Clouston Syndrome (Hidrotic Ectodermal Dysplasia) Is Not Linked to Keratin Gene Clusters on Chromosomes 12 and 17

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Clouston syndrome is an hidrotic form of ectodermal dysplasia, inherited as an autosomal dominant trait with high penetrance. The main features of the disorder are alopecia, severe dystrophy of the nails, and palmoplantar hyperkeratosis. A molecular abnormality of keratin has long been hypothesized to be the basic defect in this disorder. We have performed linkage analyses between the disorder and markers close to the keratin gene clusters on chromosomes 12 and 17 and have excluded linkage to these candidate regions in three apparently unrelated families. In addition, linkage has been excluded to four other candidate regions including 1q21, 17q23-qter, 18q21, and 20q12. These data indicate that Clouston syndrome is not due to a defect in keratin or in a subset of keratin-associated proteins. ***Key words: hyperkeratosis/gene mapping/linkage/microsatellite markers. J Invest Dermatol 107:11–14, 1996***

Clouston syndrome (Mendelian Influence in Man (MIM) 129500) is an autosomal dominant ectodermal dysplasia characterized by the triad of nail dystrophy, alopecia, and hyperkeratosis of the palms and soles (Clouston, 1929, 1939). In contrast to the X-linked form of ectodermal dysplasia, teeth, facial appearance, and sweating are normal. The pathogenesis is unknown. In 1965, Scriver *et al* suggested that a molecular defect in keratin was the cause; this hypothesis was supported by the biochemical identification of depleted hair matrix proteins accompanied by disruption of or failure to form disulfide bonds in the remaining keratin molecules of hair (Gold and Scriver, 1971, 1972). These findings implicated a gene coding for a component of the high sulfur fraction of matrix α-keratin. Further support for a possible role of keratin came from ultrastructural studies of hair from affected individuals (Escobar *et al*, 1983), which demonstrated disorganization of fibrils with loss of cuticular cortex. In addition, other disorders with palmoplantar hyperkeratosis are caused by mutations in keratin genes, including epidermolytic palmoplantar keratoderma (Reis *et al*, 1992), nonepidermolytic palmoplantar keratoderma (Kimonis *et al*, 1994), and pachonychia congenita (McLean *et al*, 1995), and may even be allelic to Clouston syndrome. Based on these findings, candidate genes for Clouston syndrome include the keratin gene family.

Keratins are intermediate filaments that form a complex and elaborate cytoskeleton essential to the structural integrity of individual epithelial cells as well as whole tissues. Keratin intermediate filaments are heterodimers formed of one type I, or acidic, keratin protein chain and one type II, or basic, chain. The type I keratins are encoded by a gene family located on chromosome 17q21.1-q21.2 (Romano *et al*, 1988; Rosenberg *et al*, 1988; Savtchenko *et al*, 1990), and the type II keratin gene family is localized to chromosome 12q11-q13 (Romano *et al*, 1988; Yoon *et al*, 1994).

In order to determine whether defects in keratin proteins are associated with Clouston syndrome, we analyzed the cosegregation of highly polymorphic microsatellite markers near the keratin gene clusters with the Clouston syndrome phenotype in three unrelated families.

Four additional candidate regions (1q21, 17q23-qter, 18q21, and 20q12) were studied, including two that are implicated in disorders of palmoplantar hyperkeratosis and two that contain genes for keratin-associated proteins. Desmogleins, members of the cadherin superfamily, are adhesive proteins of the desmosome cell junctions in epithelial cells and form a 3-dimensional lattice with keratin filaments. Three desmoglein genes map to chromosome 18q12.1 (Simrak *et al*, 1994; Wang *et al*, 1994a), the region to which striate palmoplantar keratoderma has been linked (Hennies *et al*, 1995a). Keratoderma with tylosis and esophageal cancer maps to 17q23-qter, separate from the keratin gene cluster (Hennies *et al*, 1995b). Six genes expressed in terminally differentiating epidermis (trichohyalin, profilaggrin, involucrin, loricrin, small proline-rich protein, and calcyclin) map to a 2-megabase region on chromosome 1q21 (Fietz *et al*, 1992; Lee *et al*, 1993). Transglutaminases catalyze the formation of lysine isodipeptide crosslinks in proteins. TGM3 is found in terminally differentiating epidermal and hair kertinocytes and maps to chromosome 20q12 (Wang *et al*, 1995b). Markers linked to these candidate regions were studied for cosegregation with the Clouston syndrome phenotype.

