Generalized and Naevoid Epidermolytic Ichthyosis in Denmark: Clinical and Mutational Findings

Bygum, Anette; Virtanen, Marie; Brandrup, Flemming; Gånemo, Agneta; Sommerlund, Mette; Strauss, Gitte; Vahlquist, Anders

Published in:
Acta Dermato-Venereologica

DOI:
10.2340/00015555-1447

Published: 2013-01-01

Citation for published version (APA):
CLINICAL REPORT

Generalized and Naevoid Epidermolytic Ichthyosis in Denmark: Clinical and Mutational Findings*

Anette BYGUM1, Marie VIRTANEN2, Flemming BRANDRUP1, Agneta GÅNEMO3, Mette SOMMERLUND4, Gitte STRAUSS3 and Anders VAHLQUIST2

1Department of Dermatology and Allergy Centre, University Hospital, Odense, Denmark, Departments of 2Dermatology, University Hospital, Uppsala and 3Institute of Clinical Research in Malmö, Lund University, Skåne University Hospital, Malmö, Sweden, 4Department of Dermato-Venereology, Aarhus University Hospital, Aarhus and 5Department of Dermato-Allergology, University Hospital of Copenhagen, Hellerup, Denmark

A Danish–Swedish collaboration was established to identify and classify a Danish cohort of patients with epidermolytic ichthyosis, also known as epidermolytic hyperkeratosis. Patients were recruited from 5 dermatology departments in Denmark, and data were obtained using a structured questionnaire and a systematic examination together with photographs, histopathological descriptions and blood samples for mutational analysis. Sixteen patients from 12 families with generalized or naevoid epidermolytic ichthyosis and ichthyosis bullosa of Siemens were identified. Five families had mutations in K1 and 6 families had mutations in K10. Nine patients had been treated with systemic retinoids (etretinate, acitretin, isotretinoin or alitretinoin), but only 3 patients had acceptable treatment responses and chose to continue therapy. In conclusion epidermolytic ichthyosis is a rare disease with a prevalence of approximately 1 in 350,000 in Denmark and a high percentage of de novo mutations (75%). We identified 4 novel disease-causing mutations. Key words: genomic DNA sequencing; epidermolytic ichthyosis; epidermolytic hyperkeratosis; phenotypic variation.

Accepted Jun 4, 2012; Epub ahead of print Aug 29, 2012

Anette Bygum, Department of Dermatology and Allergy Centre, Odense University Hospital, DK-5000 Odense C, Denmark. E-mail: anette.bygum@ouh.regionsyddanmark.dk

Epidermolytic ichthyosis (EI; OMIM 113800), also referred to as epidermolytic hyperkeratosis or bullous congenital ichthyosiform erythroderma, is a rare keratinization disorder caused by dominant negative mutations in keratin genes KRT1 and KRT10, leading to an unstable cytoskeleton of epithelial keratinocytes and epidermal hyperkeratosis (1). In contrast to most other ichthyoses, the histopathological picture of EI is distinctive, with hyperkeratosis, acanthosis and characteristic clumping of tonofilaments, intracellular vacuolization and intra-epidermal blisters.

Children with generalized EI are usually born with erythroderma, extensive blistering and denuded skin that later changes into a more ichthyotic phenotype with rippled hyperkeratosis mainly on flexural surfaces. The bullous component gradually becomes less prominent, but patients may continuously have problems with blistering, especially after trauma or skin infections. In addition, some patients also have palmoplantar keratoderma (PPK).

The naevoid form of EI, linear epidermolytic ichthyosis (LEI), occurs as epidermal naevi with cytoskeletal abnormalities of epidermolytic hyperkeratosis representing somatic mosaicism. In case of gonadal mosaicism the offspring of the patient may develop full-blown EI. Therefore epidermolytic naevi are included in the most recent classification of keratinopathic ichthyosis (1).

Ichthyosis bullosa of Siemens (IBS) resembles EI, but has a milder phenotype with less hyperkeratosis, more superficial blistering and no PPK. Mutations in K2, a keratin expressed later during differentiation, cause IBS (1, 2).

