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Pathogenic variants in the *CYP21A2* gene cause isolated autosomal dominant congenital posterior polar cataracts

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ABSTRACT

Background: Congenital cataracts are the most common cause of visual impairment worldwide. Inherited cataract is a clinically and genetically heterogeneous disease. Here we report disease-causing variants in a novel gene, *CYP21A2*, causing autosomal dominant posterior polar cataract. Variants in this gene are known to cause autosomal recessive congenital adrenal hyperplasia (CAH).

Methods: Using whole-exome sequencing (WES), we have identified disease-causing sequence variants in two families of British and Irish origin, and in two isolated cases of Asian-Indian and British origin. Bioinformatics analysis confirmed these variants as rare with damaging pathogenicity scores. Segregation was tested within the families using direct Sanger sequencing.

Results: A nonsense variant NM_000500.9 c.955 C > T; p.Q319* was identified in *CYP21A2* in two families with posterior polar cataract and in an isolated case with unspecified congenital cataract phenotype. This is the same variant previously linked to CAH and identified as Q318* in the literature. We have also identified a rare missense variant NM_000500.9 c.770 T > C; p.M257T in an isolated case with unspecified congenital cataract phenotype.

Conclusion: This is the first report of separate sequence variants in *CYP21A2* associated with congenital cataract. Our findings extend the genetic basis for congenital cataract and add to the phenotypic spectrum of *CYP21A2* variants and particularly the CAH associated Q318* variant. CYP21A2 has a significant role in mineralo- and gluco-corticoid biosynthesis. These findings suggest that CYP21A2 may be important for extra-adrenal biosynthesis of aldosterone and cortisol in the eye lens.

Introduction

Cataract, the opacification of the eye lens, is the most common, but treatable cause of blindness in the world (https://www.who.int/publications-detail/world-report-on-vision). Congenital cataracts are detected at birth or during the first decade of life. Hereditary cataract can be isolated or be a part of other ocular defects like anterior segment mesenchymal dysgenesis, glaucoma, microcornea, or aniridia; and systemic disorders such as heart disease, diabetes, deafness, and Wolframin disease.

Congenital cataract is usually autosomal dominant, followed by recessive and X-linked inheritance. It is a clinically and genetically heterogeneous disease, displaying various phenotypes (1). Cholesterol biosynthetic pathways are linked to cataract as evidenced by lanosterol synthase gene (LSS). The full spectrum of congenital cataract causing genes can be found here (https://cat-map.wustl.edu/) (2,3). In this study, we have identified two heterozygous variants Q319X, and M257T in the *CYP21A2* gene (MIM 201910). The variant identified as Q319X (once a legacy N-terminal sequencing error is corrected), is equivalent to the Q318X variant linked to congenital

adrenal hyperplasia (CAH) (4). As previously reported, Q319X is predicted to be pathogenic at both a bioinformatic and a structural levels; while M257T, a novel but rare variant, has been predicted as a variant of uncertain significance at bioinformatic level.

CYP21A2 is a member of the cytochrome P450 (CYP450) family of enzymes, and it is also known as steroid 21-hydroxylase, important in the biosynthesis of mineralocorticoids (aldosterone) and glucocorticoids (cortisol). Like other steroid hydroxyases, it requires a heme cofactor to function as a monooxygenase and catalyse the oxidization and hydroxylation of a variety of steroid substrates at position 21 (5), such as progesterone and 17α-hydroxyprogesterone, precursors in the synthesis of aldosterone and cortisol, respectively. For this reason, *CYP21A2* is associated with CAH (6), an autosomal recessive endocrine disorder (7,8) that leads to aldosterone deficiency and in its severe form, the pathological loss of sodium ions (9). Here, for the first time, we have identified pathogenic variants in *CYP21A2* gene causing an isolated autosomal dominant posterior polar congenital cataract.

Materials and methods

Phenotyping

Patients were identified via the proband attending the Genetic Service at Moorfields Eye Hospital, London, UK. The study protocol adhered to the Tenets of the Declaration of Helsinki and was approved by UCL research ethics committee (project ID—4817/001). All family members participating in this study gave written informed consent and underwent full ophthalmic examination, including slit lamp examination. All affected individuals from two families and two isolated cases were diagnosed as having an isolated congenital cataract.

