Acta Derm Venereol 2001; 81: 163–170

INVESTIGATIVE REPORT

Phenotypic/Genotypic Correlations in Patients with Epidermolytic Hyperkeratosis and the Effects of Retinoid Therapy on Keratin Expression

MARIE VIRTANEN[1], TOBIAS GEDDE-DAHL JR.[2], NILS-JØRGEN MÖRK[2], IRENE LEIGH[3], PAUL E. BOWDEN[4] and ANDERS VAHLQUIST[1]

[1]*Department of Medical Sciences, Section of Dermatology, Uppsala University, Uppsala, Sweden,* [2]*Departments of Dermatology, Rikshospitalet and University of Oslo, Oslo, Norway,* [3]*Imperial Cancer Institute, London and* [4]*Department of Dermatology, UWCM, Cardiff, UK*

Dominant-negative mutations in the *KRT1* and *KRT10* genes cause epidermolytic hyperkeratosis, a rare form of ichthyosis sometimes associated with palmoplantar keratoderma. Although there is no permanent cure, some patients improve on retinoid therapy. More knowledge is needed, however, about the mechanism of action of retinoids and the genotypic/phenotypic correlations in this disease. Thirteen patients from 10 families with generalized disease and 2 sporadic patients with nevoid lesions were studied, probably representing most of the patients in Sweden and Norway. All patients, except one nevoid case, were known to have *KRT1* or *KRT10* mutations. Those with mutated keratin 1 (K1) invariably had associated keratoderma ($n = 6$). In contrast, only 1 of 7 patients with K10 mutations had this problem ($p = 0.0047$). Five out of 6 patients with *KRT10* mutations benefited from treatment with oral acitretin (5–25 mg/day) or topical tretinoin/tazarotene, but none of the patients with *KRT1* mutations derived any benefit. Quantitative analysis of K1 and K10 mRNA in skin biopsies obtained before and after retinoid therapy ($n = 8$) showed no consistent down-regulation of mutated keratin that would explain the therapeutic outcome. Instead, the mRNA expression of K2e (a normal constituent of the upper epidermis) diminished especially in non-responders. In contrast, K4 mRNA and protein (marker of retinoid bioactivity in normal epidermis) increased in almost all retinoid-treated patients. In conclusion, our study confirms a strong association between *KRT1* mutations and palmoplantar keratoderma. Retinoid therapy is particularly effective in patients with *KRT10* mutations possibly because they are less vulnerable to a down-regulation of K2e, potentially functioning as a substitute for the mutated protein in patients with *KRT1* mutations.

***Key words*: *mutation; KRT1; KRT10; dermatophytes; real time quantitative polymerase chain reaction; immunohistochemistry*.**

(Accepted March 22, 2001.)

Acta Derm Venereol 2001; 81: 163–170.

Marie Virtanen, Department of Dermatology, University Hospital, SE-751 85 Uppsala, Sweden.

Epidermolytic hyperkeratosis (EHK) or bullous ichthyosiform erythroderma is an autosomal dominant genodermatosis with a prevalence of 1:100,000–1:300,000 (1) and a mutation rate of 0.9 per million gametes (2). The symptoms are initially erythema, blisters, scales, and erosions on large areas of the body. With increasing age, the tendency to blistering usually subsides and the skin becomes more hyperkeratotic, especially in flexural areas (3). In addition, some patients have palmoplantar keratoderma (PPKD). Major inter-individual variations exist in the severity of EHK. Interestingly, in some patients with EHK, the skin lesions have a nevoid-like appearance with disease expression only along the lines of Blaschko. This can be explained by genetic mosaicism following a somatic mutation during embryogenesis (4, 5).

Ultrastructurally, EHK is characterized by suprabasal keratinocytolysis and clumping of tonofilaments around the nucleus (6). The tonofilaments are composed of keratins, a major epidermal structural protein belonging to a multigene family of intermediate filaments (IF). More than 30 different keratins are known, all of which fall into either of two groups, type I (K9-20) or type II (K1-8). Keratin IFs are formed from heterodimers of types I and II proteins (7). In normal epidermis, basal keratinocytes express keratin 5 (K5) and 14 (K14), whereas suprabasal cells express K1 and K10 together with K2e (in stratum granulosum) and K9 (in palmoplantar epidermis).

Mutations in keratin genes (*KRT1* or *KRT10*) cause EHK (8, 9) and these mutations have a dominant negative effect on the IF network and cause fragility of suprabasal keratinocytes (10). Analogously, mutations in *KRT5* and *KRT14* cause fragility of basal keratinocytes, leading to epidermolysis bullosa simplex (11), and mutations in *KRT 9* and *KRT2e* cause epidermolytic PPKD (12) and ichthyosis bullosa of Siemens (13), respectively. The latter can be regarded as a mild superficial form of EHK.

The most common type of alteration in EHK is a point mutation giving rise to single amino acid substitutions (8, 9). The locations of the mutations are mainly in the α helical rod domain, a region with highly conserved amino acid sequences (7). Previous studies of genotypic/phenotypic correlation in EHK have disclosed that the presence of PPKD is usually associated with a mutation in *KRT1*, although there are a few case reports of EHK patients with PPKD having *KRT10* mutations (14, 15).

EHK therapy is symptomatic and encompasses various types of emollients, antibiotics, and/or retinoids. The latter drugs, given either topically or systemically, may reduce the hyperkeratosis (16, 17), but some patients experience increased blistering and skin irritation. The reason for this is not clear, nor is the mechanism of action of retinoids in EHK. It is well known, however, that retinoids affect keratin expression in normal human epidermis where some keratins (K4, K6, K13, K19) are up-regulated (18–20), whereas others (K2e, K1, K10) are down-regulated (20) or show little, if any, response at all after treatment with retinoids (18).

