

# PHENOTYPES OF RECESSIVE PEDIATRIC CATARACT IN A COHORT OF CHILDREN WITH IDENTIFIED HOMOZYGOUS GENE MUTATIONS (AN AMERICAN OPHTHALMOLOGICAL SOCIETY THESIS)

By Arif O. Khan MD, Mohammed A. Aldahmesh PhD, and Fowzan S. Alkuraya MD

## ABSTRACT

*Purpose:* To assess for phenotype-genotype correlations in families with recessive pediatric cataract and identified gene mutations.

*Methods:* Retrospective review (2004 through 2013) of 26 Saudi Arabian apparently nonsyndromic pediatric cataract families referred to one of the authors (A.O.K.) and for which recessive gene mutations were identified.

*Results:* Fifteen different homozygous recessive gene mutations were identified in the 26 consanguineous families; two genes and five families are novel to this study. Ten families had a founder *CRYBB1* deletion (all with bilateral central pulverulent cataract), two had the same missense mutation in *CRYAB* (both with bilateral juvenile cataract with marked variable expressivity), and two had different mutations in *FYCO1* (both with bilateral posterior capsular abnormality). The remaining 12 families each had mutations in 12 different genes (*CRYAA*, *CRYBA1*, *AKR1E2*, *AGK*, *BFSP2*, *CYP27A1*, *CYP51A1*, *EPHA2*, *GCNT2*, *LONP1*, *RNLS*, *WDR87*) with unique phenotypes noted for *CYP27A1* (bilateral juvenile fleck with anterior and/or posterior capsular cataract and later cerebrotendinous xanthomatosis), *EPHA2* (bilateral anterior persistent fetal vasculature), and *BFSP2* (bilateral flecklike with cloudy cortex). Potential carrier signs were documented for several families.

*Conclusions:* In this recessive pediatric cataract case series most identified genes are noncrystallin. Recessive pediatric cataract phenotypes are generally nonspecific, but some notable phenotypes are distinct and associated with specific gene mutations. Marked variable expressivity can occur from a recessive missense *CRYAB* mutation. Genetic analysis of apparently isolated pediatric cataract can sometimes uncover mutations in a syndromic gene. Some gene mutations seem to be associated with apparent heterozygous carrier signs.

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## INTRODUCTION

In Western populations, approximately one-third of congenital/juvenile nontraumatic cataract cases are familial, with an estimated prevalence of 14 per 100,000 children aged 0 to 17 years in a Danish prevalence study.<sup>1</sup> Western series that report genetic results suggest that familial pediatric cataract is often from dominant mutations in genes that encode crystallins,<sup>2,3</sup> which compose the major protein component of the lens. However, there are multiple other genes that encode enzymes, transcription factors, and signaling factors which when mutated could also lead to cataract; in addition, there are likely also mechanisms yet to be uncovered.<sup>4</sup> Determining the genetic cause of hereditary pediatric cataract is typically difficult in Western populations for several reasons. Autosomal dominant disease, the most common recognized hereditary form, is associated with variable expressivity (different phenotypes from mutations in the same gene) and nonallelic genetic heterogeneity (the same phenotype from mutations in different genes). This lack of phenotype-genotype correlation limits the ability to select an appropriate candidate gene for testing. Families tend to be small and outbred, precluding determination of a hereditary pattern from pedigree analysis and the use of molecular strategies such as linkage analysis and homozygosity mapping to guide candidate gene selection. Unrecognized single gene mutations likely underlie a percentage of cases labelled as idiopathic, particularly recessive causes, for which documented phenotype-genotype correlations are lacking.<sup>5</sup>

Our experience with pediatric cataract families from the Arabian Peninsula suggests that homozygous recessive causes are relatively common.<sup>6,7</sup> In a genomic study of pediatric cataract in the region, we found 17 in 24 familial cases to harbor underlying homozygous recessive gene mutations; we also found homozygous recessive gene mutations in nearly half (6 of 14) of sporadic cases.<sup>7</sup> Unlike in Western societies, in the Middle East families tend to be large and consanguineous (often first cousin marriage) or endogamous (intra-tribal marriage). Large family size increases the likelihood that more than one family member will manifest a hereditary condition if one is present, which facilitates recognition of inheritance patterns from pedigree analysis. Consanguinity and endogamy increase the likelihood that both parents will be carriers for the same recessive disease gene mutation (which they each inherited from a recent common ancestor) and thus that their offspring will be affected by that recessive disease (be homozygous, or more precisely autozygous, for that recessive mutation). In this setting, homozygosity analysis is a useful technique for narrowing candidate gene selection by revealing regions of homozygosity that are present in the affected family members but not in unaffected family members. We, among several investigators, have used this approach to successfully uncover homozygous recessive gene mutations for ocular genetic disease in the region.<sup>7,8</sup>

In our prior genomic study of recessive pediatric cataract, we reported uncovered gene mutations and mutation detection rate but did not highlight cataract phenotypes associated with the mutations.<sup>7</sup> We hypothesize that there may be phenotype-genotype correlations for some gene mutations. In the current study, we document the phenotypes of recessive pediatric cataract in families for which we uncovered homozygous recessive gene mutations from 2004 through 2013 in order to assess for potential phenotype-genotype correlations.

From the Division of Pediatric Ophthalmology, King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia (Dr Khan); Department of Genetics, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia (Dr Khan, Dr Aldahmesh, Dr Alkuraya); and Department of Anatomy and Cell Biology, College of Medicine, Alfaisal University, Riyadh, Saudi Arabia (Dr Alkuraya).

## BACKGROUND: CRYSTALLINE LENS DEVELOPMENT

Crystalline lens development<sup>9</sup> begins with formation of the lens placodes of the developing forebrain bilaterally at approximately day 25 of gestation. This is followed by the invagination of the lens pit, which develops into the lens vesicle. From the posterior side of the lens vesicle, primary lens fiber cells elongate into and obliterate its lumen; this occurs between the fourth and sixth week of gestation. The primary lens fiber organelles are degraded and expelled, allowing primary lens fibers to develop an organelle-free zone and become optically clear. These original primary lens fibers form the embryonic nucleus and can be appreciated clinically within the Y-sutures of the fully developed eye. At the anterior lens epithelium, secondary fiber cells formation begins. Here cells divide and move to the lens equator and elongate to the anterior and posterior pole of the lens, surrounding the earlier fiber cells. Where the secondary lens fibers meet each other can be seen clinically as the upright anterior Y-suture and posterior upside down Y-suture of the crystalline lens. This orderly process forms the lens cortex. More complex and less well-delineated sutures form throughout life.

The developed crystalline lens can be considered to be composed of three components—the lens capsule, the anterior cuboidal epithelium, and the lens fiber cells of the nucleus/cortex. The lens capsule is a protective outer basement membrane. The cuboidal epithelium regulates the exchange of nutrients, fluids, and salts. The lens fiber cells constitute the bulk of the crystalline lens and are not found elsewhere in the body. They are packed together tightly, forming lamellae; 90% of their mass is composed of crystallins, which are highly soluble very stable proteins. The classification of crystallins as alpha, beta, or gamma was originally based on decreasing molecular weights of the native proteins; since then much more has been discovered regarding the genomic organization and expression profiles of different types of crystallins.<sup>10</sup> Although the lens fiber cells are unique to the crystalline lens, crystallin genes have evolved from other genes and some still have functions in other parts of the body. Numerous processes and thus genes other than crystallin genes are necessary for lens clarity, and these include genes encoding cytoskeletal proteins, membrane-associated proteins, cell-signaling proteins, transcription factors, and proteins involved in metabolism.<sup>4</sup> One particularly important developmental process relevant to cataract in children is lens fetal vasculature and its abnormal persistence. During its development the crystalline lens is nourished by the tunica vasculosa lentis, which is supplied posteriorly by the hyaloid artery and anteriorly from anastomoses with vessels in the pupillary membrane. By birth the tunica vasculosa lentis has regressed, but sometimes remnants persist to a variable degree, causing a spectrum of persistent fetal vasculature associated with no or mild lens opacity to cataract.<sup>11</sup>

Whether cataract is related to gene mutations, persistent fetal vascular, or other cause, descriptive terminology for pediatric cataract is based on the morphology and location of the lens opacities (Figure 1). Phenotype descriptive terms include the following:

*Whole lens:* total, Morgagnian (outer layers liquefied, nucleus intact), disklike (following resorption), persistent fetal vasculature (often with posterior plaque/membrane).

