



# Study of p.N247S *KERA* mutation in a British family with cornea plana

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**Purpose:** To report clinical and genetic findings in a white British family with autosomal recessive cornea plana (CNA2) with a negative history for consanguinity. To look for evidence of a common ancestry with previously reported Finnish CNA2 patients by studying haplotypes.

**Methods:** Clinical examination and direct sequencing of the *keratocan* (*KERA*) gene was performed in two siblings affected with CNA2 and one unaffected parent. We also studied 22 single nucleotide polymorphisms distributed in the *KERA* genomic region by direct sequencing in this family as well as in one additional Finnish patient with CNA2 and 24 white British control subjects.

**Results:** Both siblings had the homozygous c.740A>G mutation leading to a p.N247S amino acid change originally reported as the founder mutation in 35 Finnish families. Genetic characterization of genomic regions surrounding the gene revealed large linkage disequilibrium, but the presence of shared extended haplotypes between affected individuals from Finland and the United Kingdom is consistent with a recent common ancestor.

**Conclusions:** This is the first description of recessive cornea plana in a white British family and it is the second report on the p.N247S change in the *KERA* gene. Extended haplotype analysis suggests that the two geographically remote occurrences of the c.740A>G mutation may have a common origin.

Cornea plana is a rare disorder in which the cornea is flattened with a low refractive power. Other features include microcornea, central corneal opacity, a widened corneal limbus, early arcus senilis, shallow anterior chamber, iris hypoplasia, corectopia, and peripheral anterior synechiae. Closed-angle glaucoma may also be present [1-3]. It can be inherited as an autosomal dominant (CNA1, OMIM 121400) or a clinically more severe autosomal recessive trait (CNA2, OMIM 217300) [3]. CNA2 is found worldwide with a high prevalence among the Finnish population [1]. Both CNA1 and CNA2 have been mapped to the long arm of chromosome 12 (12q21). Nonsynonymous or protein-truncating mutations of the *keratocan* (*KERA*) gene (OMIM 603288) have been identified as the cause of CNA2 but not CNA1 [4,5]. In some CNA1 families, linkage to the 12q21 locus was excluded [6].

*KERA* codes for keratocan, a small-sized highly conserved leucine-rich protein that is expressed in cornea as well as in other tissues [4,7]. In *KERA* knockout mice there is a thinner corneal stroma and a narrower cornea-iris angle than in the wild type with less organized packing and larger diameters of

stromal collagen fibrils on transmission electron microscopy [8].

Overall, seven different mutations in the coding sequence of the *KERA* gene have been described in CNA2 families; 46 Finnish patients from 35 unrelated pedigrees were shown to have the c.740A>G (p.N247S) change inherited from a common founding ancestor [4]. In the same report one Chinese-American patient, born to consanguineous parents, was homozygous for p.Q174X [4]. Other sequence variants have also been reported, such as a consanguineous family originating from Bangladesh presenting with a combined phenotype of CNA2 and microphthalmia had a p.T215K change [9], one Hispanic consanguineous pedigree was shown to have p.N131D [10], while p.R313X, p.R279X, and p.C343fs were described in 14 consanguineous Arab families [11-13]. An affected member of one of the Arab families reportedly exhibited the cornea plana phenotype as well as superior pellucid marginal degeneration [12]. Bilateral progressive corneal ectasia leading to the development of a presumed unilateral hydrops and bilateral high astigmatism has also been described in recessive cornea plana [14].

We describe a white British CNA2 family with two affected members. One sibling showed typical cornea plana phenotype and the other, in addition, had high simple astigmatism. Marker haplotypes in the *KERA* genomic region were

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studied to determine if the observed p.N247S mutation was identical by descent to those in the previously described Finnish families [4].

**METHODS**

*Patients and clinical examination:* The research complied with the tenets of the Declaration of Helsinki. Appropriate consent was obtained from participating subjects. The family was white British with no known foreign ancestry. Family members reported a negative family history for consanguinity in at least two preceding generations. The proband was a 26-year-old female (individual II:3) with poor vision since birth. Her 30-year-old sister (individual II:1) had similar phenotype, but the proband's brother, nephews, and parents were all normal (Figure 1A). Standard ophthalmic examination, which included best corrected visual acuity, intraocular pressure measurement, and slit lamp biomicroscopy, was performed in the two af-

ected siblings and their mother. Anterior segment photographs were taken of the affected siblings. Corneal horizontal diameters (white-to-white), corneal topography, pachymetry, and anterior chamber depth (ACD) measurements were performed using an Orbscan II operating under software version 3.12 (Bausch & Lomb, Rochester, NY).

