A Locus for Autosomal Dominant Keratoconus: Linkage to 16q22.3-q23.1 in Finnish Families

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Purpose. The estimated world-wide prevalence of keratoconus is 50 to 230 per 100,000 in the general population. Sporadic keratoconus is the leading cause of corneal transplantation surgery in Western countries. Positive family history has been reported in 6% to 8% of patients. The purpose of this study was to map the disease locus in 20 Finnish families with autosomal dominant keratoconus, each family having two or more affected members and with no other associated genetic disease.

Methods. DNA was extracted from blood samples, collected from 42 affected and 34 unaffected family members. Genomic DNA from patients and their parents, was typed for alleles of 292 polymorphic markers. A genome-wide screening was performed to localize the disease gene. Fluorescent markers were amplified by polymerase chain reaction and separated on an automated sequencer. Allele sizes were assigned to each family member, after which LOD scores were calculated.

Results. The disease locus was mapped to chromosome 16q, between the markers *D16S2624* and *D16S3090*, with a maximum parametric multipoint LOD score of 4.10 and corresponding nonparametric score of 3.27 (NPL, $P = 0.00006$). Evidence from 20 families provided support for the linkage, consistent with a single locus for familial autosomal dominant keratoconus without heterogeneity.

Conclusions. This study is the first genome-wide linkage study to map the keratoconus gene. The results suggest that the causative gene in keratoconus is located within the 16q22.3-q23.1 chromosomal region. (*Invest Ophthalmol Vis Sci.* 2002; 43:3160–3164)

Keratoconus (KC; Mendelian Inheritance in Man [MIM] 148300; Online Mendelian Inheritance in Man (OMIM), National Institutes of Health, Bethesda, MD: available in the public domain at http://www.ncbi.nlm.nih.gov/Omim/) is a noninflammatory disorder of the cornea in which the central cornea becomes thin and conical, inducing myopia and irregular astigmatism and causing mild to marked impairment in the quality of vision.[1,2] Onset is often at puberty, although KC may commence later in life. KC is usually progressive until the third or fourth decade of life. Decreased expression of the gene may induce the subtle forms found in KC, including keratoconus forme fruste or mild irregular astigmatism.[2] These mild disease stages can be detected with videokeratography. Histopathologic findings include thinning of corneal stroma, breaks in Bowman's layer and iron deposition in the basal layers of corneal epithelium. The pathogenesis of keratoconus is unknown. Associations with Down syndrome, atopy, connective tissue disease, eye rubbing, and numerous other multisystem, ocular, or corneal disorders have been reported.[2]

KC has been diagnosed in all populations. The estimated prevalence is 50 to 230 per 100,000 in the general population.[2] Sporadic KC is the leading cause of corneal transplantation surgery in Western countries. Positive family history has been reported in 6% to 8% of patients with KC. A study by Wang et al.[3] supports an autosomal recessive inheritance model for KC, and parental consanguinity in some families has also implied autosomal recessive inheritance.[4] However, most of the published studies have suggested that the familial KC cases are due to autosomal dominant inheritance with incomplete penetrance or variable expression.[2,5]

Because of the scarcity of multiple case families, genome-wide linkage scans to localize the KC gene or genes have not yet been published. Previous mapping studies, published as abstracts, include a directed chromosome-21 scan in one family[6] and an association study in eight unrelated patients in Tasmania.[7] The chromosome-21 scan resulted in a multipoint LOD score of 2.4 for markers on 21q, and a shared haplotype, covering 2.7 Mb on chromosome arm 18p, was identified in the association study. In addition, Rabinowitz et al.[8] have excluded one of the collagen genes, *COL6A1*, as the causative gene in KC.

