Homozygosity mapping and linkage analysis demonstrate that autosomal recessive congenital hereditary endothelial dystrophy (CHED) and autosomal dominant CHED are genetically distinct

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Abstract

***Background*—Congenital hereditary endothelial dystrophy (CHED) is a corneal dystrophy characterised by diffuse bilateral corneal clouding resulting in impaired vision. It is inherited in either an autosomal dominant (AD) or autosomal recessive (AR) manner. The AD form of CHED has been mapped to the pericentromeric region of chromosome 20. Another endothelial dystrophy, posterior polymorphous dystrophy (PPM), has been linked to a larger but overlapping region on chromosome 20. A large, Irish, consanguineous family with AR CHED was investigated to determine if there was linkage to this region.**

***Methods*—The technique of linkage analysis with polymorphic microsatellite markers amplified by polymerase chain reaction (PCR) was used. In addition, a DNA pooling approach to homozygosity mapping was employed to demonstrate the efficiency of this method.**

***Results*—Conventional genetic analysis in addition to a pooled DNA strategy excludes linkage of AR CHED to the AD CHED and larger PPMD loci.**

***Conclusion*—This demonstrates that AR CHED is genetically distinct from AD CHED and PPMD.**

(*Br J Ophthalmol* 1999;**83**:115–119)

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Accepted for publication
30 July 1998

Congenital hereditary endothelial dystrophy (CHED) is characterised by bilateral, diffuse corneal clouding which impairs visual acuity. The severity of the disorder varies, but many patients require penetrating keratoplasty to restore vision.[1–3] The primary abnormality is attributed to dysfunctional endothelial cells, resulting in thickening and opacification of the cornea.[4]

Both autosomal dominant (AD) and autosomal recessive (AR) modes of inheritance have been described for CHED. Clinically, both forms of the disorder are quite similar, and, generally, the distinction between them is made by age at time of onset and presence or absence of associated symptoms.[5]

Posterior polymorphous dystrophy (PPMD) is a rare, bilateral, congenital, and usually dominantly inherited disorder,[6] characterised clinically by corneal opacifications and vesicles on the endothelial surface. It rarely produces gross visual disturbance.[7] Like CHED, PPMD is caused by abnormal endothelial cell development. PPMD and CHED are considered by some to be part of the clinical spectrum of a single hereditary endothelial dystrophy.[6 8 9] A PPMD locus has been identified on chromosome 20,[10] and subsequently, an AD CHED locus was mapped to a 2.7 cM region within this area.[3]

We undertook a study of a large inbred family with AR CHED to investigate whether the recessive form of CHED was genetically distinct from dominant CHED and clinically similar PPMD. A DNA pooling strategy for homozygosity mapping was employed in the initial analysis of these loci. Homozygosity mapping observations were subsequently confirmed using conventional linkage analysis.

The underlying principle of homozygosity mapping is that a fraction of the genome of offspring of consanguineous matings is expected to be homozygous by descent.[11] Searching for regions that are consistently homozygous among affected individuals is therefore a powerful method for mapping recessive genes.[12] The strategy of homozygosity mapping has previously been used in the mapping of Bardet–Biedl syndrome,[13] congenital muscular dystrophy,[14] Friedreich's ataxia,[15] and many more autosomal recessive diseases.[16–23]

The DNA pooling approach simplifies this search for homozygous regions. DNA from affected individuals is pooled, and a control pool is formed of DNA from unaffected family members. Pooled DNA is used as a template for polymerase chain reaction (PCR) amplification of polymorphic microsatellite markers. The number and relative frequencies of alleles in each pool is compared. When a marker is linked to a disease gene there will be a shift in allele frequencies in the affected pool towards a single allele indicating homozygosity at that locus. If a marker is unlinked both pools show a similar distribution of alleles. This approach can be used to screen the genome quickly and effectively for areas that are homozygous by descent. The homozygous regions identified are then more closely examined using conventional linkage analysis of individual family members.