*DNA preparation and keratocan mutation screening:* Genomic DNA was extracted from venous blood samples using Nucleon™ BACC3 genomic DNA extraction kit (GE Healthcare, Bucks, UK). The coding regions and exon-intron boundaries of the *KERA* gene were amplified by polymerase chain reaction (PCR) using sets of four primers (Table 1). Primers were designed with Primer 3 program. Each PCR reaction consisted of approximately 50 ng of genomic DNA, 50 pmol of each primer and 12.5 µl of ReddyMix™ PCR master mix (1.5 mM MgCl<sub>2</sub>; ABgene, Epsom, UK) and water added up to a volume of 25 µl. After purification with Montage PCR<sub>96</sub>

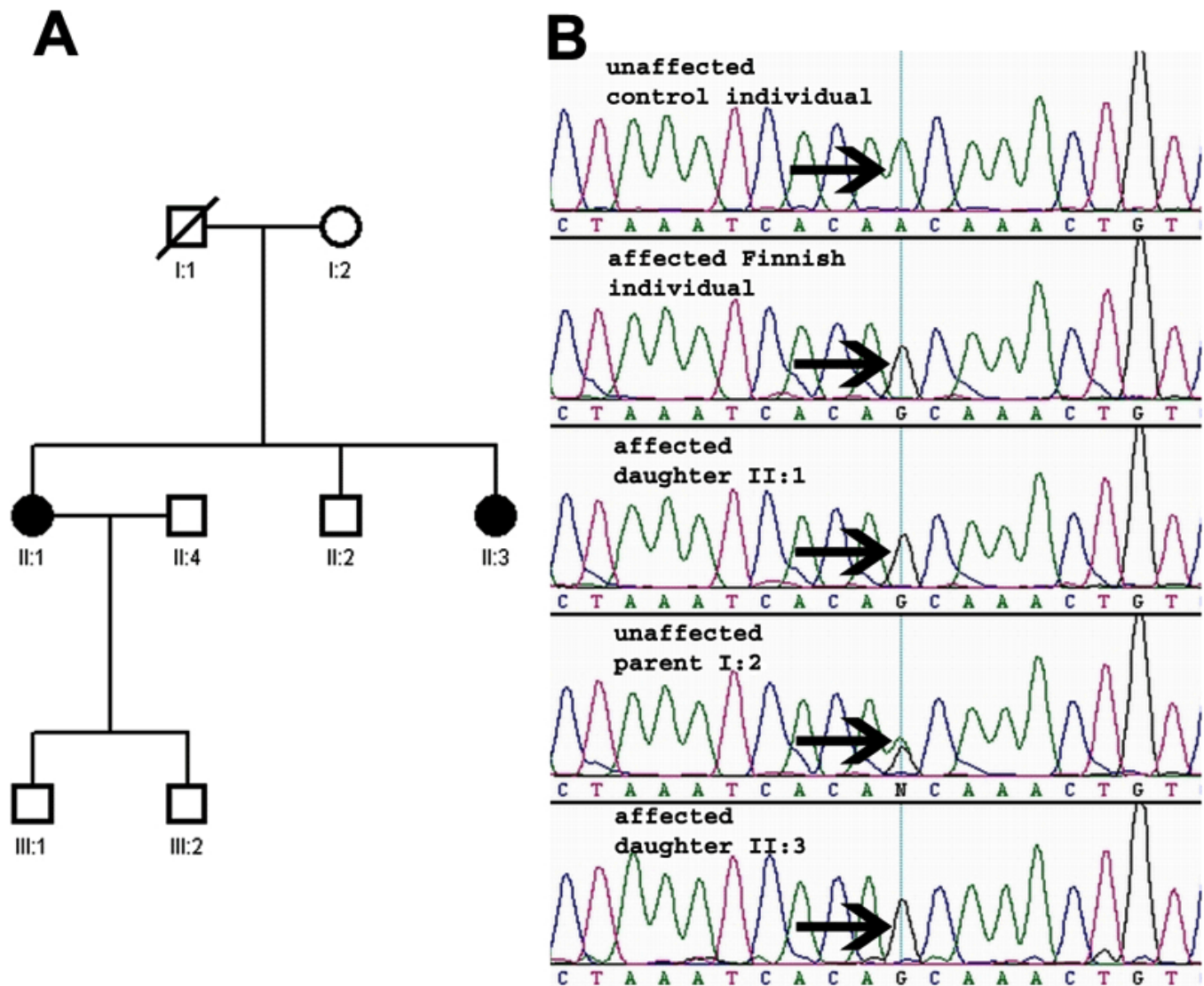


Figure 1. Pedigree of the family with recessive cornea plana and c.740A>G mutation. **A:** Two affected family members are reported in this study. **B:** Sequence data showing the homozygous c.740A>G mutation that leads to the p.N247S change in the British family, and one Finnish patient having a previously published founder effect for the same change [4].