Whole-exome sequencing (WES) and bioinformatics analysis

Whole-exome sequencing and bioinformatic analysis were performed using the Phenopolis bioinformatics platform as before (10).

Sanger sequencing

Sanger sequencing was performed to validate the variant identified by WES. Genomic DNA was amplified by PCR using GoTaq 2X master mix (AB gene; Thermo Scientific, Epsom, UK) and *CYP21A2* specific primers i) Q319*- Forward primer: *acaagcaaaaggctccttcc*; Reverse primer: *tttcctcactcatccccaac*, ii) M257T- Forward primer: *AGAGGGATCACATCGTGGAG*; Reverse primer: *ggaaggagccttttgcttgt* designed with http://bioinfo.ut.ee/primer3-0.4.0/. After validating the variant, segregation was performed in all the available family members.

The protein structure of cytochrome P450 (CYP21A2) for wt-CYP21A2, mut-CYP21A2 G319* and mut M257T was analysed using SWISSMODEL (https://swissmodel.expasy.org/interactive) (Figure 3a–d).

Results

Cataract families with bioinformatic analysis

In this study, we have investigated two families, A and B along with two isolated individuals C and D with autosomal dominant congenital cataract.

Family A consists of a three-generation British pedigree comprising 7 affected and 1 unaffected individual presenting with bilateral congenital posterior polar cataract (Figure 1a). There was no reported family or personal history of non-ocular disease. One affected individual (III:5) was sequenced by whole-exome sequencing (WES), followed by phenopolis genetic variant analysis pipeline, resulting in 356 out of a total of 2930 rare coding variants being filtered by allele frequency (Kaviar and GnomAD) and CADD > 15. The top scoring variant for CADD was a rare heterozygous variant c.955 C > T; p.Q319* in exon 8 of CYP21A2, with a score of 43.

Family B is a small Irish pedigree with posterior polar cataract, comprising 2 affected and 1 unaffected family members (Figure 1b). No reported family or personal history of non-ocular disease was found. One affected individual (II:1) was sequenced by WES. Variant annotation and tiered filtering yielded 376 out of a total of 3087 rare coding variants. Furthermore, a rare heterozygous variant c.955 C > T; p.Q319* in exon 8 of CYP21A2, with a highest CADD score of 43 was found.

Individuals C and D, both affected by congenital cataract (specific phenotype not present in clinical notes), with no personal history of non-ocular disease, underwent WES. Following this, variant analysis and tiered filtering yielded 491/3425 rare coding variants and further one rare variant c.955 C > T; p.Q319* in exon 8 of CYP21A2 (C) with CADD score of 43 and one rare variant c.770 T > C; p. M257T in exon 7 of CYP21A2 (D) with CADD score of 23.8.

Direct Sanger sequencing confirmed all the variants (Figure 2), which co-segregated in the affected family members. Pathogenicity scores of these variants are shown in Table 1.

Further we have also found 6 polymorphisms (rs61732108, rs61732108, rs61732108, rs61732108, rs147821751, and rs6455) in *CYP21A2* in our ADCC panel of 60 patients.

We have demonstrated the crystal structure of human wild type CYP21A2 (30–485) amino acids and truncated protein at position Q319*, losing 166 amino acids, where several residues are crucial for various important functions of the protein and for missense variant Met257Thr (Figure 3).
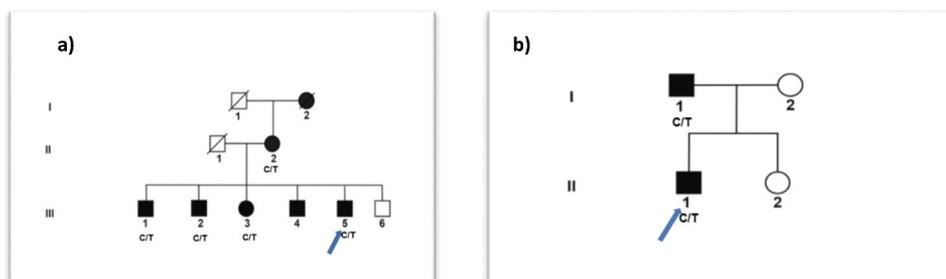
Figure 1. (a) Family A: Abridged British pedigree with posterior polar cataract; (b) Family B: Abridged Irish pedigree with posterior polar cataract. The diagonal line indicates a deceased family member. Squares and circles symbolize males and females, respectively. Open and filled symbols indicate unaffected and affected individuals, respectively. The arrow indicates the family members who participated in the WES analysis. All the available members in the family were sequenced to show the segregation.

