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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.
Missense Mutations in Keratin 17 Cause Either Pachyonychia Congenita Type 2 or a Phenotype Resembling Steatocystoma Multiplex

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Pachyonychia congenita (PC) is a group of autosomal dominant ectodermal dysplasias in which the main phenotypic characteristic is hypertrophic nail dystrophy. In the Jackson-Lawler form (PC-2), pachyonychia is accompanied by multiple pilosebaceous cysts, natal teeth, and hair abnormalities. By direct sequencing of genomic PCR products, we report heterozygous K17 missense mutations in the same conserved protein motif in a further five PC-2 families (K17 N225S in one familial and three sporadic cases; K17 Y292D in one familial case) confirming that mutations in this gene are a common cause of PC-2. We also show heterozygous missense mutations in K17 (N225H and R292H) in two families diagnosed as steatocystoma multiplex. Mild nail defects were observed in some but not all of these patients on clinical re-evaluation of these families. All the K17 mutations reported here were shown to co-segregate with the disease in the pedigrees analyzed and were excluded from 100 unaffected, unrelated chromosomes by restriction enzyme analysis of K17 genomic PCR products. We conclude that phenotypic variation is observed with K17 mutations, as is the case with other keratin disorders. Key words: genodermatoses/K17/nail dystrophy/PC-2. J Invest Dermatol 108:220–223, 1997

Keratins are major structural components of epithelial tissues. There are currently 11 keratin genes in which associated human inherited epithelial fragility disorders are known (Fuchs and Weber, 1994; McLean and Lane, 1995; Richard et al., 1995; Rugg et al., 1995). Pachyonychia congenita (PC) is a subset of autosomal dominant ectodermal dysplasias associated with hypertrophic nail dystrophy. In the PC-2 form, pachyonychia is accompanied by multiple epidermal cysts and mild focal non-epidermolytic palmoplantar keratoderma (FNNEPPK); oral mucosal lesions are rare or absent (Jackson and Lawler, 1951). Histologically, the nature of the cysts has caused confusion, as they may appear to be either keratinizing, eruptive vellus hair cysts or oil-filled steatocysts with sebaceous glands in the walls. Recently, PC-2 was reported to be linked to markers that map within the type I keratin cluster on 17q in a large Scottish kindred (Munro et al., 1994), and subsequently the causative mutation segregating in the kindred was found to be N225D in the K17 gene (McLean et al., 1995). Steatocystoma multiplex is an autosomal dominant disorder in which the main phenotype is the presence of multiple cysts. Two forms of the disease are recognized by McKusick (MIM 184510; MIM 184500) and differ by the presence or absence of natal teeth, respectively. Clinically and histologically, as the name implies, the lesions are steatocysts, but a series of recent reports have suggested an overlap with multiple vellus hair cysts, as with PC-2 (Requena and Yuz, 1991; Ohtake et al., 1992). Nail changes, which do not resemble PC, have been noted in a few steatocystoma multiplex pedigrees (Bushnell and Gorlin, 1975). Here, we describe K17 mutations in five kindreds affected by PC-2. We also report K17 mutations in two families diagnosed as steatocystoma multiplex with mild nail changes.

MATERIALS AND METHODS

Mutation Detection and Restriction Analysis. A genomic DNA fragment spanning exon 1 of the KRT17A gene was amplified by polymerase chain reaction (PCR) and directly sequenced with forward and reverse internal primers as described previously (McLean et al., 1995). This PCR is specific for the functional K17 gene (KRT17A). The mutation K17 N225S creates a new Ddel site, which was used to confirm the mutation in the affected individuals and also exclude the mutation from 50 normal unrelated individuals. PCR was performed using K17P8 and K17P10 (McLean et al., 1995) and digested with Ddel without further purification; digests were analyzed on 1.5% agarose/Trit(hydroxyethyl)methylaminoethanol-borate-eth-

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Abbreviations: FNNEPPK, focal non-epidermolytic palmoplantar keratoderma; PC, pachyonychia congenita; PC-1, Jadassohn-Llewandowsky PC; PC-2, Jackson-Lawler PC.
Figure 1. Pedigrees of families studied. Affected individuals in families PS, MJ, JB, KR, and DM present with the classic features of PC-2 (Jackson and Lawler, 1951). The probands in steatocystoma multiplex families KE and ND had multiple epidermal cysts, but nail changes were very mild or absent.

