Keratin 17 mutations cause either steatocystoma multiplex or pachyonychia congenita type 2


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Summary

Pachyonychia congenita type 2 (PC-2; Jackson–Lawler syndrome) is an autosomal dominant disorder characterized by hypertrophic nail dystrophy, mild focal keratoderma, multiple pilosebaceous cysts and other features of ectodermal dysplasia. Keratin 17 (K17) is a differentiation-specific keratin expressed in the nail bed, hair follicle, sebaceous gland and other epidermal appendages. Previously, we have demonstrated that PC-2 is caused by mutations in K17 and that similar mutations in this gene can present as steatocystoma multiplex with little or no nail dystrophy. Here, we describe three unrelated kindreds carrying K17 mutations. Two of these families have identical missense mutations (R94C) in the 1A domain of K17. However, while affected members of one kindred have the classical features of PC-2, affected persons in the other family have the steatocystoma multiplex phenotype. In a third family with PC-2, mutation N92S was detected, bringing the total number of distinct mutations reported in K17 thus far to 11. These results demonstrate that K17 mutations commonly underlie both PC-2 and steatocystoma multiplex and that the alternate phenotypes which arise from these genetic lesions in K17 are independent of the specific mutation involved.

Keratins form the intermediate filament cytoskeleton of epithelial cells. In humans, there are many different forms of epithelial cell, each with a specific pattern of keratin expression according to tissue context and state of differentiation. Specific pairs of type I and type II keratins form obligate heterodimers which undergo additional polymerization steps to produce 10 nm intermediate filaments. The higher order assembly of keratin dimers is poorly understood. However, the helix boundary motifs, short sequences located at the start and end of the central coiled-coil rod domain, have been implicated in molecular overlap interactions in filament assembly by means of their remarkable degree of evolutionary conservation, as determined by in vitro mutagenesis studies and chemical cross-linking analysis. Pathogenic mutations in human keratin disorders most often occur in the helix boundary motifs, further attesting to the structural importance of these sequences.

Pachyonychia congenita (PC) is a group of autosomal dominantly inherited diseases characterized by hypertrophic nail dystrophy accompanied by varying features of ectodermal dysplasia. Many cases of PC are accompanied by variable focal keratodermia and follicular keratosis, but two main clinical syndromes have been distinguished on the basis of other features. Gorlin et al. designated the form with oral leucokeratosis PC type 1 (PC-1), and that with multiple epidermal cysts PC type 2 (PC-2). In PC-2 focal keratodermia may be present in a milder form than in PC-1, but there are more varied ectodermal defects including natal teeth, pili torti, angular chelosis and hoarseness. Recently, the molecular basis for these two forms of PC was found...
to be mutations in keratins which are differentially expressed in epithelial cells of the nail bed and the other ectodermal structures affected in these diseases. PC-1 was found to be caused by heterozygous mutations in keratin 16 (K16) or in its expression partner, keratin 6a (K6a). Similar genetic lesions in keratin 17 (K17) were found to cause PC-2. More recently, mutations in K17 have been demonstrated in families presenting as steatocystoma multiplex. Further examination of the latter kindreds revealed subtle nail changes in some affected individuals. Similarly, K16 mutations have been identified in patients with focal non-epidermolytic palmpoplantar keratoderma, having minor nail changes which do not resemble pachyonychia. By implication, K6a mutations may also be expected to cause this phenotype, although no mutations of this type are known. No type II expression partner of K17 has been reported and to date mutations only in this protein have been described in PC-2 or steatocystoma multiplex families.

Here, we demonstrate mutations in the helix initiation motif of K17 in affected members of three unrelated families. Two of these kindreds have the hallmarks of PC-2 and one family has steatocystoma multiplex as the only phenotype.

Materials and methods

Genomic DNA was extracted from peripheral blood lymphocytes by standard methods. 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. This PCR reaction is specific for the functional K17 gene. This PCR reaction is particularly sensitive to magnesium concentration and was found to perform best with 1 mmol/L MgCl₂; however, magnesium titration is recommended. The K17 mutation N92S creates a new DdeI site which was used to confirm the mutation in the affected individuals and also to exclude the mutation from 50 normal unrelated individuals. PCR was performed using primers K17p8 and K17p10, digested with DdeI without further purification, and the digests were analysed on 1·5% agarose/TBE minigels. Mutation R94C destroys an AciI site. Nested PCR was performed initially with primer K17p8 and K17p10 (see above) and subsequently with primers K17p3 and K17p4. The secondary PCR products were exhaustively digested overnight at 37 °C with an excess of AciI to ensure complete cutting. Digests were analysed on 3·5% NuSieve agarose/TBE minigels. An uncut fragment was observed only in affected members of families 1 and 2 and not in 50 normal unrelated controls.