In this study we describe the clinical subtypes and mutational findings in a cohort of 16 Danish patients with EI/IBS.

PATIENTS AND METHODS

Patients and samples

Patients were identified through a national study of congenital ichthyosis undertaken in 2004 to 2006 by 3 of the authors (AB, AG and FB). In order to trace all patients seen in Denmark from 1984 to 2003 with a diagnosis of congenital ichthyosis, contact was established with all 6 departments of dermatology. Patients with a clinical phenotype consistent with EI or IBS were included in this study.

Written informed consent was obtained from all patients according to approval by the Danish ethics committee (jr. no. VF20040178).

A complete medical history was obtained from all patients using a structured questionnaire. In order to obtain a uniform classification, patients were systematically examined by at least 2 of 3 authors (AB, AG and FB) together with a local dermatologist to give the best clinical classification. Photographs were taken, previous histopathological descriptions were reviewed

© 2013 The Authors. doi: 10.2340/00015555-1447
Journal Compilation © 2013 Acta Dermato-Venereologica. ISSN 0001-5555

Acta Derm Venereol 93
and new skin biopsies for light or electron microscopy were taken in case of diagnostic doubt. Blood samples were obtained for DNA extraction and mutational analysis in KRT1 and KRT10. In patients where no pathogenic mutation was identified, KRT2 was also examined.

Genetic analyses
Genomic DNA (gDNA) was extracted from 2 ml ethylenediaminetetraacetic acid (EDTA) blood using E.Z.N.A. MidiKit’s (Omega Bio-Tek Norcross, GA, USA). 100 ng of gDNA was used for PCR amplification of all KRT1 exons and the hot-spot regions in KRT2 and KRT10. The DNA was added to a mix of 1×Taq buffer, 0.2 mM of each dNTP, forward and reverse primers (10 µM), 2.5 mM MgCl2, and 0.05 U Taq polymerase (Applied Biosystems, Stockholm, Sweden) in a total volume of 50 µl.

All patients were initially screened for mutations in hotspot regions of KRT1 and KRT10 by denaturing high-performance liquid chromatography (HPLC) (Transgenomic, Omaha, USA). Before the analysis the PCR fragments were partially denatured by decreasing the temperature from 94°C to 40°C during 30 min. An aliquot of PCR fragments was purified by using GTXtm PCR DNA and GelBand purification Kit (GE Healthcare, Uppsala, Sweden) followed by DNA sequencing. Automated sequencing was done using Big Dye Terminator kit and analysed on an ABIPrism 377 DNA sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA).

RESULTS
A total of 16 patients from 12 families fulfilled the inclusion criteria for EI/IBS. The symptoms varied in intensity from mild to severe and in 8 of the patients an associated PPK was observed (Table I). For 2 families, 3 affected members were found in each family. In one of the families (number 5) an affected mother and 2 children with EI were found (Fig. 1), and in the other family (number 6) EI was observed in 3 generations, starting with a naevoid lesion in the grandfather (Fig. S1; available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1447).

Ten different disease-causing mutations were detected in 11 of the 12 families (Table I). Mutations in K1 were found in 5 families, and 6 families had mutations in K10. Six of these mutations have been described previously (3–8). The remaining mutations are novel: 3 point mutations with a non-conservative amino acid substitution (family numbers 1, 2 and 11) and 1 in-frame deletion of 30 bp in KRT1 exon 2 (family number 5). Although a substitution of amino acid 486 in K1 has been identified previously (9, 10), our family number 1 has a different point mutation, resulting in a different amino acid change in this position. The other 2 point mutations (family numbers 2 and 11) are located near previously reported disease-causing mutations in KRT1 or KRT10 (4, 11). In family number 5, an in-frame deletion in KRT1 (p.His225_Phe234del) was found, which removes the last part of the L1 region and the beginning of region 1B of K1. There are no previous reports of such a mutation, but a point mutation causing PPK has been described in this region (12). The mother in family number 5 was born with erythroderma and bullae and later developed rippled hyperkeratosis in the flexural areas (Fig. 1A). She had a persistent tendency to blistering and erosions on pressure-prone areas (Fig. 1B). She gave birth to 2 children with mild erythroderma and blistering at birth changing to more hyperkeratotic skin lesions within the first year of life. They all had white spongy soles and palms at birth (Fig. 1C), later developing into PPK.