MATERIALS AND METHODS

Families were ascertained through clinical genetics centers and were examined by a clinical geneticist **(Fig 1)**. Families were identified with Clouston syndrome when 1) affected members showed at least two of the

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Abbreviation: PCR, polymerase chain reaction.

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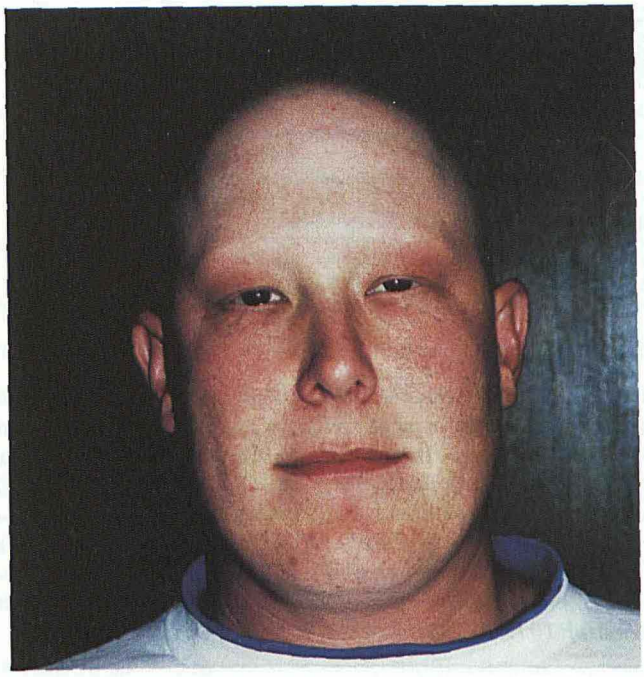

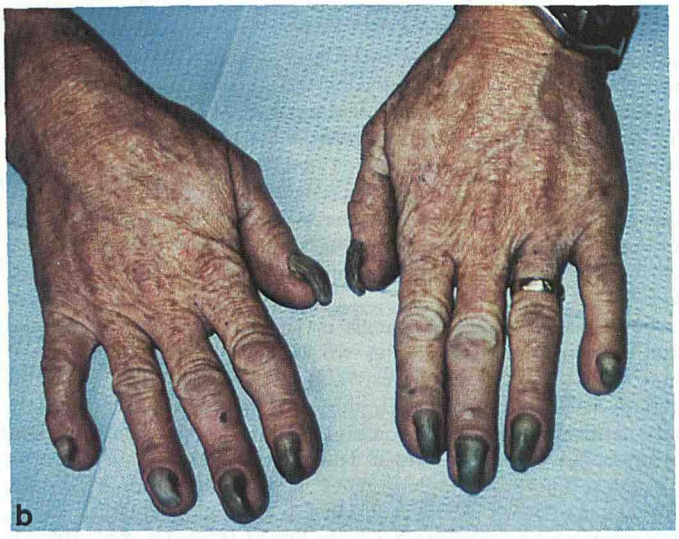

Figure 1. ***a*, absent scalp hair, facial hair and eyebrows in individual CS1 III-406.** ***b*, nail dystrophy in individual CS1 II-301.**

following features: sparse to absent scalp hair, dystrophic thickened nails, and thickening of the skin on the palms or plantar surfaces, 2) the disease followed a pattern of autosomal dominant inheritance, and 3) hypohidrosis or anhidrosis was absent.

Informed consent to participate in these studies was obtained from all participants. Fifteen milliliters of whole blood was obtained, and DNA was isolated by standard procedures (Miller *et al*, 1988). DNA was collected from a total of 35 individuals in three unrelated kindreds with a total of 18 affected individuals. The CS1 family is of Scottish and Irish descent (see **Fig 2** for pedigrees, maximum possible lod = 3.61, $\theta = 0$). The CS2 (max possible lod = 1.20) and CS3 (max possible lod = 1.51) families are of French-Canadian descent and share a surname, though they are not known be related.

For linkage analysis, polymorphic markers linked to the keratin gene clusters and adjacent regions on chromosomes 12 and 17 and those linked to chromosomes 1q21, 17q23-qter, 18q21, and 20q12 were studied. All markers are polymerase chain reaction (PCR)-based short tandem repeat, microsatellite markers (Gyapay *et al*, 1994). Selected markers had an average heterozygosity of 76%.

