human, tissue-specific differences in the function of *MYO7A* might result in specific mutations having different effects in the eye but similar effects in the inner ear. It seems likely, however, given (*a*) the wide range of *MYO7A* mutations identified for both nonsyndromic deafness and USH and (*b*) the results reported in the present study, that genetic background effects have some role to play in determining the development and severity of nonsyndromic and syndromic hearing loss.

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Haplotype Analysis in Icelandic Families Defines a Minimal Interval for the Macular Corneal Dystrophy Type I Gene

To the Editor:

Macular corneal dystrophy (MCD [MIM 217800]) is a rare autosomal recessive disorder that is clinically char-

acterized by progressive corneal stromal clouding and central corneal thinning in both eyes (Ehlers and Thorkild 1978; Klintworth 1980, 1994; Donnenfeld 1986). Depending on the population, MCD comprises 10%–75% of the corneal dystrophies requiring penetrating keratoplasty (Jonasson et al. 1989, 1996; Santo et al. 1995). In Iceland, for example, MCD is the most frequent condition necessitating penetrating keratoplasty, and it accounts for almost one-third of all corneal grafts performed there (Jonasson et al. 1996). At least two varieties of MCD have been identified, on the basis of whether antigenic keratan sulfate (aKS) is identified in the corneal stroma and serum by an enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies (Yang et al. 1988). In MCD type I, neither the cornea nor the serum contains appreciable levels of aKS. In contrast, MCD type II patients have detectable aKS in the cornea and serum (Yang et al. 1988). Recently, an additional immunophenotype of MCD (type IA) has been described in families from Saudi Arabia (Klintworth et al. 1997). In these individuals, aKS immunoreactivity is not detected in both serum and corneal stroma, but the accumulations within the keratocytes react with the anti–keratan sulfate antibody (Klintworth et al. 1997).

In a previous linkage study, we localized the MCD type I gene to an interval of ~7 cM on the long arm of chromosome 16 (16q22), flanked by the markers D16S512 and D16S518 (Vance et al. 1996). Analysis of several MCD type II families by means of the identical microsatellite markers used for MCD type I provided a maximum LOD score of 2.5. Given the prior finding that MCD type I is linked to these identical markers, this result suggests that the MCD type II gene may be linked to the same small region as is MCD type I (Vance et al. 1996). Moreover, elsewhere we have reported an unusual family in which both MCD type I individuals and MCD type II individuals coexist in a single sibship and share one identical disease haplotype, again suggesting the possible allelic manifestation of MCD types I and II (Liu et al. 1998). Here we report haplotype analysis of 10 Icelandic families, narrowing the interval for the MCD type I gene to a very small region, <1 cM. In addition, several MCD type II individuals were found to share one of the MCD disease haplotypes, supporting our previous observations that these different MCD immunophenotypes may be allelic (Vance et al. 1996; Liu et al. 1998).

Twelve MCD individuals from 10 Icelandic families were used for haplotype analysis. The diagnosis of MCD was based on a combination of the clinical presentations and the typical histopathologic features of the dystrophy in corneal tissue obtained after penetrating keratoplasty (Klintworth 1994). To determine the specific immunophenotypes, serum levels of aKS were determined by means of a well-established ELISA and an anti–keratan sulfate monoclonal antibody (5-D-4) (ICN Biomedical) directed against a highly sulfated epitope present on keratan sulfate chains (Thonar et al. 1985, 1986). The immunohistochemical evaluation of the excised corneal tissue used the same antibody on pathologic corneal tissue (Yang et al. 1988).

Constitutional DNA from each individual was extracted as described elsewhere (Pericak-Vance et al. 1988) and was genotyped with 16 polymorphic makers in the MCD region. Microsatellite repeats were amplified by PCR as described elsewhere (Ben Othmane et al. 1993). PCR products were electrophoresed on 6.5% polyacrylamide gels. Gels were then stained with SyberGreen (Molecular Probes) and were detected by a FluorImager SI (Molecular Dynamics) or Hitachi FMBio II (Hitachi Software; Engineering America). To ensure accuracy of allele analysis, PCR products from all affected individuals were analyzed side-by-side on the same gel, and DNA from control individuals (CEPH 952645 and CEPH 952646) was amplified and analyzed at the same time, for comparison of different gels. Haplotype analysis was performed as described elsewhere (Ben Othmane et al. 1993). Allele frequencies were determined by use of DNA extracted from the blood of 50 unrelated Icelandic controls. All marker data were entered into a database and were managed by means of the PEDIGENE system (Hayes et al. 1995).