RESULTS

Case Reports. Pedigrees examined in this study are shown in Fig 1. Two familial cases and three sporadic cases of PC-2 were analyzed, and two familial cases were diagnosed as steatocystoma multiplex. Figure 2a shows the characteristic hypertrophic dystrophy of the fingernails in the proband of PC-2 family MJ. Similar nail changes were observed in the affected individuals from the other PC-2 kindreds (PS, JB, KR, and DM). FNEPPK and multiple epidermal cysts were observed in the PC-2 affected individuals. Pili torti or protruberent eyebrows and natal teeth were found in all affected members of these families. In contrast, the fingernails of individual I in steatocystoma multiplex family KE showed no hyperkeratosis but a few splinter hemorrhage-like lesions (Fig 2b). She had been troubled by hypertrophic toenails, which had been removed. Her daughter, II, had cysts and normal fingernails but slight distal subungual hyperkeratosis of the great toenails. This individual also had protruberent eyebrows. While other affected family members had reported liver cysts, there was no family history of nail changes, keratodema, or natal teeth. Examination of individuals II, II, and III from steatocystoma multiplex family ND (Fig 1) showed a severe phenotype consisting of myriads of cysts in the groin, perineum, axillae, trunk, and face. The back of the proband (III1) is shown in Fig 2c. Histologic examination of tissues from the proband showed sebocytes within an epithelial wall characterized by slight epidermolytic hyperkeratosis (not shown). Nail changes were completely absent in the proband; however, individual III1 had slight thickening of the thumb nails and III4 had thinned fingernails but normal toenails. There was no family history of dental teeth, and some members had mild FNEPPK. In neither steatocystoma multiplex family had nail changes been noticed by the original dermatologists until they were specifically sought. Cysts in PC-2 and steatocystoma multiplex families were indistinguishable clinically and histologically.

Mutations in the Rod Domain of K17 in Five PC-2 Kindreds. There are three K17 genes in the human genome, designated KRT17A, KRT17B, and KRT17C. The former is the functional gene encoding the K17 protein, and the latter two genes are highly homologous intron-containing pseudogenes (φ denotes a pseudogene) (Troyanovsky et al., 1992). A PCR was devised to amplify only the functional gene (McLean et al., 1995). Direct sequencing of specific KRT17A PCR products revealed heterogeneous missense mutations in the helix initiation motif of K17 in affected members of all families reported here. Affected members of family PS were found to be heterozygous for the purine transition A<A>G in K17, producing the predicted amino acid change N25S. An identical mutation was found in the three sporadic cases analyzed from families MJ, JB, and DM. This mutation creates a new Del6 site, and this was used to confirm the mutation in affected individuals and exclude the presence of the mutation in unaffected family members as well as 50 normal, unrelated individuals (Fig 3). In the sporadic cases, no evidence of non-paternity was observed when the families were genotyped with several microsatellite probes, confirming that these are de novo mutations (not shown). In family KR, a heterogeneous transition T>440G producing the amino acid change Y98D in K17 was found in affected individuals. This mutation does not alter any known restriction site, but a new forward primer (K17KR) was made containing a 2-bp mismatch, which, in combination with the mutation, creates a Sac1 site. Digestion of these PCR products derived from affected individuals revealed an additional mutant band that was not present in the unaffected member of family KR or 50 normal, unrelated individuals (Fig 3).