 ISSN 0001-5555

The aim of this study was to examine whether there is a genotypic/phenotypic correlation in Scandinavian patients with EHK and if the type of mutation (*KRT1* or *KRT10*) determines the patient's response to retinoids, our working hypothesis being that retinoids might down-regulate the mutated keratin gene in some patients with EHK and thus improve the cytoskeletal stability of the differentiated keratinocytes.

MATERIAL AND METHODS

Patients

Fifteen patients with EHK were included in the study, 13 with generalized and 2 with nevoid forms. Table I shows the age and sex distribution of the patients with generalized EHK. The case of one patient, aged 3 years (no. 6) had been published clinically as epidermolysis bullosa simplex with tylosis (21) and another (no. 5) was pictured at age 6 (22). An 18-year-old girl and a 6-year-old boy represented the nevoid cases. All patients come from Sweden and Norway, although one boy (no. 2) was originally adopted from a South American family. Three patients with generalized disease that belonged to the same family (nos. 11–13) and another patient (no. 6) had prenatal diagnoses of affected foetuses (23), whereas, to our knowledge, the remaining patients had no family history of EHK. The diagnosis was confirmed by histopathology and in most cases also by electron microscopy (23). The patients were examined by two of the authors (MV and AV) using a standardized protocol which notifies the grading (+ to ++++) of symptoms and the extent of the disease. After informed consent, 5–10 ml EDTA blood was collected from all the patients for subsequent DNA extraction. In the case of nevoid EHK and in all patients treated with retinoids (see below), shave biopsies were also taken from lesional skin for RNA extraction.

When indicated, and after informed consent, topical or oral retinoids were given to the patients. The following topical retinoids were used: 0.05% tretinoin (Aberela®, Janssen-Cilag) and 0.05% tazarotene (Zorac®, Allergan). The creams were applied 1–2 times per day on defined and restricted areas (severe skin lesions, avoidance of flexural areas). Some patients alternatively received oral acitretin (Neotigason®, Hoffman-LaRoche) 5–25 mg/day. The effect of treatment was evaluated using photography and a standardized protocol after one month and at variable intervals thereafter, depending on the type of treatment and the response to therapy. Before and after one month of treatment, punch and shave biopsies were taken from lesional skin after infiltration with lidocaine-adrenaline. The shave biopsies, typically consisting of 1–2 cm^2 of ~80% pure epidermis (24), were immediately frozen and kept frozen pending RNA extraction. The punch biopsies were snap frozen and kept at –70°C until processed for immunohistochemistry. The local ethics committees at the Universities of Uppsala and Oslo approved the study.

DNA analysis and mutation screening

Genomic DNA or cDNA from the patients was amplified with regular polymerase chain reaction (PCR) (25). The primers used were HK10p1 (5′-AGG AGA TGG TGG CCT TCT CTC TGG-3′) and HK10p2R (5′-GCA TAG TGA ACA GCC ACA TTG TGC-3′) for analysing *KRT10* (exon 1). The specific primer pairs for analysing other exons in *KRT1* and *KRT10*, will be described elsewhere.

An aliquot of each PCR reaction was used for direct manual sequencing by the dideoxy chain termination method (USB Sequenase Version 2.0, Amersham International, Slough, UK). The *KRT10* primer used to sequence exon 1 was HK10p3R (5′-TAA GAT TCA TCT GTC TGG-3′). The others primers will be described elsewhere. The reactions were analysed on 6% polyacrylamide denaturing gels.

RNA preparation and reverse transcription (RT)

Total RNA was extracted from shave biopsies by homogenization in TRIZOL reagent (Life Technologies, Gibco BRL, Täby, Sweden). First strand cDNA was synthesized from 3 μg total RNA in a 30-μl reaction mixture containing oligo-d$(T)_{15}$ primer and M-MLV reverse transcriptase (Life Technologies, Stockholm, Sweden). After the reaction, 30 μl RNase-free water was added to a final volume of 60 μl.

Real time quantitative PCR (TaqMan)

Quantitation of specific mRNAs was accomplished by real-time PCR (26) as previously outlined (20, 27). The PCR reaction mix contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 5.0 mM $MgCl_2$, 0.4 mM of each dNTP, and 1.25 U Ampli Taq Gold polymerase (Perkin-Elmer). One microlitre of the RT reaction was added to a final volume of 25 μl. TaqMan probes, which contained FAM as 5′-reporter and TAMRA as 3′-quencher, and oligonucleotide primers were designed with the Primer Express program (PE-Applied Biosystems, Foster City, CA). The sequences of probes and primers are given in a previous publication (20). The final concentrations of the probes and primers (PE Applied Biosystems, Cheshire, and UK) were 0.2 μM and 0.4 μM, respectively. The PCR reaction were performed for 40 cycles on an ABI Prism 7700 Sequence Detector (PE Applied Biosystems), each cycle consisting of 15 s at 94°C and 30 s at 60°C. A standard curve was generated for each template by amplifying known amounts of a PCR product at the same time as the sample. The standard curves were linear over the range of concentrations studied (data not shown). The expression of the different keratins in each sample was normalized to the expression of ß-actin.

Immunostaining of keratins

Skin biopsies were kept frozen at –70°C. Sections 6 μm in thickness were fixed in 100% ice-cold acetone. Non-specific binding was blocked with 10% normal horse serum (Vector Laboratories, Burlingame, CA). The primary antibodies were diluted 1:10 (K1, LHK1, LL017) (28), 1:20 (K4, 6B10, BiogenesisDorset, UK) (29) and 1:1000 (K2e, IL39). The antiserum against K10 (LH2) (28, 30) was used undiluted. The antibodies were applied overnight at 4°C. Detection of the keratin antibodies by biotinylated anti-mouse IgG (1:200, Vector Laboratories) was followed using the Vectastain ABC kit (Vector Laboratories) and 3-amino-9-ethylcarbazole (Sigma, Stockholm, Sweden).

Statistics

Statistical significance was assessed by the Wilcoxon paired *t*-test and Fisher's exact test.

