Clinical and laboratory study

KERATIN 5 ASSOCIATED RESTRICTION FRAGMENT LENGTH POLYMORPHISM IN PATIENTS WITH PALMOPLANTAR KERATODERMA OF UNNA-THOST TYPE

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ABSTRACT

Hereditary palmoplantar keratodermas of the Unna-Thost type (HPPK-UT) are relatively frequent among the Slovenian and Croatian population. Members of two Slovenian families (33 persons altogether) were investigated for RFLP (restriction fragment length polymorphism). RFLP of the patients DNA was studied by applying the following endonucleases: Hind III, Taq I, EcoRI, Sac I, BamHI and Msp I. Only digestion with Msp I produced four polymorphic fragments which were identified as a, b, c and d alleles of keratin 5. 14 out of 15 affected persons expressed the d allele in a heterozygous or homozygous way. In a number of unaffected members the d allele was also present.

KEY WORDS

Keratin 5, restriction fragment length polymorphism, palmoplantar keratoderma, Unna-Thost type, Msp I

INTRODUCTION

Hereditary palmoplantar keratodermas (HPPK) are disorders of keratinization characterized by a thickened horny layer on palms and soles. Various types of HPPK are distinguishable by clinical symptoms, associated abnormalities and the mode of inheritance. HPPK of the Unna-Thost (UT) type is the most frequent, its prevalence is 1:40.000 in Northern Ireland (1), 1:12.000 in Slovenia (2) and 1:25.000 in Croatia (3). In Sweden at Westerbotten county a prevalence of 1:300 among adolescents (4) and at Norbotten county of 1:200 among school children were registered (5). The cause of HPPK - UT is not known, however dominant inheritance and circumscribed distribution allow the possibility that this disease is due to a mutation in a structural protein specially expressed in palmar and plantar epidermis. Likely candidates for such proteins are keratins.

Keratins are intermediate filament proteins which constitute the cytoskeleton of all epithelial cells. Over 30 such proteins are known and each is coded by a separate gene. They can be subdivided into two distinct classes on the basis of their migration in two dimensional electrophoresis, their expression in different cell types and of their sequence homology (6). Type I keratins (K10 - K19 are smaller (40-56.5 kD) and

This work was supported by the U.S. Joint Board project HSS JF 915 "Genetic Studies on Hereditary Disorders of Keratinization" which is gratefully acknowledged.

relatively acidic (pI 4.5-5.5), whereas type II keratins (K1 - K9) are larger (53-68 kD) and more basic (pI 5.5-7.5). Usually keratins are expressed as specific pairs consisting of one type I and one type II polypeptide, both of which are essential for filament formation (7,8) and as such characterize biochemically the type of epithelial differentiation. For example K5 and K14 are synthesized in the basal cell layer of all stratified squamous epithelia, while in the course of stratification differentiating epidermal cells express K1 and K10. Suprabasal maturing cells of nonkeratinizing squamous epithelia express K4 and K13, while in a number of epidermal skin diseases K6 and K16 are expressed suprabasally (9). Expression of keratins is often much greater in differentiating cells than in basal cells (10).