Results

Case reports

The pedigrees of the three families studied, designated 1, 2 and 3, each with hallmarks of an autosomal dominant mode of inheritance, are shown in Fig. 1.

Family 1. The proband is a 39-year-old American caucasian woman who had a history of subcutaneous yellow nodules on the flexor surfaces of the arms, abdomen and legs since puberty. She also had thickened nails of the feet. Histological examination of a biopsied cyst revealed that this was a steatocyst (data not shown) and a diagnosis of Jackson–Lawler form of PC was made. Her son, aged 12 years, had similar nail dystrophy (Fig. 2a) and was also seen to be developing steatocystoma multiplex (Fig. 2b).

Family 2. The index case is a 41-year-old Dutch caucasian woman who presented at an out-patient clinic because of what she described as ‘acne present from puberty’. The number of lesions had increased with age; they were also present on the abdomen, arms and legs. Of great concern to her was the fact that her daughter of 4 years and son of 11 years of age were developing similar skin problems. On examination, we noted multiple nodules of varying diameter spread over the face, neck, trunk and extremities (Fig. 2c). These were most widespread in the mother (not shown). None of the affected persons showed any nail changes (Fig. 2d), or any other skin, hair or mucosal abnormalities.

Family 3. The proband in family 3 was an 18-year-old British caucasian who presented with rough skin due to follicular keratoses, and thickened nails and plantar skin. Although she did not have clinically obvious

Figure 1. Pedigrees of the pachyonychia congenita type 2 (PC-2) and steatocystoma multiplex families. The arrow indicates the proband in each kindred.
cysts, and had mild hyperkeratosis of the buccal mucosa, other family members had abnormal nails, blistering of feet and multiple cysts. The proband’s mother was examined. She gave a history of painful keratoses and blistering on the feet, with thickened nails, yellowish cysts on the trunk and limbs, and recurrent flexural abscesses. She also had multiple milia. Histological examination of an excised lesion from the labium majus revealed an epidermoid cyst (data not shown). This family has been previously reported as having PC with hidradenitis suppurativa.17

Mutation analysis and confirmation

Exon 1 of the K17 gene was amplified from affected persons and normal unrelated controls using PCR primers and conditions which we have previously shown to be specific for the functional K17 gene (KRT17A) and which do not amplify either of the two K17 pseudogenes (ψKRT17B and ψKRT17C).12,14 Direct automated sequencing of these PCR products revealed heterozygous missense mutations in the 1A domain of K17 in all three families, as shown in Figure 3. Although unrelated and with differing phenotypes, families 1 and 2 had the identical pyrimidine transition mutation 428C→T, predicting amino acid change R94C, at the tenth amino acid of the helix initiation motif. This mutation abolishes a recognition site for the restriction enzyme AcI, which was used to confirm the mutation in the affected individuals of both families (not shown). This test was also used to exclude mutations in this codon from 50 normal unrelated persons, as described previously.14 This is the first report of this particular mutation in K17.

In family 3, the heterozygous purine transition mutation 423A→G was detected in the proband (Fig. 3), predicting amino acid substitution N92S, at the eighth residue of the helix initiation sequence. The same mutation was detected previously in four unrelated PC-2 families.14 This sequence alteration creates a new recognition site for the restriction enzyme DdeI, which was used to confirm the mutation in the proband and exclude it from 50 normal individuals, as shown previously.14
Discussion

Keratin 17 mutations in pachyonychia and steatocystoma multiplex families

By direct sequencing of K17 genomic PCR products, we have identified missense mutations in three unrelated pedigrees, making a total of 11 K17 mutations in PC-2 and steatocystoma multiplex families published to date.\textsuperscript{12,14} Mutation R94C was detected in two unrelated families and is a novel mutation in K17, although an analogous mutation has been reported in other keratins.\textsuperscript{7} This mutation is likely to have arisen via the 5’ methyl-cytosine deamination mechanism,\textsuperscript{18} which accounts for the prevalence of mutations in this codon of type I keratins.\textsuperscript{7,19} Two possible arginine substitutions can result from deamination mutation of this codon, and the other one, R94H, has been reported in K17 previously.\textsuperscript{14} The mutation N92S has been reported previously by this group in a further four PC-2 kindreds.\textsuperscript{14} All three mutations occur in the 1A domain of the K17 polypeptide, within the helix initiation motif, the site where all reported mutations in this gene occur.\textsuperscript{7} This sequence is thought to be involved in end-to-end overlap interactions in the assembly of keratin filaments,\textsuperscript{14} and is the site where most mutations in type I keratins have been found.\textsuperscript{5} Therefore, these mutations are predicted to be detrimental to the keratin cytoskeleton, producing cell fragility and hyperkeratosis in tissues expressing K17. These epithelia include the nail bed, hair follicle and sebaceous gland,\textsuperscript{16,20} explaining the phenotypes of nail dystrophy and steatocystoma multiplex seen in these patients.

