Clinical and Molecular Characterization of a Family With Autosomal Recessive Cornea Plana

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Background: Autosomal recessive cornea plana is characterized by a flattened corneal surface associated with hyperopia and various anterior segment abnormalities. Mutations have been detected in the keratocan gene (KERA), a member of the small leucine-rich proteoglycan family.

Objective: To clinically and molecularly characterize a consanguineous family of Hispanic origin in which 3 individuals are affected with cornea plana.

Methods: Clinical ophthalmic examination, including corneal topography and axial eye length measurement, was performed on 7 family members. Molecular analysis of KERA was performed on DNA from each family member who had been examined.

Results: All 3 affected individuals showed extreme flattening of the cornea (<36 diopters [D]), normal axial eye lengths, and hyperopia greater than 6.25 D (spherical equivalent). Anterior segment abnormalities included scleralization of the cornea and central iris strands to the corneal endothelium. Affected individuals were homozygous for a novel mutation in KERA. The sequence change was found in exon 2, which results in an asparagine to aspartic acid change at codon 131. This amino acid change occurs within a highly conserved leucine-rich repeat of keratocan.

Conclusions: The cause of disease in this family is likely to be a mutation in exon 2 of KERA. Other mutations in KERA known to cause cornea plana also fall within the region encoding the leucine-rich repeat motifs and are predicted to affect the tertiary structure of the protein.

Clinical Relevance: This is the first report of the identification of a mutation within KERA in a family of Hispanic origin with autosomal recessive cornea plana. Although the vast majority of cases of cornea plana are in individuals of Finnish descent, this report demonstrates the occurrence of the disease in other populations.


CORNEA PLANA CAN BE INHERITED AS EITHER A Milder, AUTOSOMAL DOMINANT DISEASE (CNA1; MENDELIAN INHERITANCE OF MAN 121400) OR AS A MORE SEVERE, AUTOSOMAL RECESSIVE FORM (CNA2; MENDELIAN INHERITANCE OF MAN 217300). THE CNA2 FORM IS A RARE DISORDER IN WHICH THE CURVATURE OF THE CORNEA IS FLATTENED, RESULTING IN A DECREASE IN THE REFRACTIVE POWER OF THE CORNEA. IN ADDED, CORNEA PLANA IS CHARACTERIZED BY A HAZY CORNEAL LIMBUS AND PERIPHERAL SCLERALIZATION OF THE CORNEA, OFTEN WITH DEEP CENTRAL CORNEAL OPACITIES. TAHVAANAINEN ET AL REPORTED THE INITIAL LINKAGE OF CNA2 TO A 10-CENTIMORGAN (CM) INTERVAL ON CHROMOSOME 12q22 IN A FINNISH PEDIGREE. LINKAGE DISEQUILIBRUM MAPPING ON 32 FINNISH FAMILIES SHOWED AN ALLELIC ASSOCIATION BETWEEN THE POLYMORPHIC MARKER D12S351 AND CNA2, ALLOWING THE DISEASE INTERVAL TO BE REFINED TO 1 CM. AUTOSOMAL DOMINANT CORNEA PLANA (CNA1) HAS BEEN DESCRIBED IN A CUBAN FAMILY. THE GENE RESPONSIBLE FOR CNA1 WAS MAPPED BY LINKAGE ANALYSIS IN THIS FAMILY TO A REGION ON CHROMOSOME 12, WHICH INCLUDED THE LOCUS CAUSING CNA2. THIS SUGGESTED THAT CNA1 AND CNA2 MAY BE ALLELIC. HOWEVER, LINKAGE ANALYSIS ON 2 FINNISH FAMILIES WITH CNA1 EXCLUDED THE AREA ON CHROMOSOME 12 AS CONTAINING THE RESPONSIBLE GENE(S) IN THESE FAMILIES, INDICATING THAT THERE IS GENETIC HETEROGENEITY FOR THE AUTOSOMAL DOMINANT FORM OF THIS DYSTROPHY. PELLEGATA ET AL IDENTIFIED MUTATIONS WITHIN THE KERATOCAN GENE (KERA; MENDELIAN INHERITANCE OF MAN 603288) THAT WERE RESPONSIBLE FOR CAUSING CNA2, AND THEY SHOWED THAT THE LARGE NUMBER OF CNA2 CASES PREVIOUSLY REPORTED IN THE FINNISH POPULATION WERE Owing TO A FOUNDER EFFECT. MORE RECENTLY, A NOVEL MUTATION WAS IDENTIFIED IN EXON 2 OF KERA IN A BANGLADESHI FAMILY WITH BOTH CNA2
and a mild form of microphthalmia. No mutations in KERA have been reported in CNA1 families.