We could not identify a mutation explaining the naevoid skin lesions in the father of patient IX with EI due to KRT10 c.1333G>A. However, only blood leukocytes were available for analysis. Patient XVI, clinically and histopathologically diagnosed as IBS (Fig. S2; available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1447), had no identifiable KRT1, KRT2 or KRT10 mutation, except for a previously reported polymorphism, an 18-bp deletion in exon 1 of KRT2 (13).

DISCUSSION
We identified 16 Danish patients with generalized or naevoid EI and IBS, corresponding to a prevalence of approximately 1 in 350,000 in Denmark. This is 3 times higher than the estimated prevalence in other Scandinavian countries (14), but similar to prevalence estimates of 1 in 100,000–300,000 reported in the literature (15, 16).

The patients with generalized EI typically had disease onset from birth, with erythroderma and blisters with denuded skin areas (Fig. S3; available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1447). Some patients subsequently developed mild flexural involvement, while others had generalized erythroderma with blistering/erosion tendency continuing in adulthood, but overall symptoms improved with age. A few patients had very severe PPK with contractures (Fig. S4; available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1447).

In this study, the percentage of de novo mutations was very high; 75% compared with a previously reported spontaneous mutation rate of about 50% (15–18). The majority of reported mutations were heterozygous missense mutations. When mutations are located at the conserved helix boundary motifs, the helix initiation or termination peptides and the non-helical H1 domain of K1 and K10, they will result in severe EI (17, 19). In family number 5 with a moderate EI phenotype, a partial deletion of L1 and 1B region in K1 was identified. Insertion/deletion mutations and splice site-defects or dinucleotide alterations in KRT10 have also been described and more than 100 mutations in KRT1 and KRT10 have already been reported (17, www.interfil.org).
All 6 patients with K1 mutations had PPK, while only 1 out of 6 patients with K10 mutation had PPK. This is in accordance with earlier published studies (15, 17, 20). The absence of PPK in patients with mutation in K10 is explainable by palmoplantar expression of K9, which is a functional substitute for K10. In our study 2 unrelated females (patient XIII and XIV) had the same mutation in K10, but only one of them had a mild PPK, suggesting that other genetic or environmental factors also influence the phenotype.

LEI is manifested as streaks of hyperkeratosis following the lines of Blaschko. LEI is caused by somatic mutations in K10, or rarely K1, arising post-zygotically during embryogenesis (21–23). Mutations have been demonstrated in keratinocytes from affected skin, whereas the mutations are absent in non-lesional skin and blood

