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CLINICAL REPORT

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Primary congenital glaucoma due to paternal uniparental isodisomy of chromosome 2 and *CYP1B1* deletion

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Abstract

Background: *CYP1B1* variants and deletions are the most common cause of primary congenital glaucoma (PCG).

Methods: We investigated an individual with PCG from the Australian and New Zealand Registry of Advanced Glaucoma. We performed sequencing of the *CYP1B1* gene, followed by Multiplex Ligation-dependent Probe Amplification and SNP array.

Results: We identified a homozygous deletion of the *CYP1B1* gene by Multiplex Ligation-dependent Probe Amplification and confirmed that the father was heterozygous for a *CYP1B1* deletion but the mother had normal gene copy number. SNP array identified paternal uniparental isodisomy of the entire chromosome 2.

Conclusions: This study is the first report of a homozygous *CYP1B1* whole gene deletion due to paternal uniparental isodisomy of chromosome 2 as a cause of PCG. These results illustrate the importance of genetic testing in providing appropriate genetic counseling regarding the risks of recurrence.

KEYWORDS
childhood glaucoma, chromosome 2, *CYP1B1*, gene deletion, primary congenital glaucoma, uniparental disomy

1 | INTRODUCTION

Primary congenital glaucoma (PCG, MIM 231300) is a significant cause of irreversible blindness in children and results from developmental defects of the aqueous humor outflow pathway structures. It is characterized by increased intraocular pressure (IOP), buphthalmos, optic disc cupping, corneal edema, enlarged corneal diameter, and/or Haab's striae (Thau et al., 2018). A high incidence has been found among some populations with founder effects or a high rate of consanguinity (Slovakian Roma 1/1,250 (Gencik, 1989), Saudi Arabian 1/2,500 (Bejjani et al., 2000)) but incidence is lower in Western countries, including Australia (1/30,000 births) (MacKinnon, Giubilato, Elder, Craig, & Mackey, 2004).

PCG is most commonly associated with autosomal recessive variants in *CYP1B1* on chromosome 2p21 (MIM 601771) (Stoilov, Akarsu, & Sarfarazi, 1997). Mice deficient in CYP1B1 have developmental defects of the ocular drainage structures resembling those reported in individuals

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with PCG, supporting loss of function as a mechanism of *CYP1B1* variants causing PCG (Libby et al., 2003). Over 200 pathogenic sequence variants have been reported in *CYP1B1* (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=CYP1B1), however deletions of the gene have seldom been reported (Damjanovich, Baldwin, Lewis, & Bayrak-Toydemir, 2013; Kelberman et al., 2011; Milla et al., 2013; Stoilov, et al., 1997). In this study, we report an individual with PCG and a homozygous *CYP1B1* deletion arising from paternal uniparental isodisomy of chromosome 2.

2 | CASE REPORT

The study was conducted in accordance with the revised Declaration of Helsinki and participants were recruited with the approval of the Southern Adelaide Clinical Human Research Ethics Committee. The proband and his family members were enrolled as part of The Australian and New Zealand Registry of Advanced Glaucoma and gave their informed consent (Souzeau et al., 2012). The proband was a 15-year-old boy and the second child of nonconsanguineous Indian parents. He presented with bilateral significant corneal clouding, enlarged cornea 12 mm horizontally and raised IOP at birth. Right trabeculectomies were performed at 4 weeks and 3 months of age, however this eye became densely amblyopic. His better left eye had a trabeculectomy with Mitomycin C antimetabolite at age 32 months. Sequential left then right Ahmed tube surgery took place at age 4 and 5 years. These surgeries all took place in India. His family moved to Australia when he was 11 years. At that time his right eye was blind with perception of light, he had IOP of 30 mmHg, band keratopathy, a small pupil and dense cataract limiting the view of his optic nerve (Figure 1). The better left eye has nystagmus, vision of 3/15, IOP in the mid-teens on maximal topical medical therapy and since age 13 also acetazolamide 250 mg bd. His most recent field test had generalized reduced sensitivity, with a left nasal step. Both parents and his older sister were examined and were unaffected.

