Linkage of the Epidermolytic Hyperkeratosis Phenotype and the Region of the Type II Keratin Gene Cluster on Chromosome 12

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Bullous congenital ichthyosiform erythroderma (epidermolytic hyperkeratosis) is a severe, generalized, lifelong disease of the skin. As in epidermolysis bullosa simplex, intraepidermal blisters and clumping of keratin intermediate filaments are characteristic. We report here linkage of the inheritance of this disease to the region of chromosome 12q containing the genes encoding type II keratins. This suggests that keratin gene mutations may underlie this complex hyperproliferative and hyperkeratotic phenotype. *J Invest Dermatol 99:524–527, 1992*

Epidermolytic hyperkeratosis is a rare hereditary disease characterized clinically by blisters, severe scaling, and redness of the skin—hence the clinically descriptive term bullous congenital ichthyosiform erythroderma (BCIE) (McKusick #11380). Histologically it is characterized at the light microscopic level by vacuolization of suprabasal cells and massive thickening of the stratum corneum—hence the term "epidermolytic hyperkeratosis" (EHK) [1,2]. The generalized redness and blistering usually are manifest at birth. The hyperkeratosis, which is the most troublesome feature throughout life, begins later; and the variation in the height of the scale along normal skin markings produces a heaped-up, ridgelike appearance, particularly in the bends of the elbows and knees, that has called forth the descriptive term "porcupine man" [3–5]. The rate of new cell formation is abnormally high, and keratinocytes traverse the living epidermis from the basal layer to the stratum corneum in as little as 4 d, a journey that takes 2 weeks in normal skin [1,6].

BCIE is inherited as an autosomal dominant trait, is fully penetrant, and affects one in several hundred thousand persons. Occasionally the same histologic findings are limited to more discrete areas, e.g., only the palms and soles [7]. Several kindreds have been reported in which the first affected, presumably a mosaic for the new mutation, had linear or patchy lesions and produced children with generalized BCIE [8–10].

The characteristic epidermal abnormality detected by electron microscopy in BCIE is clumping of the keratin intermediate filaments in the suprabasal keratinocytes [11,12]. Such clumping is "almost identical" to the aggregation of keratin intermediate filaments (IF) that occurs in the epidermal basal cell of patients with the Dowling-Meara form of epidermolysis bullosa simplex (EBS) [11]. In both diseases, the IF aggregates contain the keratins normally present in those cells—keratins 5 and 14 in the basal cells of Dowling-Meara EBS and keratins 1 and 10 in the suprabasal cells of BCIE [13,14].

During the past year, we and others have reported evidence that the inheritance of epidermolysis bullosa simplex is linked to genes encoding keratins of the basal cells, and keratin gene point mutations have been identified in patients from four kindreds [15–21]. Because some of the changes in the suprabasal keratinocytes in BCIE resemble those in the basal keratinocytes in EBS, we have used linkage analysis to test whether keratin gene mutations also might underlie BCIE.

MATERIALS AND METHODS

Collection of blood samples after informed consent was obtained; preparation of DNA, and Southern analysis were as described [15]. The DNA probe and enzyme combinations used were as follows: on chromosome 12, WEAV214 and Hinf I (COL2A1) [22], pCMM1,2 and Taq I (D12S15) [23], p9F11 and Taq I (D12S4) [24], pEFD33.2 and Msp I (D12S14) [25], and pYNH15 and Msp I (D12S17) [26]; on chromosome 17, pCMM86 and Hinf I (D17S74) [27]. The microsatellite repeat at the D17S579 locus was analyzed following PCR amplification [28]. Two-point linkage analysis was calculated with LIPED [29], and multipoint linkage was calculated using LINKAGE [30] and published genetic map distances (male and female recombination averaged) [31].

RESULTS

We studied three families with generalized BCIE. In all affected individuals, onset of disease was very early in life, skin abnormalities (hyperkeratosis and blistering) persisted throughout life, microscopic changes in the skin were typical of EHK, and segregation was compatible with a fully penetrant, autosomal dominant trait in all three kindreds (Fig 1). DNA samples from all available members were genotyped at polymorphic loci on chromosomes 12q and 17q, sites to which keratin genes, including the genes encoding keratins 1 and 10 respectively, have been mapped [32–39].