*Central:* lamellar (shell), pulverulent (fine dots grouped together), nuclear, oil-droplet (galactosemia), cortical, cerulean (blue-dot).

*Anterior:* pyramidal, polar, capsular, subcapsular, lenticonus, cortical.

*Posterior:* Mittendorf dot (persistent fetal vasculature), polar, capsular, subcapsular, lenticonus, capsule defect, cortical.

*Forms:* punctate, fleck, sutural, coralliform (crystalline), wedge-shaped, cortical rider (radial linear spoke), persistent fetal vitreous (posterior plaque/membrane).

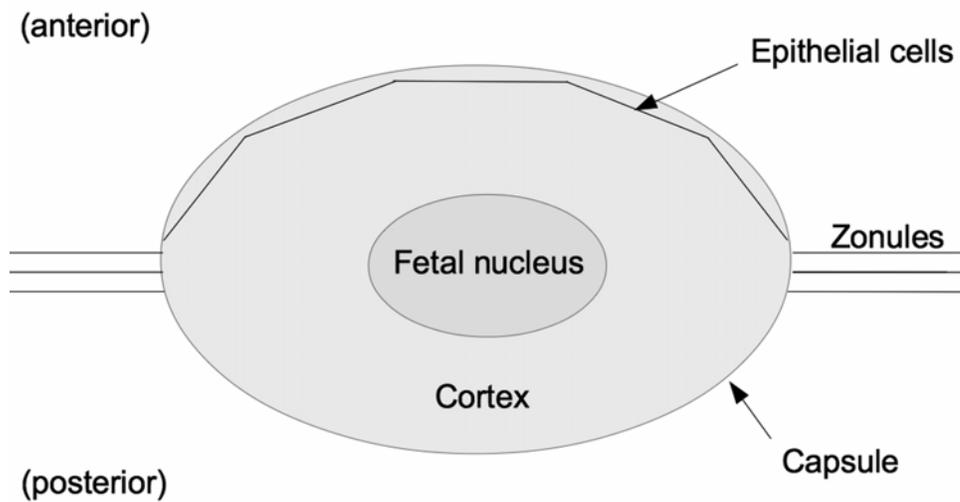


FIGURE 1

Simplified schematic of crystalline lens morphology.

## METHODS

Institutional review board (IRB) approval (of both the King Khaled Eye Specialist Hospital and the King Faisal Hospital and Research Center) was granted for this clinical and genetic study of pediatric cataracts. Approval was prospective for the genetic analysis; for clinical data, collection was both retrospective and prospective.

The families of children (younger than 18 years old) referred to one of the authors (A.O.K., from 2004 through 2013) who had nontraumatic and apparently nonsyndromic congenital/juvenile cataract, who underwent genetic testing, and for whom underlying recessive mutations were found are included in this study. These families had been referred for clinical care, were offered genetic testing under an IRB-approved protocol, agreed to participate, underwent informed consent, and were found to have associated recessive mutations. Available affected and unaffected family members of probands were tested and clinically examined whenever possible, at a minimum by slit-lamp examination. The basic strategy for genetic analysis, homozygosity-mapping-guided candidate gene selection and sequencing,<sup>12-14</sup> has been previously detailed.<sup>7</sup> Briefly, genotyping and homozygosity scanning was performed using the Axiom SNP Chip (Affymetrix, Santa Clara, California) for genome-wide genotyping of DNA extracted from venous blood; autoSNPa (<http://dna.leeds.ac.uk/autosnpa/>) was used for homozygosity analysis. Exome sequencing was performed using the TruSeq Exome Enrichment Kit (Illumina, San Diego, California). Captured libraries were sequenced on an Illumina HiSeq 2000 Sequencer, and reads were mapped against UCSC hg19 (<http://genome.ucsc.edu>) by BWA (<http://bio-bwa.sourceforge.net>). Single nucleotide polymorphisms and indels were detected by SAMtools (<http://samtools.sourceforge.net>). Primers for candidate gene confirmatory sequencing were designed using Primer3 for polymerase chain reaction with added M13 tails that were used for capillary sequencing. Primers were designed to cover all coding exons and at least 120 base pairs of the flanking introns.

For each family, all available affected and unaffected relatives of the proband were tested to confirm expected segregation analysis. Before a mutation was considered pathogenic, it was (1) confirmed to segregate in affected family members only (except for Family 16, which was a simplex case, but the unaffected parents were confirmed as heterozygous for the mutation), (2) confirmed not to be present in at least 100 ethnically matched controls (often more), and (3) predicted to be pathogenic by bioinformatic programs such as Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2>) and SIFT ([http://sift.jcvi.org/www/SIFT\\_enst\\_submit.html](http://sift.jcvi.org/www/SIFT_enst_submit.html)). Details for several of the mutations in this report were previously published.<sup>7</sup>

In the case of a recurrent *CRYBB1* deletion, after phenotype-genotype experience gained from previous cases, direct candidate gene sequencing was performed based on the clinical phenotype for some families.

## RESULTS

Our cohort includes 26 consanguineous families with homozygous recessive mutations in 15 genes (identified genes and mutations are summarized in Table 1). All families had more than one affected individual except Family 16, in which only one child was affected. All affected individuals were bilaterally affected. Genetic results for 21 of these families have been reported, and for 14 of these, phenotypes were described<sup>7,15-21</sup> as noted in Table 2. The 5 families (13, 14, 16, 24, and 26) and 2 genes (*LONP1*, *WDR87*) novel to this report are noted in Tables 1 and 2.

**TABLE 1. IDENTIFIED GENES WITH HOMOZYGOUS MUTATIONS AND ASSOCIATED WITH PEDIATRIC CATARACT**

GENE	OMIM NUMBER	REFERENCE NUMBER	FULL NAME	MAJOR KNOWN FUNCTION
<i>CRYBB1</i>	600929	NM_001887.3	Crystallin beta-B1	Lens crystallin
<i>CRYAA</i>	123580	NM_000394.2	Crystallin alpha-A	Lens crystallin
<i>CRYAB</i>	123590	NM_001885.1	Crystallin alpha-B	Lens crystallin
<i>CRYBA1</i>	123610	NM_005208	Crystallin beta-A1	Lens crystallin
<i>FYCO1</i>	607182	NM_024513	FYVE and coiled coil domain containing 1	Autophagy
<i>AGK</i>	610345	NM_018238.3	Acylglycerol kinasae	Mitochondrial acylglycerol kinase
<i>CYP27A1</i>	606530	NM_000784.3	Cytochrome P450, subfamily 27A, polypeptide 1	Cholesterol metabolism
<i>BFSP2</i>	603212	NM_003571.2	Beaded filament structural protein 2	Lens-specific filament-like protein
<i>EPHA2</i>	176946	NM_004431.3	Ephrin receptor ephA2	Receptor tyrosine kinase
<i>GCNT2</i>	600429	NM_001491.3	Glucosaminyl (N-acetyl) transferase 2, I-branching enzyme	Blood group I-antigen formation

TABLE 1. CONTINUED

GENE	OMIM NUMBER	REFERENCE NUMBER	FULL NAME	MAJOR KNOWN FUNCTION
<i>ARK1E2*</i>	...	NM_001040177	Aldo-keto reductase family 1, member E2	Aldo-keto reductase
<i>CYP51A1</i>	601637	NM_000786	Cytochrome P450, family 51, subfamily A, polypeptide 1	Cholesterol biosynthesis
<i>LONPI*</i>	...	NM_004793	LON protease 1	ATP-dependent protease
<i>RNLS*</i>	609360	NM_001031709.2	Renalase	Amine oxidases
<i>WDR87*</i>	...	NM_031951	WD repeat domain 87	?