The lack of genome-wide linkage studies clearly indicates that families with multiple cases of KC are difficult to ascertain. In an epidemiologic study, reported by Ihalainen,[9] 19% of the KC cases investigated in northern Finland were found to be familial. In 24 of 28 multiple-case families the pattern of inheritance was autosomal dominant. The use of family data from a homogeneous, isolated population increases the odds of finding linkage between chromosomal markers and a genetically heterogeneous disorder. The Finns are a well-known sample population with a clear founder effect.[10,11] In this study, we present the results of a genome-wide linkage analysis that is based on the family data collected from 1964 to 1984 in Oulu in northern Finland.[9]

Methods

Patients and Clinical Studies

The study included analysis of blood samples obtained from 20 KC-affected families from northern Finland: seven families with two affected siblings, two with three affected siblings, one with four affected

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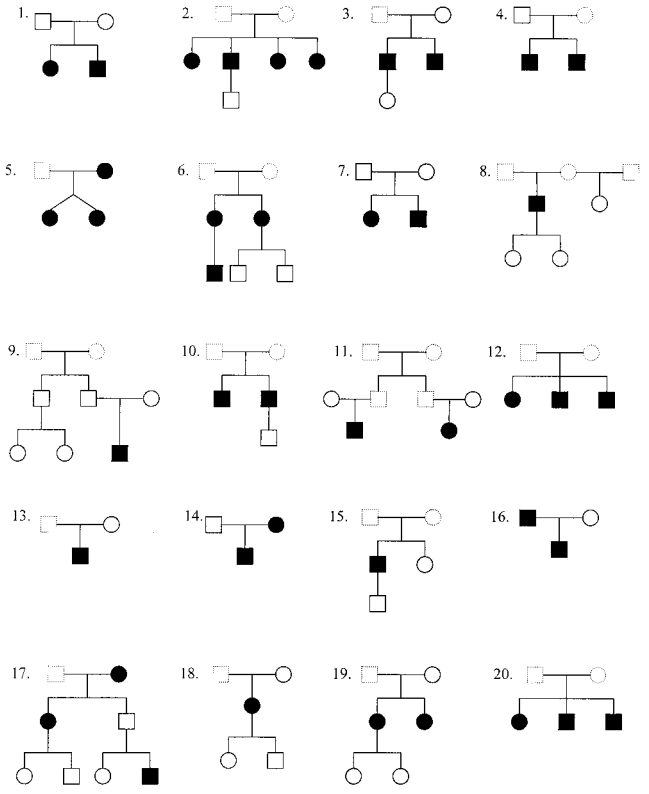

FIGURE 1. The partial pedigrees of the Finnish families affected by KC, showing the family members who participated in the study (samples were not obtained from the members marked with *dotted line*).

siblings, four with both an affected parent and child, one with affected first cousins, and one with both an affected parent and monozygotic twins. The remaining four families were also multiple-case families, but samples were obtainable only from one patient along with unaffected members (Fig. 1). The high frequency of multiple-case families in the Oulu region indicates that KC has an enrichment in northern Finland. Genealogical studies were not initiated, but to our knowledge the families are not closely related, although they may share a common ancestor if traced back a few centuries, a feature that is typical of Finnish families originating from a restricted geographical region. The Oulu area belongs to the late-settlement region and was inhabited in the late 15th century by a small number of people from southern and western Finland. Population expansion began in the 17th century.

We performed an ophthalmic reexamination of patients with KC and their first-degree relatives, using both clinical and videokeratographic evaluation. Inclusion criteria were the following: central or paracentral stromal thinning and conical protrusion of the cornea and other classic signs of KC and videokeratographically evident KC or corneal penetrating keratoplasty performed because of KC; and no clinical signs of KC in the slit lamp examination but abnormal corneal topography (KC forme fruste) according to the guidelines of Rabinowitz,[12] including central steepening, inferior–superioir dioptric asymmetry, difference between right and left central corneal power, and skewing of the steepest radial axes above and below the horizontal meridian. All the patients were more than 35 years of age, except one who aged 15 years (KC forme fruste). No other genetic disease was segregated in the families.

DNA Extraction

The research adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants, in accordance with the requirements of the University of Helsinki, Department of Medical Genetics Ethics Committee. Genomic DNA was isolated by standard methods from venous blood, collected from 42 affected and 34 unaffected family members.