Cleanup Kit (Millipore, Billerica, MA), samples were sequenced on an automated sequencer using dye-terminator chemistry under standard conditions with primers identical to those for genomic amplification (Applied Biosystems, Foster City, CA). Montage SEQ<sub>96</sub> Sequencing Reaction Cleanup Kit (Millipore) was used for purification according to the manufacturer's instructions. Sequence data were aligned and analyzed by DNASTAR Lasergene sequence analysis software (DNASTar, Inc., Madison, WI) followed by manual inspection for base changes and comparison with database reference sequence NCBI accession NM\_007035. Ninety-four unrelated white British control individuals were screened for the identified disease-causing mutation by direct sequencing. The DNA of these subjects was purchased from the European Collection of Cell Cultures (ECCAC, Porton Down, UK).

**Selection of polymorphisms and genotyping:** Thirty-three variations found on the Single Nucleotide Polymorphism dbSNP database across 7.64 kb of the *KERA* genomic sequence were genotyped in the proband, an unaffected parent, and one Finnish subject with CNA2 previously described by Pellegata et al. [4]. All alleles were determined by direct sequencing of 290-492 bp long PCR fragments as described above. A list of the polymorphisms and appropriate primer sequences are shown in Table 2. NCBI accession NT\_019546 was used as the reference sequence.

To characterize linkage disequilibrium (LD) blocks in the *KERA* genomic region, we performed extended genotyping of 22 polymorphisms (one insertion/deletion polymorphism and 21 single nucleotide polymorphisms) by direct sequencing, not only in the British CNA2 family and Finnish CNA2 patient, but also in a subset of 24 white British control individuals (the same subjects were used for mutation screening). This was followed by haplotype comparison of a region spanning both sides of the *KERA* genomic sequence for a total of almost 280 kb. The genotyped polymorphic variants, their position, and the primers used for amplification of PCR fragments

**TABLE 1. PRIMER SEQUENCES USED FOR THE AMPLIFICATION OF *KERA* CODING REGION**

Exon number	Primer sequences (5'-3')	Amplicon size (bp)
2	TGTTGACATATTTTCACCTCTTCC	402
	TCAAATGGCTTTTCAGGAATG	
	GAGGTC TCAAAGAAATTCCTGCT	441
	GCATATTCCTCAGGGCATTCC	
3	GGACAATGCCTTTCAAAGAGAC	441
	GGGCAACACATTTGCTCTTC	
3	TTGGGGGAAACAGATAGG	466
	GAAAATGGTGGCCGAGAGC	

Four sets of primers were designed to cover the entire coding region within exons 2 and 3 of the *KERA* gene. The length of the PCR products ranged from 402 to 466 bp.

are shown in Table 3. Selection of these polymorphisms was made to provide haplotypic information for wider regions of the chromosome and was made in ever increasing distances from *KERA* using the information from the HapMap publicly accessible databases.

**Haplotype Analysis:** Based on information about individual genotypes, haplotypes for each subject were reconstructed using PHASE [15], whereas the phylogenetic tree was obtained using PHYLIP (version 3.2) [16] software and the upgraded package (version 3.6) as distributed by the author.

**RESULTS**

The clinical characteristics of the proband were consistent with cornea plana (Table 4). Both corneas appeared thin and flat, but simulated keratometry could only be obtained from

**TABLE 2. SIMPLE GENETIC POLYMORPHISMS GENOTYPED ACROSS THE *KERA* GENOMIC SEQUENCE IN THE PROBAND, AN UNAFFECTED PARENT, AND ONE FINNISH SUBJECT**