ATP, adenosine triphosphate; OMIM, On-Line Mendelian Inheritance in Man.

\*The Saudi family is the only family ever reported with mutation in this gene; *LONPI* and *WDR87* are novel to this report.

The gene mutations and associated clinical findings for the 15 families are summarized in Table 2. Examples of clinical phenotypes and pedigrees are provided in Figures 2 through 8.

Families 1 through 10 all had the same *CRYBB1* deletion (c.171delG; p.Asn58Thrfs\*107). The 16 affected children had congenital central pulverulent lens opacity that was visually not significant and showed no evidence for progression over several years of follow-up. Heterozygous carriers had few discrete punctate lens opacities in the nucleus that may have represented carrier signs, as they were not present in examined genetically confined noncarriers. Clinical examples are shown in Figure 2. Pedigrees and additional information regarding these families, including analyses showing that the *CRYBB1* deletion represents a founder mutation, were previously published.<sup>7,18</sup> Central pulverulent cataract is apparently relatively specific for this founder *CRYBB1* deletion on the Arabian Peninsula, and there seem to be carrier signs in those heterozygous for the mutation.

Family 11, a nuclear family with 12 siblings, had *CRYAA* mutation (c.160C>T; p.Arg54Cys). The three homozygous children from this nuclear family (the 5th, 6th, and 12th siblings) had undergone bilateral lens aspiration with anterior vitrectomy within the first few months of life for congenital total white cataract with microcornea. Two developed aphakic glaucoma a few years postoperatively. Three asymptomatic genetically confirmed heterozygous carriers (both parents and the 11th sibling) had few but discrete punctate lens opacities within the lens nucleus that were not present in the six siblings who were noncarriers for the mutation. These potential carrier signs were difficult to photograph, but an example was previously published, as was genetic analysis of this family.<sup>15</sup> This family is an example of potential carrier signs in recessive pediatric cataract.

Family 12, a nuclear family with seven siblings, had *CRYAB* mutation (c.166C>T; p.Arg56Trp). The mother had had cataract surgery within the first few months of life, suggestive of dominant inheritance, but homozygosity analysis uncovered segregation of the homozygous *CRYAB* mutation with the phenotype, confirming pseudodominant rather than dominant inheritance.<sup>16,17</sup> The four siblings homozygous for the mutation and their mother, also homozygous for the mutation, showed variable expressivity, a phenomenon previously well described for dominant cataract but not for recessive cataract. Three of these five individuals had had bilateral cataract surgery—the mother (within the first few months of life for a cataract phenotype that was not documented), the first child (at 16 months of age for dense white cataracts), and the second child (at 6 years of age for bilateral nuclear cataract). The other two individuals homozygous for the mutation were asymptomatic but had lens opacities—the fourth sibling (nuclear and cortical opacities by slit-lamp examination at 8 years old, Figure 3) and the seventh sibling (fine dots in the retinoscopy reflex at 1 year of age). Members of this family who had had cataract surgery and were left aphakic later developed retinal degeneration, which may have been related to impaired *CRYAB* function in the retina.<sup>17</sup> Genetic analysis for this family was previously published<sup>16,17</sup> (sibling IV.5 was incorrectly marked with an asterisk<sup>17</sup>). Genetically confirmed heterozygous carriers were clinically normal. This family highlights the potential for marked variable expressivity in recessive pediatric cataract from mutations in this gene.

Family 13, a nuclear family with six siblings novel to this report (Figure 4), had the same gene mutation as family 12 (c.166C>T; p.Arg56Trp). Three children (the first, second, and sixth) had had bilateral juvenile cataract surgery for cataract phenotypes described as “total white.” Surgeries were at age 11 years, 1 year, and 1 year, respectively. One asymptomatic child, the third sibling, was homozygous for the mutation and did have lens opacities by slit-lamp examination at age 16 years. These were few in number and central punctate, linear, or wedge-shaped opacities (Figure 5). Only one of the confirmed heterozygous carriers, the mother, had lens opacities, which were few in number, central, and punctate or wedge-shaped (Figure 5). Like the other family with this *CRYAB* mutation (Family 12), this family highlights the potential for marked variable expressivity in recessive pediatric cataract from this gene mutation.

Family 14, a nuclear family with 13 siblings novel to this report (Figure 4), had *CRYABI* mutation (c.585\_588del; p.195\_196del). Two boys (the fourth and 13th siblings) had had lens aspiration and anterior vitrectomy in both eyes within the first few months of life for congenital total white cataract. Four asymptomatic family members (the mother and two siblings, the sixth and ninth) were heterozygous for the mutation but were unavailable for clinical examination.

TABLE 2. SUMMARY OF GENE MUTATIONS, PHENOTYPES, AND APPARENT CARRIER SIGNS

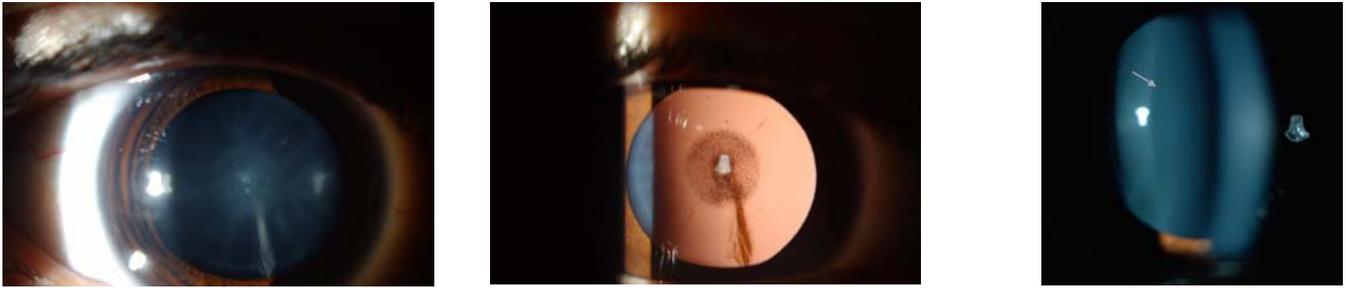
ID	GENE	HOMOZYGOUS RECESSIVE MUTATION	CASES	CARRIERS	BILATERAL PHENOTYPE*	ONSET†	FAMILY SOURCE (REFERENCE)	CARRIER SIGNS?*	COMMENTS
1-10	<i>CRYBB1</i>	c.171delG (p.Asn58Thrfs*107)	16	12	Central (nuclear) pulverulent*	Congenital	7,18	Yes*	Typically need refraction, not surgery, in early childhood
11	<i>CRYAA</i>	c.160C>T (p.Arg54Cys)	3	3	Total white with microcornea*	Congenital	15	Yes*	
12	<i>CRYAB</i>	c.166C>T (p.Arg56Trp)	5	4	Minimal to total white	Juvenile	16,17	No	Marked variable expressivity
13	<i>CRYAB</i>	c.166C>T (p.Arg56Trp)	4	3	Minimal to total white	Juvenile	This study	Unclear	Marked variable expressivity
14	<i>CRYBA1</i>	c.585_588del; (p.195_196del)	2	4	Total white	Congenital	This study	No	
15	<i>FYCO1</i>	c.2505del (p.Ala836ProfsX80)	2	2	Posterior capsular defect*	Congenital defect	7	No	Congenital defect and later cataract; some lens material in anterior vitreous
16	<i>FYCO1</i>	c.T449C (p.Ile150Thr)	1	2	Posterior capsular defect*	Congenital defect	This study	No	Congenital defect; later cataract
17	<i>AGK</i>	c.424-3C>G	3	3	Total white	Congenital	19	?‡	
18§	<i>CYP27A1</i>	c.1263+1G>A	3	2	Fleck with capsular opacity*	Juvenile	7	Yes*	
19	<i>BFSP2</i>	c.598_599dup; p.Ala201ArgfsX19	3	5	Fleck with cortical clouding*	Juvenile	21	Unclear	
20	<i>EPHA2</i>	c.1405T>C (p.Tyr469His)	2	2	Persistent fetal vasculature*	Congenital	7	Unclear	OD lens resorbed; OS dense posterior lens plaque
21	<i>GCNT2</i>	c.1040A>G (p.Tyr347Cys)	2	3	Total white	Congenital	7	Yes*	Unilateral retinal fold in one child
22	<i>ARK1E2</i>	c.582+1G>A	3	2	Total white	Congenital	7	Yes*	
23	<i>CYP51A1</i>	c.C829T (p.Arg77Cys)	2	1	Total white	Congenital	7	?	
24	<i>LONP1</i>	c.2014C>T (p.Arg72Cys)	3	3	Nuclear	Congenital	This study	Unclear	
25	<i>RNLS</i>	c.215_216delinsT (p.Lys72Ilefs*10)	3	2	Total white	Congenital	7	?	
26	<i>WDR87</i>	c.G856T (p.Glu286X)	2	2	Total white	Congenital	This study	?	