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Abbreviations:

BCIE: bullous congenital ichthyosiform erythroderma
EBS: epidermolysis bullosa simplex
EHK: epidermolytic hyperkeratosis
IF: intermediate filaments

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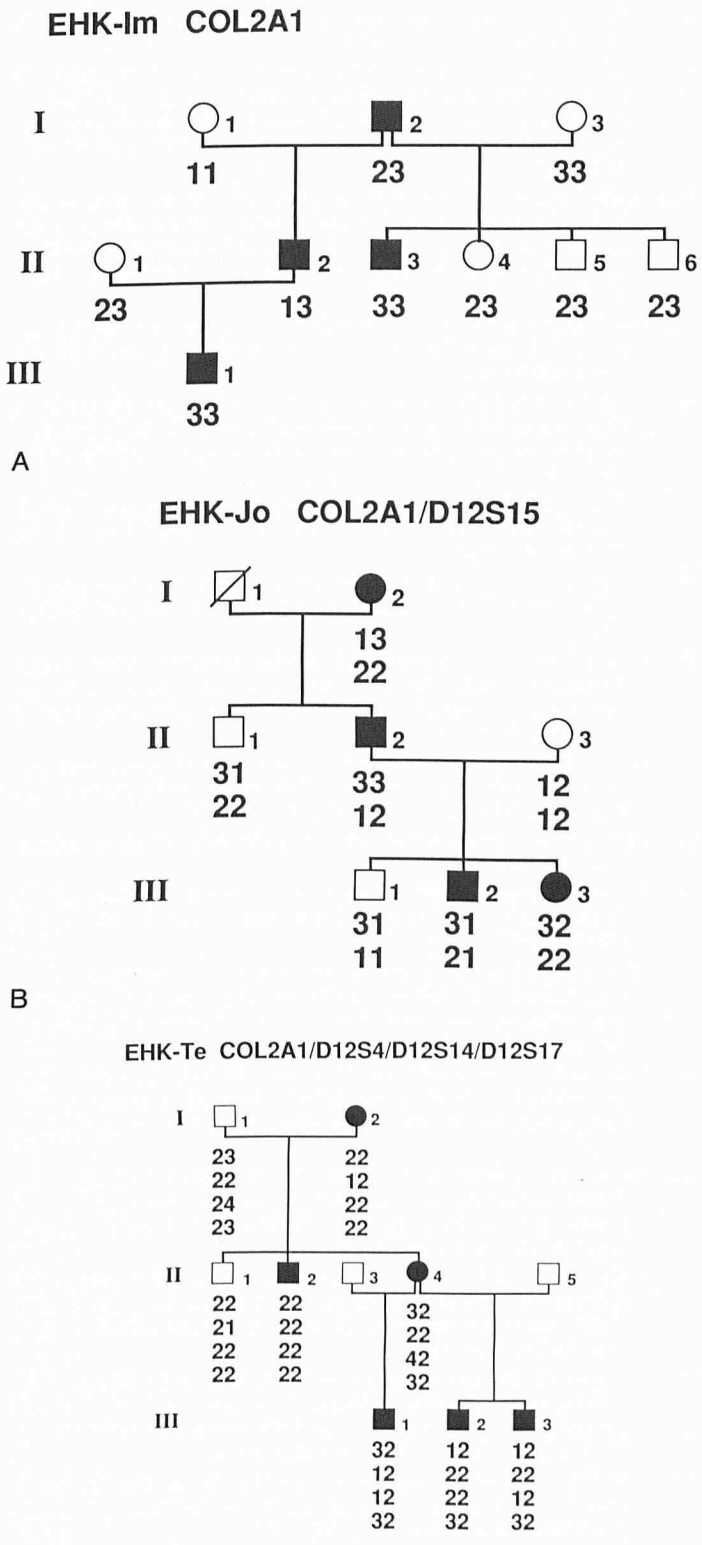

Figure 1. Pedigree of EHK-Im *(A)*, EHK-Jo *(B)*, and EHK-Te *(C)* families. *Solid symbols,* individuals clinically affected with generalized bullous congenital ichthyosiform erythroderma. Results of analysis of chromosome 12 polymorphic loci that were useful for analysis in each family are shown; results that did not increase further the informativeness of the meioses are omitted. In all instances, results at the less informative alleles were compatible with those shown. In *A*, alleles of COL2A1 are indicated. In *B*, alleles of COL2A1 *(upper)* and D12S15 *(lower)* are indicated. In *C*, alleles of *(top to bottom)* COL2A1, D12S4, D12S14, and D12S17 are indicated. The genetic span of these five loci is estimated at 4cM [31].