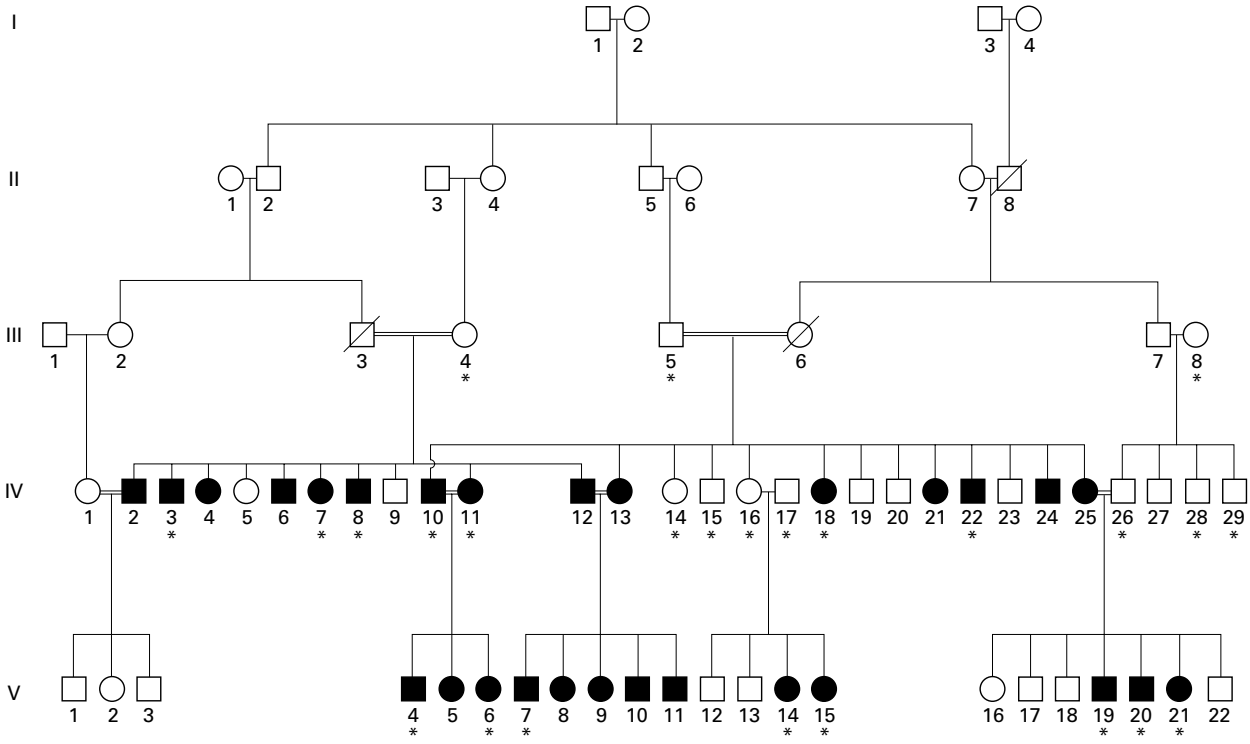

Figure 1 Pedigree drawing of family affected with autosomal recessive congenital hereditary endothelial dystrophy. Filled symbols indicate clinically affected individuals, open symbols indicate unaffected individuals or those whose disease status was not established. DNA was available from the individuals indicated by an asterisk; all these individuals were examined by an ophthalmologist.

Materials and methods

GENOTYPING

Blood samples were obtained from 15 individuals affected with AR CHED and 11 unaffected family members. Genomic DNA was extracted from these samples using standard protocols.[24 25] Equal molar amounts of DNA from affected family members were pooled and, similarly, DNA from unaffected members was pooled to form a control group. PCR was carried out on pooled DNA samples and also on all individual samples. A negative PCR control, containing water instead of DNA was included for each marker. Sequences for oligonucleotide primers flanking the five microsatellite markers studied were obtained from Généthon and the Collaborative Human Linkage Centre (CHLC) (http://www.chlc.org) databases. The PCR products were denatured, electrophoresed on 6% polyacrylamide gels, and visualised using silver staining.[26]

LINKAGE ANALYSIS

Two point linkage analysis was carried out using FASTLINK (v3.0p) and LINKAGE (v5.2) programs. The disease frequency was estimated to be 1:10 000 and the allele frequencies for the markers were obtained from the published CEPH databases (http://www.cephb.fr). Owing to the complexity of the pedigree multipoint analysis was not performed. The two point LOD scores generated using the five markers were significant enough to allow the exclusion of the entire PPMD locus. The AD CHED locus was excluded independently by three separate microsatellite markers.

