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Confirmation and Refinement of the Heterozygous Deletion of the Small Leucine-rich Proteoglycans Associated with Posterior Amorphous Corneal Dystrophy

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Abstract

Purpose: To present the clinical and cytogenetic features of a previously unreported family with posterior amorphous corneal dystrophy (PACD) associated with a heterozygous deletion of the small leucine-rich proteoglycan genes on chromosome 12.

Methods: Clinical characterization was performed using slit lamp biomicroscopic and optical coherence tomography (OCT) imaging. Genomic DNA was collected from affected and unaffected family members, and a cytogenomic array was used to identify copy number variations (CNV) present in the PACD locus.

Results: Three members of a Guatemalan family presented with clinical characteristics consistent with PACD: bilateral posterior stromal lamellar opacification, decreased corneal curvature and iridocorneal adhesions. OCT imaging demonstrated decreased corneal thickness and hyperreflectivity of the posterior third of the corneal stroma. CNV analysis confirmed the presumed clinical diagnosis of PACD by revealing a 0.304 Mb heterozygous deletion in the PACD locus on chromosome 12 that included the four small leucine-rich proteoglycan (SLRP) genes (*KERA*, *LUM*, *DCN*, and *EPYC*) deleted in each of the PACD families in which CNV analysis has been reported.

Conclusions: This is the first report of the OCT appearance of PACD and the second confirmation of a heterozygous deletion of chromosome 12q21.33 as the cause of PACD, highlighting the utility of array-based cytogenomics to confirm the suspected clinical diagnosis of PACD. As the smallest previously reported pathogenic deletion was 0.701 Mb, the 0.304 Mb deletion we report is the smallest identified to date and reduces the size of the PACD locus to 0.275 Mb.

Keywords

posterior amorphous corneal dystrophy; PACD; small leucine-rich proteoglycans; SLRPs; copy number variation; CNV; optical coherence tomography; OCT

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

INTRODUCTION

Posterior amorphous corneal dystrophy (PACD [MIM 612868]), a rare autosomal dominant dystrophy, is characterized by posterior stromal lamellar opacification, decreased central corneal thickness, and reduced corneal curvature. Additional clinical features may include peripheral corneal scleralization and iris abnormalities, including iris coloboma, corectopia, iris atrophy, and iridocorneal adhesions [1–11].

We previously reported linkage of PACD in a large pedigree to a region on chromosome 12q21.33 that spans 3.5 Mb [1]. After whole exome sequencing of all protein coding genes failed to demonstrate a potentially pathogenic mutation, we hypothesized that PACD could be associated with a structural genomic change present in the chromosome 12 locus [1, 11]. A cytogenomic array used to identify copy number variations (CNV) present in the PACD locus revealed a 701 kb heterozygous deletion in this family and 701 kb and 1.32 Mb heterozygous deletions in two other unrelated families [1, 11, 12]. Subsequently, PACD was reported in association with a de novo heterozygous deletion (1.67 Mb) in the PACD locus, providing the first independent confirmation of the association of PACD with deletion of the small leucine-rich proteoglycan (SLRP) genes (keratocan (*KERA* [MIM 603288]), lumican (*LUM* [MIM 600616]), decorin (*DCN* [MIM 125255]), and epiphykan (*EPYC*, also known as *DSPG3* [MIM 601657])), as well as *CCER1* and *LINC00615* [12].

Herein, we present a previously unreported pedigree with PACD, only the fifteenth reported to date. We describe the findings of corneal optical coherence tomography (OCT) imaging in PACD for the first time, as well as the use of CNV analysis to identify a heterozygous deletion in the PACD locus on chromosome 12, refining the disease-causing locus.

MATERIALS AND METHODS

Ethics statement

The standards set forth by the Declaration of Helsinki were utilized in the treatment of the subjects reported in this study. The Institutional Review Board at the University of California, Los Angeles (UCLA IRB 11–000020) provided approval of the study, and all subjects provided written informed consent.

Patient identification and DNA collection

After each individual provided informed consent, slit lamp biomicroscopy and corneal topography were performed to determine affected status in regards to PACD. The affected status was determined based on the presence of one or more of the following clinical characteristics: partial or complete posterior lamellar corneal opacification; scleralization of the peripheral cornea; significant corneal flattening (average keratometry value of <39.0 D in each eye); decreased central corneal thickness (<500 microns in each eye); and iris abnormalities (coloboma, corectopia, atrophy, iridocorneal adhesions). DNA was collected using saliva collection kits (Oragene; DNA Genotek Inc., Kanata, ON, Canada).