RESULTS

Patient characteristics: no clear phenotypic/genotypic correlations except for keratoderma

A summary of the clinical features of the patients with generalized EHK listed by order of age and type of keratin mutation (K1 or K10) is presented in table I. Brief reports of some of the mutation analyses have been given previously.[1,2,3] The mutations were either confirmations of a previous hotspot residue (no. 156 in K10) (31–33) or new deletions, splice site and point mutations that will be presented in detail elsewhere. As seen from table I, there were more males than females and the age distribution was markedly skewed with a majority of patients born during the 1980s. The skin symptoms varied in intensity from mild to severe, and in 7 of the patients an associated PPKD was observed. Mutations in K1 were found in the unrelated patients, nos.1–6 having mild to severe EHK always associated with PPKD (Figs 1A and B). In the

[1]Three novel heterozygous point mutations in keratin genes (KRT1 and KRT10) in families with epidermolytic hyperkeratosis (abstract). J Invest Dermatol 1999; 113: 497.

[2]Two splice site mutations and one deletion mutation in the KRT1 gene of Scandinavian families with epidermolytic hyperkeratosis (abstract). J Invest Dermatol 1999; 113: 497.

[3]Absence of exon 6 in the KRT1 gene from a patient with epidermolytic hyperkeratosis (abstract). J Invest Dermatol 2000; 155: 575.

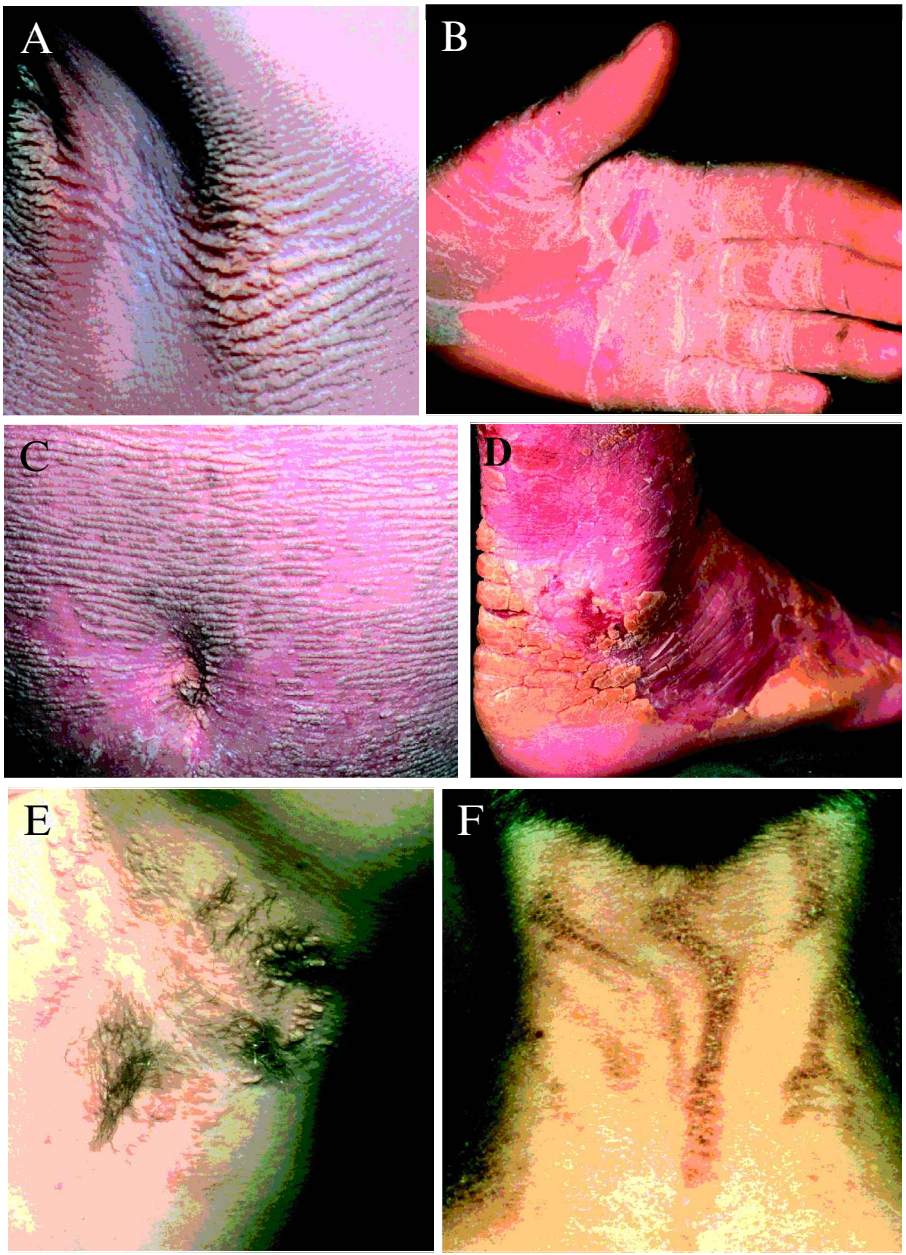

Fig. 1. Examples of skin symptoms due to mutations in *KRT1* (A, B) and *KRT10* (C, D, F). (A) Warty hyperkeratosis in the axilla of patient 1; (B) diffuse keratoderma in patient 5 (note the shaved areas of the proximal palm produced by the patient); (C) generalized erythema and hyperkeratosis on the abdomen of patient 10; (D) intense erythema and superficial blistering on the ankle of patient 8 (note the normal sole); (E) verrucous hyperkeratosis in the left axilla of patient 15, and (F) striate lesions in the neck of patient 14, both suffering from nevoid EHK