The initial framework marker order of cen-D16S3033-D16S512-D16S3018-D15S515-D16S3097-D16S3101-D16S3125-D16S518-tel was based on the Généthon genetic map (Dib et al. 1996) and the Whitehead physical map. However, for fine mapping of the order of microsatellite markers used in this study, we constructed a YAC contig to span the MCD region (fig. 1). The CEPH YAC megabase library on 230 96-well plates (Cohen et al. 1993), available from the Duke Center of Human Genetics, was the source for contig formation. DNAs from these plates were pooled on the basis a two-dimensional PCR screening strategy (Amemiya et al. 1992; Ben Othmane et al. 1995). Microsatellite markers were then screened by PCR through the first dimension, which comprised 23 pools of 10 plates each. The corresponding second-dimension pools (comprising 20 pools of rows or columns of 23 plates each) were subsequently screened to identify the YACs positive for the specific marker. PCR-positive clones were cultured, and YAC DNA was extracted from these cultures. The presence of markers then was verified by reamplification of the sequences from the extracted DNAs. The PCR reaction was performed by means of a Hybaid OmniGene thermocycler, as described elsewhere (Ben Othmane et al. 1993). The markers D16S2624, D16S738, afm112xh2, D16S3115, D16S3118, D16S3083, JSB16A, and D16S266 were ordered by

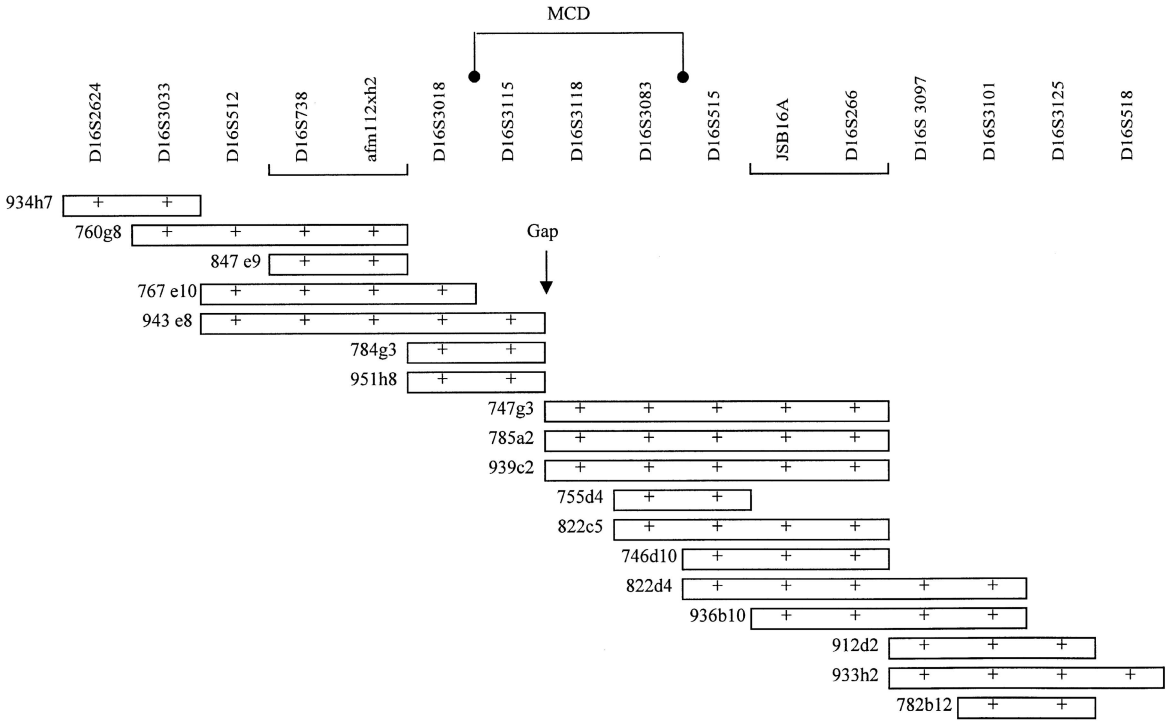

Figure 1 YAC contig of MCD region. Markers were ordered on the basis of combined physical mapping data; square brackets denote that marker order is unknown.

physical mapping on the constructed YAC contig (fig. 1). However, at this time, D16S3115 and D16S3118 are not connected by any YAC, leaving a gap between these two markers.