\*Indicates unusual distinct bilateral phenotype (column 6) or noted carrier signs (column 9).

†Congenital refers to opacities noted since birth; juvenile refers to symptoms first noted in the latter first or early second decade of life.

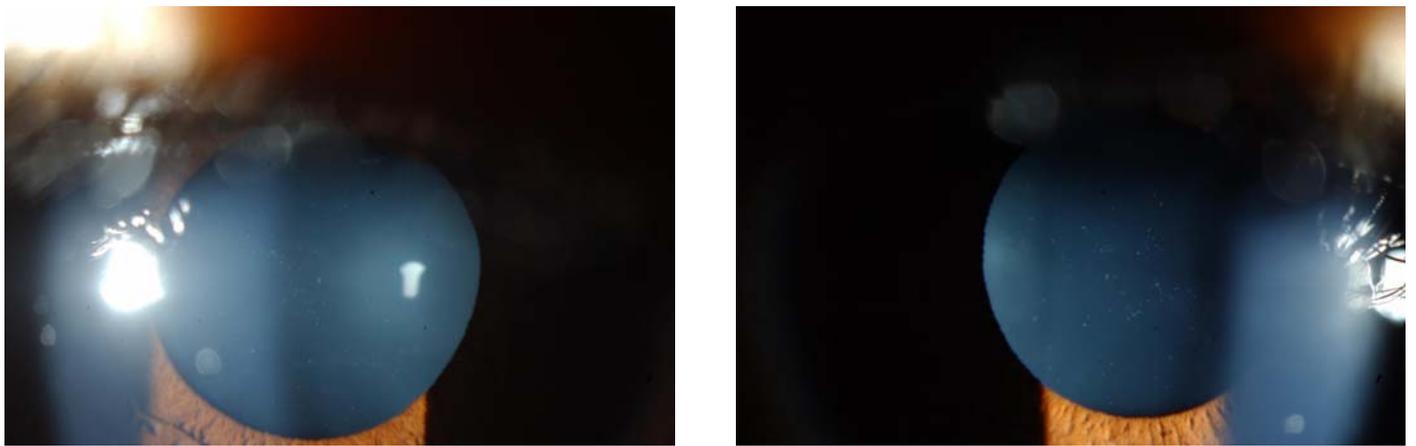
‡Carriers not examined.

§Only Family 18 had a syndromic phenotypic, as detailed in the text.



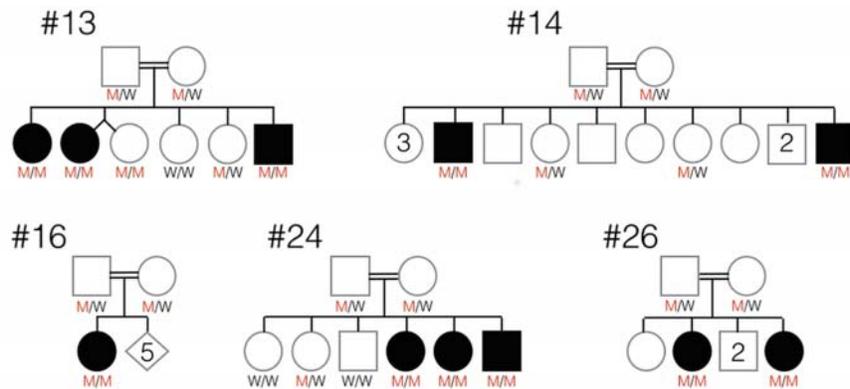
**FIGURE 2**

Left and Middle, Right eye of an affected boy (from Family 3) showing central pulverulent cataract associated with homozygosity for a founder *CRYBB1* deletion. There is an inferior liner cortical rider. Right, A 10-year-old boy (from Family 10) heterozygous for the *CRYBB1* deletion had a few punctate lens opacities in the nucleus and cortex; one is visible in the image (arrow).



**FIGURE 3**

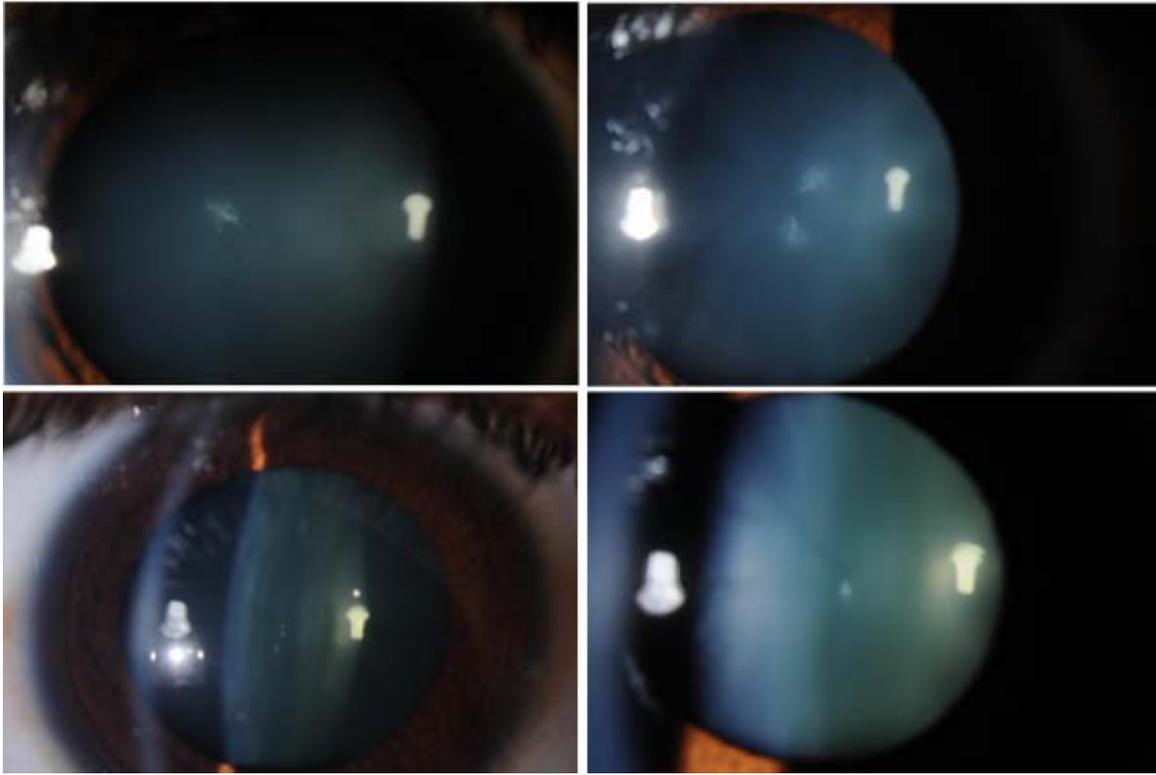
The fourth sibling in Family 12 was an asymptomatic 8-year old girl homozygous for the *CRYAB* mutation. Multiple scattered nuclear and cortical dot opacities can be appreciated by slit-lamp examination in both her right eye (on left) and left eye (on right).