Polymorphism number	rsID	Primer pairs (5'-3')	Amplicon size (bp)
1	rs10859101	CAATAAAAAGGACACATTGTGTATG TTTGCAGTTTAAAGTGGATTCA	423
2	rs11105952	same as rs10859101	410
3	rs2464171	CATATFCCCATTGCTCTCG TCAAACCTGTACCAACACAGC	
4	rs2701166	TTGGGGGAAACAGATAGG GAAAATGGTGGCCGAGAGC	465
5	rs2245775	GGCTTGAGTTCTTTGTCATTCA TCCCCAACAAGTTCAG	488
6	rs1920771	same as rs2245775	419
7	rs2268580	same as rs2245775	
8	rs2268579	same as rs2245775	415
9	rs1920772	same as rs2245775	
10	rs2735341	same as rs2245775	419
11	rs1920773	CCAGAATCCCACAAAATGC CATACAGGAGAGAGGCCAGTT	
12	rs2540006	same as rs1920773	415
13	rs3216597	GAACCTGCTTAAGCAATATGATGGA TCACAGGCTTTATCAGCCAAT	
14	rs17018619	TGCAATAGCCAGAACCTGAG TGTTTCTCAGGGAACACAG	475
15	rs11105956	TGTTTAGGAGTGAGGCAGCA TTGCTCTGGGCAAAATATCC	404
16	rs2041711	GTTGTGCTGCCTGGGTATTA ATTGTCCTGGGGCTTTTA	410
17	rs2735337	GGGACTTACAGGCAATCGTC AGCAGTCCCAGTGGAAAA	492
18	rs2735336	same as rs2735337	481
19	rs2540004	same as rs2735337	
20	rs734722	ATCTGCAGCACCTTACCTT GTTTACAGCATCAGCTGTCA	481
21	rs1920775	same as rs734722	441
22	rs737111	GGACAATGCCTTTCAAAGAGAC GGGCAACACATTTGCTCTTC	
23	rs2735335	GAGGTC TCAAAGAAATTCCTGCT GCATATTCCTCAGGGCATTCC	441
24	rs2735334	same as rs2735335	402
25	rs2540003	same as rs2735335	
26	rs12320366	TGTTGACATATTTTCACCTCTTCC TCAAATGGCTTTTCAGGAATG	402
27	rs2735333	same as rs12320366	431
28	rs2701164	TTGAGAGTTGAGCTAGAGAATAACA TAGGCACCATATGCAAAGCA	
29	rs1990550	GAGCCTGGTCAATCCATTA TGCCTTCCAGGAATACACC	290
30	rs1990549	same as rs1990550	489
31	rs2735330	TCAGGAACAGATAAAATATGTCCA TTCCACTTTGACAGGGCTTC	
32	rs1990548	same as rs2735330	489
33	rs2540002	same as rs2735330	

Record identifiers (rsID) of 33 nucleotide variations found in the Single Nucleotide Polymorphism dbSNP database with primer sequences used for amplification of sequenced PCR products, as well as their size are shown (range 290 to 492 bp).

the left eye (Figure 2C). There was axial full thickness scarring in the right eye and bilateral mild peripheral corneal vascularization around a widened corneal limbus. Both anterior chambers were shallow. There was no other evidence of anterior segment dysgenesis. Intraocular pressures and fundus examination were normal. Individual II:1 (Figure 1A) also had poor vision since birth. She had less severe hypermetropia than her sister but higher astigmatism (Table 4). Both of her eyes had an abnormally broad limbus zone and arcus with

more pronounced corneal thinning than her sister and mild bilateral anterior stromal scarring (Figure 2D,E). The rest of the clinical examination was normal.

Sequencing of the *KERA* gene in the affected individuals revealed homozygous c.740A>G substitution in exon 2 leading to asparagine to serine amino acid change at codon 247 (Figure 1B). Consistent with autosomal recessive cornea plana, the parent was heterozygous for this change (Figure 1B). The mutated allele was absent from 94 control subjects (188 chro-