3 | GENETIC ANALYSIS

Genomic DNA was extracted from venous blood of the proband, his parents, and his sister. *CYP1B1* sequencing was performed on the proband's DNA through the NATA (National Association of Testing Authorities) accredited laboratories of SA Pathology at Flinders Medical Centre (Adelaide, Australia) as previously described (Souzeau, Hayes, Zhou, et al., 2015). Amplification of the two coding exons of *CYP1B1* failed, suggesting a potential gene deletion (data not shown).

CYP1B1 was then analyzed for copy number variation by Multiplex Ligation-dependent Probe Amplification (MLPA) using the SALSA P128 Cytochrome P450 probemix (MRC Holland, Amsterdam, The Netherlands) according to the manufacturers' protocol. MLPA analysis of *CYP1B1* indicated an abnormal copy number in the proband's DNA, characteristic of a homozygous deletion of the entire gene. MLPA analysis of both parents was then performed, and revealed in the father the loss of a single allele of *CYP1B1* but indicated a normal copy number for all exons of *CYP1B1* in the mother (Figure 2).

To investigate the extent of the *CYP1B1* gene deletion in the proband and the possibility of uniparental disomy (UPD), a SNP array (Illumina Infinium CytoSNP-850K BeadChip) was conducted. Array result of the proband revealed an interstitial homozygous deletion of 119kb on chromosome 2q22.2 (chr2: 38 239 356–38 358 664, GRCh37/hg19 assembly) encompassing the entire *CYP1B1* gene as well as the *RMDN2* gene (MIM 611872). The *RMDN2* gene has not been associated with any clinical phenotype. Paternal isodisomy for the entire chromosome 2 was identified, confirming that the homozygous *CYP1B1* deletion in the child originated from

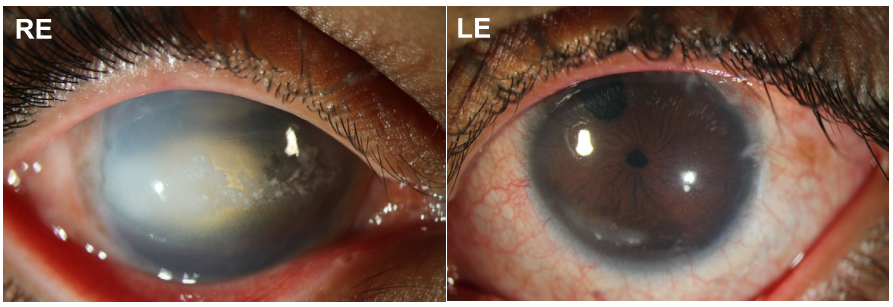

FIGURE 1 Slit lamp photographs of the proband. Right eye (RE) showing central calcified band keratopathy, unreactive pupil with surgical iridectomy occluded by cataract. Left eye (LE) showing iridectomy (iris resection in the superior part of the iris) from previous trabeculectomy, constriction of the pupil due to intraocular lowering topical therapy and limbal scars due to surgery (at the corner of the cornea and the sclera)