Elucidating of keratin functions has been hampered by lack of known genetic disease involving an identified keratin mutation. During the last few years however important new data concerning the genetics of keratin were accumulated. Hereditary epidermolysis bullosa simplex (EBS) arises from basal cell cytolysis. Coulombe et al. (11) demonstrated that a perturbation of basal cell keratin filament network was responsible for the clinical manifestations of EBS. In two patients with EBS of Dowling-Meara type a point mutation in the critical region of the K14 gene was observed: Arg 125 to Cys mutation. The same genetic defect in transfected keratinocytes resulted in a disrupted keratin network formation and in a perturbarted filament assembly. In a family with EBS of Koebner type Bonifas et al. (12) were able to map the defect to chromosome 17 by lod score calculation and to identify the mutation in the base pair 3542 of the responsible gene. Lane et al. (13) described in a family with EBS of Dowling-Meara type a Glu to Gly mutation in the helix-termination peptide of keratin 5. It is worth to mention that Vassar et al. (14) by inserting a truncated K14 protein (missing 135 amino acid residues at the C-terminal) into mice succeeded to produce epidermal blistering in transgenic offspring which was similar to EBS lesions. The micromorphologic investigation revealed a disruption of keratin network assembly, presence of clumped keratin and cytolysis of basal cells. Complementary in vitro filament assembly studies on bacterially produced human K5, K14, and the truncated mutant K14 showed that addition of as little as 1 % of the mutant K14 to the assembly mixture caused a detectable alteration in filament formation (14). Further transgenic studies confirmed the dominant behavior of mutations such as the above mutation in K14, and provided strong evidences as to which human skin diseases might be candidates for natural mutations not only in K14 but also in other genes such as K5. More recently another hereditary epidermal disorder has been traced to mutations in keratin genes: epidermolytic hyperkeratosis (EH) which is due to mutations in the K1 and K10 keratin genes (15, 16, 17, 18).

Therefore we examined the possibilitiy that HPPK-UT mutation maps in the type II keratin gene cluster, by examining genetic linkage between HPPK-UT and a polymorphic marker in the K5 keratin gene.

The human K5 keratin gene encodes a 58 kD protein which belongs to the basic Type II keratin family. The protein is polymorphic and at least two allelic variants were detected (19,20). The gene itself is approximately 5 kb long, contains nine exons and is located on the chromosome 12 (21,22). In the present study we are reporting some evidence about polymorphism within the coding region of the gene itself.

MATERIALS AND METHODS

Blood samples were taken from members of two Slovenian families affected by HPPK-UT as well as from non-affected and nonrelated controls. Eight patients and nine non-affected relatives belonged to family SO from Eastern Slovenia (Celje area). Another seven patients and nine non-affected persons were members of family FA from Western Slovenia (Ajdovščina area).

High molecular weight DNA was prepared from nuclei of leukocytes. Samples of the whole blood were first lysed by adding an equal volume of solution containing 0.32 M sucrose, 10 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$ and 1 % Triton X-100. The nuclei were then collected by centrifugation and the DNA was purified by incubating overnight at 37°C in a buffer solution of 10 mM Tris-HCl, 400 mM NaCl and 2 mM EDTA (pH 8.2) containing 1 % sodium dodecyl sulfate (SDS) and 0.15 mg/ml Proteinase K. Proteins were salted out by 6M NaCl and DNA was precipitated from the supernatant by adding two volumes of ice-cold absolute ethanol (23).

Digestions of the DNA with restriction endonucleases such as Hind III, Taq I, Eco RI, Sac I, Bam HI and Msp I were carried out observing the conditions recommended by the manufacturer (Gibco BRL). The digested DNAs were fractioned by electrophoresis in 0.8 % agarose gel.

After electrophoresis the digested DNA was denatured in the gel by submersing in 0.4M NaOH and blotted onto nylon membranes (NEN DuPont) by capillary transfer (24). PKA_1 probe containing a 1.68 bp DNA fragment of the K5 gene (PKA_1-b probe) was labelled with alpha/^{32}P/-dCTP to a specific activity of 3×10^8 cpm/µg by random primer extension method (25) using the Amersham Multiprime labelling kit.

Membranes with restricted and denatured DNA fragments were hybridized to radioactively labelled PKA1-b probe in a standard hybridization solution containing 50 % formamide