Corneal imaging

Anterior segment optical coherence tomography corneal imaging (vertical cross section images and corneal pachymetry measurements) (Optovue, Fremont, California) was performed on affected family members. Corneal topographic imaging (NIDEK OPD-Scan III, Jacksonville, FL) was performed on both affected and unaffected individuals.

Copy number variation analysis

Genomic DNA isolated from saliva samples provided by affected and unaffected family members was submitted to the UCLA Technology Center for Genomics & Bioinformatics. Genomic DNA was hybridized to the CytoScan™ HD array (Thermo Fisher Scientific, Waltham, MA), containing over 2.6 million SNP and copy number probes. Data analysis was performed using the Chromosome Analysis Suite (ChAS) software, version 3.2 (Thermo Fisher Scientific, Waltham, MA). Analysis setup within the ChAS included the na36.r1 reference model and na.36 annotation database. Analysis was performed using the hg38 human reference genome with default settings.

RESULTS

Clinical characterization

Four family members were clinically evaluated (Figure 1: individuals II-1, II-2, III-1, III-2), and three were diagnosed with PACD based on the presence of bilateral posterior stromal lamellar opacification and significant corneal flattening (Table 1, Figures 2–3). The 5-year-old proband (Figure 1: III-2) demonstrated diffuse irregular posterior stromal lamellar opacification OU, inferotemporal iridocorneal adhesion, mild temporal iris atrophy and nasal corectopia OD. Corneal topographic imaging demonstrated flattening OU (average keratometry values of 37.73 D OD, 40.53 D OS), and OCT imaging of both corneas demonstrated diffuse stromal thinning and significant posterior stromal hyperreflectivity. Ultrasound pachymetry confirmed decreased central corneal thickness (485 µm OD, 427 µm OS). The proband's 14-year-old brother (Figure 1: III-1) also demonstrated incomplete central and peripheral posterior corneal stromal opacification OU, corresponding to posterior stromal hyperreflectivity seen on OCT imaging. Corneal topographic imaging demonstrated even flatter corneas than the proband (average keratometry values of 35.65 D OD, 36.14 D OS) but less corneal stromal thinning (central corneal thickness 502 µm OD, 499 µm OS). The proband's 50-year-old father (Figure 1: II-2) also presented with diffuse, incomplete posterior corneal opacification, flattened corneal curvatures (38.85 D OD, 38.66 D OS) and decreased central corneal thickness (453 µm OD, 521 µm OS). As observed on OCT imaging of the proband and his brother, decreased corneal thickness and posterior stromal hyperreflectivity were observed on OCT imaging of the proband's father. The proband's 39-year-old paternal aunt (Figure 1: II-1) demonstrated clear corneas with normal corneal curvature in each eye (42.46 D OD, 42.07 D OS). All four individuals provided DNA for genetic analysis.

Copy number analysis identifies a refined heterozygous deletion interval

Cytogenomic array analysis of the proband's genomic DNA sample identified a 0.304 Mb heterozygous deletion (hg38, chr.12: 90,914,489–91,218,206) in the region of chromosome 12q21.33 containing four SLRP genes (*KERA*, *LUM*, *DCN*, and *EPYC*) as well as *CCER1* (coiled-coil glutamate rich protein 1) and *LINC00615* (long intergenic non-protein coding RNA 615) (Figure 4: III-2). The identical and nearly identical (hg38, chr.12: 90,914,489–91,207,189) deletion were identified in the proband's affected father (Figure 4: II-2) and brother (Figure 4: III-1), respectively, but were not present in his unaffected paternal aunt (Figure 4: II-1).

DISCUSSION

We report the identification of a 0.304 Mb hemideletion in a previously unreported pedigree with PACD that reduces the common PACD interval to a 0.275 Mb region that includes *CCER1*, *LINC00615*, *KERA*, *LUM*, *DCN*, and *EPYC*. Four of these genes, *KERA*, *LUM*, *DCN*, and *EPYC*, are members of the SLRP protein family, and all except for *EPYC* are expressed in the cornea and have been reported to play a role in corneal development, structure, function, and transparency [11]. Although *CCER1*, which is also not expressed in the cornea, is a protein-coding gene, the function of the encoded protein is currently unknown [11, 13]. *LINC00615* is a gene that transcribes a non-protein-coding RNA and because little information is known about the function of the gene, its significance in the pathogenesis of PACD is unknown and is difficult to determine.