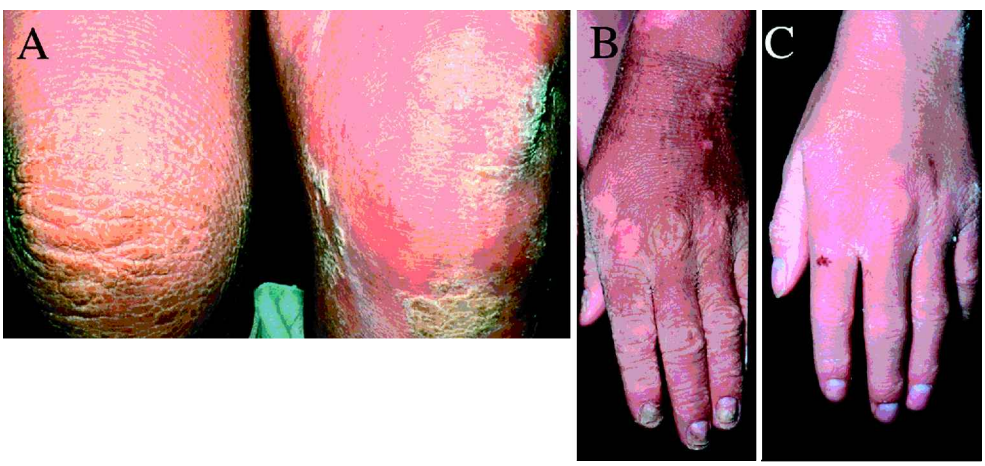

Fig. 2. Examples of successful effects of treatment in epidermolytic hyperkeratosis. (A) Result of daily application of tretinoin 0.05% to the left knee of patient 12 for 4 weeks (the right knee received a bland emollient). (B) The left hand of patient 9 before treatment; note the diffuse hyperkeratosis on the back of the hand and the onychomycosis of the nails; (C) the same hand after 3 months of oral terbinafin (for onychomycosis) followed by 4 weeks of oral acitretin 20 mg/day.

Table I. *The clinicogenetic features and response to retinoid therapy in patients with generalised epidermolytic hyperkeratosis with or without palmoplantar keratoderma (PPKD).*

Pat. no.	Sex/age	Degree of hyperkeratosis (0–4)					Erythema	Site of erosions	Mutated keratin gene	Retinoid treatment	Effect
		Flexural areas	Elbow, knee	Torso	Neck	PPKD					
1	M/12	2	2	0	2	1	–	–	K1, Point mutation[a]	Tazarotene	Neg
2	M/12	2	3	2	3	4	Localized	Widely spread	K1, Point mutation[a]	Tazarotene	Neg
3	M/18	1	1	0	1	2	–	Legs	K1, Splice site[a]	Tazarotene	Neg
4	M/18	2	2	1	1	1	Localized	Feet, hands	K1, Splice site[a]	–	
5	M/31	4	3	1	3	4	Localized	Neck, feet, hands	K1, Deletion[a]	Acitretin 10 mg/d, tazarotene	Neg
6	F/41	2	2	1	0	3	Localized	Torso, hands, arms, legs	K1, Deletion[a]	Acitretin 10 mg/d, tazarotene	Neg
7	M/6	2	3	2	2	2	Generalized	Buttock, feet	K10, Point mutation[a]	–	
8	M/10	4	4	3	2	0	Generalized	Torso, arms, feet	K10, Deletion[a]	Acitretin 5 mg/e.o.d. Tretinoin	– /+
9	M/12	3	4	2	3	0	Generalized	Torso, hands, feet, neck	K10, Point mutation[b]	Acitretin 20 mg/d, tretinoin	++[c]
10	F/16	1	4	3	1	0	Generalized	Widely spread	K10, Point mutation[b]	Acitretin 25 mg/d	++[c]
11	F/16	4	4	3	2	0	Generalized	Arms, legs, torso, hands	K10, Point mutation[b]	Tretinoin	+[c]
12	F/48	3	3	2	2	0	Generalized	Arms, legs, torso, hands	K10, Point mutation[b]	Acitretin 10 mg/d, tazarotene	++[c]
13	F/74	3	2	2	1	0	Localized	Feet	K10 Point mutation[b]	Acitretin 20 mg/d	+[c]

[a] For details, see footnotes under Results.
[b] The following hotspot mutations were found: no. 9 R156S, no. 10 R156G and nos. 11–13 R156H.
[c] (+) moderate or (++) good effect from retinoid therapy.

remaining 5 families with 7 cases of generalized EHK (nos.11–13 from the same family), mutations in K10 were found. In general, the patients with K10 mutations had a tendency toward more severe disease compared with those with K1 mutations (Figs 1C and D). However, only one of the patients with K10 mutation had associated PPKD ($p = 0.0047$).

A *KRT10* point mutation (R156H) was also found in cDNA from lesional skin of one boy with nevoid EHK with lesions extending over the neck, axilla and legs (Fig. 1F). The other patient with equally typical epidermolytic lesions along the lines of Blaschko (Fig. 1E) failed to show any *KRT1* or *KRT10* mutation when cDNA from lesional skin was sequenced. This patient was included in the study because of her typical morphology and excellent response to topical tretinoin therapy (see below).

In 4 of the patients with generalized EHK (nos. 2, 4, 8 and 9), long-standing abnormalities of the nails were noted. One of the patients (no. 8) also had a fine scaling in his palms that resembled a mild PPKD. However, fungal cultivation revealed dermatophytes in the nails of 3 patients (nos. 4, 8 and 9) and *Candida albicans* in the palms and nails of patient 2. After oral anti-fungal treatment for 3 months, all patients showed normal nails and the scaliness of the palms had vanished in patient 8.

The clinical results of retinoid therapy are related to the genotype of EHK

Thirteen patients (11 with generalized and 2 with nevoid EHK) were clinically examined before and after retinoid therapy, which was given either topically as tretinoin or tazarotene ($n = 9$), or systemically as acitretin ($n = 7$) for at least 4 weeks. The results of retinoid therapy in patients with generalized EHK are summarized in table I. A reduction of hyperkeratosis was usually seen after 4 weeks, but some patients showed skin irritation and increased blistering. Interestingly, the outcome of retinoid therapy seemed to be related to the patient's genotype. Thus, whereas none of the patients with K1 mutations benefited from treatment with retinoids, all except one of the patients with K10 mutations gained from the therapy. Examples of the good effects of retinoids in K10 patients are shown in Fig. 2. The only exception to the rule was patient 8 who, despite initial improvement of hyperkeratosis when using topical tretinoin, subsequently developed blisters around his ankles and feet when treated with oral acitretin. Both patients with nevoid EHK responded well to topical tretinoin, showing markedly reduced hyperkeratosis and minor skin irritation, with the exception of in the groin.