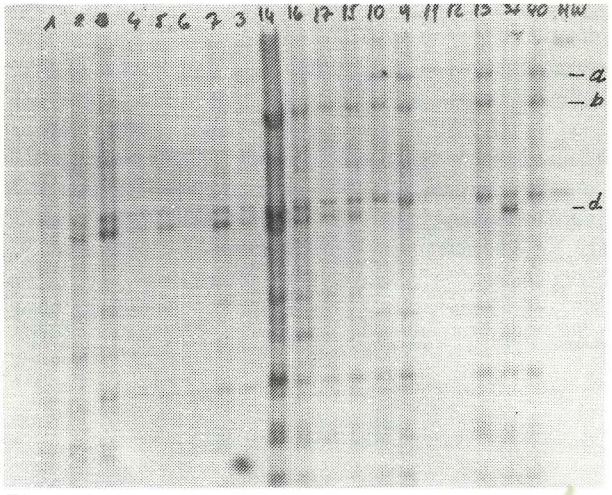

Figure 1:
Southern blots of restricted DNAs as individualized by autoradiography. Familly SO.

(Amersham Hybridization Protocols) at 42°C for 48 hours. After hybridization the membranes were washed once in 2xSSC/0.1 % SDS for 5 minutes at room temperature, and a second time in the same solution at 65°C for 15 minutes. Visualization was performed by autoradiography using a X-ray film (X-Omat, Kodak), after two and seven days exposure times.

RESULTS

In order to search for K5-gene polymorphisms first six DNA samples from unaffected unrelated individuals were digested with six different restriction endonucleases and hybridized to PKA1 probe. Five of the enzymes gave non-polymorphic band patterns, but only digestion with Msp I produced polymorphic fragments of which the total number was four and with the lengths of 8.7, 7.2, 6.4 and 3.2 kb respectively. The fragments were assigned as a, b, c and d alleles (Figures 1 and 2).

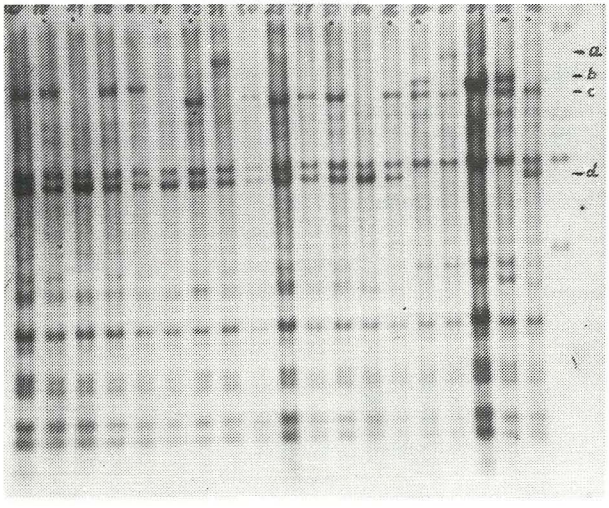

Figure 2:
Southern blots of restricted DNAs as visualized by autoradiography. Familly FA.

Two families with hereditary PKK were then screened for the presence of Msp I RFLP. The genotypes of both the families are presented. In the family SO (Eastern Slovenia) the eight affected members displayed b/d and a/d heterozigous or d/d homozigous genotype (Fig 3). In the family FA (Western Slovenia), six out of seven affected members expressed the d allele. Details are presented in Figure 4.

DISCUSSION

It is difficult to find out exactly who was the first to describe the polymorphism of keratin molecules. It seems however that the introduction of sodium dodecyl sulfate polyacrylamide gel electrophoresis as well as of modern chromatographic methods in the protein biochemistry were helpful in achieving essential progress in this direction. Wild and Mische described in 1986 the occurrence of keratins K4a and K4b as well as K5a and K5b as conspicuous doublets in the stratified epithelium lining the upper digestive tract in some individuals, while in others only one of these variants was present. They assumed that a polymorphism of the respective keratin genes was responsible for this heterogeneity (26). Later on the same authors made a similar observation analyzing the keratins of human epidermis by SDS PAGE and immunoblotting: basic keratins K1a, K1b, K5a and K5b, and acidic keratins K10a and K10b appeared either as doublets or as one or the other variant. In rare cases other acidic keratins tentatively designated as K10d and K10c were also observed (20). In 1992 Korge et al. (27) confirmed the existence of the above mentioned four variants of K10, which represent four differently sized alleles. In order to answer the question whether these K10 variants are due to multiple genes or different alleles within the human population or are caused by different posttranslational RNA processing, they have looked for the nature of the polymorphism by analyzing respective gene sequences. Their investigation showed that the polymorphism was located in the V2 subdomain of the K10 molecule. According to their experience the size polymorphism of the K10 gene is inherited as a normal Mendelian trait, and the differently sized products obtained by PCR amplification most likely represent different alleles of a single-copy gene per haploid genome.

