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We hope that making available the relevant information on Pachyonychia Congenita will be a means of furthering research to find effective therapies and a cure for PC.
Genetic Linkage of the Keratin Type II Gene Cluster with Ichthyosis Bullosa of Siemens and with Autosomal Dominant Ichthyosis Exfoliativa

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Ichthyosis bullosa of Siemens is an autosomal dominant disease characterized by mild hyperkeratosis and blistering. Autosomal dominant ichthyosis exfoliativa is a recently described disease with clinical features similar to ichthyosis bullosa of Siemens, but in contrast to ichthyosis bullosa of Siemens no histologic signs typical for epidermolytic hyperkeratosis are observed. We used linkage analysis to test whether keratin gene mutations might underlie both diseases. This analysis showed linkage of both disorders with the region of chromosome 12 in which the keratin type II gene cluster is located. The keratin type I gene cluster on chromosome 17 is excluded. These data, combined with clinical observations, strongly suggest that the genes coding for keratin 1 or keratin 2e, both expressed in the suprabasal compartment of the epidermis and located in the type II gene cluster, are candidate genes for ichthyosis bullosa of Siemens and ichthyosis exfoliativa. J Invest Dermatol 103:282–285, 1994

Epidermolytic hyperkeratosis is a histopathologic characteristic of a variety of monogenic keratinization disorders comprising bullous congenital ichthyosiform erythroderma of Brocq (BCIE), epidermolytic palmo-plantar keratoderm of Vörner (EPPK), ichthyosis hystrix of Curth-Macklin (IHCM), and ichthyosis bullosa of Siemens (IBS). The term epidermolytic hyperkeratosis is also used to refer to the diseases.

For two of these disorders there is convincing evidence for mutations in keratin genes. In patients with BCIE, mutations have been found in the genes coding for keratin 1 (K1) or K10 [1–4] and in case of EPPK in the gene coding for K9 [5,6]. On the other hand, IHCM seems not to be due to a defect in a keratin, because the loci of both the type 1 and type II keratin gene cluster (17q12–q21 and 12q11–13, respectively) have been genetically excluded [7]. So far, there have been no clues about the defect causing IBS.

IBS has been described as a bullous form of ichthyosis distinct from BCIE [8–10], and in IBS blistering and hyperkeratosis are milder. IBS can be further differentiated from BCIE by the absence of congenital erythroderma. Light- and electron-microscopic examination show the features of epidermolytic hyperkeratosis consisting of coarse keratohyaline granules, intracellular vacuolization, and perinuclear clumping of tonofilaments. In IBS these features are confined to the stratum granulosum and the upper part of the stratum spinosum, whereas in BCIE these findings are present in the whole suprabasal compartment. It has been hypothesized that BCIE and IBS are allelic disorders [11]. Autosomal dominant ichthyosis exfoliativa (IE) is a recently described type of bullous ichthyosis described in one family with clinical symptoms very similar to IBS, but the histologic features of epidermolytic hyperkeratosis were absent [12]. Both IBS and IE show autosomal dominant inheritance.

We addressed whether IBS and IE are due to keratin gene defects. Linkage analysis was performed in the kindred with IBS that has been described previously [8] and in the kindred with IE, described by Vakilzadeh and Kolde [12], employing polymorphic markers from (the close vicinity of) the type I and II keratin cluster. In both families, co-segregation of the disease with the type II keratin gene cluster was observed.

MATERIALS AND METHODS

Patients Family 1 has been reported as suffering from ichthyosis bullosa of Siemens [8]. The affected individuals had brownish rimmed hyperkeratosis and superficial blistering since early childhood. Blistering was more pronounced during hot and humid weather and could be provoked by mild trauma. Erythroderma had never been present in any of the affected individuals. Skin lesions were localized especially on the extensor surfaces of arms and legs and around the umbilicus, knees, and ankles. In the hyperkeratotic regions superficially denuded areas were present. Occasionally, fresh blisters ranging in size from 0.5 to 2 cm appeared. Light-microscopic examination of multiple biopsies of hyperkeratotic areas showed an acanthotic epidermis with orthohyperkeratosis. In the stratum granulosum and the upper part of the stratum spinosum coarse keratohyaline granules, intracellular vacuolization, and hyperchromatic pyknotic nuclei were present (Fig 1a). On ultrastructural examination keratinocytes in the upper spinous layer displayed aggregates of tonofilaments forming V shapes or shells around the nucleus.