**FIGURE 4**

The pedigrees of families novel to this report (Families 13, 14, 16, 24, and 26) are shown with allele status of mutated gene in tested family members. M, mutant allele, W, wild-type allele.

Blackened symbols represent clinically symptomatic individuals. See text for further details.



**FIGURE 5**

The third sibling in Family 13 was an asymptomatic 16-year-old girl homozygous for the *CRYAB* mutation. She had few central punctate, linear, or wedge-shaped lens opacities in both her right (top left) and left (top right) eyes. Only one of the confirmed heterozygous carriers, the mother, had lens opacities, which were also few in number, central, and punctate or wedge-shaped in both her right eye (bottom left) and left eye (bottom right).

Family 15, a nuclear family with five siblings, had *FYCO1* mutation (c.2505del; p.Ala836ProfsX80). The proband was the fourth sibling and was homozygous for the mutation. He presented with developmental complete white cataract at 4 months old (Figure 6). It was only intraoperatively that a bilateral preexisting posterior capsular defect was evident. His older sister (the second sibling), homozygous for the mutation, had had cataract surgery for developmental cataract between 1 and 2 years of age, but preoperative details of the cataract phenotype were not available. The asymptomatic parents, heterozygous for the mutation, were clinically normal. Genetic analysis of this family was previously published.<sup>7</sup> This family highlights an association between bilateral posterior capsular defect and recessive *FYCO1* mutation.



**FIGURE 6**

Nonspecific white cataract is seen preoperatively in boy (from Family 15) with homozygous recessive *FYCO1* mutation. Although not visible preoperatively, bilateral posterior capsular defects were evident intraoperatively.

Family 16, a nuclear family with six siblings novel to this report (Figure 4), had a different mutation in *FYCO1* (c.T449C;p.Ile150Thr). The affected girl, homozygous for mutation, had bilateral posterior lenticonus, which required surgery at the age of 4 years because of significant whitening of the lenses. Intraoperatively the lenses seemed of abnormal liquefied consistency and connected to vitreous. The parents, heterozygous for the mutation, were normal. Like the other family with *FYCO1* mutation (Family 15), this family highlights an association between bilateral posterior capsular defect and recessive *FYCO1* mutation.

Family 17, a nuclear family with four siblings, had a splice mutation in *AGK* (c.424-3C>G), the gene associated with the autosomal recessive mitochondrial disorder Sengers syndrome.<sup>19</sup> The three siblings homozygous for the mutation (first, third, and fourth siblings) had had bilateral lensectomy and anterior vitrectomy within the first few months of life for congenital complete cataract. Systemic reassessment of the siblings homozygous for the mutation after the genetic diagnosis did not reveal any signs of systemic disease.<sup>19</sup> Clinical ophthalmic examination of heterozygous carriers of the mutation was not performed. Details of clinical assessment and genetic analysis were previously reported.<sup>19</sup> This family highlights the potential for recessive pediatric cataract being from mutation in gene associated with systemic disease.

Family 18, a nuclear family with seven siblings, had a splice mutation in *CYP27A1* (c.1263+1G>A), the gene associated with the autosomal recessive lipid storage disease cerebrotendinous xanthomatosis.<sup>7,20</sup> The three siblings homozygous for the mutation had juvenile cataract that started as flecklike opacities before age 10 years (Figure 7). They also had had infantile intractable diarrhea and later developed cognitive decline, systemic features of cerebrotendinous xanthomatosis. The parents, heterozygous for the mutation, had few central punctate opacities around the Y-sutures. Details of this family and of the genetic analysis have been previously reported.<sup>7,20</sup> Like Family 17, this family highlights the potential for recessive pediatric cataract resulting from mutation in a gene associated with systemic disease.

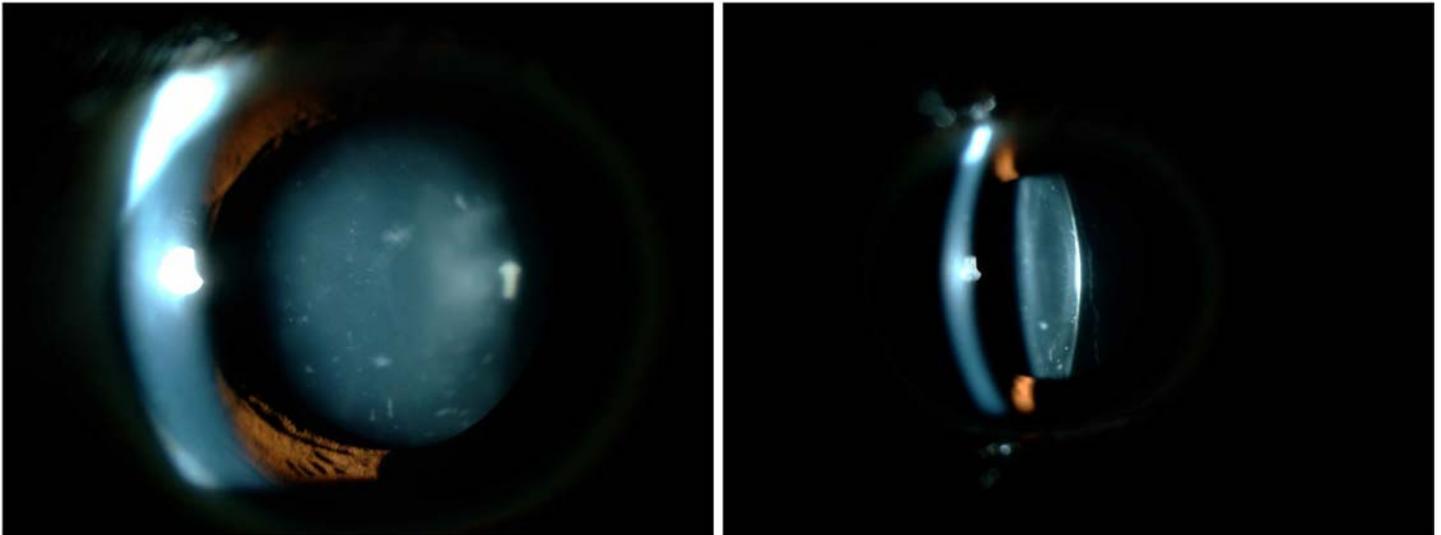


FIGURE 7

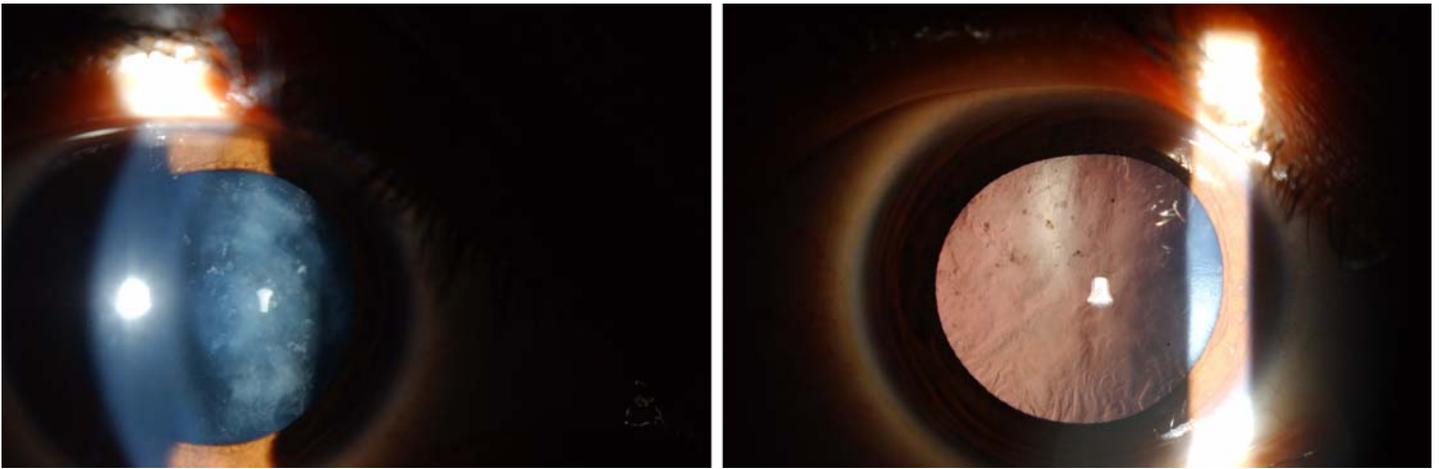
A unique pattern of juvenile fleck opacities and posterior capsular cataract in the right eye of girl (from Family 18) found to have homozygous recessive *CYP27A1* mutation and eventually diagnosed with cerebrotendinous xanthomatosis.