In our investigation we have looked for the possible existence of restriction fragment length polymorphisms within

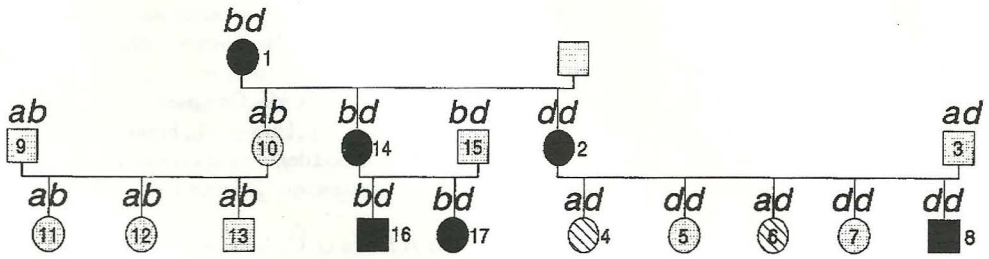

Figure 3:
Geneological tree and inheritance of the K5 polymorphic alleles in family SO.

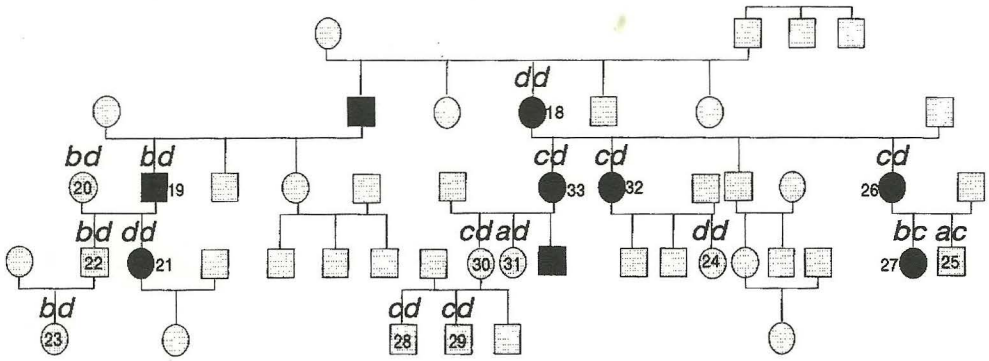

Figure 4:
Geneological tree and inheritance of the K5 polymorphic alleles in family FA.

the coding region of the K5 gene.Results obtained in this study indicate that K5 is also present, like K10, in four allelic modifications. It is interesting to mention that allele d was found in almost all persons affected with HPPK type Unna-Thost (in 14 out of 15 patients). As this allele was present also in certain unaffected individuals it can be assumed that the respective gene is not characteristic for HPPK-UT.

Furthermore, in three individuals, 24, 27 and 31 of family FA, the disease does not segregate with the K5 gene alleles. We conclude from these preliminary results that the mutation causing HPPK-UT is probably not linked with the basic keratin genes on chromosome 12.

Further investigation, in order to confirm the above results and to find possibly the informative polymorphisms associated with pathological phenotype (s), has to include much greater number of hereditary PKK cases. To define precisely the nature of the polymorphisms found in this investigation PCR amplification and sequence analyses of the DNAs should be performed. And finally, extensive analyses such as cloning and sequencing of keratin cDNAs, such as K5 and K14 cDNAs from skin mRNAs, in a number of patients will be needed before the etiology of the disease is fully understood.

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