The core protein of a major corneal keratan sulfate proteoglycan is encoded by KERA. Although KERA is abundantly expressed in the cornea and sclera, it is expressed at lower levels in skin, ligament, cartilage, arteries, and striated muscles. The gene spans 7.65 kilobases of genomic DNA, consists of 3 exons with complementary DNA of 2160 base pairs (bp), and encodes a protein of 352 amino acids. Exon 1 is untranslated while exon 2 contains the start codon and an N-terminal signal peptide followed by a highly conserved region containing 10 leucine-rich repeat (LRR) motifs.

In this study, we investigated a consanguineous family of Hispanic origin in which 3 individuals are affected with cornea plana. Seven family members were examined clinically and molecular analysis was performed on their DNA. A novel nucleic acid change was found in KERA that results in an amino acid change in a highly conserved portion of the LRR motif of the protein. This study contributes additional support to the occurrence of CNA2 in individuals with no Finnish ancestry.

**METHODS**

**PATIENTS**

A 4-generation, consanguineous, Hispanic pedigree from northern Mexico (Figure 1) in which 3 of the 5 children were affected with cornea plana was ascertained for a genetic study that conformed to the tenets of the Declaration of Helsinki. A full ophthalmic examination was performed on all 7 family mem-
bers from the third and fourth generations and included the following: cycloplegic refraction, slitlamp examination, intraocular pressure measurement, optic disc assessment, corneal topography, and A-scan ultrasonography to determine axial length.

DNA ANALYSIS

Blood specimens were obtained from all family members who were examined, and genomic DNA was extracted from peripheral blood leukocytes using the Nucleon II kit (Scotlab Limited, Strathclyde, Scotland) according to the manufacturer’s instructions. Polymerase chain reaction amplification of KERA was performed using intronic oligonucleotide primers (Table) to amplify the coding region. Because exon 2 is 893 bp long, it was amplified in 3 overlapping segments. The purified polymerase chain reaction samples were sequenced bidirectionally on an automated sequencer (ABI 3100; Applied Biosystems, Foster City, Calif) using the ABI Prism Ready Reaction Dye Terminator cycle sequencing kit (FS kit; Applied Biosystems) following the manufacturer’s protocol.

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Abbreviations: bp, base pair; T_a, annealing temperature.

RESULTS

CLINICAL ANALYSIS

Case IV:1

The first affected child was a 12-year-old girl who had a best-corrected visual acuity of 20/60 OD and 20/70 OS with cycloplegic refraction of +5.00 + 2.50 × 135 OD and +6.50 + 2.00 × 052 OS. Extraocular motility, confrontation fields, pupillary reaction, intraocular pressures, and fundus examination results were normal. Slitlamp examination showed peripheral scleralization of the cornea in each eye. There were also central and peripheral iris strands in the cornea in each eye. Humphrey topography results showed simulated keratometric readings of OD 27.62 D at 88°, OD 30.87 D at 178°, OS 25.12 D at 84°, and OS 32.62 D at 174°. A-scan measurements found equal axial lengths of 23.55 mm OU.