Microsatellite and Linkage Analysis

A genome-wide screening, with 292 highly polymorphic autosomal microsatellite markers spanning the entire genome except for chromosomes X and Y, with average spacing of 11.7 centimorgans (cM, modified from CHLC Screening Set 6; obtained from the Cooperative Human Linkage Center; National Institutes of Health, Bethesda, MD; http://gai.nci.nih.gov/CHLC/), was performed to localize the disease gene. Genomic DNA from family members was amplified by polymerase chain reaction (PCR), using fluorescently labeled markers. PCR products were separated on an automated sequencer (model 377; Applied Biosystems, Foster City, CA) before analysis on computer (Genescan 2.2 and Genotyper 2.0 software; Genescan was developed by Christopher Burge, Stanford University, Stanford, CA, and is provided to academic users at no fee by the Massachusetts Institute of Technology, Cambridge, MA, at http://mit.edu./Genescan; Genotyper 2.0; Applied Biosystems). Parametric (LOD scores) and nonparametric (NPL scores) multipoint linkage analysis for the genome wide screen were performed with the Genehunter program (version 1.2; provided in the public domain by the Laboratory of Statistical Genetics, Rockefeller University, New York, NY; available at linkage.rockefeller.edu/).[13] Nonparametric linkage analysis concentrates on allele-sharing between family members with the same phenotype. Pair-wise linkage calculations were performed with the MLINK program of the FASTLINK package (version 4.1P; FASTLINK is provided in the public domain by the National Institutes of Health and is available at ftp://fastlink.nih.gov/pub/fastlink/fastlink.tar.Z/).[14] Heterogeneity testing was performed with the Genehunter program. Allele frequencies of the microsatellite markers were obtained from the CEPH database (provided in the public domain by Fondation Jean Dausset—Centre de'Etude du Polymorphisme Humain (CEPH; Center for the Study of Human Polymorphism) Paris, France; available at www.cephb.fr/cgi-bin/wdb/ceph/systeme/form. The frequency for the putative KC gene was set at 0.0015.[9] No sex difference was assumed. Based on the family data, the penetrance of the disease in Finnish KC-affected families was estimated to be 50%[9] and was set thus with one liability class. The disease phenotype was analyzed as an autosomal dominant trait, but because in some small families the mode of inheritance was not obvious, we also included nonparametric linkage analysis, which is independent of the model of inheritance. Haplotypes were constructed manually.

To check the two previously assigned potential loci for KC on chromosome arms 18p and 21q, we analyzed these chromosomes first and also genotyped additional chromosome-18 and-21–specific markers. The primer sequences for these markers were obtained through the Genome database (data not shown; the Genome database is provided in the public domain by the Hospital for Sick Children, Toronto, Ontario, Canada, and is available at http://www.gdb.org).

RESULTS

No evidence for linkage between KC and the markers on chromosome arms 18p or 21q was observed. The multipoint LOD scores were negative throughout both chromosomal regions (Fig. 2).

Significant exclusion was obtained for all markers, except for four regions on chromosomes 2, 10, 15, and 16 with LOD scores exceeding 1.5 (Fig. 2). Additional markers flanking the four peak regions were genotyped and increased multipoint LOD scores were obtained only for markers on chromosome arm 16q. The highest initial LOD score on chromosome arm 16q was obtained with the genome-wide panel marker *D16S518*. The flanking dinucleotide repeat markers *D16S2624*, *D16S3033*, *D16S2642*, *D16S512*, *D16S3018*, *D16S3115*, *D16S3118*, *D16S3083*, *D16S3090*, *D16S3138*, and *D16S3029* were used