Except for one previously unpublished dinucleotide-repeat marker (JSB16A [forward primer, 5′-CCA TAG AAG ACT AGT AGG CA-3′; reverse primer 5′-TCA ATA GCT TGG AGG TTA G-3′; product size 204–230 bp]), the primer sequences for all markers were obtained through the Genome Database interactive network. JSB16A was isolated, by means of an indirect sequencing method, from a P1-derived artificial chromosome (PAC) clone 134d23, which was obtained by screening the PAC library filters (Genome Systems) by use of D16S266. The indirect sequencing method is a modification of that previously reported by Browne and Litt (1992) and Loeb et al. (1994) and consisted of two sequencing steps. In short, the first sequencing was performed on purified PAC DNA, by use of CA-repeat primers (CA_{10}A/G/T-A, CA_{10}A/G/T-C, CA_{10}A/G/T-G, CA_{10}A/G/T-T, AC_{10}C/G/T-A, AC_{10}C/G/T-C, AC_{10}C/G/T-G, and AC_{10}C/G/T-T). A reverse primer then was designed from the successful first sequences and was used to sequence back to span the CA-repeat region. A forward primer then was obtained from the second sequencing.

The MCD haplotypes of the 12 Icelandic individuals (8 MCD type I and 4 MCD type II) from 10 families are shown in figure 2. Among the 24 MCD-bearing chromosomes, five different haplotypes (haplotypes 1–5) were found that spanned the MCD type I region. Three of these were found in MCD type I patients, whereas two were found exclusively in MCD type II families. Given the known homogeneity of the Icelandic population (Jonasson et al. 1996; Vance et al. 1996), the multiple haplotypes for each type were unexpected. Haplotype 1 was the major haplotype, presenting, in its complete form, in four families, three of which contained MCD type I patients and one of which was a MCD type II family. Haplotype 2 was present in two MCD type I patients from the same family. Haplotypes 3 and 3a, which differ only in having one marker outside the MCD region, were detected in three MCD type II patients from three different families. Haplotypes 4 (MCD type I) and 5 (MCD type II) differed significantly from the existing haplotypes and were each found in one family. The different MCD type I haplotypes may represent different mutation events in the population, which seems unlikely, or some or all of the haplotypes may reflect a single mutation, with the ancestral recombinations rendering the shared region too small to be seen by the current resolution of polymorphic markers. Further genotyping in the minimal candidate region should clarify this point.

Haplotype Number	1	1	1	1	1	1a	1b	1c	1d	1e	1f	1g	1h	1i	1j	2	2	2	3	3	3	3a	4	5
D16S2624	140	140	140	140	140	140	140	140	140	140	140	148	148	140	136	136	136	136	140	140	140	144	136	136
D16S3033	136	136	136	136	136	136	136	136	136	136	136	134	134	136	136	136	136	136	128	128	128	128	136	138
D16S512	199	199	199	199	199	199	199	199	199	199	205	205	205	199	199	203	203	203	205	205	205	205	203	205
D16S738	128	128	128	128	128	128	128	128	128	128	126	126	126	128	128	128	128	128	126	126	126	126	128	126
afm112xh2	168	168	168	168	168	168	168	168	168	168	174	168	168	168	168	168	168	168	168	168	168	168	168	168
D16S3018	266	266	266	266	266	266	266	266	266	266	272	266	266	266	266	258	258	258	258	258	258	258	272	276
D16S3115	260	260	260	260	260	260	260	260	260	260	256	260	260	260	260	258	258	258	260	260	260	260	260	258
D16S3118	123	123	123	123	123	123	123	123	123	121	123	123	123	123	123	123	123	123	123	123	123	123	123	123
D16S3083	211	211	211	211	211	211	211	211	207	209	211	211	211	211	211	207	207	207	205	205	205	205	205	205
D16S515	242	242	242	242	242	242	242	242	234	226	242	242	242	242	242	228	228	228	242	242	242	242	238	238
JSB16A	204	204	204	204	204	204	204	222	226	204	204	204	204	204	204	216	216	216	204	204	204	204	204	216
D16S266	96	96	96	96	96	96	100	96	96	96	96	96	96	96	96	96	96	96	96	96	96	96	96	96
D16S3097	198	198	198	198	198	198	198	210	214	200	198	198	198	198	210	208	208	208	198	198	198	198	200	200
D16S3101	163	163	163	163	163	161	167	161	163	161	163	159	159	159	161	165	165	165	161	161	161	161	161	161
D16S3125	207	207	207	207	207	209	187	187	193	207	207	207	207	207	187	193	193	193	187	187	187	187	187	187
D16S518	272	272	272	272	272	272	282	268	276	272	272	276	276	276	280	272	272	272	268	268	268	268	286	272
Family No	1	2	2	3	4	1	5	6	7	8	3	5	5	5	9	9	9	9	10	10	8	6	7	4
Patient No	1	2	2	3	4	1	5	6	7	8	3	5	9	9	10	10	11	11	12	12	8	6	7	4
MCD type	I	I	I	I	II	I	I	II	I	II	I	I	I	I	I	I	I	I	II	II	II	II	I	II