Case IV:2

The second affected sibling was a 10-year-old boy who had a best-corrected visual acuity of 20/80 OD and 20/60 OS with cycloplegic refraction of +6.00 + 1.75 × 010 OD and +7.50 + 0.25 × 010 OS. Extraocular motility, confrontation fields, pupillary reaction, intraocular pressures, and fundus examination results were normal. Slitlamp examination showed peripheral scleralization of the cornea in each eye with many central and peripheral iris strands to the cornea resulting in corectopia (Figure 2A). The patient’s corneas were too irregular for the Humphrey machine to measure. Keratometer readings were less than 36 D in each eye. Axial lengths were 26.54 mm OD and 25.05 mm OS.

Case IV:3

The third and last affected child was an 8-year-old girl. Her best-corrected visual acuity was 20/50 OU. Cycloplegic refraction was +6.00 + 2.25 × 065 OD and +8.50 + 1.75 × 095 OS. Extraocular motility, confrontation fields, pupillary reaction, intraocular pressures, and fundus examination results were normal. Slitlamp examination revealed peripheral scleralization of the cornea in each eye with no iris strands in the right eye but with a few iris strands to the cornea in the left eye (Figure 2B). Humphrey topography results (Figure 2C) showed simulated keratometric readings of OD 31.50 D at 30°, OD 33.75 D at 120°, OS 26.62 D at 160°, and OS 33.50 D at 70°. Axial lengths were 23.33 mm OD and 23.55 mm OS.

Cases III:1, III:2, IV:4, and IV:5

Individuals III:1, III:2, IV:4, and IV:5 all had normal ophthalmic examination results (Figure 2D). There was no evidence of decreased visual acuity. The spherical equivalent of the cycloplegic refraction of the 5 unaffected family members who were examined ranged from −0.25 to +1.00 D. No unaffected individual who was examined had greater than 0.50 D of astigmatism. Simulated keratometric readings ranged from 41.37 to 46.12 D. Axial eye lengths ranged from 22.91 to 23.59 mm. There were no anterior segment or fundus abnormalities.

DNA ANALYSIS

Sequencing of KERA (GenBank accession number NM_007035) revealed a single-nucleotide substitution in exon 2 that segregated with the disease phenotype (Figure 1). The mutation was an adenine to guanine change at nucleotide 391 (A391G) that resulted in an amino acid substitution of asparagine to aspartic acid in codon 131 (Asn131Asp). Both parents and unaffected children were heterozygous (A/G); however, all 3 affected siblings were homozygous (G/G) for this change (Figure 1). There were 130 control individuals (260 chro-
mosomes) of mainly British white descent who were tested for this sequence change; no alterations were detected, suggesting that this sequence change is unlikely to be a common polymorphism, although an ethnically matched control population was not able to be ascertained.

ALIGNMENT OF CONSERVED RESIDUES

Further evidence that this sequence change may be responsible for causing cornea plana was obtained from a multiple sequence alignment using CLUSTALW, which was performed to determine if this residue was conserved. The Asn131Asp substitution occurs within the third LRR motif of keratocan, which is a highly conserved residue within the LRR. The CLUSTALW alignment (Figure 3) indicates that this residue is highly conserved in keratocan from human, bovine, mouse, quail, and chick.

Similarly, the mutations previously identified as a cause of recessive cornea plana also affect highly conserved residues. The Thr215Lys change seen in a Bangladeshi family occurs at a highly conserved threonine residue in the seventh LRR. The Asn247Ser change, seen in the Finnish population, involves the substitution of the conserved asparagine residue (LXXLXLXXNXL) within the eighth LRR. A third identified mutation is a nonsense mutation (Gln174Xaa) that likely results in the production of no encoded protein, secondary to nonsense-mediated decay of RNA (Figure 3).

COMMENT

Cornea plana is a rare corneal dystrophy that displays both phenotypic and genetic heterogeneity. The autosomal dominant form of the disease, CNA1, is a relatively mild disorder with little loss of visual acuity. The refractive power of the cornea is reduced to 38 to 42 D. The autosomal recessive form of this dystrophy, CNA2, results in a significant loss of visual acuity as the refractive power of the cornea is reduced to 25 to 35 D. Thus, these 2 dystrophies can be distinguished based on phenotype, as CNA2 presents as a more severe disease. All affected individuals in the presented pedigree have a refractive power lower than 36 D. Although the genes responsible for CNA1 and CNA2 have been linked to markers within overlapping regions on chromosome 12q22, mutations within KERA have only been identified in CNA2 families.