PCR was performed in a total volume of 7 μl with 14 ng of genomic DNA, 3.5 pmol of each primer, 1.5 mM $MgCl_2$, 200 μM dNTPs, 50 mM

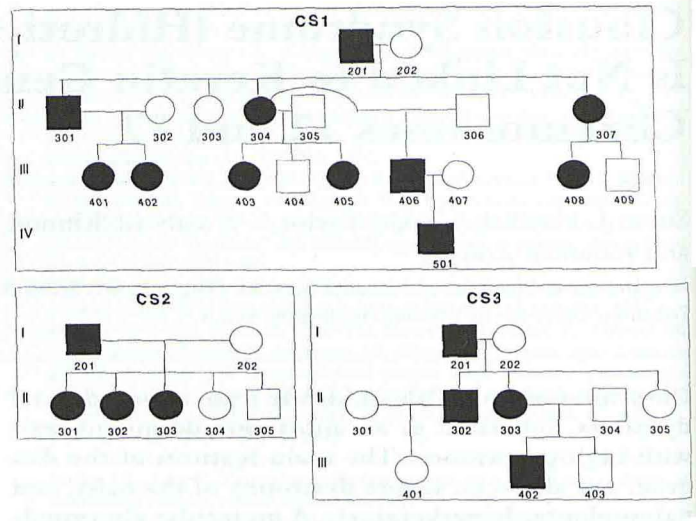

Figure 2. Pedigrees of the families studied.

KCl, 10 mM Tris-Cl, pH 8.5, and 0.175 U of *Taq* polymerase (Amplitaq). In a typical experiment, two-temperature "touchdown" PCR amplification was performed in a Perkin-Elmer/Cetus System 9600 thermocycler, using an initial annealing temperature of 65°C, which was decreased by one degree in each of the first 15 cycles, then maintained at 50°C for 20 more cycles. Each cycle consisted of a 15-s, 94°C denaturing step and a 30-s annealing/extension step. This was followed by a final 5-min extension step at 72°C, and the temperature was then reduced to 15°C until the reaction tubes were removed from the apparatus.

After PCR amplification, products were resolved on DNA sequencing gels containing 5.6 M urea and 32% formamide, transferred to nylon membranes by capillary blotting and revealed by probing the membranes with 5′-[^{32}P]-labeled $(CA)_{15}$ as previously described (Litt *et al*, 1993).

Linkage analysis was performed using the MLINK and LINKMAP options of the LINKAGE package (Lathrop *et al*, 1984; Terwilliger and Ott, 1994). Autosomal dominant inheritance with 95% penetrance was assumed using a population gene frequency of 10^{-5}. Because reliable population allele frequencies for most of the markers do not exist, they were assumed to be equal for lod score calculations. Genetic distances between the markers were based on published information (National Institutes of Health/CEPH Collaborative Mapping Group, 1992; Gyapay *et al*, 1994) or were calculated using genotypes in the CEPH database v7.0. These genetic distances agreed with those calculated using genotypes of our families. When pairwise analysis was uninformative, multipoint analysis was performed. Because of the possibility of locus heterogeneity, linkage analysis was performed independently on each family.

RESULTS

Table I shows combined lod scores obtained in the three families using the designated markers. Individual family data gave no evidence for linkage to the regions studied. These data exclude close linkage to markers in the regions spanning the keratin gene clusters and additional candidate regions on chromosomes 1q21, 17q23-qter, 18q21, and 20q12. Using the criterion that a lod score ≤ -2 excludes linkage together with the published linkage maps of the regions (Gyapay *et al*, 1994; Yoon *et al*, 1994), we can exclude each of the regions studied.

DISCUSSION

Based on biochemical and pathologic studies, keratin defects have been implicated in the etiology of Clouston syndrome. Therefore the genes encoding keratin proteins are candidate genes in this disease. Additional candidates include genes for other disorders of hyperkeratosis, with which Clouston syndrome might be allelic, and regions to which genes that encode keratin-associated proteins have been mapped. The objective of our study was to investigate co-segregation of Clouston syndrome with highly polymorphic markers tightly linked to these regions. Linkage was excluded to the keratin gene clusters on chromosomes 12 and 17 in three