**TABLE 3. GENOTYPING OF 22 POLYMORPHISMS LOCATED IN THE *KERA* GENOMIC REGION**

Polymorphism number	rsID	Chromosome position/location within a gene	Primer pairs (5'-3')	Amplicon size (bp)	CNA2 alleles	Alleles/number of controls	Alleles/number of controls	Alleles/number of controls
1	rs6144808	89920657..89920675 (plus) DSPG3 intron 1-2	AGCTGGTAGCTTTCCTCCA CCAATGCCTAAGCCACACTG	415	del/del	del/del 1	del/ins 12	ins/ins 11
2	rs2701166	89969434 (minus) KERA intron 2-3	TGGGGGAAACAGATAGG GAAAATGGTGGCCGAGAGC	465	t/t	c/c 11	c/t 12	t/t 1
3	rs2245775	89969619 (minus) KERA intron 2-3	GGCTTGAGTTCTTTGTCATTCA TCCCCCAACAAAAGTTGAG	488	t/t	t/t 21	t/a 1	a/a 2
4	rs1920771	89969680 (plus) KERA intron 2-3	same as rs2245775		t/t	t/t 24	c/t 0	c/c 0
5	rs2268580	89969685 (minus) KERA intron 2-3	same as rs2245775		t/t	t/t 23	t/c 1	c/c 0
6	novel 1	89969853 (minus) KERA intron 2-3	same as rs2245775		a/a	a/a 23	a/g 1	g/g 0
7	rs2041711	89972017 (minus) KERA intron 2-3	GTTGTGCTGCCTGGGTATTA ATTGTCCTGGGGCTTTTA	410	g/g	g/g 21	g/t 1	t/t 2
8	rs734722	89973043 (minus) KERA intron 2-3	ATCTGCAGCACCTTCACCTT GTTTCAGGCATCAGCTGTCA	481	g/g	g/g 22	g/a 2	a/a 0
9	rs1920775	89973205 (plus) KERA intron 2-3	same as rs734722		g/g	g/g 24	g/a 0	a/a 0
10	rs737111	89973486 (minus) KERA intron 2-3	GGACAATGCCTTTCAAAGAGAC GGGCAACACATTTGCTCTTC	441	t/t	t/t 24	g/t 0	g/g 0
11	rs12320366	89974115 (plus) KERA exon 2	TGTTGACATAATTTACACCTCTCC TCAAATGGCTTTTCAGGAATG	402	c/c	c/c 23	c/t 1	t/t 0
12	rs2735333	89974121 (minus) KERA exon 2	same as rs12320366		a/a	a/a 22	a/g 0	g/g 2
13	rs1990550	89975774 (minus) KERA 5' UTR- exon 1	GAGCCTGGTCAATCCATTA TGCCCTCCAGGAATACACC	290	a/a	a/a 22	a/c 1	a/a 1
14	rs1990549	89975775 (minus) KERA 5' UTR- exon 1	same as rs1990550		g/g	g/g 22	g/a 1	a/a 1
15	rs1990548	89976072 (minus) KERA 5' UTR- exon 1	TCAGGAACAGATAAAAATATGTCCA TTCCACTTTGACAGGGCTTC	489	a/a	a/a 23	a/c 1	c/c 0
16	novel 2	89976073 (minus) KERA 5' UTR- exon 1	same as rs1990548		t/t	t/t 23	t/c 1	c/c 0
17	novel 3	89996330 (minus)	AGGCAGCAGTCTGTCAGGTT GTCCCTTGATAAAGATGAAAGCAAA	401	t/t	t/t 20	c/t 3	c/c 1
18	rs1920726	89996495 (minus)	same as novel 3		g/g	g/g 22	g/a 0	a/a 2
19	rs3759222	90031084 (minus)	TCTCAGCTCCATCCTGTTCA TGGTATTATAACAGACCTCAAATGC	402	c/c	c/c 22	a/c 0	a/a 2
20	rs516115	90081423 (minus) DCN intron 4	GGATTATGCATGCTGTGCTCT GGTTGGTCATTTCCTTGT	449	a/a	a/a 17	g/a 7	g/g 0
21	rs566806	90083328 (minus) DCN intron 3	TCCTACTTCAATATGAAACATGAAAA TTGTGGCAGGATCTTGAAAA	433	c/c	c/c 17	c/t 7	t/t 0
22	rs826778	90199860 (plus)	TTCCATAGCACAGGACCA CA TTGGGAACCGTCTTTCTCG	467	a/a	a/a 15	c/a 8	c/c 1

Results obtained in British and Finnish CNA2 patients and in 24 white British control individuals (48 chromosomes) are shown. SNP database record identifiers (rsID; when available) are shown in the second column and the chromosomal location is correlated to NCBI Build 36.1 human genome released update.

mosomes). Genotyping of 33 known simple genetic polymorphisms across the *KERA* gene (Table 2) in both the British and Finnish patients, as well as the unaffected parent, revealed that these polymorphisms were identical except for the disease-causing mutation. Further characterization of alleles in the *KERA* genomic region in the patients, their available parent, and healthy controls is shown in Table 3 while the reconstructed haplotypes are shown in Table 5.