Pellegata et al identified mutations in KERA that have been shown to cause CNA2 in a large cohort of Finnish patients in which there was a founder effect and in an American patient of Chinese origin.
Asn247Ser and Glu174stop changes, respectively. More recently, a Bangladeshi family with CNA2 was identified with a novel Thr215Lys mutation in KERA. This family had a broader phenotype in which a mild form of microphthalmia cosegregated with cornea plana. These findings implicated keratocan in a wider role in the formation of the structure of the eye and an involvement in ocular development. The affected individuals described in the Hispanic pedigree showed no evidence of microphthalmia.

Corneal strength, transparency, and curvature are determined by stromal keratocytes and depend on an organized stromal extracellular matrix that includes uniformly small collagen fibril diameter (22.5-35 nm) and regular interfibrillar spacing (42-44 nm). Proteoglycan-collagen and collagen-collagen interactions have been implicated in the assembly of the different levels of stromal organization. Proteoglycans are macromolecules composed of a protein core with covalently linked glycosaminoglycan side chains. The corneal proteogly-

**Figure 3.** A CLUSTALW alignment of the human keratocan protein with that of bovine, mouse, quail, and chick. The mutation detected in this family (substitution of asparagine to aspartic acid in codon 131) is shown by the green arrow whereas the red arrows indicate mutations previously reported as causing cornea plana. These arrows delineate regions of the protein that are highly conserved across species. The red line highlights the first 20 amino acids that form the signal peptide, and the blue lines show positions of the leucine-rich repeat regions from numbers 1 to 10.
Keratocan is expressed almost exclusively in the adult by corneal keratocytes and is often used as an in vitro marker for keratocyte differentiation. Keratocan expression is maintained at a constant level throughout development, suggesting that keratocan may have a unique function in maintaining corneal physiology. There is also a transient expression by different sublineages of neural crest cells. As with other members of the keratan sulfate proteoglycan subgroup of the small leucine-rich proteoglycan family, keratocan includes a series of LRR motifs flanked by hypervariable N- and C-terminal regions. The regular LRR motif arrangement is thought to be important in the spacing of collagen fibrils on which corneal transparency depends. A partial 3-dimensional model for keratocan indicates that the LRR motifs form a series of parallel β-strands that stack into an arched β-sheet array.6 The mutation noted in the Hispanic family changes a conserved amino acid in the third LRR of keratocan, which may disrupt the stacking or spacing of the β-strands. Keratocan has also been found to be up-regulated specifically in the stroma of keratoconus corneas, which supports its role in the determination of corneal shape.21

Knockout mice have been created for lumican, mimecan, and keratocan, helping to elucidate the role of these keratan sulfate proteoglycans in corneal physiology. Lumican knockout mice have the most severe phenotype with corneal opacity, disorganized collagen fibrils, and thinner corneal stroma. Mimecan knockout mice have slightly larger but uniform collagen fibrils with a transparent cornea. Keratocan knockout mice have clear corneas with a thin corneal stroma, narrower cornea-iris angle, abnormal collagen fibril spacing, and larger stromal fibril diameters.23

The homozygous mutation described in this study, Asn131Asp, is in a family of Hispanic origin in which there are 3 affected and 2 unaffected children from a consanguineous marriage. The mutation affects the asparagine residue in the third LRR, which is highly conserved across species. This substitution to an aspartic acid residue may lead to the destabilization of the protein structure or may alter the ability of the mutant keratocan to interact with other proteins such as collagen.8,9 This report, together with the reports of mutations in a Bangladeshi family and a Chinese individual, contributes to the knowledge of the function of keratocan and also demonstrates the occurrence of CNA2 in other populations.

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