The gene responsible for Clouston hidrotic ectodermal dysplasia maps to the pericentromeric region of chromosome 13q

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Hidrotic ectodermal dysplasia (HED), Clouston type, is an autosomal dominant skin disorder which is most common in the French-Canadian population and is characterized by hair defects, nail dystrophy and palmoplantar hyperkeratosis. Biophysical and biochemical studies conducted in HED suggested a molecular abnormality of keratins. We tested eight French-Canadian families segregating HED for linkage to microsatellite markers flanking the known keratin genes and were able to exclude linkage to these loci. Therefore, a genome-wide search for the HED gene was initiated. The first lod score above 3.00 was obtained with the marker D13S175 located in the pericentromeric region of chromosome 13q (Z_{max} = 8.12 at zero recombination). The cumulative lod scores were above 3.00 for six other markers in the region. A multipoint linkage analysis using the markers D13S175, D13S141 and D13S143 gave a maximum lod score of 11.12 at D13S141 with the one-lod-unit support interval spanning a 12.7 cM region which includes D13S175 and D13S141. Haplotype analysis allowed us to establish D13S143 as the telomeric flanking marker for the HED candidate region.

INTRODUCTION

Hidrotic ectodermal dysplasia (HED), or Clouston syndrome, is a rare autosomal dominant genodermatosis transmitted with complete penetrance and variable clinical expressivity (1). It was first described by Nicolle and Hallipré in a French-Canadian family in 1895 (2). The hereditary nature of HED was established only in 1929 when Clouston described the disorder in five generations of a large French-Canadian family living in Québec (3). To date, most of the families segregating this condition are of either French or French-Canadian origin, suggesting a common ancestor (4). Occurrence in families of other ethnic origins such as Chinese (5), Japanese (6), British (7) and African-American (8) has also been reported. There is no obvious genetic origin common to all cases.

The main features of HED are dystrophy of the nails that tend to be hypoplastic and deformed with increased susceptibility to paronychial infections, defects of the hair that range from brittleness and slow growth rate to total alopecia and moderate to severe palmoplantar hyperkeratosis with reduced keratinocyte desquamation (6,9). Variable and infrequent features reported with HED include ocular abnormalities, sensorineural deafness, hyperpigmentation of the skin, mental retardation and skeletal anomalies such as polydactyly and syndactyly (10). In contrast to the X-linked anhidrotic type of ectodermal dysplasia, facial appearance, tooth growth and sweating capacity are normal in HED (9).

Biophysical and biochemical studies conducted in HED suggest depletion of a component of the high-sulfur portion of the matrix proteins related to a disruption of disulfide bond formation in the remaining keratin. The hair is thin with abnormal swelling capacity, reduced tensile strength, reduced birefringence under polarized light, looser and disorganized structure by light microscopy and decreased levels of cysteine and disulfide bonds (11). The diminished cysteine content was also shown in a biochemical study of keratin in the nails of HED patients (12). Ultrastructural findings reveal disorganization of hair fibrils with desquamation of the cuticle that could be due to the reduction of disulfide bonds (3). All these abnormal changes are suggestive of a molecular defect involving the keratin proteins. Point mutations in keratin genes have been described in many ectodermal dysplasias, such as epidermolysis bullosa simplex (13), epidermolytic hyperkeratosis (14), and pachyonychia congenita (15), stressing their importance in maintaining the structural integrity of and providing mechanical strength to the epithelial cells (16).

In the present study, we report the chromosomal localization of the gene responsible for HED to the pericentromeric region of chromosome 13q using linkage analysis of nine microsatellite markers in eight French-Canadian families.