Retinoid therapy alters the keratin gene expression in lesional skin but there is no distinct relation to clinical effects

Shave biopsies were obtained before and after retinoid treatment in 9 patients with generalized EHK, of whom 4 carried K10 mutations and responded well to therapy. The remaining patients carried a K1 mutation and were regarded as non-responders in the clinical trial (see above). Using real-time PCR, the mRNA expression of 5 different keratins (K1, K2e, K4, K10, and K13), cellular retinoic acid-binding protein 2 (CRABP2) and a housekeeping gene (ß-actin) was analysed

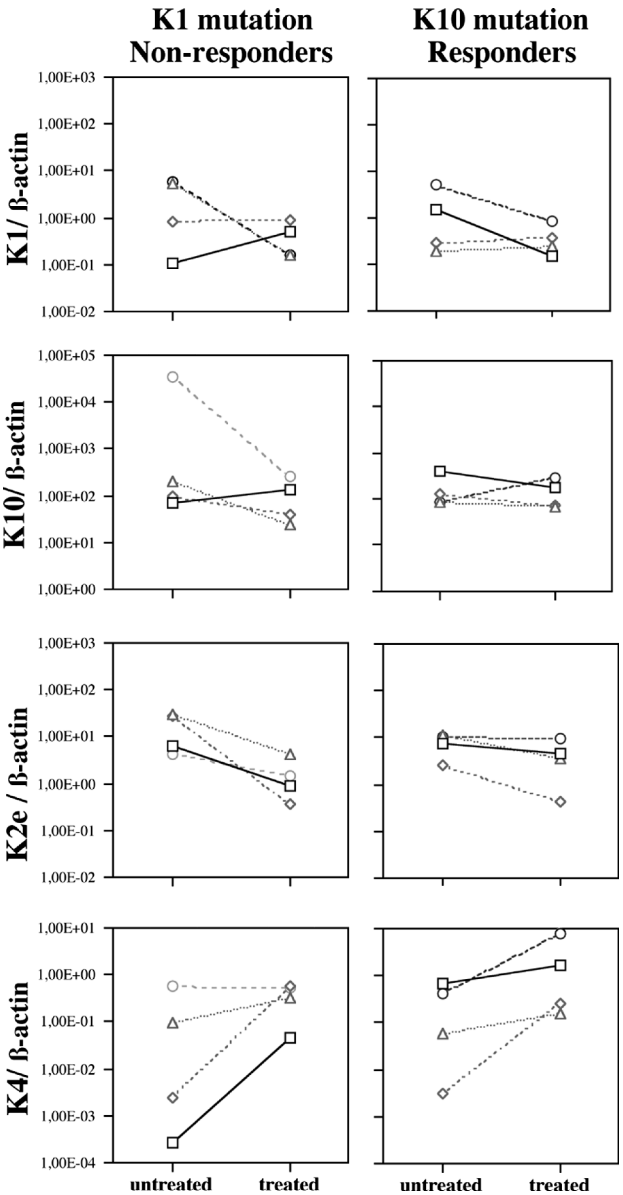

Fig. 3. mRNA expression of K1, K10, K2e and K4 (as a ratio to ß-actin) before (untreated) and after retinoid therapy (treated) in patients ($n = 8$) with either *KRT1* or *KRT10* mutations. Patient are indicated by the following symbols: ◇ = 1, ○ = 2, △ = 3 and □ = 5, non-responders; ◇ = 8 (tretinoin), ○ = 12, △ = 10 and □ = 9, responders.

in all samples. Fig. 3 shows the K1, K10, K2e and K4 results (expressed in relation to ß-actin mRNA) for the two groups of patients (one of the K1 patients had to be excluded for technical reasons). It can be seen that K1 was markedly (10–100-fold) down-regulated by retinoid therapy in two patients in each of the two groups, whereas K10 was markedly down-regulated in only one of the non-responders in the K1 group. The other patients showed little or no change in the K1/K10 transcript levels, which argues against the hypothesized correlation between retinoid responsiveness and down-regulation of the mutated keratin gene. On the other hand, the K2e transcript decreased significantly after retinoid therapy in the group as whole ($p = 0.0078$; $n = 8$). Interestingly, K2e was clearly down-regulated in all non-responders (K1 mutation), but in only one of the responders, possibly indicating that a reduction of K2e protein by retinoids may further aggravate the IF instability in keratinocytes expressing a K1 mutation. K1 and K2e are both type II keratins and may replace one another when binding to K10. A down-regulation of K2e mRNA by retinoids has been previously described in normal skin (20).

In keeping with our previous observations in normal epidermis (20), K4 was up-regulated by retinoids in all but one patient ($p = 0.0156$) with no clear differences between the groups. Considerable amounts of K4 mRNA were also present in untreated lesional skin, in contrast to the situation in normal skin (20). This was also the case with mRNA for CRABP2 and K13, which only marginally or inconsistently increased during retinoid therapy (data not shown).

Immunohistochemical visualization of keratin expression before and after retinoid therapy

Using specific antibodies against K1, K2e, K4, and K10, keratin expression in the epidermis was examined in biopsies taken before and after therapy in 8 patients (4 responders and 4 non-responders). Before therapy, K1, K2e, and K10 were uniformly present in suprabasal cell layers (not shown) whereas K4 was only found in stratum granulosum of some patients, notably in the "responders" (Fig. 4). After therapy, the only conspicuous change in keratin expression was increased staining for K4. This was most apparent in patients carrying a *KRT10* mutation (responders) who showed a band-like staining in the upper epidermis (Fig. 4) similar to that reported in normal, retinoid-treated skin (20). In contrast, only two of the non-responders (nos. 1 and 5) showed faint K4 staining in the upper epidermis after retinoid treatment. The other two K1 patients (nos. 3 and 5) remained negative for K4.