The nature of cysts associated with keratin 17 mutation

Although usually referred to as steatocystoma multiplex, the heterogeneous nature of the cysts in PC-2 has caused confusion. Reviewing previous reports of either epidermoid cysts or steatocysts, Clementi et al. suggested that the two types might be genetically distinct forms of PC-2.\textsuperscript{21} However, within a single large pedigree,\textsuperscript{22} and indeed in a single individual, we have critically examined multiple cysts and found some to be true steatocysts and others keratinous cysts, some of which contained vellus hair (Munro CS et al., unpublished data). Milia, flexural abscesses identical to hidradenitis suppurativa\textsuperscript{17} and scrotal and vulvar cystomatosis also form part of the syndrome. Eruptive vellus hair cysts and steatocystoma multiplex may be variants of a single entity,\textsuperscript{23} and the combination has also been reported with PC.\textsuperscript{24} Thus, the cysts associated with K17 mutations are highly heterogeneous, but form part of the spectrum of follicular hybrid cysts.\textsuperscript{25} The reasons for cyst formation are unknown but are presumably related to hyperkeratosis producing infundibular or ductal occlusion. The histological type of cyst may be determined by the initial state of development of hair or sebaceous gland in the pilosebaceous unit, or the site of obstruction.

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Diagnostic criteria in pachyonychia congenita and steatocystoma multiplex

The patients here were diagnosed as PC-2 or steatocystoma multiplex on clinical and pathological grounds prior to molecular genetic analysis. However, classification on the basis of clinical features may be misleading in individual cases, as for example in the case of family 3 who had no cysts but did have mild oral lesions. Although the latter are primarily a feature of PC-1, these can be seen in PC-2, reflecting some K17 expression in the oral mucosa. Hoarseness may occur in either syndrome, and suggests laryngeal mucosal involvement. K17 is presumably also among the keratins expressed in association with developing teeth, although how this results in premature eruption is not known. We have previously shown that some cases diagnosed both clinically and histologically as pure steatocystoma multiplex are due to K17 mutation. It is likely that K17 mutations are responsible for a proportion of cases which otherwise pass clinically as steatocystoma multiplex, but in which mild nail changes in patients or relatives are overlooked. The size of this proportion is unknown but is possibly small, as we have failed to find mutations in other cases of steatocystoma multiplex and of eruptive vellus hair cysts.

Two phenotypes arising from one keratin 17 mutation

Although two of the families examined here share the same K17 mutation, R94C, these differ in phenotype in that affected members of family 1 have the typical PC-2 nail dystrophy and epidermal cysts, whereas affected persons in family 2 have no nail changes and steatocystoma multiplex is the only phenotype (Fig. 2). Previously, we have shown that families with very similar mutations in K17 could give rise to these similar but distinct phenotypes, specifically mutations R94H and N92H in steatocystoma multiplex families and mutations N92D, N92S and Y98D in PC-2 kindreds. Phenotypic variation is common in other keratin diseases. From our previous studies of keratin diseases, we felt that this is not closely related to genotype, but we could not rule out the possibility that these two phenotypes might be related to the specific mutations involved. However, here we see both phenotypes arising from the same mutation, R94C, confirming that the variable phenotype phenomenon is not due to the specific K17 mutation alone, but must depend on a combination of other factors. The identity of these modifiers is unknown but is likely to be a combination of both genetic and environmental factors. Certainly, trauma is a factor in producing hyperkeratosis in keratinizing disorders and so life-style variables may well influence nail dystrophy. In transgenic animals, genetic background influences the outcome of targeted keratin mutations, but the identification of specific accessory genes in humans is difficult, due to the size of pedigrees required to study the segregation of distinct phenotypes.

This study demonstrates that at least some families with steatocystoma multiplex may have K17 mutations, so that molecular analysis is an option to be considered for this disorder. PC-2 is considered by some authors to be much less prevalent than PC-1, and one explanation for this is the possibility that many K17 mutations may present as steatocystoma multiplex without pachyonychia. It should be emphasized, however, that we have previously examined the K17 gene in sporadic cases diagnosed as steatocystoma multiplex or eruptive vellus hair cysts without identifying mutations. Thus, there may be other factors, genetic or otherwise, involved in these epidermal cyst disorders.

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