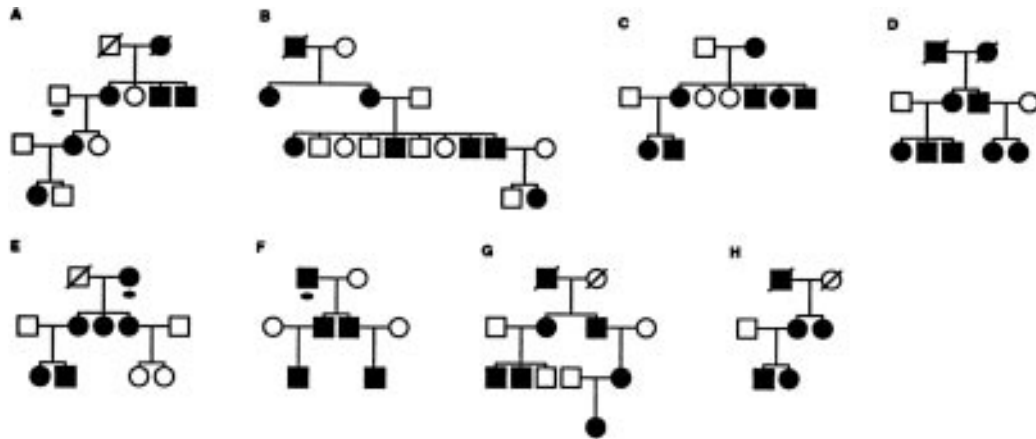**Figure 1.** Pedigrees of the HED families used in this study. DNA is available from all live members shown except those indicated by black ovals.

RESULTS

Two-point linkage analysis

Eight French-Canadian families segregating HED were studied (Fig. 1). They included 45 affected among a total of 76 participants. First, we sought linkage of HED to the chromosomal regions harboring the candidate keratin genes. Epidermal soft keratin genes have been found in clusters on two chomosomes: the type I acidic cluster on chromosome 17 and the type II basic cluster on chromosome 12 (17,18). Many of the hair keratin genes have been isolated and characterized but not mapped yet (19,20); however, there is evidence from linkage data in mice that they are associated with the epidermal keratin genes (21). Other chromo-

somes that have been suggested as the sites of more distantly related keratin genes, by *in situ* hybridization techniques, include chromosomes 14, 15 and 21 (18). Genes encoding the cuticle's ultrahigh-sulfur proteins have been localized to two clusters on chromosome 11 (22). We started by typing the HED pedigrees with polymorphic microsatellite markers localized to these chromosomal regions and found no evidence of linkage to any of them. Consequently, we initiated a general genome search using polymorphic markers spaced, on average, 20 cM apart. After excluding ~10% of the genome by typing 27 polymorphic markers, we found linkage to the pericentromeric marker D13S175 with a maximum combined two-point lod score of 8.12 at $\theta = 0$. Family B, by itself, gave a maximum lod score of 3.52 at $\theta = 0$ (Table 1).

Table 1. Two-point lod scores between HED and nine markers mapped to the pericentromeric region of chromosome 13q at various recombination fractions

| Marker	Family	Lod score (Z) at recombination fraction (Θ)							Z_{max}	Θ
		0.00	0.05	0.10	0.15	0.20	0.25	0.30		
D13S1316	B	0.08	0.07	0.05	0.04	0.03	0.02	0.01		
	total	3.83	3.41	2.98	2.55	2.12	1.68	1.24	3.83	0.00
D13S1236	B	0.12	0.10	0.08	0.07	0.05	0.03	0.02		
	total	$-\infty$	2.22	2.46	2.32	2.03	1.67	1.27	2.46	0.10
D13S175	B	3.52	3.24	2.95	2.65	2.33	2.00	1.65		
	total	8.12	7.39	6.61	5.80	4.95	4.10	3.23	8.12	0.00
D13S1275	B	$-\infty$	1.37	1.45	1.39	1.27	1.12	0.93		
	total	$-\infty$	4.14	4.41	4.15	3.68	3.08	2.41	4.42	0.09
D13S292	B	$-\infty$	1.74	1.79	1.71s	1.56	1.37	1.15		
	total	$-\infty$	3.01	3.67	3.67	3.40	2.97	2.43	3.71	0.12
D13S283[a]	B	$-\infty$	-1.16	-0.42	-0.07	0.12	0.21	0.25		
	total	$-\infty$	-1.92	-0.01	0.76	1.06	1.11	1.00	1.11	0.22
D13S141	B	1.21	1.10	0.99	0.88	0.76	0.63	0.51		
	total	4.00	3.54	3.08	2.61	2.16	1.71	1.28	4.00	0.00
D13S143	B	$-\infty$	2.00	2.03	1.93	1.75	1.54	1.29		
	total	$-\infty$	7.14	6.83	6.18	5.37	4.45	3.48	7.14	0.05
D13S115	B	$-\infty$	1.45	1.53	1.47	1.35	1.19	1.00		
	total	$-\infty$	4.00	4.34	4.15	3.72	3.16	2.52	4.34	0.10