DISCUSSION

We have reason to believe that our study represents virtually all patients with generalized EHK living in Sweden and Norway, with a total population of about 13 million people. If we add one male in his 20s who was unavailable for clinical trials, this gives an EHK prevalence of about 1 per million, clearly lower than previous indirect estimates (1). The explanation is obviously the lack of milder multigeneration EHK families and the low fertility of the more severe dominant mutants. However, if cases of nevoid EHK are included, the prevalence will be higher, especially as the two cases identified in this study are probably a gross underestimate of the true occurrence of genetic mosaicism for *KRT1* and *KRT10*, since milder forms are unlikely to be referred to dermatologists and thus never correctly diagnosed.

Surprisingly, many of our patients (73%) were below the age of 20 and 87% probably represent *de novo* mutations. Previous investigators have found about 50% *de novo* mutations (1).

The clinical features of EHK in Scandinavia are variable, ranging from a mild flexural involvement to a disabling disease with generalized hyperkeratosis, erythema and erosions. As many as 4 of the patients had chronic onychomycosis that was readily cured by oral antifungal agents. An intrinsic propensity for mycosis in EHK patients might be related to the hyperkeratosis *per se*, but may also reflect a general barrier

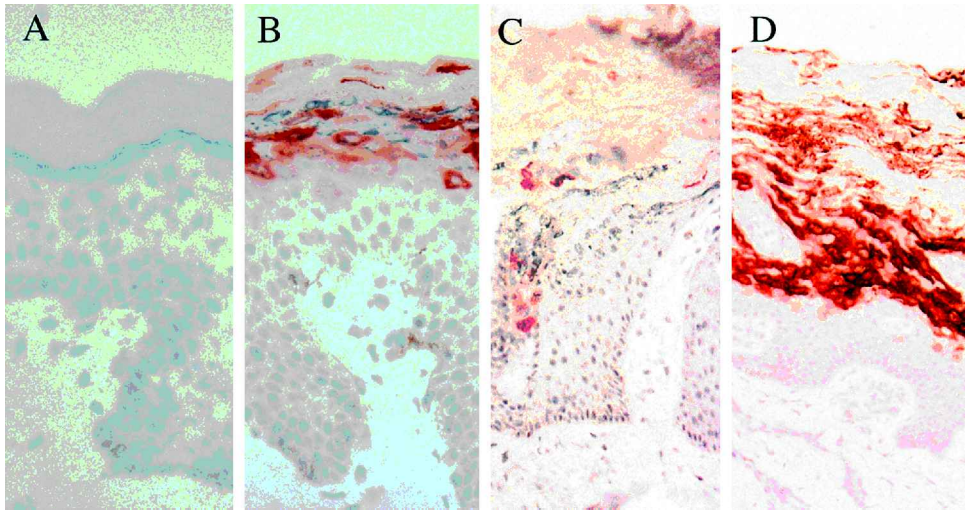

Fig. 4. Retinoids increase K4 expression in the upper epidermis independent of the therapeutic results. (A) and (B) show the immunohistochemistry of skin biopsies obtained from a non-responder (no. 1) before and after treatment with topical tazarotene for 4 weeks. (C) and (D) show the corresponding results in a responder (no. 10) before and after treatment with oral acitretin for 4 weeks. (Note the focal staining for K4 before treatment followed by massive up-regulation of the staining after therapy).

function problem. Interestingly, K1 and K10 are also expressed in the nail folds (34), which may be crucial to a defence against invading microbes.

Although the erythema tended to be more severe in patients with K10 mutations, there was no clear-cut correlation between the clinical features and the genotype, except for the predominance of PPKD in the K1 group and a better therapeutic response to retinoids in the K10 group. The association of *KRT1* mutations with PPKD, although not a strict one, has also been noted by others (3). The almost invariable absence of PPKD in K10 patients is probably explained by the co-expression in palmoplantar epidermis of an alternative type I keratin, K9, which might compensate for mutated K10 molecules in the formation of keratin heterodimers. However, one of our patients (no. 7) with a K10 point mutation had a moderate PPKD, which confirms previous exceptions to the rule (14, 15).

The response of EHK to retinoid therapy is notoriously difficult to predict (16, 17, 35, 36). A low dosage regime is recommended because the keratolytic and irritant effects of retinoids can be detrimental in EHK. Although oral treatment is preferred in generalized EHK, more localized forms of the disease, including nevoid EHK, may respond well to topical retinoids. We found that oral acitretin and topical retinoids were particularly effective in patients with *KRT10* mutations. Topical retinoid therapy must, however, be individually tailored to different parts of the body, with less frequent applications in flexural areas and on the face. Prescribed in this way, we find that tazarotene is especially useful in EHK, but some patients prefer tretinoin and adapalene (AV, unpublished observations).