Family 19, a nuclear family with nine siblings, had *BFSP2* mutation (c.598\_599dup; p.Ala201ArgfsX19). The three sisters homozygous for the mutation had juvenile cataract with unusual flecklike opacities when symptomatic and were examined at approximately 11 years of age (Figure 8). The father, heterozygous for the mutation, had had cataract surgery in his early 40s, but the cataract phenotype was not documented. The mother, heterozygous for the mutation, was unavailable for clinical examination, as were the three siblings heterozygous for the mutation. Clinical examples and genetic analysis of this family were previously published.<sup>21</sup> This family highlights an unusual phenotype associated with an unusual gene mutation.

Family 20, a nuclear family with 11 siblings, had *EPHA2* mutation (c.1405T>C; p.Tyr469His). Two brothers homozygous for the mutation, the 10th and 11th siblings, had had lens aspiration and anterior vitrectomy for bilateral congenital cataract associated with bilateral persistent fetal vasculature within the first few months of life. The cataract was described as disklike following resorption. Three older sisters were affected by history but were not available for examination or testing. The mother, heterozygous for the mutation, had few small punctate lens opacities, but the father was unavailable for clinical examination. Genetic analysis of this family was previously published.<sup>7</sup> This family highlights an association between bilateral persistent fetal vasculature cataract and *EPHA2* mutations.

Family 21, a nuclear family with three siblings, had *GCNT2* mutation (c.1040A>G; p.Tyr347Cys). The two siblings homozygous for the mutation, the first and third siblings, had had lensectomy and anterior vitrectomy within the first month of life for complete

white cataract noted in the first week of life. The parents and the second sibling (10 years old), heterozygous for the mutation, had few small punctate central lens opacities that were difficult to photograph. Genetic analysis of this family was previously published.<sup>7</sup> This family is an example of potential carrier signs for recessive cataract.



**FIGURE 8**

A unique pattern of juvenile flecklike opacities and “dirty” cortex in the right eye of girl (from Family 19) found to have homozygous recessive *BFSP2* mutation.

Family 22, a nuclear family with three siblings, had a splice mutation in *ARKIE2* (c.582+1G>A). The three children, all homozygous for the mutation, had had bilateral lens aspiration and anterior vitrectomy with the first several weeks of life for the diagnosis of congenital complete cataract. The parents, heterozygous for the mutation, had few small central punctate opacities that were difficult to photograph. Genetic analysis of this family was previously published.<sup>7</sup> This family is an example of potential carrier signs for recessive cataract.

Family 23, a nuclear family with one daughter, had *CYP51A1* mutation (c.C829T; p.Arg77Cys). The father was affected, suggesting dominant inheritance, but genomic analysis confirmed homozygous *CYP51A1* mutation to segregate with the phenotype and thus confirmed pseudodominance. The father had had cataract surgery in early childhood, but the cataract phenotype was not available. The child had congenital nuclear cataract for which she underwent bilateral lens aspiration and anterior vitrectomy in the first month of life. Heterozygotes for the mutation were not available for clinical examination. Genetic analysis of this family was previously published.<sup>7</sup>

Family 24, a nuclear family of six siblings novel to this report (Figure 4), had *LONPI* mutation (c.2014C>T; p.Arg72Cys). The three children homozygous for the mutation, the fourth, fifth, and sixth siblings, had undergone bilateral lens aspiration and anterior vitrectomy within the first few months of life for what was documented as congenital nuclear cataract. Of the individuals heterozygous for the mutation, the parents and the second sibling, only the father had few small central punctate lens opacities. This family represents a potential novel candidate gene for recessive pediatric cataract.

Family 25, a nuclear family of seven children, had *RNLS* mutation (c.215\_216delinsT; p.Lys72Ilefs\*10). The three siblings homozygous for the mutation had undergone bilateral lens aspiration and anterior vitrectomy within the first few months of life for the diagnosis of congenital cataract. The individuals heterozygous for the mutations, three siblings and both parents, were not available for clinical examination. Genetic analysis for this family was previously published.<sup>7</sup>

Family 26, a nuclear family of five siblings novel to this study (Figure 4), had *WDR87* mutation (c.G856T; p.Glu286X). The two sisters homozygous for the mutation, the second and fifth siblings, had had bilateral lens aspiration and anterior vitrectomy in the first month of life for congenital complete cataract. The parents, both heterozygous for the mutation, were not available for clinical examination. This family represents a potential novel candidate gene for recessive pediatric cataract.

## DISCUSSION

Our phenotypic series, the largest of genetically confirmed recessive cataract to date, is composed of 26 consanguineous families with recessive pediatric cataract found to have underlying homozygous recessive mutations in 15 different underlying mutated genes, 11 of which are noncrystallin genes. For most genes, the associated phenotype was nonspecific congenital white cataract; however, some gene mutations were associated with apparently distinct phenotypes that we report here for the first time, eg, *FYCO1* mutations associated with posterior capsular defects. We also document marked variable expressivity for juvenile cataract from a recessive *CRYAB* mutation in two families, mutations in recessive syndromic genes in two families for which affected children were initially considered nonsyndromic, and apparent heterozygote carrier signs for several families.

*CRYBB1* encodes one of the beta-crystallins, beta-crystallin B1, and was the most commonly mutated gene in our series. All 10 families (Families 1 through 10)<sup>18</sup> found to have *CRYBB1* mutations had a specific homozygous deletion c.171delG

(p.Asn58Thrfs\*107) and the same phenotype of central pulverulent cataract, a correlation we have previously reported<sup>18</sup> (Figure 2). This phenotype was not seen with a different genotype, which indicates that on the Arabian Peninsula central pulverulent cataract is relatively specific for the *CRYBB1* homozygous deletion. These affected Saudi families shared a common haplotype, indicative of a single founder effect on the Arabian peninsula.<sup>18</sup> This finding of a single founder effect is in contrast to other pediatric cataract genotypes we describe in the current study and our past experience with most recessive ocular genetic disease in the Middle East, for which multiple mutations in multiple genes are common.<sup>8</sup> Why a founder effect for central pulverulent cataract exists on the Arabian Peninsula is unclear. Potential explanations include a relatively low mutation rate in *CRYBB1*, other mutations in *CRYBB1* causing phenotypes that are not suspected to be related to *CRYBB1*, or an unknown advantage to the carrier state. Fine lenticular opacities noted in those heterozygous for the mutation may have represented a carrier state.<sup>18</sup> The same recessive *CRYBB1* deletion was reported as a cause of recessive cataract in two Negev desert Bedouin families, but it is unclear whether that phenotype was the same phenotype as we describe.<sup>22</sup> An additional recessive *CRYBB1* mutation was reported in a Somali family, and the associated pediatric cataract phenotype was also described as pulverulent.<sup>23</sup> In other populations, dominant *CRYBB1* mutations have been reported and were associated with a variety of often nonspecific pediatric cataract phenotypes.<sup>24-28</sup> Regarding the phenotype of central pulverulent cataract, outside of the Arabian Peninsula it has been associated with dominant mutation in multiple genes, such as *BFSP2*,<sup>29</sup> *CRYBA1*,<sup>30</sup> *CRYBB1*,<sup>24</sup> *CRYBB2*,<sup>31</sup> *CRYGC*,<sup>32</sup> *GJA3*,<sup>33</sup> and *GJA8*.<sup>34</sup>