LD was strong over the extended genomic region. All three affected individuals shared exactly the same haplotype (Table 5) for a 279 kb long genomic interval between the rs6144808 and rs826778 markers. Unaffected controls typically had a lower degree of LD with haplotype continuity broken beyond rs2701166 and rs516115. Organization of haplotypes in phylogenetic trees (Figure 3) shows the haplotype from the parent containing the mutation and the CNA2 patients forming a

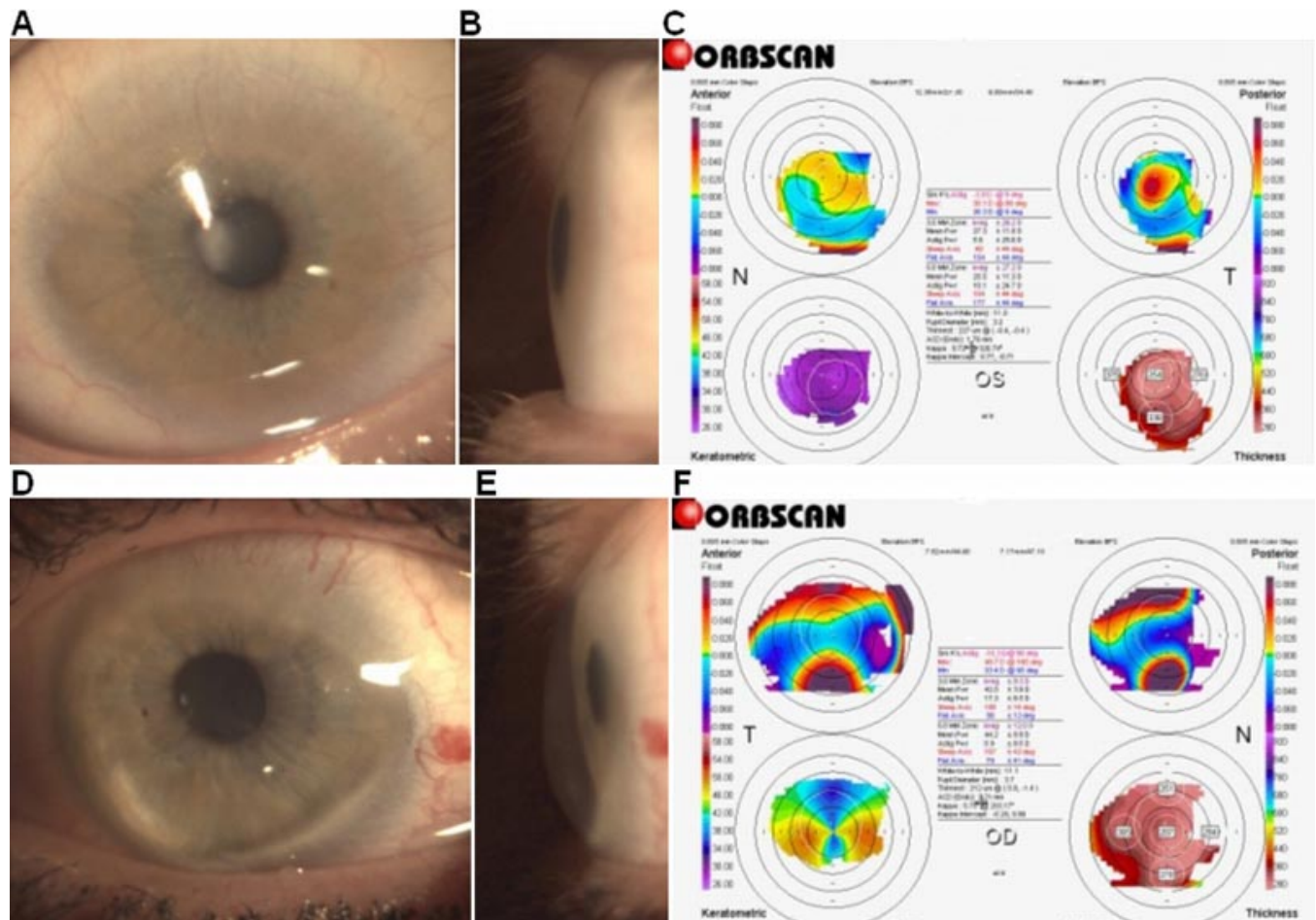


Figure 2. Clinical findings in cornea plana family members. The left eye of individual II:3 shows a diffuse opacity across the central cornea (A), with (B) a typically flat cornea seen in profile. C: An Orbscan II map of the same eye (individual II:3). D: The right eye of individual II:1 shows an arcus with a widened limbus with mild stromal opacity. Corneal flattening is less obvious than in individual II:3 (E). An Orbscan II map of II:3 shows marked against-the-rule astigmatism (F).