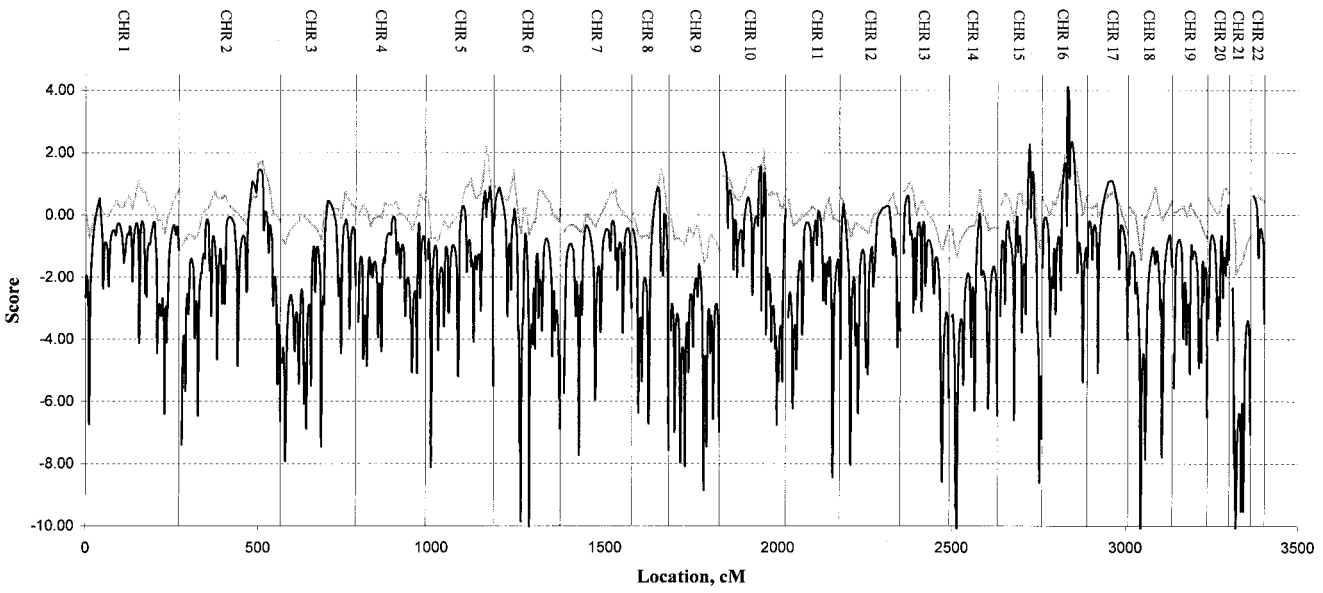

FIGURE 2. Linkage results of the genome scan for KC with 292 autosomal markers in 20 pedigrees. *Black line*: multipoint LOD scores; *gray line*: NPL scores. The figure illustrates the approximate genomic coverage and was constructed from the values obtained in Genehunter. The lines at chromosomal boundaries are discontinuous.

to obtain a denser marker map of the region. The order of markers was obtained from the physical map by Liu et al.[15] and the GenBank genomic sequences (GenBank, the genetic sequence database, is provided in the public domain by the National Institutes of Health, Bethesda, MD; available at http://www.ncbi.nlm.nih.gov/Genbank/) The use of additional markers resulted in the maximum multipoint LOD score of 4.11 and NPL (assuming no fixed model of inheritance of KC) of 3.27 (approximate $P = 0.00006$, Fig. 3). No heterogeneity was observed. A maximum two-point LOD score of 2.31 (at $\theta = 0.001$) was obtained between KC and the marker *D16S3115* (Table 1).

The allele data, which were obtained using 14 markers from the region *D16S771-D16S3029*, was used to construct disease haplotypes. Recombinations were observed in families 2, 6, 11, and 19 (Table 2). Based on the haplotype data, the KC gene was localized between the markers *D16S2624* and *D16S3090*, a region of approximately 6.9 cM.

DISCUSSION

The results of the present genome-wide linkage study suggest that the causative gene in KC is located within the 16q22.3-q23.1 chromosomal region, in an interval of approximately 6.9 cM. Three genes with defects resulting in different corneal disorders are located on or near the KC candidate region and are thus worth checking more thoroughly. The lecithin-choles-