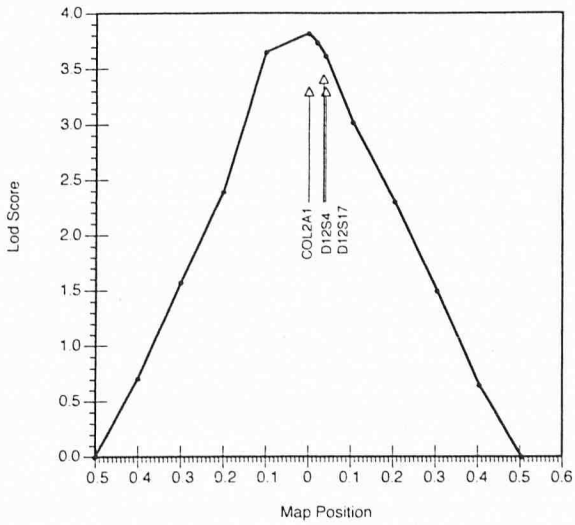

Figure 2. Multipoint analysis of linkage between BCIE and the region of chromosome 12q around the type II keratin gene cluster. The map position of COL2A1 is taken as 0, and genetic distances from that locus are indicated (percent recombination is assumed to be equivalent to cM distance; no correction for interference has been made).

Inheritance of the disease in all three kindreds was linked to the region of the type II keratin gene cluster on chromosome 12q with no recombinants with a combined lod score of 3.82 (Fig 2). Analysis of polymorphic loci in the region of the type I keratin gene cluster on chromosome 17q provided evidence against linkage in all three families (Table I).

DISCUSSION

The odds favoring linkage of this disease to chromosome 12q (lod score = 3.82) are nearly tenfold higher than the usual threshold for significant linkage (i.e., 3.00). Thus it is highly likely that the gene(s) whose mutations underlie BCIE in these three families is located on chromosome 12q. It should be stressed that the genes encoding the retinoic acid receptor γ and, no doubt, other epidermally expressed genes, also map to this region of chromosome 12q [40]. However, taken together with the report of keratin 1 in the intermediate filament aggregates in suprabasal keratinocytes of patients with BCIE, our findings suggest that mutations of the keratin 1 gene are likely to underlie BCIE in these three families.

By analogy with findings in EBS, in which mutations of the genes encoding either member of the heterodipolymer can cause the disease, it seems likely that mutations of the gene encoding keratin 10, the keratin 1 "partner," might underlie BCIE in families other than

Table I. Pairwise Lod Scores Between EHK and Chromosome 17q Loci[a]

	Lod Score at θ of			
	0.001	0.01	0.05	0.10
Im				
D17S74	−4.50	−2.51	−1.19	−0.68
D17S579	−7.50	−4.50	−2.44	−1.59
Jo				
D17S74	−1.80	−0.81	−0.16	−0.06
Te				
D17S74	−7.50	−4.50	−2.46	−1.63
D17S579	−4.62	−2.78	−1.44	−0.97

[a] Paternal and maternal segregation are combined, and autosomal dominant inheritance with full penetrance and no sporadic cases have been assumed.

the three reported here. Furthermore, the gene encoding keratin 9, a type I keratin whose expression is limited to the suprabasal keratinocytes of the palms and soles, would seem a good candidate for the site of mutations underlying those cases of EHK limited to these sites—the Vörner type of hereditary palmoplantar keratoderma [7,41].

Experimental identification of the functions of cytoskeletal intermediate filaments has been very difficult [21,42]. The finding that dominant negative keratin gene mutations cause EBS has established the importance of intermediate filaments in ensuring the mechanical stability of the epidermis. The abnormalities in BCIE—hyperproliferation, vasodilation, and massive hyperkeratosis (far greater than the palmar stratum corneum thickening seen in some patients with Dowling-Meara EBS [43])—suggest, if mutations of keratin genes indeed do underlie BCIE, that IF may subserve yet other functions.

At present, the only technology available for prenatal diagnosis of this devastating disease is fetal skin biopsy [11,12,44]. Irrespective of the exact nature of the mutation, localization of the disease gene will allow DNA-based prenatal diagnosis to supplement or, depending on the family, to replace that invasive technology.

Note Added in Proof: Subsequent to submission of this manuscript, data favoring linkage of one large family with EHK to chromosome 12q has been published (Compton JG, DiGiovanna JJ, Santucci SK, Kearns RS, Amos CI, Abangan DL, Korge BP, MacBride OW, Steinert PM, Bale SJ: Linkage of epidermolytic hyperkeratosis to the type II keratin gene cluster on chromosome 12q. Nature Genet 1:301–305, 1992).

Also published has been a report of development of a transgenic mouse carrying a mutant keratin 10 with EHK-like skin abnormalities (Fuchs E, Coulombe PA: Of mice and men: genetic skin diseases of keratin. Cell 69:899–902, 1992).

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