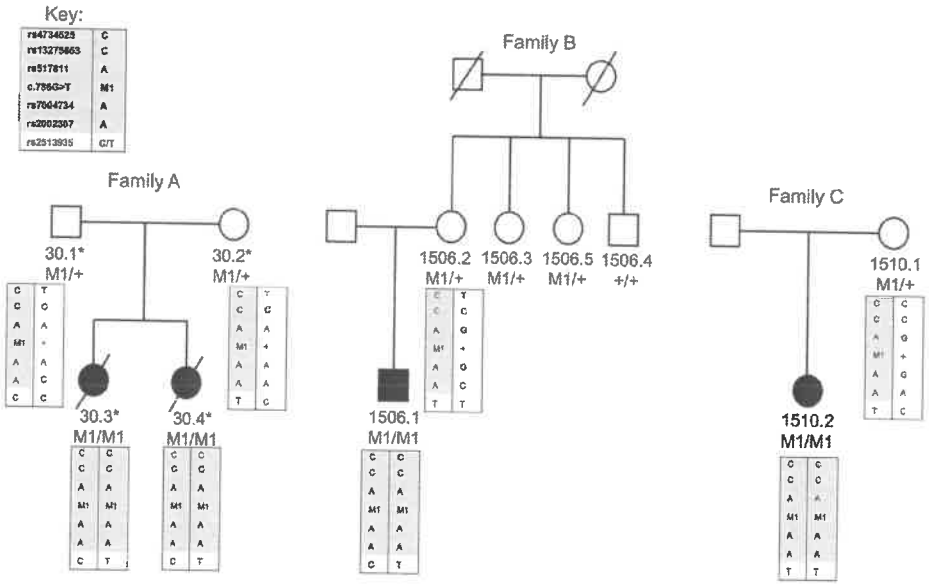


FIGURE 1 Pedigrees of three unrelated families showing cosegregation of the *RRM2B* c.786G>T variant on an identical haplotype. Shaded symbols indicate affected individuals. Identifier codes show individuals from whom biological material is available, and those selected for whole-exome sequencing are noted with an asterisk. Segregation of mutation(s) in the families is indicated +/+ homozygous for wild-type allele; M1/+, heterozygous; M1/M1, homozygous c.786G>T

missense mutation was also the highest-ranked common variant between the affected siblings when their variant call format (vcf) files were analyzed using Exomiser (Smedley et al., 2015). Exomiser comprises a suite of algorithms that prioritize WES variants based on clinical data, model organism phenotype, and protein interaction data. For this analysis, the Human Phenotype Ontology phenotypes HP:0000510 (rod-cone dystrophy), HP:0000407 (sensorineural deafness), and HP:0000124 (renal dysfunction) were used, with an autosomal recessive (homozygous and compound heterozygous) mode of inheritance. Ultimately, the availability of DNA samples from two new unrelated cases and their family members (Figure 1; Family B and Family C) allowed cosegregation analysis which eliminated the five other WES candidate variants; only *RRM2B* c.786G>T segregated appropriately with the expected autosomal recessive inheritance pattern of disease.

The c.786G>T; p.(Glu262Asp) variant in Exon 7 of *RRM2B* occurs at genomic position 8: 103226285 (Human reference genomic sequence hg19). This variant was not reported in the ExAC (Lek et al., 2016; accessed June 21, 2018), gnomAD v2.11 (Karczewski et al., 2019) or Ensembl release 99 (GRCh37 and GRCh38.p13 versions; Cunningham et al., 2019; accessed February 27, 2020) browsers, and is, therefore, presumably novel. Sanger sequencing confirmed: (a) the presence of the homozygous c.768G>T in four affected individuals from three unrelated families (Figure S1); (b) the absence of the variant in 34 healthy controls from the Afrikaner population group; and (c) an identical haplotype comprising six single nucleotide polymorphisms spanning 908 kb upstream and 618 kb downstream of the *RRM2B* mutation, indicating a probable founder effect (Figure 1; Table S2).

The Residual Variation Intolerance Score of −0.6047 for *RRM2B* (http://genic-intolerance.org/index.jsp) indicates that this gene is intolerant to variation. Furthermore, the amino acid in question is highly conserved across species (Figure S2a). Applying the ACMG guidelines for variant interpretation (Richards et al., 2015) and utilizing Varsome (Kopanos et al., 2019), the homozygous *RRM2B* c.786G>T variant satisfied the following criteria for evidence of pathogenicity: (a) PM2: absent from controls; (b) PM3: observation of the homozygous variant in multiple unrelated patients with the same recessive phenotype; (c) PP2: missense variant in a gene that has a low rate of benign missense variation, that is, 25 pathogenic missense variants versus four benign variants; and (d) PP3: 10 pathogenic predictions from DANN, GERP, dbNSFP, FATHMM, LRT, MetaLR, MetaSVM, MutationAssessor, MutationTaster, PROVEAN, and SIFT (vs. no benign predictions). The combination of two moderate and two supporting criteria allows the classification of the variant as "likely pathogenic" (Richards et al., 2015).