TABLE 4. CLINICAL EXAMINATION OF TWO SIBLINGS WITH CORNEA PLANA

Individual	Corneal horizontal diameter (mm)		Best corrected visual acuity		Simulated keratometry (D)		Refractive errors (D)		Thinnest pachymetry (µm)		Anterior chamber depth (mm)	
	RE	LE	RE	LE	RE	LE	RE	LE	RE	LE	RE	LE
II:1	11.1	11.4	6/45	6/45	49.7 at 180° 33.4 at 90°	57.1 at 2° 37.0 at 92°	+0.50/-5.00x115°	-5.50/-3.50x70°	212	107	3.21	3.25
II:3	-	11.0	6/21	6/21	-	30.1 at 99° 26.3 at 9°	+14.00/-2.00x130°	+6.50/-1.00x130°	-	227	-	1.76

Biometric characteristics, best corrected visual acuity, and refractive error of affected family members. The corneal horizontal diameter, simulated keratometry, and thinnest pachymetry of the right eye of individual II:3 are not shown due to the insufficient quality of the obtained Orbscan. Abbreviations: RE, right eye; LE, left eye.

TABLE 5. HAPLOTYPES ON AND AROUND *KERA* GENE

Individual	rs6144808	rs2701166	rs2245775	rs1920771	rs2268580	novel 1	rs2041711	rs734722	rs1920775	c.740A>G	rs737111	rs12320366	rs2735333	rs1990550	rs1990549	rs1990548	novel 2	novel 3	rs1920726	rs3759222	rs516115	rs566806	rs826778	Haplotype recorded as		
proband_a	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
proband_b	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Finnish_a	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Finnish_b	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
parent_a	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
parent_b	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
1_a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	8
1_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	5
2_a	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
2_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
3_a	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
3_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	2	2	1	1	9	
4_a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
4_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	4	
5_a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
5_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
6_a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
6_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
7_a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
7_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	2	2	2	2	2	6
8_a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
8_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
9_a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
9_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	4	
10_a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
10_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
11_a	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
11_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
12_a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
12_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	4	
13_a	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
13_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
14_a	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
14_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	5	
15_a	2	2	2	1	1	1	2	1	1	1	1	1	2	2	2	1	1	1	2	2	1	1	1	1	7	
15_b	2	2	2	1	1	1	2	1	1	1	1	1	2	2	2	1	1	1	2	2	1	1	1	1	7	
16_a	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
16_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	2	2	2	2	2	6	
17_a	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
17_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
18_a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
18_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	5	
19_a	2	2	2	1	1	1	2	1	1	1	1	2	1	1	1	1	2	2	2	1	1	1	1	1	10	
19_b	2	2	2	1	1	1	2	2	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	11	
20_a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
20_b	2	2	2	1	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	12
21_a	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
21_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
22_a	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
22_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
23_a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
23_b	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
24_a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
24_b	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3

Haplotypes for the genomic region of the *KERA* gene obtained by genotyping 22 nucleotide variations (results extrapolated to number 1 or 2) were reconstructed in the proband, parent, a member of a previously described CNA2 Finnish pedigree [4], and 24 white British controls. Twelve different haplotype types were recorded in total.