As the remaining SLRPs (minus *EPYC*) have known functional roles in the cornea, we consider PACD to be secondary to heterozygous insufficiency of one or more of these three corneal expressed SLRPs.

The role of SLRPs in the cornea has been widely investigated in animal models and in humans and has generally demonstrated important functional roles for *KERA*, *LUM* and *DCN* [14–18]. Loss-of-function mutations in *DCN* have been associated with congenital stromal corneal dystrophy (CSCD [MIM 610048]), which presents with bilateral congenital corneal opacification [19–23]. In addition, electron tomography of CSCD corneas has revealed abnormal stroma with thicker collagen fibrils, suggesting that *DCN* plays a significant role in maintaining organization of the corneal extracellular matrix [24]. Mutations in *KERA* result in autosomal recessive cornea plana (CNA2 [MIM 217300]), which is characterized by overlapping clinical features with PACD, including decreased corneal curvature, corneal opacification and reduced corneal thickness [25, 26]. While a distinct corneal dystrophy has not been associated with coding region mutations in *LUM*, single nucleotide polymorphisms (SNPs) within the non-coding (e.g., promoter and untranslated regions) regions of *LUM* have been correlated with high myopia (MYP3 [MIM 603221]) [27]. However, based on the corneal phenotype observed in mice deficient for each of the SLRPs and the observed interaction between the SLRPs expressed in the cornea, *LUM* deficiency is likely necessary and possibly sufficient to cause PACD [11]. Genetic analysis of additional PACD pedigrees in which heterozygous deletions are observed excluding one or more of the genes in the refined common interval may eventually provide evidence for *LUM* or another SLRP as the primary genetic basis of PACD. Alternatively,

PACD may continue to be associated in each pedigree with a heterozygous deletion of each of the SLRP genes on chromosome 12.

We report the smallest deletion to date associated with PACD, reducing the size of the PACD locus to 0.275 Mb, defined by the 5' breakpoint (90,914,489 bp) of the family we report here and the 3' breakpoint (91,189,180 bp) of a previously reported family (Table 2: Family 3) [11]. While we report exact breakpoint positions, we caution against accepting the reported breakpoints as precise positions due to the relatively poor resolution of array-based techniques. The breakpoints derived from array-based systems are an approximation, and the accuracy of the predicted breakpoint is dependent on the density of probes interrogating the region of interest. As such, we suspect the difference in the breakpoints between the proband, his father and brother to be due to the technical limitations of the array.

We also present the initial report of the appearance of PACD on OCT corneal imaging. While the stromal opacification appears incomplete and to involve only the pre-Descemet stromal lamella on slit lamp examination, the posterior stromal hyperreflectivity observed on OCT imaging appears to be relatively evenly distributed across the cornea and to involve the posterior third of the stroma. Serial corneal OCT imaging of the affected individuals in this and other families with PACD will be a useful means to determine whether the posterior stromal lamellar opacification and corneal stromal thinning are stable or progressive over time, and to characterize the degree of phenotypic variability of the posterior stromal opacification in PACD.

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Figure 1.

Pedigree of a family with PACD. Filled symbols represent affected individuals, open symbols represent unaffected individuals, and the arrow indicates the proband. Asterisk (*) represents individuals from whom gDNA was isolated for this study.

Figure 2.

Clinical features of a family with PACD. Slit lamp photomicrographs of the 5-year-old proband's (Fig 1: III-2) right eye demonstrates iridocorneal adhesion (A) and left eye demonstrates central posterior stromal opacification (B). Corneal topographic imaging of the right eye demonstrates a reduced corneal curvature with an average keratometry value of 37.73 D (C). Slit lamp photomicrographs of the proband's 14-year-old brother's (Fig 1: III-1) right (D) and left (E) eyes demonstrates lamellar posterior stromal opacification. Corneal topographic imaging of the right eye demonstrates significant corneal flattening with an average keratometry value of 35.65 D (F). Slit lamp photomicrographs of the proband's 50-year-old father's (Fig 1: II-2) right (G) and left (H) eyes demonstrates incomplete lamellar posterior stromal opacification. Corneal topographic imaging of the right eye demonstrates a decreased corneal curvature with an average keratometry value of 38.85 D (I).