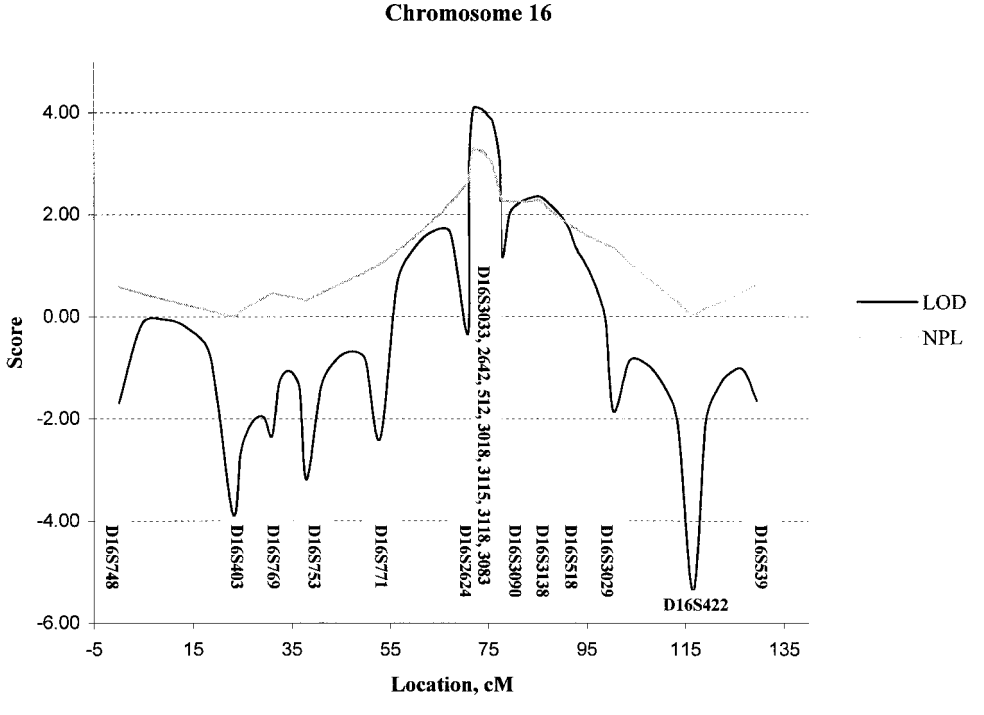

FIGURE 3. Linkage results with chromosome-16–specific markers. The peak multipoint LOD score of 4.1 was obtained at the marker locus *D16S3018*. *Black line*: multipoint LOD scores; *gray line*: NPL scores.

TABLE 1. Two-Point LOD Scores for Chromosome 16 Markers within and Flanking the Critical Region for KC

	Recombination Fraction, Θ							
Locus	**0.0**	**0.001**	**0.01**	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**
D16S771	−3.61	−3.22	−1.96	−0.70	−0.21	0.09	0.10	0.04
D16S2624	0.33	0.62	1.30	1.69	1.61	1.14	0.61	0.19
D16S3033	0.61	0.61	0.60	0.56	0.49	0.32	0.16	0.04
D16S2642	2.09	2.08	2.02	1.74	1.41	0.83	0.38	0.10
D16S512	1.49	1.48	1.44	1.24	1.00	0.60	0.28	0.07
D16S3018	2.00	2.00	1.94	1.70	1.40	0.83	0.38	0.10
D16S3115	2.31	2.31	2.24	1.94	1.58	0.93	0.43	0.11
D16S3118	1.02	1.01	0.98	0.85	0.69	0.40	0.18	0.05
D16S3083	1.56	1.55	1.50	1.29	1.04	0.60	0.27	0.06
D16S3090	−1.54	−1.39	−0.85	−0.28	−0.06	0.05	0.04	0.01
D16S3138	1.43	1.43	1.40	1.27	1.08	0.70	0.35	0.10
D16S518	1.51	1.51	1.47	1.30	1.10	0.69	0.35	0.11
D16S3029	−2.87	−2.57	−1.48	−0.40	−0.03	0.13	0.09	0.03

terol acyltransferase gene (*LCAT*) is responsible for fish-eye disease with corneal lesions (MIM 136120).[16] The tyrosine aminotransferase (*TAT*) gene, causes tyrosinemia, which is characterized by herpetiform corneal ulcers (MIM 276600).[17] Recently, the carbohydrate sulfotransferase 6 gene (*CHST6*) was identified as the causative gene for macular corneal dystrophy types I and II (*MCD*, MIM 217800).[18] Autosomal recessive MCD is characterized by progressive clouding of corneal stroma. In MCD type I, neither cornea nor serum contains appreciable levels of antigenic keratan sulfate (aKS), whereas in MCD type II aKS is present in cornea and serum. Homozygous inactivating mutations were detected in the coding region of *CHST6* in patients with MCD type I, whereas in patients with MCD type II DNA rearrangements were found in the upstream region of the same gene. MCD has not been reported to be associated with KC. The 6.9-cM KC candidate gene region also includes several other mapped genes, none of which was an obvious functional candidate (Fig. 4).