Alpha-crystallin is the largest protein component of the mammalian lens and is composed of two gene products, alpha-A (encoded by *CRYAA*) and alpha-B (encoded by *CRYAB*).<sup>10</sup> In addition to its refractive function, alpha-crystallin has a molecular chaperone role, protecting lens cells from stress-related cell death.<sup>10</sup> Family 11<sup>15</sup> had microcornea and congenital white cataract that segregated with a homozygous recessive c.160C>T (p.Arg54Cys) mutation, which we have previously reported as the second recessive mutation in this gene.<sup>15</sup> This was the only family in our series with associated microcornea; however, microcornea with congenital cataract is a well-recognized nonspecific phenotype that has been associated with dominant and recessive mutations in multiple genes.<sup>35</sup> Microcornea in cases of congenital cataract may be an inductive effect on the cornea from the abnormally developing lens rather than a direct result of the mutated gene on the cornea. In Family 11, carrier signs were distinct in affected heterozygotes.<sup>15</sup> The first reported recessive *CRYAA* mutation was described by Pras and colleagues,<sup>36</sup> who did not provide the specifics of the cataract phenotype. Two dominant *CRYAA* mutations have also been reported, and although phenotypes were not always detailed, some had microcornea or iris coloboma.<sup>37-39</sup>

While both alpha-crystallin components—alpha-A, encoded by *CRYAA*, and alpha-B, encoded by *CRYAB*—are found in tissues other than the lens, *CRYAB* expression is more widespread, including in the retina, where it has an antiapoptotic function.<sup>10</sup> Families 12<sup>16,17</sup> and 13 (novel to this report) both harbored the same homozygous recessive *CRYAB* mutation (c.166C>T; p.Arg56Trp) with a juvenile cataract rather than a congenital cataract phenotype. To the best of our knowledge, these are the only two recessive *CRYAB*-related cataract families published to date. We previously highlighted affected individuals from Family 12 who were left aphakic after cataract surgery because they years later developed retinal degeneration, whereas affected individuals who were left phakic or pseudophakic did not.<sup>17</sup> Decades of unfiltered light through the aphakic pupil may have led to retinal degeneration in the context of retinal *CRYAB* mutation, as the normal encoded protein has an antiapoptotic function in the retina. Potential carrier signs were not definitively seen in either family; only the mother in Family 13 had asymptomatic lens opacities. However, variable expressivity among those homozygous for the mutation was clearly documented for both families. In both families those children who presented with juvenile white cataract presented in the first or second decade of life. Lens opacities were likely present and less significant before presentation, but because the symptomatic children were not examined before presentation, the presymptomatic lens opacity phenotype is unknown. In addition, we did identify asymptomatic children from both families who were homozygous for the mutation and had lens opacities that very well may later develop into symptomatic cataract (Figures 3 and 5). Dominant *CRYAB* mutations have been associated with different forms of cataract with or without cardiac and/or skeletal myopathy,<sup>10,40</sup> but no individual in Family 12 or 13 had clinical evidence for skeletal or cardiac myopathy when evaluated with knowledge of this possibility in mind. Alpha-B accumulation is also associated with a broad range of neurological disease,<sup>10</sup> but no individual in Family 12 or 13 had clinical evidence of neurological disorders; moreover, *CRYAB* mutations are not known to cause neurological disease.

The fourth of the four mutated crystallin genes found in our series (in addition to *CRYBB1*, *CRYAA*, and *CRYAB*) was *CRYBA1* (c.585\_588del; p.195\_196del). By alternative splicing, *CRYBA1* encodes both beta-A1-crystallin and beta-A3-crystallin.<sup>10</sup> Worldwide, numerous dominant families with variable cataract phenotypes have been reported to date, often related to splice mutations.<sup>30,41-53</sup> To the best of our knowledge, Family 14 (novel to this report) is the second to be reported with a recessive *CRYBA1* mutation, the first being a recently reported developmentally delayed child with bilateral congenital cataract described as nuclear, cortical, and lamellar.<sup>53</sup> In Family 14, the phenotype of bilateral congenital white congenital cataract was nonspecific and there was no evidence for neurological delay. As virtually all previous mutations in this gene have been dominant, it would have been very interesting to assess for carrier signs in heterozygotes from this family with this novel *CRYBB1* mutation; however, they were not available for slit-lamp examination.

Autophagy is important for lens fiber maturation and formation of the lens fiber's organelle-free zone.<sup>54</sup> *FYCO1* encodes a binding protein involved in intracellular transport of autophagocytic vesicles.<sup>54</sup> Recessive mutations in the gene are rare but are responsible for up to 10% of recessive cataract in Pakistan (12 reported Pakistani families to date), with the phenotype mentioned as bilateral nuclear cataracts but not detailed.<sup>54</sup> In addition, an affected Arab Israeli family has also been reported,<sup>54</sup> as well as one child noted to have posterior lenticonus.<sup>53</sup> Families 15<sup>7</sup> and 16 (novel to this report) represent two additional families with private homozygous recessive *FYCO1* mutations and are the first to be reported with detailed phenotypes. Homozygous individuals from both families who were carefully examined before and at the time of surgery clearly had bilateral posterior capsular abnormalities, but these were only evident

at the time of surgery after lens material was aspirated because of the complete lens opacities preoperatively (Figure 6). The younger child from Family 15 (*FYCO1*: c.2505del; p.Ala836ProfsX80) presented at 4 months old; the older sibling had had cataract surgery between 1 and 2 years of age and was aphakic when evaluated by us. The child from Family 16 (*FYCO1*: c.T449C; p.Ile150Thr) presented at 4 years of age. We are aware of only one other gene that has been associated with bilateral capsular defects—recessive mutations in *TDRD7*, which encodes an RNA granule component.<sup>55</sup> Carrier signs were not seen in either Family 15 or Family 16.

Families 17<sup>19</sup> and 18<sup>7,20</sup> both had not been considered syndromic before our analysis uncovered homozygous recessive mutations in the syndromic genes *AGK* and *CYP27A1*, respectively, as we have previously reported.<sup>7,19,20</sup> Recessive *AGK* mutations cause Sengers syndrome, a mitochondrial depletion syndrome characterized by congenital cataracts, hypertrophic cardiomyopathy, and skeletal muscle disease.<sup>56</sup> In Family 17 three siblings had congenital cataract and an underlying homozygous recessive *AGK* mutation (c.424-3C>G), but recall and systemic assessment did not reveal any evidence for cardiomyopathy, skeletal disease, or any other extraocular issues.<sup>19</sup> The phenotype of congenital white cataract was not specific, and carrier signs were not assessed. Recessive *CYP27A1* mutations cause cerebrotendinous xanthomatosis, a neurodegenerative storage disease that is characterized by intractable infantile diarrhea, juvenile cataract, and neurological decline in early adulthood, which is preventable by early treatment.<sup>57</sup> In Family 18 three siblings had juvenile cataract and an underlying known homozygous recessive *CYP27A1* mutation (c.1263+1G>A). Recall and reassessment revealed a history of severe intractable diarrhea in infancy and recent neuropsychiatric issues in the affected siblings.<sup>20</sup> The juvenile cataract seemed unique, characterized early by flecks and anterior and/or posterior capsular opacities (Figure 7).<sup>20</sup> Dot opacities between the Y-sutures in the parents seemed to represent heterozygous carrier signs.<sup>20</sup>

*BFSP2* encodes a lens cytoskeletal protein, and dominant mutations in the gene have been associated with congenital and juvenile cataract described as lamellar, sutural, spokelike, and pulverulent.<sup>29,53,58-61</sup> Family 19<sup>21</sup> represents the only recessive mutation in *BFSP2* (c.598\_599depAGGC; p.Ala201GlyfsX6) associated with cataract to the best of our knowledge. In our experience the phenotype was very unique. Curved flecklike opacities were present throughout the lens, with a “dirty” appearance to the lens substance (Figure 8). The father was pseudophakic, having undergone bilateral cataract surgery at 40 years old. His preoperative cataract phenotype was not documented and may or may not have been related to his carrier state for this mutation. The mother was unavailable for clinical examination to confirm the possibility of carrier signs.