a)
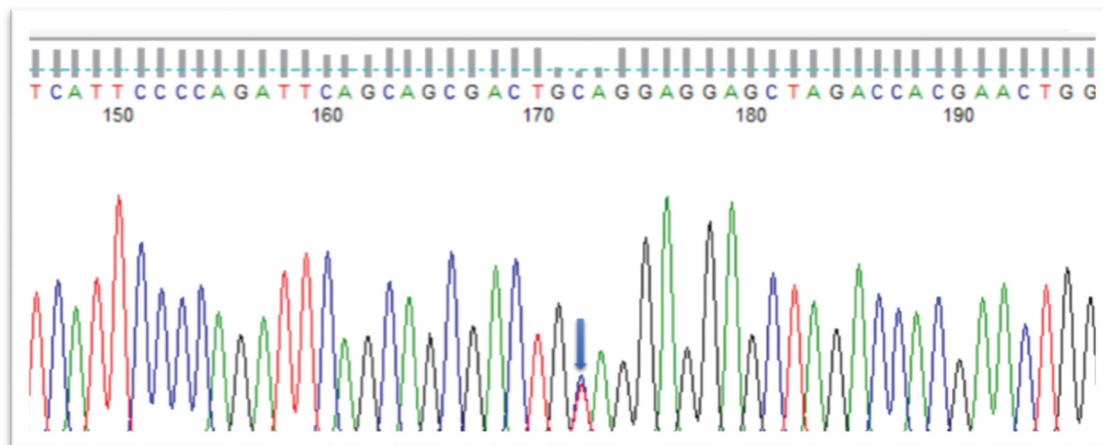
b)
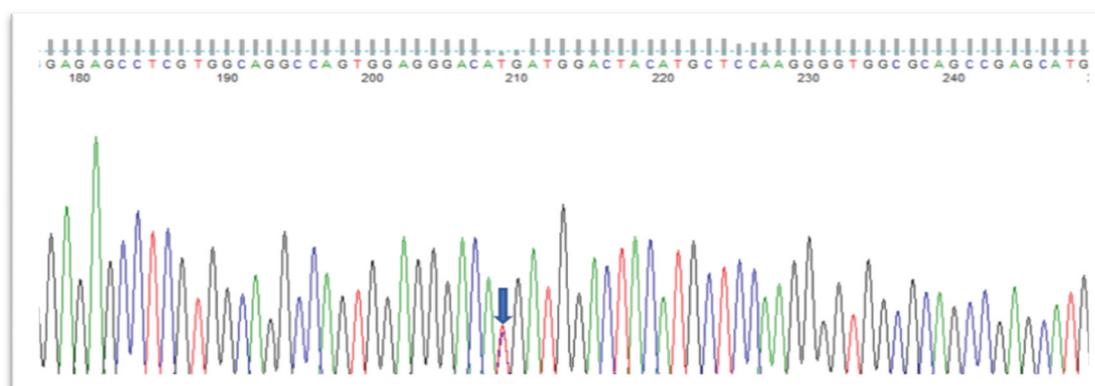
Figure 2. (a) Sequence analysis of *CYP21A2*: nonsense variant c.955 C > T in all affected member of family A, family B with autosomal dominant posterior polar cataract and an isolated individual C with congenital cataract; (b): missense variant c.770 T > C in individual D with congenital cataract.

Discussion

Cytochrome (CYP) P450 is a superfamily of 18 members encoding 57 genes in the human genome (11). It has a wide spectrum of functions such as synthesis of steroid hormones (e.g., oestrogen and testosterone), fatty acids, and sterols (cholesterol and bile acids). CYP enzymes are crucial for the synthesis and breakdown of non-protein ligands which bind to receptors or activate G protein-mediated pathways regulating growth, differentiation, apoptosis, homeostasis, and other neuroendocrine functions (12).