Figure 2 Haplotypes of 12 MCD individuals from 10 Icelandic families. From top to bottom, markers are listed from centromere to telomere. The major ancestral founder haplotypes and the identifiable cosegregating traces are shaded or boxed. On the basis of ancestral recombinations, the MCD type I locus has been inferred to be flanked by markers D16S3115 and D16S3083.

Among the haplotypes derived from ancestral haplotype 1, the disease chromosomes could be distinguished from the putative haplotype 1 by one or two recombination events. Interestingly, three individuals with MCD type II (patients 4, 6, and 8) were found to share the same haplotype, haplotype 1, as was seen in MCD type I patients. In earlier studies, we had suggested that MCD types I and II may be linked to the same small region of chromosome 16 and thus may be allelic (Vance et al. 1996; Liu et al. 1998). The finding in the current study further supports these previous observations. However, although suggestive, they do not prove that these two immunophenotypes are allelic. Since this overlap in haplotypes could be secondary to chance, we also haplotyped 21 Icelandic controls, for these same markers. None of the five MCD haplotypes was found in these control individuals. Interestingly, allelic heterogeneity for the 5q31-linked corneal dystrophies has been reported elsewhere (Munier et al. 1997). It has been found that mutations originating from a single gene for keratoepithelin (βig-h3) (Skonier et al. 1992) are responsible for four corneal dystrophies, including granular dystrophy (Groenouw type I), Reis-Bucklers, lattice type I, and Avellino (Munier et al. 1997). Finally, it is possible, although less likely, that MCD types I and II share the same two mutations and that the currently noted differences are not due to different alleles but are secondary to either epistasis or modifying genes. Final knowledge of the actual relationship between MCD types I and II awaits identification of the MCD types I and II gene(s).

If one uses only MCD type I patients, then haplotype 1f shows an ancestral recombination with D16S3115 and thus is the centromeric boundary for the MCD type I locus. Moreover, haplotype 1d shows a recombination with D16S3083, on the telomeric side. The MCD type I locus thus can be refined to the DNA region between markers D16S3115 and D16S3083, which corresponds to an approximate genetic distance of <1 cM (Dib et al. 1996). Interestingly, if MCD types I and II are indeed allelic, then haplotype 1e from an individual with MCD type II would localize D16S3118 as the telomeric marker for the MCD gene.

One rare allele identified the haplotype as well. The 242-bp allele with marker D16S515, which existed in 17 (70.8%) of the 24 disease haplotypes (fig. 2), was found in only 9 of 100 Icelandic normal controls' chromosomes. The 123-bp allele with D16S3118 and the 96-bp allele with D16S266 existed in 23 (95.6%) of the 24 disease haplotypes (fig. 2). However, these two alleles are common alleles in the normal Icelandic population, with frequencies of .64 and .51 respectively.

Unfortunately, the YAC contig still has a gap between D16S3115 and D16S3118 (fig. 1). Efforts currently are being conducted to cross the minimal candidate region for MCD (D16S3115-D16S3083) with a PAC/BAC (bacterial artificial chromosome) contig.

In summary, this study indicates that the MCD type I gene is localized between markers D16S3115 and D16S3083, which corresponds to a genetic distance of <1 cM. This represents a significant refinement of the MCD candidate interval and should facilitate the ultimate cloning of the MCD gene. In addition, it suggests either that multiple mutations may have led to MCD type I in Iceland or that MCD is due to an extremely

old mutation, with multiple recombinants having occurred since its inception.