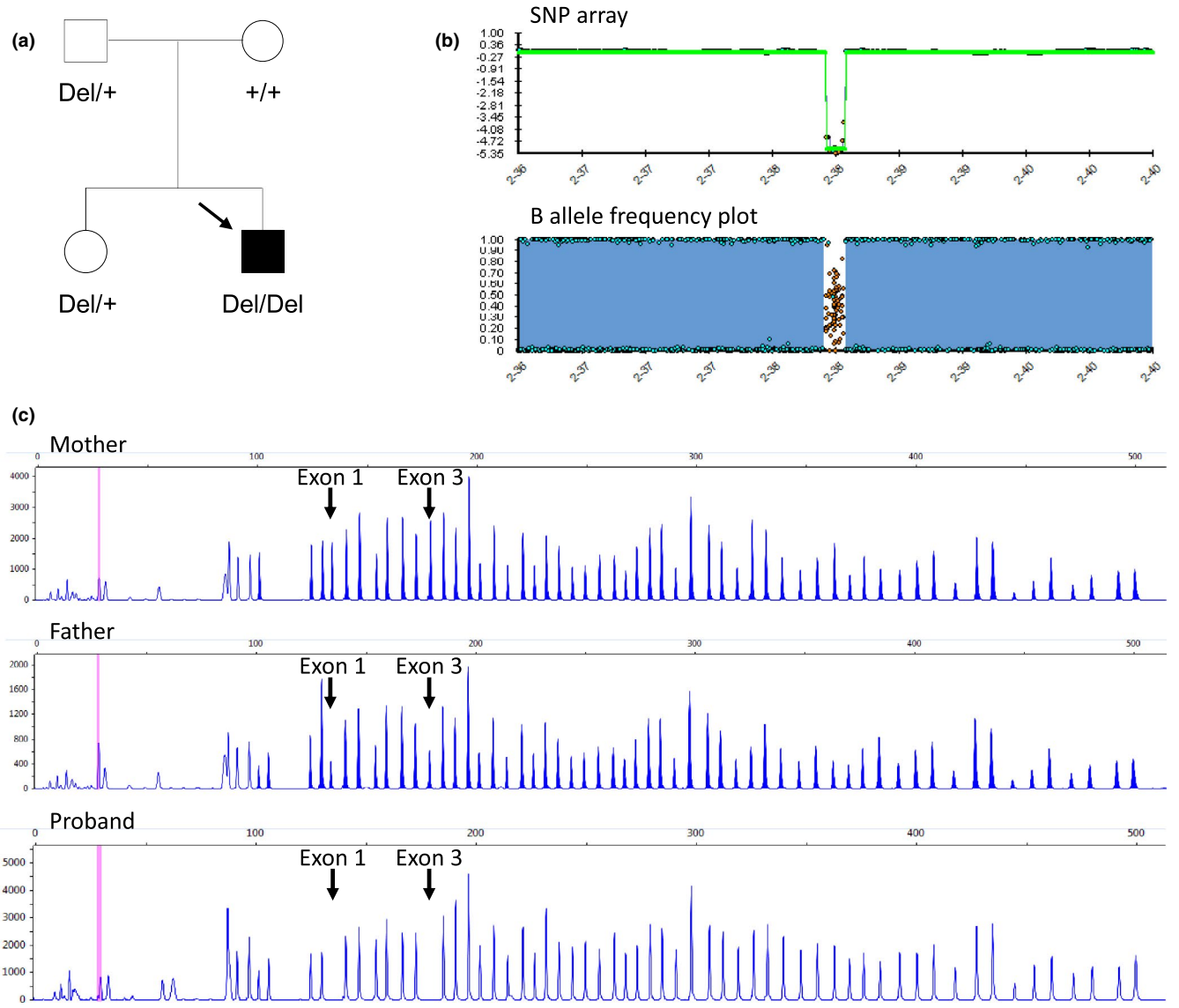

FIGURE 2 Pedigree and genetic analysis. (a) Pedigree of the family. The solid symbol indicates primary congenital glaucoma, the *CYP1B1* deletion is represented by "Del" and the wild type allele by the "+' sign. (b) Single nucleotide polymorphism (SNP) array confirming the homozygous *CYP1B1* deletion (top panel) and B allele frequency plot showing complete homozygosity on chromosome 2, corresponding to uniparental isodisomy (bottom panel). The B allele frequency is a normalized measure of the allele frequency ratio of the two alleles: values of 0 or 1 indicate areas of homozygosity, whereas a value of 0.5 indicates areas of heterozygosity. (c) MLPA analysis showing normal *CYP1B1* copy number in the proband's mother (upper panel), heterozygous *CYP1B1* deletion in the proband's father (middle panel) and homozygous *CYP1B1* deletion in the proband (bottom panel). The arrows show the two probes in exons 1 and 3, while the other peaks represent unrelated probes to other genes used as internal controls

a single parent (Figure 2). Chromosome 2 has not been assessed for the presence of other homozygous variants.

4 | DISCUSSION

UPD is a rare event that arises when an individual with a diploid genome carries both homologs of a pair of chromosomes from one parent (uniparental heterodisomy) or both copies of a single parental chromosome (uniparental isodisomy) (Engel, 1980). UPD can result in clinically recognizable phenotypes through imprinting defects, mosaicism, or unmasking of homozygosity for pathogenic variants inherited from one parent in autosomal recessive genes. Paternal UPD2 is relatively rare and has generally been reported following genetic analysis of individuals homozygous for variants in autosomal recessive genes consistent with their phenotype (Baskin, Geraghty, & Ray, 2010; Chavez, Valdez, & Vilchis, 2000; Lopez-Garrido, Campos-Mollo, Harto, & Escribano, 2009; Shen et al., 2018; Thompson et al., 2002). One of these cases involved a homozygous *CYP1B1* missense variant due to paternal UPD2 in a patient with PCG (Lopez-Garrido et al., 2009).