Table 1. Summed Lod Scores for the CS Families at Markers Near the Keratin Genes and Other Candidate Loci[a]

cM	Marker	Lod score at theta = 0.00	0.01	0.05	0.10	0.20	0.30	0.40	Candidate Genes
1	D1S534	−6.69	−2.45	−1.26	−0.63	−0.05	0.05	0.15	Trichohyalin, profilaggrin, involucrin, loricrin
4	D1S442	−12.34	−4.45	−2.35	−1.27	−0.27	0.11	0.17	
	D1S305	−18.83	−6.94	−3.73	−2.30	−1.03	−0.48	−0.20	
<1	D12S368	−17.23	−6.16	−2.97	−1.57	−0.38	0.04	0.11	- KRT7
2	D12S262	−8.71	−1.15	0.23	0.70	0.88	0.67	0.32	- KRT1, KRT2, KRT5, KRT6a, KRT6b
3	D12S103	−13.88	−5.04	−2.66	−1.59	−0.64	−0.23	−0.05	- KRT4, KRT8, KRT18
	D12S90	−11.28	−2.79	−0.99	−0.19	0.39	0.45	0.29	
6	D17S783	−6.56	−2.60	−1.56	−1.01	−0.47	−0.21	−0.08	
	D17S798	−23.07	−8.04	−4.13	−2.38	−0.80	−0.12	0.11	
9	D17S250	−6.54	−2.23	−1.34	−0.90	−0.45	−0.22	−0.08	
	D17S800	−11.34	−3.71	−1.56	−0.70	−0.02	0.18	0.17	
3	KT10	−12.61	−6.06	−3.10	−1.85	−0.72	−0.22	−0.01	- KT10
	KRT9	−21.37	−8.69	−4.68	−2.83	−1.09	−0.30	0.02	- KRT9
3	D17S791	−29.29	−9.02	−4.88	−3.00	−1.25	−0.43	−0.06	
	D17S579	−23.86	−10.90	−5.77	−3.56	−1.51	−0.55	−0.10	
8	D17S806	−31.51	−10.61	−5.49	−3.30	−1.31	−0.41	−0.02	
8	D17S787	−15.74	−7.60	−4.30	−2.75	−1.24	−0.50	−0.12	
9	D17S808	−15.08	−2.15	−0.11	0.62	1.00	0.88	0.51	
4	D17S795	−18.11	−4.66	−2.03	−1.04	−0.27	−0.02	0.03	
11	D17S949	−21.78	−4.99	−1.86	−0.59	0.35	0.54	0.38	
2	D17S785	−11.81	−1.90	−0.47	0.07	0.39	0.35	0.19	- TOCG
11	D17S937	−6.79	−1.59	−0.72	−0.32	0.00	0.09	0.08	
	D17S784	−6.62	−6.41	−4.71	−3.15	−1.60	−0.80	−0.32	
2	D18S56	−12.27	−6.05	−3.15	−1.91	−0.78	−0.26	−0.03	
2	DD18S457	−5.32	−2.53	−1.20	−0.69	−0.27	−0.09	−0.01	- Striate PPK
2	D18S456	−17.93	−9.43	−6.10	−4.07	−2.08	−1.01	−0.37	
	D18S57	−19.72	−9.91	−5.54	−3.57	−1.69	−0.74	−0.23	
12	D20S112	−5.87	−1.46	−0.50	−0.07	0.23	0.23	0.13	
9	D20S106	−18.75	−8.02	−4.18	−2.53	−1.07	−0.44	−0.14	- TGM3
	D20S107	−19.27	−6.04	−2.90	−1.55	−0.43	0.00	0.09	

[a] Genetic distances between adjacent markers are listed under "cM" (centiMorgans).

families. Furthermore, no evidence for linkage was found to markers near genes implicated in two other disorders of hyperkeratosis, striate palmoplantar keratoderma and keratoderma with tylosis and esophageal cancer, or in regions to which genes for keratin-associated proteins localize.

Clouston's original family with hidrotic ectodermal dysplasia was French-Canadian, as are the CS2 and CS3 families, though none of these families is known to be related to the others. The third family we studied is of Scottish and Irish descent. Therefore, we have excluded the keratin gene clusters in families from two distinct ethnic groups. As in all genetic disorders, the possibility of nonallelic genetic heterogeneity must be considered in Clouston syndrome.

Several additional keratin-associated matrix proteins have been described, and the genes for these were mapped (Fratini *et al*, 1993; Rogers and Powell, 1993; Chou *et al*, 1994). Though the original hypothesis of a keratin gene mutation causing Clouston syndrome is not supported by our data, other candidate genes await investigation.

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