Family 2 has been reported as suffering from ichthyosis exfoliativa [12]. The patients showed dark grey hyperkeratotic lesions with denuded areas. Superficial blistering occurred spontaneously especially during the summer but occurred also after trivial trauma. There was no history of erythroderma in any of the patients. Histologic examination of multiple biopsies of hyper-
keratotic areas showed acanthosis and orthohyperkeratosis of the epidermis (Fig 1b). The keratinocytes in the granular and upper spinous layer showed marked edema with indistinct boundaries, the nuclei of the cells were small and hyperchromatic, and the cells contained a few small keratohyaline granules. The electron microscopic findings have been described previously: “the cells of the granular and spinous layer were characterized by marked intra-

cellular edema; the number of tonofilaments and keratohyaline granules were markedly reduced with no grouping of the tonofilaments” [12].

Linkage Analysis DNA of patients was isolated from peripheral blood according to the method of Miller et al [13]. For the type II keratin cluster we tested polymorphisms in the genes coding for K1 (KRT1) [14], K4 (KRT4) [15], D12S96, and collagen type IIA1 (Col2A1) [16]. A map of the relevant region is given in Fig 2 (GDB [17]). For K1 the polymorphic glycine-rich carboxyl-terminal domain was amplified with the forward (5'-GGTTTCCTGGCGGCTGCCTAC-3') and reverse (5'-AGAGAGCTTGCGTCCTCCGC-3') primers. Two hundred nanograms of genomic DNA were amplified in a 15-μL polymerase chain reaction mix with 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.005 mM dithiothreitol, 0.001% (w/v) gelatin, 1.5 μCi α²PdCTP, 250 μM of each dATP, dGTP, dTTP, 3.0 μM CTP, 75 ng of each of the primers, 10% (v/v) glycerol, and 3% (v/v) formamide. After an initial denaturation of 5 min at 94°C, 35 cycles of 1 min 94°C, 2 min 67°C, and 1.5 min 72°C were performed. The K4 polymorphism was analyzed as described by Warner et al [13], the VNTR in the Col2A1 gene as described by Wu et al [16], and the marker D12S96 (GDB) according to [18]. To test linkage with the type I keratin gene cluster, a polymorphism in the gene coding for K10 (KRT10) [19] and the nearby loci D17S250 [20] and D17S579 [21] were analyzed. The order of the markers of chromosome 17q is cen-D17S250-KRT10-D17S579 (Fig 2). The genetic distance between D17S250 and D17S579 is about 6 cM [22]. For amplification of the K10 polymorphism the following primers

Figure 2. Map of the loci surrounding the keratin gene clusters. a) On chromosome 12q; b) on chromosome 17q (GDB [17,22]). Distances are given in cM.

RESULTS AND DISCUSSION To sort out whether IBS and IE are due to mutations in keratin genes, with K1 and K10 as particularly promising candidates, markers located within, or close to, the keratin gene clusters on chromosome 12q (type II keratins) and 17q (type I keratins) are tested (Fig 2). Haplotypes are depicted in Figs 3 and 4, and in Table I the pairwise lod scores are given between the markers and IBS and IE.

In the family with IBS the lod score of 3.24 with Col2A1 clearly shows linkage of this locus and IBS. Both KRT1 and KRT4 are not completely informative in this family. However, construction of haplotypes (Fig 3) gives no indication of recombination between IBS and the region of 12q analyzed with the four markers Col2A1, D12S96, KRT1, and KRT4. These results indicate that IBS is due to a defect in a gene in the analyzed region of chromosome 12q including the keratin genes.

KRT10 shows recombinations with the disease, in persons IV.8 and V.3 (Fig 3), thereby excluding the K10 gene and other keratin genes in the type I cluster as the cause of IBS in the present family. Exclusion of the type I cluster is further substantiated by the lod score of −1.99 at θ = 0.1 with D17S579, which is at a distance between 3 and 4 cM from the keratin cluster [22]. There also is recombination with D17S250 (Z = −1.95 at θ = 0.03), which is located less than 3 cM proximal to KRT10 [22]. In a second small family with IBS, results comparable to those in the presented family have been obtained, but this family is too small to show statistically significant linkage. Combination of lod scores for both families with IBS resulted in the maximum lod score of 4.20 at θ = 0.0 with the marker Col2A1.