K17 Mutations in Two Steatocystoma Multiplex Families. K17 sequence analysis was also performed on the steatocystoma families KE and ND. In family KE, affected individuals were found...
to be heterozygous for the transversion A422C, which is predicted to produce the amino acid change N92H. Again, this mutation does not alter a restriction site, and in this case a mismatch primer was designed that creates a BspHI site in combination with the mutation. BspHI digests of this PCR product derived from affected members of family KE yielded an additional mutant band; however, this was not found in unaffected family members or in 50 normal individuals (Fig 3). Two affected members of family ND were found to carry a heterozygous purine transition G429A causing the predicted amino acid change R94H. AciI digestion of K17 PCR products derived from normal individuals cut completely; however, affected individuals were heterozygous for an uncut band due to the mutation (Fig 3). This assay was used to exclude the mutation from 50 normal unrelated individuals. This mutation occurs in the tenth residue of the K17 helix initiation peptide (R10) and is a potential CpG deamination mutation (Cooper and Krawczak, 1993). The equivalent and reciprocal CpG mutations in this codon have been found previously for other diseases of type I keratins (McLean and Lane, 1995; Shamsheer et al, 1995).

**DISCUSSION**

**PC-2 is Caused by K17 Mutation** K17 is a differentiation-specific type I keratin that is expressed in several epithelial structures, notably in nail bed, hair follicles, and sebaceous glands and other tissues (Trojanowsky et al, 1989). Here we describe seven mutations in the highly conserved helix initiation peptide motif of K17 causing two similar but distinct disease phenotypes affecting some or all of these tissues. Mutation analysis in five families with the classic PC-2 phenotype confirms that this disorder is caused by mutations in K17. All cases to date that we have diagnosed as PC-2 on the basis of pachyonychia with epidermal cysts and without oral leukokeratosis have proved to carry K17 mutations. The same point mutation (N92S) was found in four cases, three of which were sporadic. Despite the fact that the N92S mutation was found to have occurred four times in this study and previously in other keratins (McLean and Lane, 1995), this purine transition mutation cannot be explained by any obvious mechanism such as methylated CpG or CpnPG deamination (Cooper and Krawczak, 1993; Clark et al, 1995). The affected asparagine residue is highly conserved and is presumed to be critical in the function of the helix initiation peptide in filament assembly (McLean and Lane, 1995). Similarly, the mutation Y94D found in one familial case of PC-2 is a nonconservative amino acid change in the highly conserved 1A domain and therefore is predicted to disrupt keratin function.

**K17 Mutant Phenotype Can Resemble Steatoctystoma Multiplex** We also report mutations in patients diagnosed with steatoctystoma multiplex (N92H; R94H), also in the 1A domain of K17. It appears that the disorder segregating in these families is really a variant of PC-2. Both families have individuals with mild nail changes, which may distinguish these PC-2 variants from other steatoctystoma patients. Phenotypic variation is common in other keratin disorders; for example, PC-1 and a phenotypically milder disease FNEPPK are due to mutations in K16 (McLean et al, 1995; Shamsheer et al, 1995). Steatoctystoma multiplex also occurs without any other disease phenotype (L. M. Leigh and C. S. Munro, unpublished clinical observations), and there are reports of other sporadic dominant conditions that resemble steatoctystoma multiplex or PC-2, such as eruptive vellus hair cysts with (Nogita et al, 1991) or without attached sebaceous glands (Esterly et al, 1977).

We have analyzed exon 1 of K17 in one sporadic case of steatoctystoma multiplex and a sporadic case of eruptive vellus hair cysts and found no sequence changes (data not shown). In both instances the distribution of cysts was widespread rather than nevoid and occurred with no other clinical phenotype. One explanation is that steatoctystoma multiplex is genetically heterogeneous; however, all K17 mutations reported to date occur in the highly conserved 1A domain, whereas in epidermolysis bullosa simplex, mutations in the L12 domain or internal rod domain mutations in K5 or K14 result in milder phenotypes (McLean and
Lane, 1995). Analogous mutations in K17 might lead to milder phenotypes related to PC-2 or steatocystoma multiplex, e.g., steatocystoma multiplex with natal teeth. In view of this, K17 is a good candidate for these and other mechanisms of the gene are being analyzed currently. Linkage and/or mutation analysis in large families with steatocystomas or eruptive vellus hair cysts as the only phenotype is necessary to reveal whether these diseases are due to mutations in K17 or in other genes.

REFERENCES