[a]$Z_{max} = -2.64$ at $\Theta = 0.04$
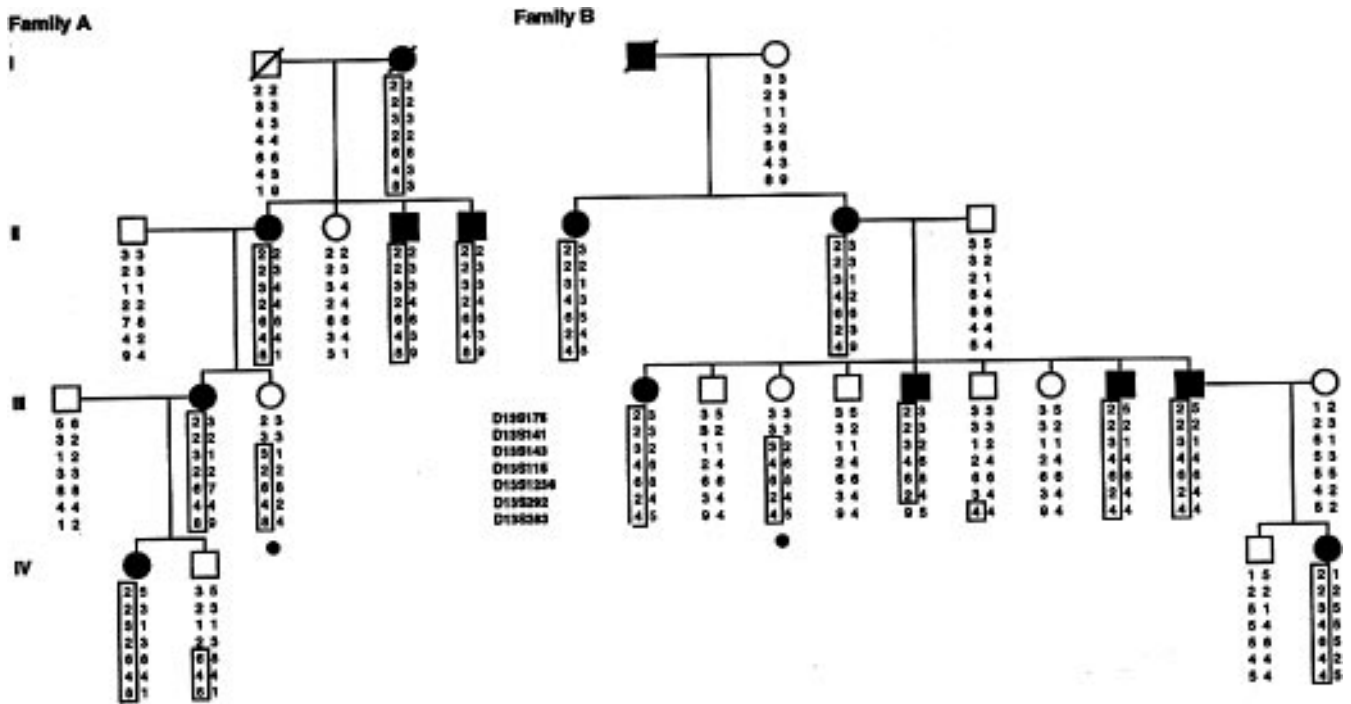
Figure 2. Pedigrees of two HED families with their haplotypes showing two recombinants, individuals III-3 in family A and III-3 in family B, that would place the HED locus proximal to D13S143 (●). The phasing of haplotypes was inferred from the available genotypic data. Haplotypes that most likely contain the mutant HED gene are boxed. The order of the markers used in this analysis was obtained from a YAC contig established in this region (26).