Results

Family A is a large five generation inbred family with a total of 28 affected members (Fig 1). The pattern of segregation of the disease in this family is that of an autosomal recessive mode of inheritance. The presence of multiple consanguineous relationships is in keeping with recessive transmission. Blood was available from 15 affected and 11 unaffected individuals; 13 of the 15 members affected with CHED are offspring of consanguineous relationships.

Five polymorphic microsatellite markers on chromosome 20 were studied. Initially two markers flanking (D20S96, D20S98) and one marker within (D20S195) the 30 cM PPMD locus were examined. Pooled DNA was genotyped by PCR for each of these markers. Similar alleles were observed at each of these loci in both DNA pools (Fig 2).

To confirm this observation, individual DNA samples from family A were studied by conventional linkage analysis, and pairwise LOD scores generated. These results are presented in Table 1. Taking a LOD score of <-2 as evidence for exclusion, the disease locus in this family was excluded from a 14.3 cM region flanking the D20S96 microsatellite marker (Fig 3). Analysis of D20S195 indicated that the AR CHED gene was not within 34.3 cM of this locus. A further 6.6 cM region flanking D20S98 was also excluded (Fig 3). These data exclude the disease locus from the entire PPMD locus. This region includes the AD CHED locus.

The AR CHED family was then analysed at two of the most tightly linked markers to the dominant CHED gene: D20S114 and

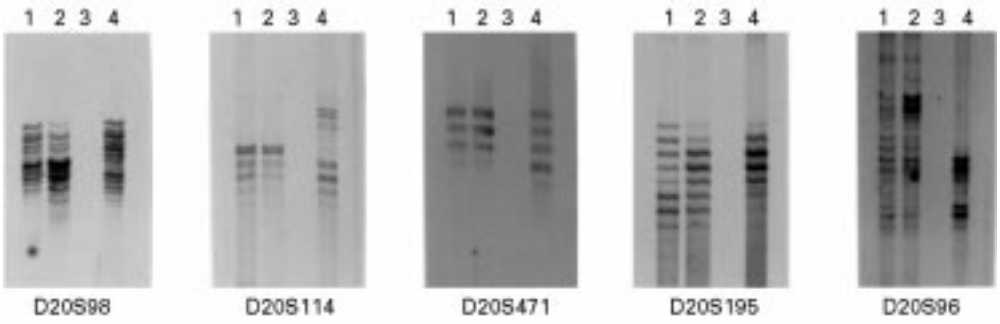

Figure 2 Silver stained polyacrylamide gels of the PCR products of pooled DNA at the five polymorphic microsatellite markers closely linked to the dominant congenital hereditary endothelial dystrophy locus. In all cases lane 1 is pool of DNA from unaffected individuals, lane 2 is pool of DNA from affected individuals, lane 3 is negative PCR control, and lane 4 is DNA from CEPH individuals 1331-01 and 1331-02 combined. There is no reduction in the number of alleles in pooled DNA from affected individuals compared with pool of unaffected DNA, indicating no increased homozygosity in this region.

