Coexistence of macular corneal dystrophy types I and II in a single sibship

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Abstract
Background—Macular corneal dystrophy (MCD) is an inherited autosomal recessive disorder that has been subdivided into two primary immunophenotypes, MCD types I and II. The MCD type I gene has been localised previously to chromosome 16q22 and suggestive evidence provided that MCD type II gene is also linked to this region. Here an unusual family is reported where both MCD types I and II are found in a single sibship.

Methods—Immunoreactivity to an antikeratan sulphate monoclonal antibody (5-D–4) was evaluated in patients’ serum and in corneal tissue obtained at keratoplasty. Chromosomal haplotypes were constructed using microsatellite repeat markers spanning the region of the MCD type I locus.

Results—Immunological studies demonstrated that two of the affected siblings have MCD type II while one has MCD type I. Haplotype analysis suggests that all three affected sibs inherited one identical parental haplotype. However, the two MCD types differ in their alternative chromosome with both MCD type II children sharing an identical haplotype, different from their MCD type I sibling.

Conclusion—The findings in this study support the hypothesis that the genes for MCD types I and II co-localise to the same region of chromosome 16 and are likely to be due to allelic manifestations of the same abnormal gene.

(Macular corneal dystrophy (MCD) is an autosomal recessive disorder that comprises 10–75% of the corneal dystrophies requiring penetrating keratoplasty, depending on the population. Patients develop irregular, cloudy regions that begin axially in both corneas. These areas progressively merge until eventually the entire corneal stroma opacifies. Immunological evaluation of antigenic keratan sulphate (AgKS) in serum and corneal tissue has shown that cases of MCD can be subdivided into two basic immunophenotypes. In MCD type I, AgKS immunoreactivity is absent from both serum and corneal tissue. Detectable, and often normal, serum AgKS levels and corneal accumulations that react with a monoclonal anti-keratan sulphate (KS) antibody characterise MCD type II. Recently, MCD type IA, an additional subclass of immunoreactivity, has been described in families from Saudi Arabia. In these individuals, AgKS immunoreactivity is absent from both serum and corneal stroma, but accumulations within the keratocytes react with the anti-KS antibody.

In a previous linkage study, we localised the MCD type I locus to chromosome 16q22. Analysis of several MCD type II families provided a maximum lod score of Zmax = 2.5 to the chromosome 16 microsatellite D16S518, one of the flanking markers for MCD type I. In that report, as well as in the one of Jonasson and colleagues, patients with both MCD type I and MCD type II were observed in a large 15 generation pedigree. However, none was observed in the same sibship and a chance occurrence could not be excluded.

Here, we report the finding of both MCD type I and type II individuals in a single sibship of an American family. Together, these findings provide support for the hypothesis that these antigenically different forms of MCD may be due to defects in the same genetic locus.

Materials and methods
After obtaining informed consent from participants, venepuncture was used to collect blood for DNA and AgKS analysis. The serum levels of AgKS were determined using a well-established enzyme linked immunosorbent assay (ELISA) and an anti-KS monoclonal antibody (5-D–4) (ICN Biomedical) directed against a highly sulphated epitope present on KS chains. The immunohistochemical evaluation of the excised corneal tissue used the same antibody on pathological corneal tissue.

For haplotype analysis, the microsatellite marker order cen-D16S514-D16S421-D16S512-D16S3101-D16S3125-D16S516-D16S504-tel was used with D16S512 and D16S518 being the flanking markers for MCD type I. The markers D16S266 and D16S395 were ordered using a YAC (yeast artificial chromosome) contig that we have constructed to span the MCD region (data not shown). The primer sequences for all markers were obtained through the genome database interactive network (see http://gdwww.ncbi.nlm.nih.gov/). Amplification of microsatellite repeats was performed by polymerase chain reaction (PCR) using a Hybaid OmniGene thermocycler. After electrophoresis, gels were stained with SyberGreen (Molecular Probes) and detected by a FluorImager SI (Molecular Dynamics). Haplotype analysis was conducted as previously described. All pedigree and marker data were fed into a database and managed using the PEDIGENE system.
Results

The pedigree is summarised in Figure 1. Individuals 2, 3, and 4 were born in the late 19th century and had corneal opacities. Individual 2 died at 78 years of age without having had a corneal transplant. Individual 3 underwent a penetrating keratoplasty, but no material was available for study. The children of individual 2 reported that their mother and uncles had an identical corneal disorder to themselves and were examined by ophthalmologists who confirmed that they all had the identical disorder. However, these ophthalmologists are now dead and owing to the span of time involved, written records confirming these reports are not available. The father (individual 1), who had no symptoms or signs of any corneal diseases, died when he was 77 years old. All three siblings (individuals 5 (aged 86), 6 (aged 80), and 7 (aged 75)) were diagnosed as having MCD. Individuals 5 and 6 have undergone penetrating keratoplasty and the diagnosis of MCD was confirmed histopathologically on the excised corneal tissue. Two of the siblings (5 and 7) were diagnosed as having MCD type II with detectable levels of AgKS in serum. The serum AgKS levels in individuals 5 and 7 were 68 ng/ml and 140 ng/ml respectively (normal individuals without eye or joint disease have serum AgKS levels of 112–617 ng/ml). In a previous study, the glycosaminoglycans and proteoglycans had been analysed in the corneal tissue from individual 5 (case VC, family 88 in Klintworth and Smith). Retrospectively, these biochemical findings were consistent with individual 5 having MCD type II. The serum AgKS level in the third sibling (individual 6) was not above background (<9 ng/ml) and the corneal tissue was negative by immunohistochemical staining; hence individual 6 has MCD type I. There is no known consanguinity in the family. No individual affected with MCD has transmitted MCD or any corneal disease to their offspring, except individual 2 (Fig 1).

Haplotype analysis of individuals 5, 6, and 7 was performed with 10 markers spanning the MCD type I region. Analysis suggests that all three patients inherited a single identical chromosomal haplotype denoted as “A”. Both MCD type II siblings (individuals 5 and 7) inherited the same “B” chromosome while their MCD type I sibling (individual 6) inherited a third haplotype “C” (Fig 2). No haplotype inconsistencies were observed for the three siblings in over 100 microsatellites tested.

Discussion

The pedigree reported here demonstrates the coexistence of MCD types I and II in the same sibship. This extends a previous observation of patients with MCD types I and II appearing in a large inbred pedigree. Unfortunately, we lack data definitively

Figure 1 The family pedigree showing the coexistence of macular corneal dystrophy (MCD) types I and II in the same sibship. Arrow indicates proband.

Figure 2 Haplotype analysis of individuals 5, 6, and 7 from Figure 1. A, B, and C denote different chromosomal haplotypes. The names of microsatellite repeat markers are indicated in the left hand column adjacent to the haplotypes. The alleles are designated in terms of molecular weight. Arrow indicates proband. Haplotypes of individuals 1 and 2 are inferred.
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