Functional candidate genes for KC have been proposed based on histologic findings. These include corneal collagens, proteinases, proteinase inhibitors, and interleukin-1–associated proteins. Only *COL6A1* (MIM 120220) on chromosome arm 21q has been excluded as the causative gene in autosomal dominant KC.[8] The expression levels of a number of degradative enzymes and protease inhibitors have been compared in normal and KC corneas. For example, the expression of matrix metalloproteinase (MMP)-2 (MIM 120360) in KC-affected cornea has been actively studied.[5,19,20] Because KC is considered to be genetically heterogeneous, the results of expression studies depend strongly on the genetic background of the cornea donors. Ihalainen et al.[5] found an increase in type IV collagenolytic (or MMP-2) activity in primary KC corneal cultures of Finnish patients. The MMP-2 gene maps to 16q21.[21] It has now been excluded as the causative gene for KC, because it was mapped outside the candidate gene region (data not shown).

A future goal is to narrow the candidate gene interval by linkage disequilibrium studies involving both additional markers and patients. In the present study, the patients in each family shared a common disease haplotype in the 6.9-cM interval, but the haplotypes of these patients varied among families. It is probable that linkage disequilibrium can be detected by using a denser marker map of the region, as has been shown in two other linkage studies of Finnish families.[22] Even though it is not yet known whether the gene on chromosome 16 is defective in patients with KC worldwide, identifying the gene in a Finnish KC subtype improves the understanding of the

TABLE 2. Summary of the Haplotype Data of the KC Candidate Region

		Affected Patients			
Distance between Markers (cM)	**Marker**	**Family 2**	**Family 6**	**Family 11**	**Family 19**
	D16S771	NA	*	*	*
1.8	*D16S2624*	NA	*	o	*
1.0	*D16S3033*	o	o	NA	o
0.25	*D16S2642*	o	o	NA	o
0.25	*D16S512*	o	o	NA	o
0.5	*D16S3018*	o	o	o	o
0.7	*D16S3115*	o	o	o	o
0.7	*D16S3118*	o	o	NA	o
1.5	*D16S3083*	o	o	o	o
2.0	*D16S3090*	NA	o	*	o
7.5	*D16S3138*	o	o	NA	o
7.5	*D16S518*	o	o	NA	o
7.5	*D16S3029*	*	o	*	o
16.0	*D16S442*	*	NA	*	o

Data show the observed recombinations in disease haplotypes of four families. The families did not share a common haplotype. The KC gene is localized between *D16S2624* and *D16S3090*. *, allele segregating with normal phenotype; o, allele segregating with disease phenotype; NA, data not available.

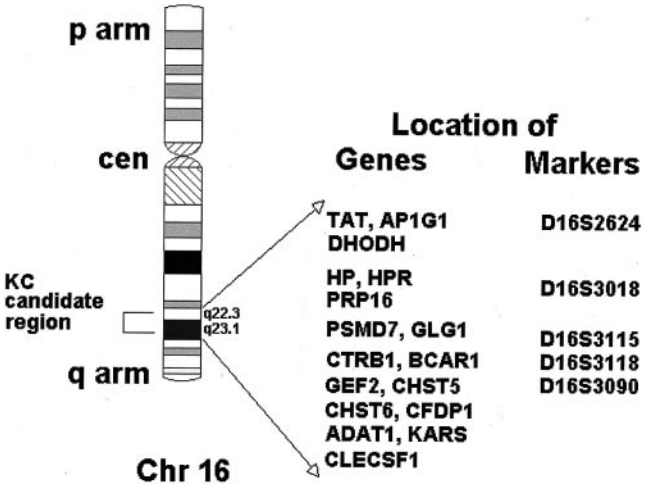

FIGURE 4. A karyogram of chromosome 16, showing the KC candidate region and the genes localized to this region (according to the National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD; available in the public domain at http://www.ncbi.nlm.nih.gov/).

pathogenesis of this complex trait and may provide insight into the cause of the more common sporadic form.

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