Table 1 Two point LOD scores for linkage between recessive congenital hereditary endothelial dystrophy and five microsatellite markers on chromosome 20

	Recombination fraction (θ)							
Marker	*0.00*	*0.01*	*0.05*	*0.10*	*0.20*	*0.30*	*0.40*	*θ min*
D20S98	−999.99	−3.959	−1.394	−0.414	0.265	0.322	0.175	0.033
D20S114	−999.99	−5.458	−2.164	−0.892	0.041	0.250	0.183	0.054
D20S471	−999.99	−5.290	−2.082	−0.922	−0.110	0.092	0.084	0.052
D20S195	−999.99	−12.862	−6.277	−3.667	−1.459	−0.555	−0.172	0.165
D20S96	−999.99	−8.154	−3.009	−1.133	0.224	0.535	0.375	0.071

D20S471. Again, pooled DNA samples from affected and unaffected individuals of family A demonstrated a similar allele distribution when examined at these loci (Fig 2). Two point analysis of the disease gene and D20S114 excluded linkage of the AR CHED gene from a 10.8 cM region flanking this marker. Analysis of the D20S471 polymorphism allowed exclusion of an additional, overlapping the 10.4 cM region (Fig 3). This confirms that the AR CHED disease gene is not linked to the AD CHED locus.

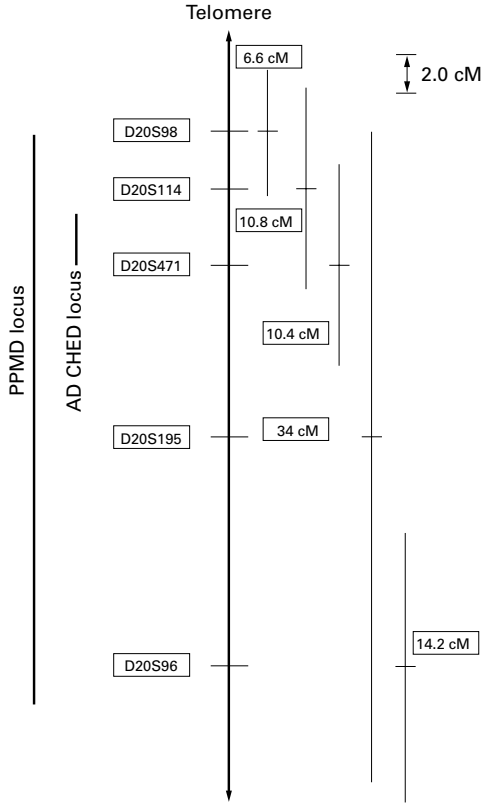

Figure 3 Map of the pericentromeric region of chromosome 20 indicating the position of the microsatellite markers analysed and the genetic distances from which linkage has been excluded.

We have therefore demonstrated the exclusion of the recessive CHED gene from the entire interval between D20S96 and D20S98 and thus from both the PPMD and AD CHED loci. These data suggest that recessively inherited CHED is genetically distinct from the dominant form, and also from the clinically related posterior polymorphous dystrophy.

Discussion

In this first genetic study of autosomal recessive CHED, we have shown in a five generation consanguineous pedigree that the disease locus is outside the pericentromeric region of chromosome 20 to which the dominant CHED gene and PPMD gene have both been mapped.

CHED and PPMD are dystrophies, which affect the corneal endothelium. These disorders have three common features: (1) there is stromal and epithelial oedema due to the breakdown of normal endothelial cell function; (2) the endothelial cells observed are abnormal in appearance, reduced in number, or absent; and (3) dysfunctional endothelial cells cause a thickening of Descemet's membrane.[7]

Maumenee in 1960 was the first to suggest that a congenital endothelial anomaly was the primary defect in CHED.[1] The barrier and pump functions of the corneal endothelium are vital components of the system, which ensures a clear cornea necessary for sharp vision.[5] Alterations in function or loss of these cells are associated with corneal dysfunction and loss of clarity.[5 10]

The clinical presentation of CHED has been well described.[1–4 27–33] The ground glass, bluish-white opacity observed in CHED is due to diffuse oedema of the corneal stroma, which results from dysfunctional endothelial cells.[1] It is believed that the autosomal dominant and recessive forms are clinically distinct, and may be distinguished based on age at time of onset and the presence or absence of accompanying signs and symptoms.