RRM2B encodes for the p53-inducible ribonucleotide reductase small subunit (P53R2) protein. The P53R2 structure of the wild-type form (Smith et al., 2009) was retrieved from the Protein Data Bank (PDB-ID: 3HF1). The Glu262Asp mutation was performed and assessed using Dynamut (Rodrigues, Pires, & Ascher, 2018). Pymol (v1.7.2.1; https://pymol.org/2/; The PyMOL Molecular Graphics System, version 1.2r3pre, Schrödinger, LLC) was used for molecular visualization. The Glu262 variant lies within helix G of the P53R2 monomer (Figure S2 B). The predicted change in Gibbs free energy ($\Delta\Delta G$) resulting from the Glu262Asp variant is −0.642 kcal/mol, indicating an overall destabilizing effect. This prediction is supported by structural evidence based on the loss or weakening of notable polar contacts between this residue and others within their helical cluster (Figure S2c,d). The shorter side chain of the Asp262 residue would not be able to establish the contacts that the longer Glu262 side chain is able to create. The hydrogen bond between Glu262 and Tyr164 is a defining feature of P53R2, which distinguishes it from RRM2 (Smith et al., 2009). Disruption of this bond may lead to destabilization of the hydrogen bond network within the helical cluster, which may have knock-on effects for the stability of the P53R2 dimer, and thus the formation of the functional heterotetrameric ribonucleotide reductase enzyme, that is, 2-P53R2/2-RRM1.

All nine exons of the *RRM2B* gene were subsequently sequenced using M13-tailed primer pairs (Table S3) and Big Dye® Direct cycle sequencing kit (Thermo Fisher Scientific, Waltham, MA) in 46 patients with related phenotypes. This cohort was selected from the UCT inherited retinal disease (IRD) repository and database, which archives biological material and demographic and clinical data from patients with IRDs in South Africa (Rebello, Greenberg, & Ramesar, 2002). The cohort selected for this investigation comprised $n = 40$ patients diagnosed with Usher syndrome, and $n = 6$ patients with IRD and renal disease, with or without hearing loss. The homozygous c.786G>T variant was identified in a further two unrelated probands with rod-cone dystrophy/retinitis pigmentosa, hearing loss, and Fanconi-type renal disease (Table S4). The same haplotype was confirmed in these probands. Therefore, we report a total of five families with the *RRM2B* mutation and identical phenotype.

RRM2B encodes a subunit of ribonucleotide reductase, playing an essential role in dNTP supply for DNA replication and repair, and mitochondrial DNA (mtDNA) synthesis. In humans, *RRM2B* expression is ubiquitous and occurs at high levels in skeletal tissue (Cho et al., 2015). In vitro analysis has shown reduced expression under oxidative stress is associated with greater DNA damage and a decrease in relative mitochondrial mass, suggesting a protective role in mitochondrial homeostasis and oxidative stress-induced DNA damage (Cho et al., 2015).

Mutations in nuclear genes associated with mtDNA maintenance, such as *RRM2B*, can cause mtDNA depletion (Bourdon et al., 2007) and/or the accumulation of multiple mtDNA deletions (Pitceathly et al., 2011, 2012). In the case of *RRM2B* pathology, such deletions and depletion are normally most evident in muscle, resulting in severe muscle pathology (Finsterer & Zarrouk-Mahjoub, 2018; Penque et al., 2019; Pitceathly et al., 2012). Interestingly, none of the patients in this cohort presented with myopathic features (Table S5). Furthermore, while ocular involvement is common in mitochondrial diseases, *RRM2B* mutations are most frequently associated with ophthalmoparesis and ptosis resulting from muscle weakness around the eye (Pitceathly et al., 2012), while in this cohort the ocular involvement was predominantly retinal (rod-cone dystrophy). Death in early adulthood due to renal failure makes it impossible to ascertain whether our patients may eventually

develop myopathic features, such as ptosis and progressive external ophthalmoplegia, later in life.

Therefore, to establish whether the *RRM2B* c.786G>T variant has a potentially subclinical functional biological effect on muscle mitochondria, a biopsy was performed on individual 1510.2 of Family C (with the patient's assent and guardian's consent; UCT Human Research Ethics HREC/REF675/2015) for histological and genetic investigations. This patient was aged 13 years at the time of the biopsy. DNA was extracted from the muscle tissue and assessed for large mtDNA rearrangements using three different primer sets flanking the commonly deleted regions of the mtDNA genome (Damas, Carneiro, Amorim, & Pereira, 2014; Damas, Samuels, Carneiro, Amorim, & Pereira, 2014) as well as by assessing the copy number ratio of the commonly deleted *MT-ND4* region (in the major arc) to that of the *MT-TL1* gene using droplet digital polymerase chain reaction (ddPCR). In addition, mtDNA content was quantified to detect depletion by assessing the ratios of mtDNA (*MT-ND4* and *MT-TL1*) to nuclear DNA (*B2M*) using ddPCR. No evidence of deletions or depletion could be identified. However, microscopy revealed clear morphological evidence of a mild mitochondrial abnormality (Figure 2).