To confirm the initial linkage and to define the HED locus further, pairwise linkage analyses were performed with eight additional markers mapped to the pericentromeric region of chromosome 13. Five of these microsatellites were obtained from Genethon: D13S1316, D13S1236, D13S1275, D13S292 and D13S283; the other three were physically mapped by somatic cell hybridization: D13S141 and D13S143 to 13q11–q12.1 (23) and D13S115 to 13pter–q12.1 (24). Table 1 summarizes the two-point lod scores for the nine markers at various recombination fractions. Lod scores >3.00 were obtained for all of the markers except D13S1236 and D13S283. D13S1236 was not informative in this set of families and D13S283 which maps 5 cM telomeric to D13S175 (25) excluded linkage to HED up to $\theta = 0.04$ ($Z_{max} = -2.64$). Three markers showed no recombination with a maximum combined lod score >3 at $\theta = 0$: D13S1316, D13S175 and D13S141. D13S175 gave the highest two-point lod score in this study of 8.12 at $\theta = 0$.

Haplotype and multipoint linkage analyses

In order to define the smallest interval containing the HED locus, we analyzed the eight families for recombination events between marker loci and HED. Since no single chromosome 13 map contains all the markers studied, we first constructed the haplotypes using only the ordered Genethon markers as follows: D13S1316-D13S1236-D13S175-D13S1275-D13S292-D13S293 (Genethon). Using this order, we detected three double recombinants in three families (data not shown). Our data suggested an order consistent with physical data for a 6 Mb YAC contig established in the 13q pericentromeric region spanning the region

between D13S175 and D13S221, establishing the following order for the markers we typed: cen–D13S175-D13S141-D13S143-D13S115-D13S1236-D13S292-D13S283–tel (26). D13S1316 and D13S1275 were not included in the haplotype analysis because their position has yet to be determined. We identified eight informative recombination events in five families (data not shown). Figure 2 shows the two critical crossovers that would place the HED locus centromeric to D13S143 (individuals III-3 in family A and III-3 in family B). In addition, we found that all 45 affected individuals share a common haplotype (2 2 3) for the markers D13S175, D13S141 and D13S143.

To estimate the most likely position of the HED gene, we performed multipoint linkage analysis using the genetic map: D13S175-2 cM-D13S141-6 cM-D13S143. The recombination fractions between these markers were determined from pairwise linkage analysis in the eight HED kindreds (data not shown). The maximum multipoint lod score attained was 11.12 at D13S141. However, if we drop 1 lod unit below the maximum, the support interval would span a 12.7 cM region, 6.3 cM centromeric to D13S175 and 4.4 cM telomeric to D13S141 (Fig. 3).

DISCUSSION

By using linkage analysis in eight French-Canadian families segregating HED, we were able to map the responsible gene to the pericentromeric region of chromosome 13q. Strong evidence for linkage to HED was obtained with seven polymorphic markers mapped to this region of which D13S175 generated the highest cumulative two-point lod score ($Z_{max} = 8.12$ at $\theta = 0$). Data from haplotype analysis showed that D13S143 is the telomeric
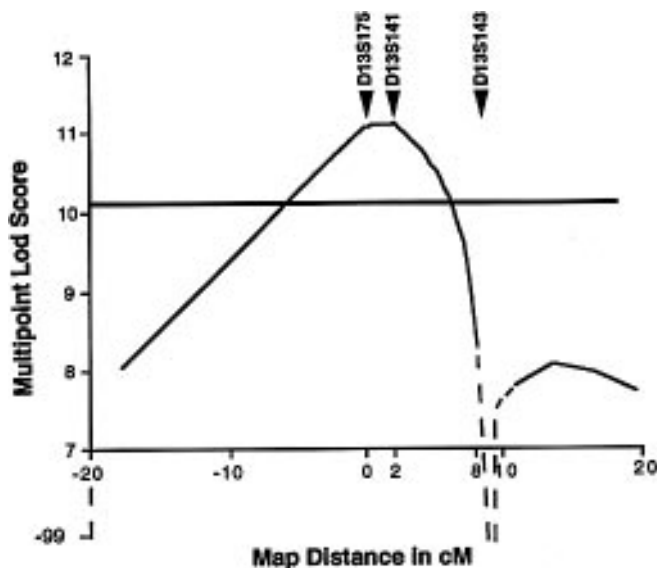
Figure 3. Multipoint linkage analysis of HED with the markers D13S175, D13S141 and D13S143. The solid line represents the 1 lod-unit support interval for the placement of the HED locus. The genetic distance between markers was determined from two-point linkage analysis in the eight HED families. The map distance was calculated using the Haldane mapping function.

flanking marker for the candidate region of the HED gene. In the absence of more centromeric markers, we could not define a proximal limit to this region. However, multipoint linkage analysis places the HED locus at the most 6.3 cM centromeric to D13S175.