In the family with IE, the lod score of 3.60 with Col2A1 at θ = 0.0 indicates linkage between IE and the Col2A1 gene. At θ = 0.2 the lod score is 2.28, suggesting linkage also with the keratin genes, which are at a distance of about 15 cM from the Col2A1.
gene (GBD [17]). The three markers closer to or in the keratin gene cluster on chromosome 12 have a very limited informativity (Table I, Fig 4). There is no recombination between IE and these markers. The highest lod score of 1.48 was obtained with D12S96, which is located about 5 cM proximal to the keratin gene cluster. As is shown for IBS, IE is indicated to be due to a defect in a gene in the region of the Col2A1 gene. Also for IE the defect may be in a keratin gene. The markers D17S250 and D17S579, flanking KRT10, both recombine with IE in persons III.5 and V.1 (Fig 4). Accordingly, haplotype analysis reveals that the interval of D17S250-KRT10-D17S579 is excluded from linkage with IE, which is reflected in a maximal lod score of -4.88 for this interval obtained by multipoint analysis. The positive lod score obtained with KRT10 is due to the fact that the recombinations in III.5 and V.1 are not visible because the affected parent of those people is homozygous for KRT10.

The results from linkage analysis show that among other genes in the region of the Col2A1 gene, members of the type II keratin gene cluster are candidates for both IBS and IE. So far, mutations found in keratin genes were in regions coding for the evolutionary conserved domains of the proteins, i.e., 1) the helix initiation peptide at the end of helix domain 1A, 2) the helix termination peptide at the end of helix 2B, 3) the H1 subdomain of type II keratins, and 4) the L12 linker region ([3,4] and references therein). Sequence analysis gave no indications for mutations in the H1 region, the A1 rod domain, the L12 linker region, or the helix termination peptide of K1 in the present families. However, a mutation outside the highly conserved parts of K1 may be even more likely regarding the mild phenotypes of IBS and IE [2,24,25]. On the other hand, the genetic defect may be present in the K2e gene, which is expressed suprabasally in the epidermis from the third or fourth cell layer onwards [26]. This expression pattern exactly coincides with the occurrence of the lesions in patients with IBS and IE, i.e., in the upper part of the spinous layer and the granular layer. Mutation analysis in the K2e gene in patients of the present families is underway.

We thank Dr. D. Mischke for making its KRT4 marker available before publication and Prof. B. Lane and Dr. M. McLean for discussion. For practical assistance we thank E.M.A. Boender-van Rossum and S.D. van der Velde-Visser.

REFERENCES

Table I. Pairwise lod Scores Between IBS, IE, and Loci Within or Close to the Keratin Gene Clusters

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<td>∞</td>
<td>0.98</td>
</tr>
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</table>

* The first three columns give the lod scores calculated for the family with IBS, the last three columns those for the family with IE. Statistically significant lod scores are obtained in both families with the marker Col2A1. The region surrounding the keratin type 1 cluster on 17q is excluded.

ANNOUNCEMENT

The annual meeting of the British Society for Investigative Dermatology will be held on 6–7 April 1995 at the University of Oxford. Abstracts are invited for oral and poster presentations at this meeting and awards will be made for the best presentations. The meeting will include overseas guest speakers: Professor Nishikawa talking about “Mechanisms in pemphigus” and Dr. George Guidice, talking on “Mechanisms of blistering.” Additionally a local expert will talk on “The role of super antigens in skin diseases” and abstracts on this topic and the blistering diseases are particularly invited. Young Investigators may apply for bursaries to attend assistance at this meeting provided they are co-presenters. There are two BSID Young Investigator Awards, one for the UK and one for Eastern Europe and the developing world. Details of how to apply for bursaries and fellowships, and abstract forms, are available from Dr. Graham Sharpe at the University of Liverpool, Department of Dermatology, P.O. Box 147, Liverpool L69 3BX, Telephone: 051 706 4033, Fax: 051 706 5842. The deadline for receipt of abstracts is 18 November 1994.
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