Figure 3.

Anterior segment OCT images of a Guatemalan family with PACD. The 5-year-old proband's (Fig 1: III-2) left cornea demonstrates reduced corneal stromal thickness with hyperreflectivity of the posterior third of the stroma (A). The proband's 50-year-old father's (Fig 1: II-2) left cornea displays diffuse thinning with a hyperreflective posterior stroma, most prominent in the central portion of the cornea (B). The proband's 14-year-old brother's (Fig 1: III-1) right cornea displays diffuse stromal thinning and hyperreflectivity of the

posterior third of the stroma, most prominent in the central cornea (C). In all three individuals, the anterior two thirds of the cornea appear normal.

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Figure 4.

Copy number analysis of a region of chromosome 12 in a Guatemalan family with PACD. Four individuals (1 unaffected, 3 affected) were analyzed for CNV within the PACD locus on chromosome 12 (hg38). While the unaffected individual (II-1) did not demonstrate a change in copy number in the PACD locus, a nearly identical heterozygous deletion (approximately 0.3 Mb) was identified in three affected individuals (II-2, III-1 and III-2). A schematic of the genes within the region of the heterozygous deletion is shown at the bottom. Signals for individual probes within the interrogated chromosome 12 region are plotted as a weighted Log2 ratio, and their distribution along this genomic region is interpreted as either no change (blue line, 2 copies) or as a heterozygous deletion (red line, 1 copy).

Table 1.

Clinical features of members of a Guatemalan family with PACD

Patient	Sex	Eye	Corneal topography			Clinical Exam			
			Steep K	Flat K	Average K	Corneal exam	Corneal Pachymetry	Iris exam	
II-1	F	OD	43.05	41.87	42.46	Clear		Normal	
		OS	42.72	41.41	42.07	Clear		Normal	
II-2	M	OD	40.86	36.84	38.85	Diffuse incomplete posterior corneal opacification		453	Normal
		OS	39.52	37.79	38.66	Diffuse incomplete posterior corneal opacification		521	Normal
III-1	M	OD	36.64	34.65	35.65	Incomplete central and peripheral posterior corneal stromal opacification		502	Normal
		OS	36.80	33.48	36.14	Incomplete central and peripheral posterior corneal stromal opacification		499	Normal
III-2	M	OD	38.62	36.84	37.73	Diffuse irregular posterior stromal lamellar opacification		485	Iridocorneal adhesion, nasal corectopia
		OS	41.26	39.80	40.53	Diffuse irregular posterior stromal lamellar opacification		427	Normal

Table 2.

Summary of hemizygous deletions identified in reported pedigrees with PACD

Family	Genome Coordinates (hg38, ch.12)	Size (Mb)	Probe Count	Gene Count	Genes
1 ^[11]	90,488,060–91,189,204	0.701	637	6	<i>LINC00615</i> , <i>CCER1</i> , <i>EPYC</i> , <i>KERA</i> , <i>LUM</i> , <i>DCN</i>
2 ^[11]	90,666,390–91,973,988	1.308	1,176	6	<i>LINC00615</i> , <i>CCER1</i> , <i>EPYC</i> , <i>KERA</i> , <i>LUM</i> , <i>DCN</i>
3 ^[11]	90,488,060–91,189,180	0.701	636	6	<i>LINC00615</i> , <i>CCER1</i> , <i>EPYC</i> , <i>KERA</i> , <i>LUM</i> , <i>DCN</i>
4 ^{this study}	90,914,489–91,218,206	0.304	392	6	<i>LINC00615</i> , <i>CCER1</i> , <i>EPYC</i> , <i>KERA</i> , <i>LUM</i> , <i>DCN</i>
5 ^[12]	90,899,856–92,570,724 [*]	1.671	N/A	11	<i>LINC00615</i> , <i>CCER1</i> , <i>EPYC</i> , <i>KERA</i> , <i>LUM</i> , <i>DCN</i> , <i>C12orf79</i> , <i>BTG1</i> , <i>RPL21p106</i> , <i>CLLU10S</i> , <i>CCLU1</i>

^{*} Genome coordinates were converted from hg19 to hg38 using the NCBI Genome Remapping Service