Since the biochemical defect in HED is believed to involve keratins, exclusion of linkage to all chromosomes known to harbor the keratin genes i.e. chromosomes 11, 12, 14, 15, 17 and 21 was unexpected. However, not all keratin genes have been mapped and the HED locus could still correspond to an unlocalized keratin. Another hypothesis has been proposed to explain all types of ectodermal dysplasias, including HED, where the disease is caused by a disturbed mesoderm–ectoderm interaction during morphogenesis of the ectodermal tissues (27,28). Given this hypothesis, it may be significant that two forms of non-syndromic neurosensory deafness, recessive DFNB1 (29) and dominant DFNA3 (30), also map to the region containing the HED locus and show linkage to D13S175, D13S143 and D13S115. These two diseases result from an endocochlear defect and the responsible genes may code for one of the proteins involved in cochlea structure and function. Because cochlea development is influenced and controlled by a series of inductive ectoderm–mesoderm reciprocal interactions (31) and because hearing loss has been reported in a few cases of HED (9), it is possible that these three diseases are caused by different mutations in the same gene or in related genes found in a cluster.

The candidate region for the HED gene contains three expressed sequence tags (ESTs) (D13S183E, D13S502E and D13S837E) and an α-tubulin gene (TUBA2) (26). These ESTs will be further analyzed to establish their possible role as candidate genes for HED. TUBA2 is unlikely to be involved in this disease since its expression is most probably testis-specific (26). We will continue to reduce the candidate region by typing new markers and collecting additional HED families. Further-

more, since a founder effect has been suggested by the high prevalence of HED in the French-Canadian population, we may be able to use linkage disequilibrium mapping to narrow down the HED candidate region.

MATERIALS AND METHODS

HED families

Diagnosis of HED was established when individuals presented with various degrees of hair abnormalities, nail defects and hyperkeratosis of palms and soles. Eight French-Canadian families participated in this study consisting of 76 individuals that included 45 affected (Fig. 1).

DNA analysis and genotyping

From each consenting member, 20 ml blood was obtained: 10 ml was used to establish Epstein–Barr virus transformed lymphoblastoid cell lines and the other 10 ml was used for direct DNA extraction using standard procedures (32). PCR amplifications for the microsatellite markers were carried out in a total volume of 14 µl containing: 40 ng of genomic DNA; 200 µM of dCTP, dGTP and dTTP; 25 µM dATP, 125 ng of each primer; 1.5 µCi [^{35}S]dATP, 0.5 U *Taq* DNA polymerase (Perkin Elmer) and 1.25 µl *Taq* buffer with 15 mM MgCl$_2$ (Perkin Elmer). For the Genethon markers, PCR conditions consisted of 35 cycles with 45 s at 94°C, 30 s at 57°C and 45 s at 72°C for each cycle. For D13S141, D13S143 and D13S115, PCR conditions were as previously published (23,24). For D13S143, the primers' sequences were as described for marker ca016(A) (23). The PCR products were run on denaturing 6% poyacrylamide gels in 1× TBE for 2–3 h. After drying, the gels were exposed to X-ray films for 2–3 days at room temperature. An M13 sequencing ladder was used to determine the alleles' sizes for the genotypes. For D13S175, allele 2 is 103 bp; for D13S141, allele 2 is 125 bp and for D13S143 allele 3 is 128 bp.

Linkage analysis

Two-point and multipoint linkage analyses were performed using the MLINK and the LINKMAP programs of the LINKAGE 5.1 package (33,34). The mode of inheritance was considered to be autosomal dominant with complete penetrance and an estimated gene frequency of 0.00001. Recombination fractions were assumed to be equal in females and males. The allele frequencies in the French-Canadian population for all markers were obtained from the genotypes of 18 unaffected spouses participating in this study.

ABBREVIATIONS

HED, hidrotic ectodermal dysplasia; cM, centimorgan; kb, kilobase pairs; PCR, polymerase chain reaction; bp, base pairs.

ACKNOWLEDGEMENTS

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