In the more common AR CHED, corneal clouding is observed at birth, or within the neonatal period, with minimal progression over time. The most frequently seen additional sign is nystagmus, which is presumably caused by the severe corneal clouding present from early in life. In contrast, in AD CHED, opacification is not usually seen until the first year or two, and often the initial evidence of the disorder is the appearance of accompanying signs such as photophobia and epiphora. These signs diminish as the corneal clouding progresses. Nystagmus is rarely observed.[27]

PPMD is a corneal dystrophy most commonly inherited in an autosomal dominant fashion, but autosomal recessive transmission has been observed.[9] The characteristic histological feature is epithelialisation of the endothelium. These epithelial-like cells produce collagen, which causes thickening of Descemet's membrane as in CHED.[34] However, PPMD rarely impairs vision.

Both PPMD and CHED demonstrate a primary dysfunction of the corneal endothelium. During normal development, the corneal endothelium produces a thick basement membrane, Descemet's membrane. In both dominant and recessive forms of CHED and in PPMD, this membrane is greatly thickened. Normally, fetal corneal endothelium secretes a banded membrane which forms the anterior banded zone (ABZ) of Descemet's membrane. This layer does not increase during life. At birth, a putative terminal differentiation occurs which is evident in the change to the secretion of a non-banded zone (PNBZ) and a dramatic reduction in cell density. This zone is laid down throughout life.[5] In PPMD and AD CHED, the PNBZ is thin and multilamellar, implying that onset of the dystrophy is at the putative terminal differentiation shortly before birth. The ABZ is normal, suggesting that the earlier stage of development is normal.[8] Furthermore, the thickening of Descemet's membrane is caused by the laying down in a disorganised fashion (bird's nest pattern) of a reactive posterior collagenous membrane (PCL). In AR CHED, however, the ABZ is thick possibly due to growth at the accelerated prenatal phase continuing after birth without the usual slowing down. Descemet's membrane is thickened in a homogeneous fashion by accumulation of normally constituted material in the posterior zone of Descemet's membrane. There is no evidence of the bird's nest pattern.[2] These differences suggest that perhaps AD CHED and PPMD share a common basic defect, which is supported by the linkage of both dystrophies to the same region of chromosome 20[3 10] and that the AR CHED defect is distinct, possibly involving a locus critical in a different stage of endothelial cell development and regulation. The data presented here demonstrate that AR CHED is distinct genetically, which supports this proposal of an alternative defect.

There are therefore clinical, ultrastructural, and genetic differences which delineate recessive CHED from its dominantly inherited counterpart and the clinically related PPMD. However, the underlying anomaly in all these diseases is abnormal endothelial cell development. As the endothelial cells produce the Descemet's membrane, any abnormality in the function of the endothelium is reflected in the state of the Descemet's membrane. This may explain the broad spectrum of clinical pictures seen in all of these disorders.[2]

As AR CHED is genetically distinct from AD CHED, genetic analysis could be used to clearly define the mode of inheritance particularly in sporadic cases of CHED. Further studies need to be carried out to establish whether a locus other than that identified on chromosome 20 exists for AD CHED.

The DNA pooling approach to homozygosity mapping has proved to be a fast and efficient way of genetic screening in this large consanguineous family. No significant reduction in the number of bands was detected in the pool of affected DNA samples when compared with that of the unaffected samples for the five microsatellite markers analysed. However, the frequencies of the alleles observed were not always equal between the two pools. In both D20S98 and D20S96; in particular, there did appear to be a stronger intensity of one allele, but as there were multiple additional alleles present in the pool, it was felt that this did not represent a significant shift to homozygosity. The greater number of DNA samples in the pool of affected individuals would also be expected to result in a difference in intensity of bands between the pools. Conventional linkage analysis confirmed the data obtained in the analysis of five microsatellite markers presented here.

A genome wide search using the DNA pooling approach to homozygosity mapping is currently under way to identify the loci involved in AR CHED. Potential loci identified by homozygosity mapping can be confirmed by genotyping the individual family members.

M Callaghan and CK Hand together carried out all the laboratory studies.

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