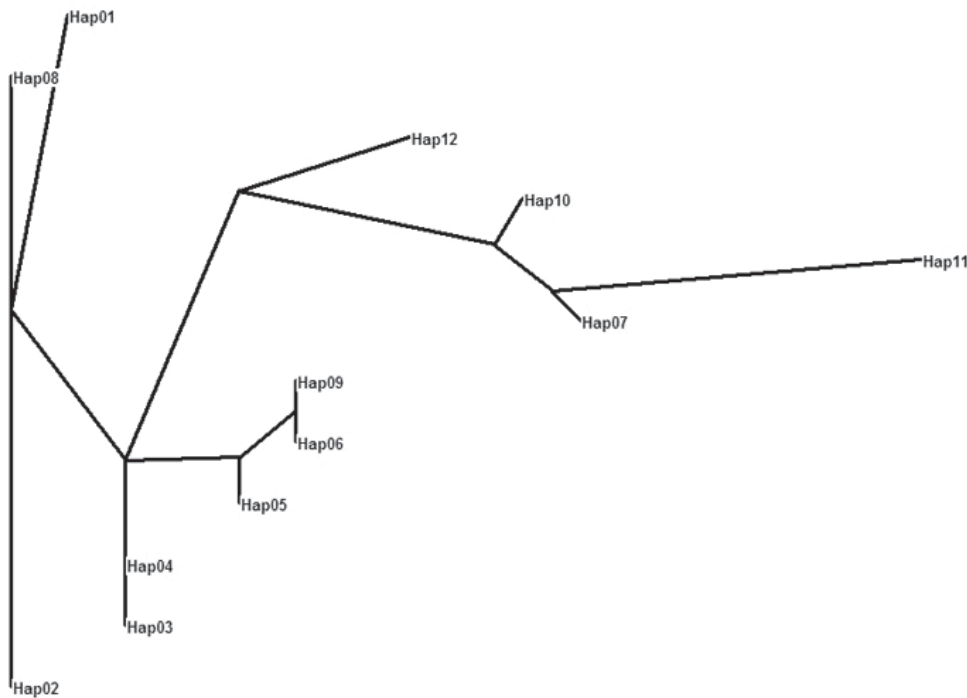


Figure 3. Phylogenetic tree of the observed haplotypes. The tree is constructed from haplotypes of individuals from the British CNA2 pedigree, a published Finnish CNA2 patient [4] (all with p.N247S change), and 24 healthy white British controls. Haplotype numbers are the same as in Table 5.



Figure 4. Heatmap plot of linkage disequilibrium (LD) over the *KERA* gene and surrounding genome in the control study panel. Graphical representation of the linkage disequilibrium (LD) over the *KERA* gene and flanking region in the control panel is shown. The color filling of the cells represents the pairwise LD measured as  $D'$ . The bright-red color of the cells is indicative of the high LD, while the dark-blue colors represent the least LD. Numbers inside the cells, when present, show the exact value of  $D'$  if it is not 1. Uninformative polymorphisms were not included.

separate branch. The individual alleles in the diseased individuals remain in phase over the entire genomic interval studied. LD is not as strong and shows clear signs of decay at the extremities of the same interval in the controls' haplotypes (the pairwise LD between the two furthestmost markers in our genotyped interval is only a modest  $D' = 0.13$ ; Figure 4). Although not fully conclusive proof, this contrast is suggestive of a relatively recent common ancestor of all CNA2 cases of Finnish and British origin.

## DISCUSSION

Autosomal recessive cornea plana is a very rare disorder. It is most common in Finland where 78 cases have been identified. This represents the majority of cases reported worldwide [1]. We describe a new CNA2 family of British descent exhibiting a nonsynonymous amino acid substitution at codon 247 in the *KERA* gene. Prior to this study, this mutation has only been described in a cohort of 35 Finnish pedigrees (Figure 1B) [4]. To our understanding, this is the first case of a p.N247S substitution outside Finland. The clinical characteristics of the two affected patients were consistent with CNA2. High astigmatism associated with superior pellucid marginal degeneration has previously been observed in one patient out of six affected members from a Saudi Arabian family with CNA2 [12]. Although we are unsure of the mechanism leading to the atypical topography pattern in patient II:1, there were no clinical features of pellucid marginal degeneration. Both patients had considerably less than average central corneal thickness measurements, but this did not increase toward the periphery. High astigmatism has also been reported in another Arab patient with progressive bilateral corneal ectasia [14]. Since serial observations have not been performed, we cannot comment whether there has been a progression of the corneal changes to possible ectasia in patient II:1.

As patients with p.N247S changes have previously only been reported from a confined geographical area, we tried to determine whether there was a recent shared ancestry between the affected individuals from Finland and the United Kingdom. For this purpose, we attempted to collect haplotype information through the genotyping of single nucleotide polymorphisms. This class of molecular markers has the advantage of being more frequent than variable number tandem repeats and is transmitted from one generation to another with few, if any, modifications. The entire genomic region around *KERA* shows a generally high level of LD. By examining the single nucleotide polymorphisms and their combination in haplotypes, we found haplotypes containing the disease-causing change were perfectly conserved across the studied interval while the majority of haplotypes in healthy controls were not. A differential conservation of haplotypes adjacent to susceptibility loci and their susceptibility to natural selection has been observed in many occasions [17-21]. There may be two explanations for the observed conservation of both the structure and length of a specific haplotype: recent common ancestry or positive selection. Positive selection often results in extension of the size of the haplotype containing the beneficial allele. This possibility is unlikely since the extended

haplotypes were observed in people affected with serious vision impairment.

Although no conclusive proof can be produced with regards to the identity of descent by the cases examined here, the presence of haplotype sharing between affected individuals of both British and Finnish origin is suggestive of a common ancestor.

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