CYP21A2 gene consists of 10 exons, and it is a part of the RCCX module (**R**P2-**C**4B-**C**YP21A2-TN**X**B) located on chromosome 6p21.3 (13). *CYP21A2* shares 98% sequence homology with a non-functional pseudogene (*CYP21A1P*) that is arranged in tandem at the same site (14). Where truncations occur, such as R316X (15) and Q318X, the genotype-phenotype correlation can be complicated by the gene duplication despite enzyme inactivation, due to the removal of the heme binding domain by these truncations (9,16). The severe salt wasting phenotype requires all alleles to be affected (9). Within the spectrum of observed phenotypes associated with CAH, there is a report of keratoconus being linked to non-classical CAH (17).

The expression of CYP450 family members has also been identified in ocular tissues; CYP46A1 (24-hydroxycholesterol) has been identified in the brain (18) and retina (19); while CYP1B1 variants are responsible for primary congenital glaucoma (20) and CYP4V2 variants cause Bietti corneo-retinal crystalline dystrophy and retinitis pigmentosa (21,22).

CYP21A2 is expressed in lens cells (4) suggesting that congenital variants in *CYP21A2* can present with, albeit rare, eye phenotypes as observed for multiple other genes associated with both syndromic and non-syndromic (isolated ocular) disorders. The lens is an eye tissue that actively synthesizes sterols (23,24) and steroid medication is a well-established cause of cataract, presenting characteristically as a posterior subcapsular cataract (25,26).

CYP21A2 is a key player in the biosynthesis of aldosterone and cortisol. Aldosterone is expressed in the lens (27). This mineralocorticoid is important in the modulation and regulation of ion channels (28) and could therefore contribute to volume regulation and ion transport in the lens. Cholesterol-dependent transmembrane channel protein, TRPV4, acts as a mechanosensor and is highly expressed in lens epithelial cells and differentiating fiber cells, which are also responsible for volume regulation and ion

a) Wt. CYP21A2 Q319

b) Mut. CYP21A2 Q319*

c) Wt. CYP21A2 M257

d) Mut CYP21A2 T257
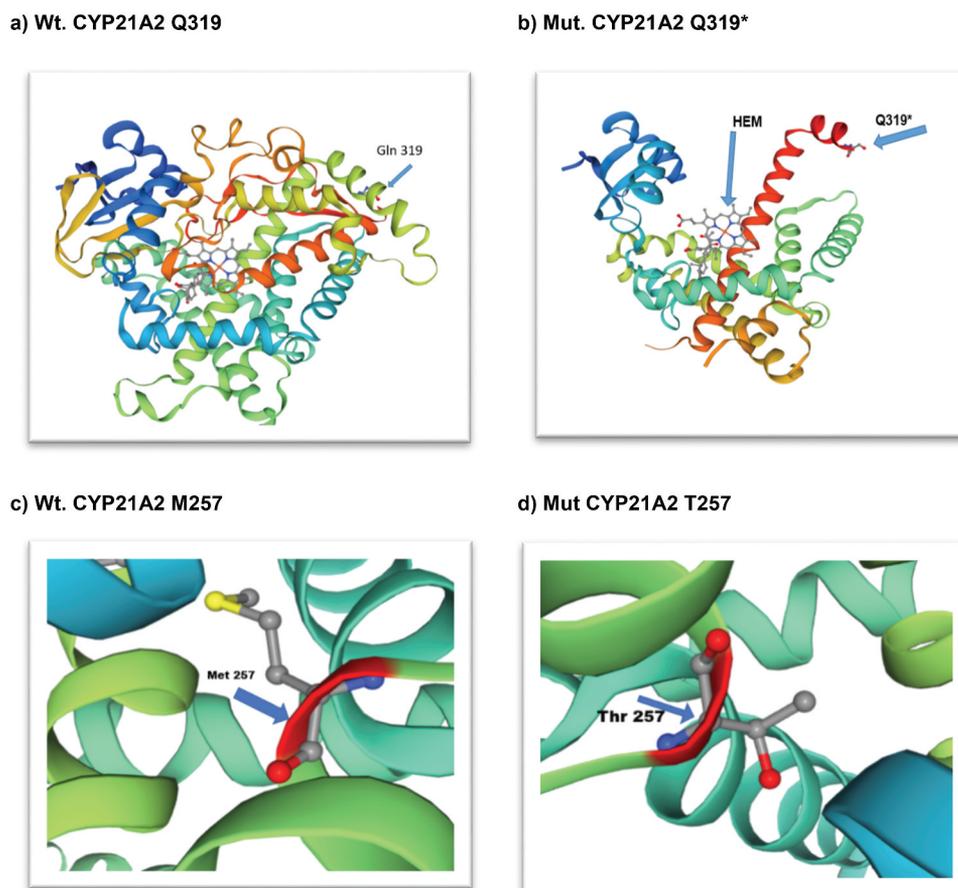
Figure 3. Structural view of human cytochrome P450 21A2 hydroxyprogesterone complex (CYP21A2): X-ray diffraction, 3.31 monomer 1x HEM (https://swissmodel.expasy.org/interactive); (a) wild-type CYP21A2 (30–485 amino acids); (b) mutant stop codon amino acid at 319 (Glutamine); (c) wild-type amino acid Methionine at position 257; (d) mutant missense amino acid Threonine at position 257.