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Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim

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Heterogeneity in World Distribution of the Thermolabile C677T Mutation in 5,10-Methylenetetrahydrofolate Reductase

To the Editor:

Hyperhomocysteinemia now is recognized as an independent risk factor for vascular disease (Clarke et al. 1991) and defects of the neural tube (Mills et al. 1995).

Table 1

World Distribution of MTHFR T677

Population	No. of Chromosomes	Mutant Allele	
		No.	Frequency ± Standard Error
European:			
Italian (Calabria)	96	43	.448 ± .051
Spanish[a]	66	36	.545 ± .061
Sub-Saharan African:			
Dendi	24	0	0
Bariba	26	2	.077 ± .052
Berba	32	3	.094 ± .051
Fon	96	8	.083 ± .028
Total[b]	178	13	.073 ± .019
Asian:			
Tharu[a]	108	21	.194 ± .038
Chinese[a]	24	9	.375 ± .099
Indonesian	98	2	.020 ± .014
Amerindian:			
Cayapa[a]	114	49	.430 ± .046
Mixed:			
Ethiopian:			
Amhara	54	4	.074 ± .033
Oromo	54	3	.055 ± .031
Total[b]	108	7	.065 ± .024
African Ecuadoran:			
Viche[a]	82	13	.183 ± .043

[a] Testable population, except for Calabrians (Italy), in Hardy-Weinberg equilibrium.

[b] Populations were pooled when compatibility was verified by a χ^2 test of heterogeneity.

Genetic factors associated with hyperhomocysteinemia are mutations in genes coding for enzymes involved in the methionine-metabolism pathway, such as 5,10-methylenetetrahydrofolate reductase (MTHFR) and cystathionine β-synthase. Related environmental factors include low dietary intake or increased necessity of folate or vitamins B_6 and B_{12} (Fenton and Rosenberg 1995). Recently, a common thermolabile MTHFR variant, causing mild hyperhomocysteinemia in 30% of homozygotes, was detected. The C677T single-nucleotide substitution was found to cause an amino acid change, from alanine to valine (Frosst et al. 1995). The T677 allele is distributed widely among populations showing a high heterogeneity. The purpose of this study was to investigate the prevalence of the C677T MTHFR mutation among the major ethnic groups. The high prevalence of T677 homozygotes in preselected populations would result in the need for a higher dietary intake of folate, to prevent in utero neural-tube defects and long-term vascular damage (Molloy et al. 1997).

We studied a total of 437 unrelated, apparently healthy subjects from the major ethnic groups, categorized as follows: 81 Europeans (33 Spanish and 48 Italians); 89 sub-Saharan Africans (48 Fon, 13 Bariba, 16 Berba, and 12 Dendi); 115 Asians (49 Indonesians [Java], 12 Chinese [Peking area], and 54 Tharus); 57 Amerindians (Cayapa population [Ecuador]); and some mixed populations, namely, 54 Ethiopians (27 Amhara and 27 Oromo) and 41 African Ecuadorans (Viche area). All the subjects gave their informed consent for the study.

For information on the origins of the populations and their geographical distribution, see the article by Pepe et al. (1997) and the references cited therein. Genomic DNA was extracted from peripheral blood–cell lymphocytes, was amplified by PCR, and was digested with *Hin*fI restriction enzyme. The bands were visualized by ethidium-bromide staining, as described elsewhere (Abbate et al. 1998). The mutation creates a new *Hin*fI site, which is absent in the wild-type allele. Pooled frequencies were obtained as mean frequencies weighted for the sample size, after the compatibility of the allele frequencies was verified by the χ^2 test of heterogeneity. Results are reported in table 1.

Our data show a marked heterogeneity in the prevalence of the T677 MTHFR allele among the major ethnic groups surveyed and, in some cases, even *within* these groups. The frequency among Europeans ranged from .448 in Italian subjects to .545 in Spanish subjects, whereas the prevalence among Asians and Africans was within the ranges .041–.375 and 0–.094, respectively. For the Amhara and the Oromo, the frequency of the T677 allele was intermediate between that for the Europeans (~.5) and the sub-Saharan Africans (<.1); this also has been confirmed in previous studies on genetic admixture in these populations (Scacchi et al. 1994; O.R.