The lack of mtDNA deletions and depletion observed in muscle and the lack of clinical muscle pathology in this cohort is unusual for RRM2B deficiency. However, since there are clear signs of structural mitochondrial damage on histological examination, the possibility of mosaic mtDNA depletion (as observed in other mtDNA maintenance defects such as MPV17; Uusimaa et al., 2014; and POLG; Ashley et al., 2008) should be considered.

Alternatively, c.786G>T maybe a mild mutation. It has previously been reported that dominantly inherited (heterozygous) *RRM2B* mutations present with a later onset, predominantly myopathic phenotype, and recessively inherited mutations present with an early onset, severe, and multisystem disorder (Pitceathly et al., 2012). Ribonucleotide reductase activity is, therefore, impacted by both dominant negative/gain of function effects and haploinsufficiency, and the presence of a wild-type allele causes a dosage effect ameliorating the phenotype (Pitceathly et al., 2011, 2012). A mild or hypomorphic mutation, causing only a partial loss of gene function, could result in tissue-specific sensitivity. For example, the retina is a highly sophisticated, metabolically active tissue displaying cellular diversity and vast gene expression diversity, and as such is particularly susceptible to dysfunction. Therefore, while muscle tissue might cope with the limited amount of structural damage caused by a hypomorphic mutation, retinal tissue might not. This hypothesis is supported by our structural evidence which showed that, although the Glu262Asp variant leads to a destabilization effect, it may not lead to a complete knockout of the protein. The reduced stability may result in the reduced activity of the functional tetramer of 2-P53R2/2-RRM1 or reduce the rate of its formation. Additional analyses, including messenger RNA stability, western blots, and evaluating mosaic expression or depletion in other tissues, may elucidate the functional consequence(s) of this mutation.

In conclusion, we propose that this is a new phenotypic description of *RRM2B* mitochondrial pathology, in which muscle involvement is subclinical and masked by predominant ocular, auditory, and renal symptoms.

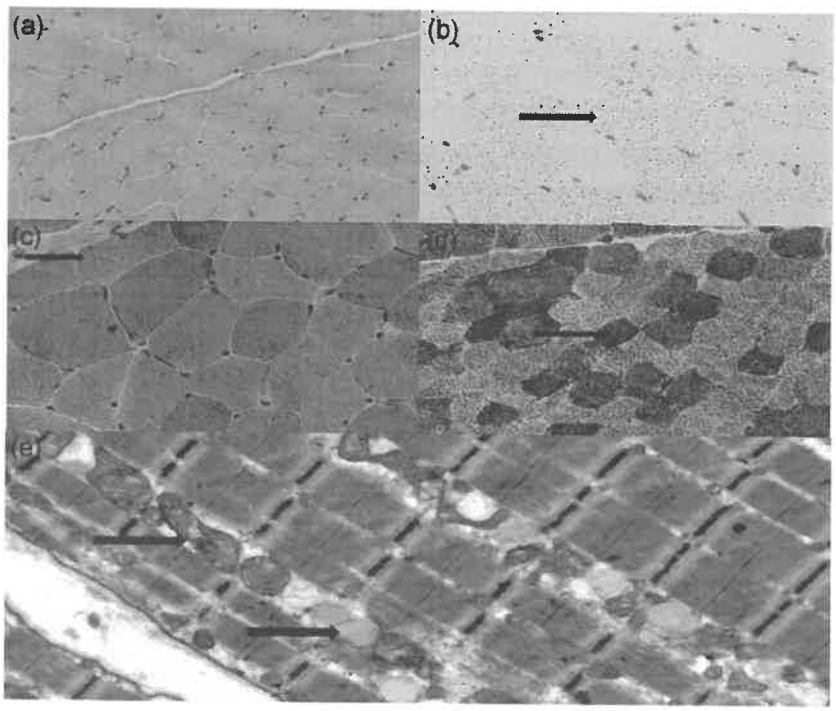


FIGURE 2 Light (a–d) and electron (e) microscopy of a muscle biopsy from homozygous individual 1510.2. The myofibres were normal on the hematoxylin and eosin stain (a). There was a significant increase in sarcoplasmic lipid in scattered myofibres (b) with coarse staining of mitochondria on the modified Gomori stain (c). A subsarcolemmal accentuation of staining was noted on the modified Gomori and NADH stains (d). Electron microscopy (e) showed scattered enlarged and elongated mitochondria (top arrow) with abnormal cristae and associated lipid droplets (bottom arrow)

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

Research data are not available, due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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