Diffuse Nonepidermolytic Palmoplantar Keratoderma Caused by a Recurrent Nonsense Mutation in DSG1

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**Background:** Mutations in genes coding for 2 desmosomal proteins, desmoglein 1 and desmplakin, have been shown to cause autosomal dominant keratoderma palmoplantaris striata.

**Observations:** We describe a family affected with a diffuse nonstriated form of palmoplantar keratoderma. Histopathologic examination of skin biopsy specimens disclosed cell-cell disadhesion in the suprabasal layers of the epidermis, as previously described in keratoderma palmoplantaris striata. We therefore genotyped all family members using microsatellite markers encompassing 3 keratoderma palmoplantaris striata-associated loci. Haplotypic analysis suggested linkage of the disease to 18q12.1, which harbors the DSG1 gene, encoding desmoglein 1. Mutation analysis eventually led to the identification of a causative recurrent nonsense mutation in this gene.

**Conclusions:** Mutations in DSG1 are not exclusively associated with striated palmoplantar keratoderma. The present study illustrates the efficacy of an integrative diagnostic approach to palmoplantar keratoderma involving clinical assessment, pathologic examination, microsatellite marker screening, and mutational analysis.

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**Inherited Palmoplantar Keratodermas (PPKs)** occur in a large group of cornification disorders characterized by extensive phenotypic heterogeneity. The Online Mendelian Inheritance in Man (OMIM) catalogue (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM) mentions more than 35 genetic diseases manifesting with prominent PPK. Over the last several years, much progress has been achieved toward a better understanding of the molecular basis of these disorders. Mutations in more than 20 distinct genes have been described in various forms of PPK. Many of these genes code for structural proteins (eg, keratins) or components of the desmosomal plaque, which are all known to play an important function during keratinocyte differentiation. The physiologic role of other molecules associated with the pathogenesis of PPK, such as connexins and secreted LY6/UPAR-related protein 1 (SLURP-1), is less well understood.

To overcome the difficulties posed by phenotypic and genetic heterogeneity in the diagnosis of inherited PPK, a number of classification schemes have been devised in which morphologic features are used to predict the underlying molecular defect. For example, the association of periodontitis and PPK is suggestive of Papillon-Lefèvre syndrome caused by mutations in the cathepsin C gene, whereas the coexistence of PPK and deafness is suggestive of a connexin gene mutation.

Keratosis palmoplantaris striata (KPS) is a rare autosomal dominant disorder characterized by linear hyperkeratotic streaks along the volar surface of the fingers and focal keratoderma over the soles. Nonsense and frameshift mutations in DSG1 and DSP encoding 2 desmosomal proteins, desmoglein 1 and desmplakin, demarcate 2 KPS subtypes, type I (OMIM 148700) and type II (OMIM 125647), respectively. Recently, a frameshift mutation affecting the keratin 1 tail domain was found to underlie KPS type III (OMIM 607654) in a large kindred of British extraction.

Keratosis palmoplantaris striata is regarded as a prototypic desmosomal genodermatosis. Indeed desmoglein 1 and desmplakin are critical components of the desmosomal plaque in the upper epidermis, and frameshift mutations at the tail...
MUTATION ANALYSIS

Genomic DNA was PCR amplified with primer pairs covering the entire coding sequence of the DSG1 gene as well as intron-exon boundaries. Polymerase chain reaction amplification was performed using Taq polymerase (Qiagen, Valencia, Calif) and Q solution according to the manufacturer’s instructions. Gel-purified amplicons were subjected to bidirectional sequencing using Big Dye Terminator (PE Applied Biosystems). To verify R26X, a 169-base pair PCR fragment, encompassing exon 2, was PCR amplified and digested with BstYI endonuclease.

RESULTS

CLINICAL FINDINGS

The proband was a 50-year-old man of Jewish Yemenite origin. From age 3 years, he had thickening of the skin of his palms and soles accompanied by painful fissures. Three of his children displayed a milder form of keratoderma, mainly evident on the soles. His grandparents and parents were unavailable for examination or DNA sampling, but the proband indicated that his maternal grandfather, but not his mother, had reportedly been affected by a similar disease. Treatment with etretinate for several months led to partial improvement of his condition but was discontinued at the patient’s request because of excessive skin dryness.

On examination, diffuse hyperkeratosis and fissuring of the volar surface of the hands and digits were observed (Figure 1A). Similar features were seen over weight-bearing areas of the soles and toes (Figure 1B). Mild onycholysis was accompanied by yellowish discoloration of most nails. Hair, teeth, mucosae, and nonpalmarplantar skin were normal.