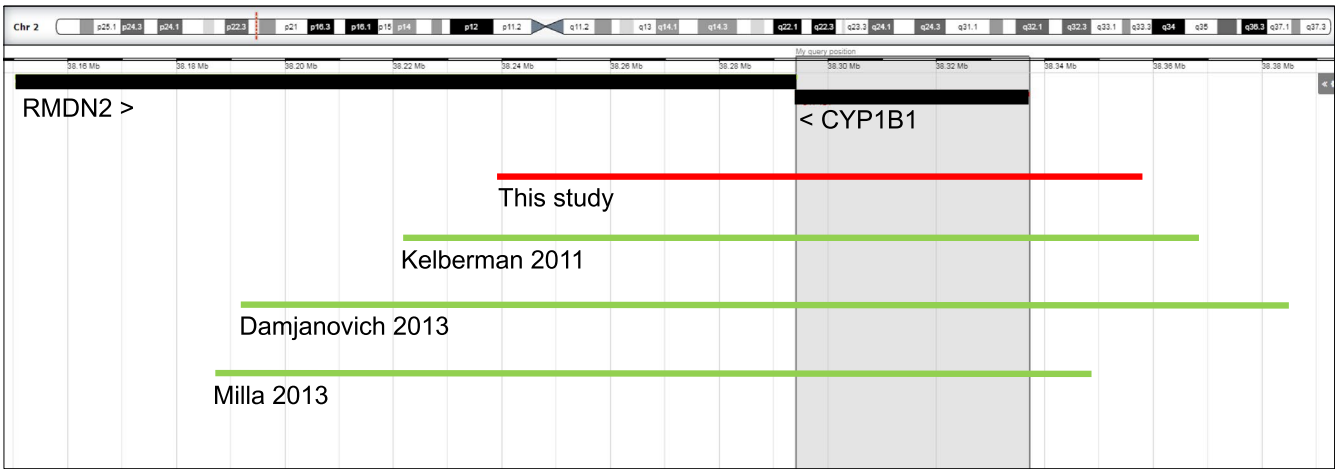

FIGURE 3 Summary of *CYP1B1* entire gene deletions. Schematic drawing of the 2p22.2 region showing known human protein-coding genes from the NCBI reference sequence (RefSeq) as seen in DECIPHER (http://decipher.sanger.ac.uk) using the GRCh37/hg19 assembly. The 119kb deletion reported in this study is shown in a solid red line (chr2: 38,239,356–38,358,664). Previously reported deletions are displayed in solid green lines (146kb, chr2: 38,222,086–38,368,231 (Kelberman, 2011); 193 kb, chr2: 38,191,823–38,385,253 (Damjanovich, 2013); 162kb, chr2: 38,187,289–38,349,505 (Milla, 2013))

We previously showed that deletion of the entire *CYP1B1* gene is not a major contributor to PCG (Souzeau, Hayes, Ruddle, et al., 2015). *CYP1B1* deletions have only been reported three times, with deletion sizes ranging from 146kb to 193kb (Figure 3) (Damjanovich et al., 2013; Kelberman et al., 2011; Milla et al., 2013). The deletion reported in this study is the smallest deletion encompassing the entire *CYP1B1* gene, with a size of 119kb. All reported deletions included part of the *RMDN2* gene that has not been associated with any clinical phenotype.

The confirmation of UPD as the cause of variant homozygosity has important implications for the family in terms of genetic counseling. The parents can be counseled that the risk of recurrence for other children is low, as opposed to the 25% risk of recurrence usually reported for variants in autosomal recessive genes such as *CYP1B1*. Similarly, the occurrence of phenotypically normal individuals with paternal UPD2 does not support a significant role for paternally imprinted genes on chromosome 2 (Keller et al., 2009; Ou et al., 2013). This is supported by the absence of additional clinical features in the proband reported in this study.

In summary, this is the first report of a homozygous *CYP1B1* whole gene deletion due to paternal UPD2 and associated with PCG. This study illustrates the importance of genetic testing in identifying the underlying molecular cause of the disease and in providing adequate genetic counseling regarding the possible recurrence risks.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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