The mechanism of action of retinoids in EHK is far from clear. Although these compounds may suppress epidermal differentiation and hyperkeratinization via binding to specific nuclear receptors (37, 38), this should not automatically improve the cytoskeletal stability in keratinocytes expressing K1 or K10 mutations. We therefore initially hypothesized that retinoids might work by down-regulating the mutated keratin in EHK and reciprocally up-regulating other wild-type keratins that might mitigate the negative effects of the mutation on IF polymerization. According to this hypothesis, the patients who respond best to retinoids should also have the largest down-regulation of the mutated keratin gene. However, our studies of keratin mRNA levels in a limited series of paired skin samples obtained before and after therapy did not show such a clear-cut difference in K1 and K10 expression between patients, with good and poor response, respectively. But, the poor responders had a marked down-regulation of the K2e gene, which may be detrimental to IF stability if there was a pre-existing, partial compensation of the K1 mutation by K2e co-expressed in the upper layers of the epidermis. K10 and K2e, on the other hand, cannot substitute for one another, but can form heterodimers instead (39). Accordingly, the different responses in K1 and K10 patients may be more related to low tolerability to retinoids in the former group than to a specific down-regulation of the mutated keratin gene in the latter group. A dose-dependent down-regulation of K2e by retinoids has been previously demonstrated in healthy skin exposed to topical tretinoin and tazarotene (20). It would have been interesting to study the dose-dependent reduction in different keratin mRNAs also in patients with EHK, but this was not possible in practice. It is interesting to note, in this context, that topical retinoids are especially effective in ichthyosis bullosa of Siemens, which can be considered a superficial variant of EHK due to K2e mutations (17, 40). We believe that such patients may present a better example of how retinoids can act by silencing a mutated keratin gene. Experiments are in progress to investigate this possibility, taking special care to ensure that the shave biopsy technique does not produce artefactual changes in the keratin expression owing to an altered cellular composition in samples obtained after therapy.

Another conspicuous finding in retinoid-treated EHK patients was the up-regulation of K4, especially in the good responders with *KRT10* mutations. This change in keratin expression was evident both by real-time PCR and on immunohistochemistry using specific keratin antibodies. Although K4 is expressed in the upper epidermis after retinoid treatment,

this keratin is unlikely to participate to a significant degree in the IF polymerization with K10, mainly because of its much lower abundance. Up-regulation of K4 mRNA is, however, a good marker for retinoid activity in normal epidermis (20) and our results show that 7 out of 8 patients had this up-regulation in lesional skin after oral or topical retinoid therapy. The reason why more patients in the K10 group had demonstrable K4 protein in the epidermis both before, and especially after retinoid therapy, is presently unclear.

In conclusion, our study emphasizes several interesting features of EHK, such as the almost dichotomous association of PPKD with K1 mutations, the propensity to onychomycosis in some patients and the beneficial effect of retinoids only in patients with K10 mutations, which might help to explain the mechanism of actions of retinoids in EHK and related diseases.

ACKNOWLEDGEMENTS

We thank all our patients for their friendly collaboration. Grants from the Swedish Medical Research Council (project no. 71X-07133-17C) and the Welander and Finsen Foundations supported this study.