*EPHA2* encodes a protein that belongs to the ephrin receptor subfamily of the protein-tyrosine kinase family, a class of proteins important in developmental cell-cell interactions, particularly in the nervous system. Dominant mutations in the gene have been implicated in autosomal dominant cortical or posterior cataract,<sup>62-64</sup> heterozygous variants have been associated with age-related cataract,<sup>65-71</sup> and recessive *EPHA2* mutations were associated with juvenile nuclear cataract in one Pakistani family<sup>72</sup> and recently with lamellar cataract in one dysmorphic child.<sup>53</sup> Family 20<sup>7</sup> represents an additional recessive case with private homozygous mutation (*EPHA2*: c.1405T>C; p.Tyr469His), making the total of reported recessive cases to date three, and our detailing the associated phenotype herein reveals interesting features. The cataract was resorbed in the setting of bilateral anterior persistent fetal vasculature. There was no retinal involvement, and the eyes were not microphthalmic. The mother of Family 20 may have had carrier signs, but other individuals heterozygous for the mutation were unavailable for examination. Persistent fetal vasculature commonly underlies unilateral congenital cataract, but bilateral cases are rare.<sup>11</sup> Moreover, when bilateral, persistent fetal vasculature cataract is often associated with frank microcornea, retinal involvement, and syndromic findings.<sup>73</sup> To the best of our knowledge, no genes have been previously associated with persistent fetal vasculature-related cataract as occurred in Family 20. Supporting our observation of this phenotype-genotype correlation is a recent study that documented ephrin-A5 as critical for the regression of the primary vitreous in mice.<sup>74</sup> Also supporting our suggestion that cataract from recessive *EPHA2* mutations can lead to persistent fetal vasculature cataract are the comments regarding the recently reported dysmorphic child with lamellar cataract and a recessive *EPHA2* mutation<sup>53</sup>; his ocular vessels were described as having “abnormal trafficking,” the vitreous was described as abnormal, and the eyes were small (hyperopic).<sup>53</sup> A related but distinct phenotype was reported in one Pakistani family, which harbored a recessive mutation in *ATOH7* associated with bilateral posterior fetal vasculature with retinal involvement.<sup>75</sup>

*GCNT2* encodes three isoforms of glucoaminyl (N-acetyl) transferase 2. The second isoform is found in the crystalline lens, whereas the third isoform is found on the surface of erythrocytes, where it converts the liner i-antigen to the branched I-antigen.<sup>76</sup> Recessive mutations in *GCNT2* have been documented to cause congenital cataract and the i-antigen erythrocyte phenotype in four Arab Israeli families and two Pakistani families.<sup>77,78</sup> Family 21 (novel to this report) represents the sixth reported *GCNT2*-related pediatric cataract family to date (c.1040A>G;p.Tyr347Cys), and the phenotype was nonspecific congenital white cataract. Previous reports mentioned the phenotype as congenital without further detail.<sup>77,78</sup> In Family 21 one child was noted to have a horizontal congenital retinal fold in one eye after the cataract was removed; this may or may not be related to the mutation. Few central dot opacities were seen in the carrier parents.

Family 23<sup>7</sup> harbored a homozygous recessive mutation in *CYP51A1* (c.C829T; p.Arg77Cys), a gene involved in cholesterol biosynthesis, and when the family’s genetic results were reported as part of a pediatric cataract cohort, it was the first time cataract was associated with this gene.<sup>7</sup> Herein we document that the phenotype of this family was nonspecific congenital white cataract and that the affected individuals were otherwise neurologically and systemically normal. Recently, a boy described as having nuclear and lamellar cataract, developmental delay, spastic diplegia, and cryptogenic neonatal liver cirrhosis was found to have compound heterozygous mutations in *CYP51A1*<sup>53</sup>; this is further validation that recessive mutations in the gene can underlie recessive congenital cataract. That boy’s systemic manifestations may have been the result of variable expressivity of the mutations or separate unrelated problems. Heterozygotes in Family 23 were not available for carrier sign assessment.

For four families in our series (22, 24, 25, 26), homozygous recessive mutations were identified in four genes that have not been otherwise associated with cataract (*ARK1E2*, *LONP1*, *RNLS*, *WDR87*, respectively, with *LONP1* and *WDR87* being novel to this

report).<sup>7</sup> None of these cataract phenotypes had unique distinctive features (all bilateral congenital total cataract). Independent confirmation in additional families is needed before these genes can be considered definitively associated with recessive cataract. *AKR1E2* encodes a member of the aldo-keto reductase superfamily. *LONP1* encodes a mitochondrial matrix protein in the Lon family of ATP-dependent proteases. *RNLS* encodes a FAD-dependent monoamine oxidase that catabolizes circulating catecholamines. *WDR87* is a protein-coding gene of unknown function. The carrier parents in Family 22 (*ARK1E2*) had several central punctate lens opacities suggestive of carrier signs. For Families 24, 25, and 26, the presence of potential carrier signs was either unclear or not assessed (Table 2).

Limitations of our study include a lack of functional work to confirm the pathogenicity of the uncovered mutations, and this is one reason we cannot conclusively state that homozygous recessive gene mutations found for only one family and not previously associated with cataract (Families 22, 24, 25, 26) were definitive causative mutations. We only looked at homozygous recessive mutations without taking into consideration the potential effects of other variants in the rest of the genome. We cannot absolutely confirm that observed lens opacities in heterozygotes from some families which we considered carrier signs were truly manifestations of carrier states for mutations, as we do not have data regarding the prevalence of such lens opacities in the general population. Our referral setting is a limitation in that it is not a multispecialty hospital; because it only accepts children with ocular disease who are otherwise generally healthy, homozygous recessive causes of cataract that also cause significant extraocular disease are not part of this study. Moreover, we are unable to comment on visual outcomes after surgery because of large variability in regard to family compliance with the operative date, postoperative refractive correction, postoperative amblyopia treatment, and follow-up appointments. This large variability confounds visual outcome data.

Genetic testing of our patient population has allowed us to make unique observations regarding phenotypes and genotypes of recessive cataract in the current study, the largest such series to date. According to our data, a majority of identifiable recessive genes are noncrystallin and most recessive gene mutations result in nonspecific pediatric cataract phenotypes. These genes could be included on future diagnostic pediatric cataract gene panels. In addition, we document specific phenotypes that are exceptional in that they seem associated with particular recessive gene mutations, information that can be useful for future directed genetic testing. The association between central pulverulent cataract and homozygosity for a regional founder *CRYBB1* deletion is an observation we have made before,<sup>18</sup> but other notable phenotypes we highlight here for the first time include bilateral posterior capsular defects (recessive *FYCO1* mutations), bilateral anterior persistent fetal vasculature-related cataract (recessive *EPHA2* mutations), and the potential for marked variable expressivity in families with recessive pediatric cataract from a recessive *CRYAB* mutation. We found recessive mutations in syndromic disease genes for two families that were initially considered to have isolated familial pediatric cataract, highlighting that the possibility should always be a consideration in children with cataract. In addition, we found apparent carrier signs for several different recessive gene mutations, which could be useful when assessing pediatric cataract families with suspected recessive inheritance.

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