Table 1. Pathogenicity scores of variants in *CYP21A2* gene in ADCC patients.

Sample	Genomic pos./Exon	Genomic coordinates (hg37)	HGVSc	HGVSp	MutationTaster/ verdict	GERP	CADD phred	SIFT	Phenotype	Origin
Fam-A-C	Chr6/Ex8	6–32008198-C-T	c.955 C > T	p.Q319*	Disease-causing /0.81-Pathogenic	5.09	43.0	Damaging	Posterior polar cataract	British, Irish and Asian Indian
Ind.-D	Chr6/Ex/7	6–32007813-T-C	c.770 T > C	p.M257T	Uncertain Significance	4.96	23.8	Damaging	Congenital cataract	British

Combined Annotation-Dependent Depletion (CADD) is score for the deleteriousness of a variant. A CADD score >15 is considered damaging; Sorts Intolerant From Tolerant (SIFT) is a score to check the deleteriousness of the amino acid substitution on the protein function; Genomic Evolutionary Rate Profiling (GERP) NR corresponds to the neutral rate conservation score of the site; * indicates the truncated protein.

exchange (29,30). Based on this, we hypothesise that CYP21A2 is likely important to the renin–angiotensin–aldosterone system (RAAS) (31) in the lens.

Nearly, 200 variants have been found in the *CYP21A2* gene causing autosomal recessive CAH; from severe classic CAH to non-classic CAH (NCCAH) with a milder phenotype (32), reflecting that other factors may be responsible for the genotype-phenotype variability in patients with CAH and NCCAH. Here, we have found pathogenic variants, p.Q319X, and one rare novel variant p. M257T in *CYP21A2* causing isolated congenital posterior polar cataract and congenital cataract with undefined phenotype, respectively. The p.Q319X variant

has been found previously in different ethnic groups in various parts of the world, displaying a wide spectrum of clinical features from severe to mild phenotypes (9). This variant p. Q319X is frequently found in the Iranian (16), Italian (33), and Brazilian (34) populations, both with CAH and NCCAH, harbouring either homozygous or compound heterozygous forms; and reported to coexist with the wildtype allele on the same chromosome, showing mild symptoms in patients (9).

In the families reported herein, the p.Q319X and p. M257T variants cause isolated congenital autosomal dominant congenital cataracts (ADCC) in European and Indian Asian ethnicities, further extending the genetic basis of

congenital cataracts. It is possible that haploinsufficiency accounts for the autosomal dominant nature of the cataract caused by these variants—although other genetic modifying factors and/or environmental influences may be involved. It is commonplace in the inherited retinal disease group of disorders that specific alleles can be associated with a huge range of differing phenotypes both within and between families, as well as causing both syndromic and non-syndromic manifestations. Moreover, the p.Q319X variant association with both syndromic recessive disease and isolated ADCC is in keeping with the phenotypes reported for certain *WFS1* variants (35).

Conclusions

Based on the data presented, we speculate that mineralocorticoids (aldosterone) and glucocorticoids (cortisol) play important roles in the physiopathology of cataract, and identify a novel cause of ADCC. As CYP21A2 is critical to their biosynthesis, this suggests the lens also actively contributes to the renin–angiotensin–aldosterone system (RAAS) of the eye. This report also alerts clinicians to the importance of a broader examination by endocrinologists, urologists and ophthalmologists for suspected CAH cases.

Acknowledgments

Author's Contribution

V.B. conceived, analyzed the data, wrote, and provided critical revision of the manuscript. A.I. provided clinical information. A.K., R.Q., and M. M. provided critical revision of the manuscript. N.P. contributed to WES data analysis.

Disclosure statement

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