REFERENCES

1. Bale SJ, Compton JG, DiGiovanna JJ. Epidermolytic hyperkeratosis. Semin Dermatol 1993; 12: 202–209.
2. Gedde-Dahl T Jr, Anton-Lamprecht I. Epidermolysis bullosa. In: Rimoin DLCJ, Pyeritz RE, eds. 1. Principles and practice of medical genetics. Edinburgh, London, Melbourne, New York: Churchill, 1996: 1225–1278 (Tab. 57.4).
3. DiGiovanna JJ, Bale SJ. Clinical heterogeneity in epidermolytic hyperkeratosis. Arch Dermatol 1994; 130: 1026–1035.
4. Moss C. Cytogenetic and molecular evidence for cutaneous mosaicism: the ectodermal origin of Blaschko lines. Am J Med Genet 1999; 85: 330–333.
5. Nazzaro V, Ermacora E, Santucci B, Caputo R. Epidermolytic hyperkeratosis: generalized form in children from parents with systematized linear form. Br J Dermatol 1990; 122: 417–422.
6. Anton-Lamprecht I. Genetically induced abnormalities of epidermal differentiation and ultrastructure in ichthyoses and epidermolyses: pathogenesis, heterogeneity, fetal manifestation, and prenatal diagnosis. J Invest Dermatol 1983; 81: 149s–156s.
7. Bowden PE. Keratins and other epidermal proteins. In: Priestley GC, ed. Molecular aspects of dermatology. Chichester: John Wiley & Sons, 1993: 19–54.
8. Chipev CC, Korge BP, Markova N, Bale SJ, DiGiovanna JJ, Compton JG, *et al.* A leucine–proline mutation in the H1 subdomain of keratin 1 causes epidermolytic hyperkeratosis. Cell 1992; 70: 821–828.
9. Rothnagel JA. Analysis, diagnosis and molecular genetics of keratin disorders. Curr Opin Dermatol 1995; 211–218.
10. Ishida-Yamamoto A, McGrath JA, Judge MR, Leigh IM, Lane EB, Eady RA. Selective involvement of keratins K1 and K10 in the cytoskeletal abnormality of epidermolytic hyperkeratosis (bullous congenital ichthyosiform erythroderma). J Invest Dermatol 1992; 99: 19–26.
11. Fuchs E. The molecular biology of epidermolysis bullosa simplex. In: Fine JDBE, McGuire J, Moshell A, eds. Epidermolysis bullosa. Clinical, epidemiologic, and laboratory advances and the findings of the National Epidermolysis Bullosa Registry. Baltimore: Johns Hopkins University Press, 1999: 280–299.
12. Bonifas JM, Matsumura K, Chen MA, Berth-Jones J, Hutchison PE, Zloczower M, *et al.* Mutations of keratin 9 in two families with palmoplantar epidermolytic hyperkeratosis. J Invest Dermatol 1994; 103: 474–477.
13. Basarab T, Smith FJ, Jolliffe VM, McLean WH, Neill S, Rustin MH, *et al.* Ichthyosis bullosa of Siemens: report of a family with evidence of a keratin 2e mutation, and a review of the literature. Br J Dermatol 1999; 140: 689–695.
14. Arin MJ, Longley MA, Anton-Lamprecht I, Kurze G, Huber M, Hohl D, *et al.* A novel substitution in keratin 10 in epidermolytic hyperkeratosis. J Invest Dermatol 1999; 112: 506–508.
15. McLean WH, Morley SM, Higgins C, Bowden PE, White M, Leigh IM, *et al.* Novel and recurrent mutations in keratin 10 causing bullous congenital ichthyosiform erythroderma. Exp Dermatol 1999; 8: 120–123.
16. Ruiz-Maldonado R, Tamayo-Sanchez L, Orozco-Covarrubias ML. The use of retinoids in the pediatric patient. Dermatol Clin 1998; 16: 553–569.
17. Hofmann B, Stege H, Ruzicka T, Lehmann P. Effect of topical tazarotene in the treatment of congenital ichthyoses. Br J Dermatol 1999; 141: 642–646.
18. Rosenthal DS, Griffiths CE, Yuspa SH, Roop DR, Voorhees JJ. Acute or chronic topical retinoic acid treatment of human skin *in vivo* alters the expression of epidermal transglutaminase, loricrin, involucrin, filaggrin, and keratins 6 and 13 but not keratins 1, 10, and 14 (published erratum appears in J Invest Dermatol 1992; 99: 145). J Invest Dermatol 1992; 98: 343–350.
19. Schon M, Rheinwald JG. A limited role for retinoic acid and retinoic acid receptors RAR alpha and RAR beta in regulating keratin 19 expression and keratinization in oral and epidermal keratinocytes. J Invest Dermatol 1996; 107: 428–438.
20. Virtanen M, Törmä H, Vahlquist A. Keratin 4 upregulation by retinoic acid *in vivo*: a sensitive marker for retinoid bioactivity in human epidermis. J Invest Dermatol 2000; 114: 487–93.
21. Gedde-Dahl T Jr. Epidermolysis bullosa. A clinical, genetic and epidemiological study. Baltimore: The Johns Hopkins Press, 1971: 34–36.
22. Gedde-Dahl T Jr. Epidermolysis bullosa simplex (intraepidermal epidermolysis bullosa) and allied conditions. In: Wojnarowska F, ed. Management of the blistering diseases. London: Chapman and Hall, 1990: 189–211.
23. Anton-Lamprecht I. The skin. In: Papadimitriou JM, Spagnolo DV, eds. Diagnostic ultrastructure of non-neoplastic diseases. Edinburgh: Churchill Livingstone, 1992: 459–550.
24. Vahlquist A. Vitamin A in human skin: II. J Invest Dermatol 1982; 79: 89–93.
25. Jones DO, Watts C, Mills G, Sharpe G, Marks R, Bowden PE. A new keratin 2e mutation in ichthyosis bullosa of Siemens. J Invest Dermatol 1997; 108: 354–356
26. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. Genome Res 1996; 6: 986–994.
27. Törmä H, Karlsson T, Michaelsson G, Rollman O, Vahlquist A. Decreased mRNA levels of retinoic acid receptor alpha, retinoid X receptor alpha and thyroid hormone receptor alpha in lesional psoriatic skin. Acta Derm Venereol 2000; 80: 4–9.
28. Leigh IM, Purkis PE, Whitehead P, Lane EB. Monospecific monoclonal antibodies to keratin 1 carboxy terminal (synthetic peptide) and to keratin 10 as markers of epidermal differentiation. Br J Dermatol 1993; 129: 110–119.
29. van Muijen GN, Ruiter DJ, Franke WW, Achtstatter T, Haasnoot WH, Ponec M, *et al.* Cell type heterogeneity of cytokeratin expression in complex epithelia and carcinomas as demonstrated by monoclonal antibodies specific for cytokeratins nos. 4 and 13. Exp Cell Res 1986; 162: 97–113.
30. Perkins W, Campbell I, Leigh IM, MacKie RM. Keratin expression in normal skin and epidermal neoplasms demonstrated by a panel of monoclonal antibodies. J Cutan Pathol 1992; 19: 476–482.
31. Rothnagel JA, Dominey AM, Dempsey LD, Longley MA, Greenhalgh DA, Gagne TA, *et al.* Mutations in the rod domains of keratins 1 and 10 in epidermolytic hyperkeratosis. Science 1992; 257: 1128–1130.

32. Chipev CC, Yang JM, DiGiovanna JJ, Steinert PM, Marekov L, Compton JG, *et al.* Preferential sites in keratin 10 that are mutated in epidermolytic hyperkeratosis. Am J Hum Genet 1994; 54: 179–190.
33. McLean WH, Eady RA, Dopping-Hepenstal PJ, McMillan JR, Leigh IM, Navsaria HA, *et al.* Mutations in the rod 1A domain of keratins 1 and 10 in bullous congenital ichthyosiform erythroderma (BCIE). J Invest Dermatol 1994; 102: 24–30.
34. De Berker D, Wojnarowska F, Sviland L, Westgate GE, Dawber RP, Leigh IM. Keratin expression in the normal nail unit: markers of regional differentiation. Br J Dermatol 2000; 142: 89–96.
35. Happle R, van de Kerkhof PC, Traupe H. Retinoids in disorders of keratinization: their use in adults. Dermatologica 1987; 175: 107–124.
36. Blanchet-Bardon C, Nazzaro V, Rognin C, Geiger JM, Puissant A. Acitretin in the treatment of severe disorders of keratinization. Results of an open study. J Am Acad Dermatol 1991; 24: 982–986.
37. Tomic M, Jiang CK, Epstein HS, Freedberg IM, Samuels HH, Blumenberg M. Nuclear receptors for retinoic acid and thyroid hormone regulate transcription of keratin genes. Cell Regul 1990; 1: 965–973.
38. Giguere V. Retinoic acid receptors and cellular retinoid binding proteins: complex interplay in retinoid signaling. Endocr Rev 1994; 15: 61–79.
39. Reichelt J, Bauer C, Porter R, Lane E, Magin V. Out of balance: consequences of a partial keratin 10 knockout. J Cell Sci 1997; 110: 2175–2186.
40. Steijlen PM, van Dooren-Greebe RJ, Happle R, Van de Kerkhof PC. Ichthyosis bullosa of Siemens responds well to low-dosage oral retinoids. Br J Dermatol 1991; 125: 469–471.