Histologic examination of a skin biopsy specimen obtained from the palmar skin revealed some papillomatosis and marked ortho hyperkeratosis in the epidermis (Figure 1C). Widening of the intercellular spaces and disadhesion of keratinocytes were observed in the upper spinous and granular cell layers (Figure 1D).

HAPLOTYPING ANALYSIS

Since the histopathologic features observed in skin biopsy specimens from the patient resembled those previously described in KPS, we considered the possibility that a mutation in a gene previously associated with this disease might underlie the diffuse PPK displayed by the proband. To assess this possibility, we established 3 panels of microsatellite markers spanning the 3-gene loci previously shown to be associated with KPS on 18q12.1 (DSG1), 6p24 (DSP), and 12q13 (KRT14). Markers were selected based on their index of heterogeneity and short distance to each of the 3 genes. We established the genotypes of each of the 7 family members at the 3 loci. Haplotype analysis revealed that all affected individuals shared a common 11.4-megabase chromosomal segment between markers D18S877 and D18S533 on 18q12.1, encompassing the DSG1 locus (Figure 2), which suggested the existence of a pathogenic mutation in this gene.
MUTATION ANALYSIS

We analyzed genomic DNA extracted from the patient's blood lymphocytes for pathogenic mutations in DSG1. All exons and intronic boundary regions of the gene were PCR amplified and directly sequenced. A single heterozygous C→T transition at complementary DNA position 76 (starting from the ATG) was identified in all affected individuals (Figure 3). This mutation results in the substitution of a stop codon for an arginine residue at position 26 of the amino acid sequence (R26X) and has been previously described in a sporadic case of striated keratoderm.a We developed a PCR–restriction fragment length polymorphism assay based on the fact that the mutation abolishes a restriction site for BsiYI endonuclease (Figure 4). Using this assay, we confirmed segregation of the mutation in the family.

Desmosomal cadherins, which include a number of desmocollins and desmogleins isoforms, are transmembranal proteins that play a critical role in cell-cell adhesion and are part of pivotal signal transduction pathways regulating cell growth and differentiation. In accordance with their pleiotropic functions, abnormal desmosomal cadherins have been linked to a growing number of inherited and acquired skin diseases. Desmoglein 1, a major component of the desmosome in the upper epidermal layers, is associated with the pathogenesis of at least 3 skin diseases: pemphigus foliaceus, staphylococcal scalded skin syndrome, and autosomal dominant KPS.

In the present study, we identified a heterozygous nonsense mutation, R26X, that causes a diffuse nonstriated form of PPK. The R26X location at the start of the DSG1 coding sequence predicts loss of function of the mutant allele due to either severe truncation or, more likely, nonsense-mediated messenger RNA decay. This mutation has previously been described in a sporadic case of European ancestry affected with typical KPS. The variable phenotypic expression of the mutation (diffuse PPK vs KPS) in 2 cases and the reported absence of any phenotype in the mother of the proband suggest the influence of epigenetic factors such as physical trauma to the palmar and plantar skin. Interestingly, our proband denied any physical activity for the past 5 years, possibly suggesting the existence of other epigenetic or genetic modifier traits.

Despite the diffuse nature of the PPK affecting our index case, histologic findings led us to focus our initial molecular analysis on genes coding for desmosomal components. It has been known for many years that, in skin biopsy specimens obtained from patients with PPK, epidermolysis changes in the upper epidermal layers very often indicate mutations in KRT9 or KRT1. Our data underscore the usefulness of another histopathologic clue for the diagnosis of PPK caused by mutations in genes encoding desmosomal proteins, namely widening of the intercellular spaces and disadhesion of suprabasal keratinocytes.

However, these histopathologic findings cannot be considered as entirely specific or sensitive for KPS; they must be interpreted with caution because similar histologic features have been reported in other inherited desmosomal disorders, and these changes were absent in a number of typical KPS cases caused by dominant mutations in DSG1 (including a patient carrying R26X). Thus, to confirm our working diagnosis and restrict our subsequent mutation analysis, we developed a screening approach based on the use of 3 panels of microsatellite mark-
ers and haplotype analysis. While a formal mutation analysis of all 3 genes involved in the pathogenesis of KPS would have entailed the sequencing of more than 50 amplicons, by using haplotype analysis as a screening tool, we identified DSG1 (comprising 15 exons only) as a target for subsequent mutation analysis. This integrative approach combining clinical ascertainment, pathologic examination, and candidate gene marker screening proved to be efficient and led ultimately to the discovery of the underlying genetic defect, despite the confusing phenotypic features displayed by the proband.

In summary, we have identified a recurrent mutation in DSG1 that causes diffuse, and not striated, PPK. Our data show that the diagnostic challenge posed by phenotypically heterogeneous PPK can be met through the use of a comprehensive clinical, pathologic, and molecular approach.

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