<table>
<thead>
<tr>
<th>Pat. No.</th>
<th>Diagnosis</th>
<th>Sex/age (years)</th>
<th>Familial (family No.)</th>
<th>Mutation</th>
<th>Protein</th>
<th>Clinical presentation</th>
<th>At birth</th>
<th>At inclusion in study</th>
<th>Histology showing EHK</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>EI</td>
<td>M/34</td>
<td>No (1)</td>
<td>KRT1 c.1457T&gt;A</td>
<td>p.Leu486Gln</td>
<td>Blistering Erosions</td>
<td>Few erosions Hyperkeratosis PPK</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>II</td>
<td>EI</td>
<td>M/20</td>
<td>No (2)</td>
<td>KRT1 c.1424T&gt;C</td>
<td>p.Leu475Proa</td>
<td>Blistering Erosions</td>
<td>Focal blisters Hyperkeratosis PPK</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>III</td>
<td>EI</td>
<td>M/27</td>
<td>No (3)</td>
<td>KRT1 c.1445A&gt;G</td>
<td>p.Tyr482Cys</td>
<td>Erythroderma Blistering</td>
<td>Erythroderma Blistering Hyperkeratosis PPK</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>IV</td>
<td>EI</td>
<td>M/40</td>
<td>No (4)</td>
<td>KRT1 c.1465G&gt;A</td>
<td>p.Glu489Lys</td>
<td>Blistering Erosions</td>
<td>Focal blisters EHK PPK</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>V</td>
<td>EI</td>
<td>F/33</td>
<td>Yes (5)</td>
<td>KRT1 c.673-702 del 30</td>
<td>p.His225_Phe 234del*</td>
<td>Erythroderma Blistering</td>
<td>Focal blisters Hyperkeratosis PPK</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>VI</td>
<td>EI</td>
<td>M/3</td>
<td>Yes (5)</td>
<td>KRT1 c.673-702 del 30</td>
<td>p.His225_Phe 234del</td>
<td>Erythroderma Blistering</td>
<td>Focal blisters Hyperkeratosis PPK</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>VII</td>
<td>EI</td>
<td>F/1</td>
<td>Yes (5)</td>
<td>NT</td>
<td>Erythroderma Blistering</td>
<td>Erythroderma Blistering Hyperkeratosis PPK</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td>LEI</td>
<td>M/65</td>
<td>Yes (6)</td>
<td>No mutation found</td>
<td>Erythroderma Blistering</td>
<td>Striate Hyperkeratosis</td>
<td>Yes</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>IX</td>
<td>EI</td>
<td>F/38</td>
<td>Yes (6)</td>
<td>KRT10 c.1333G&gt;A</td>
<td>p.Glu445Lys</td>
<td>Erythroderma Erosions</td>
<td>Focal blisters Hyperkeratosis Erosions</td>
<td>Yes</td>
<td>NT</td>
</tr>
<tr>
<td>X</td>
<td>EI</td>
<td>F/6</td>
<td>Yes (6)</td>
<td>NT</td>
<td>Erythroderma Erosions</td>
<td>Focal blisters EHK</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>XI</td>
<td>EI</td>
<td>M/18</td>
<td>No (7)</td>
<td>KRT10 c.466C&gt;T</td>
<td>p.Arg156Cys</td>
<td>Erythroderma Erosions</td>
<td>Focal blisters Hyperkeratosis Erosions</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>XII</td>
<td>EI</td>
<td>M/3</td>
<td>No (8)</td>
<td>KRT10 c.466C&gt;A</td>
<td>p.Arg156Serb</td>
<td>Erythroderma Erosions</td>
<td>Focal blisters Hyperkeratosis Erosions</td>
<td>Yes</td>
<td>NT</td>
</tr>
<tr>
<td>XIII</td>
<td>EI</td>
<td>F/44</td>
<td>No (9)</td>
<td>KRT10 c.482T&gt;C</td>
<td>p.Leu161Ser</td>
<td>Erythroderma Blistering</td>
<td>Focal blisters Hyperkeratosis PPK</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>XIV</td>
<td>EI</td>
<td>F/38</td>
<td>No (10)</td>
<td>KRT10 c.482T&gt;C</td>
<td>p.Leu161Serc</td>
<td>Erythroderma Blistering</td>
<td>Erythroderma Blistering Hyperkeratosis</td>
<td>Yes</td>
<td>NT</td>
</tr>
<tr>
<td>XV</td>
<td>EI</td>
<td>M/42</td>
<td>No (11)</td>
<td>KRT10 c.452A&gt;C</td>
<td>p.Gln151Pro</td>
<td>Blistering Erosions</td>
<td>Blistering Hyperkeratosis</td>
<td>Yes</td>
<td>NT</td>
</tr>
<tr>
<td>XVI</td>
<td>IBS</td>
<td>F/29</td>
<td>Adopted (12)</td>
<td>No mutation found</td>
<td>Unknown</td>
<td>Hyperkeratosis</td>
<td>Hyperkeratosis</td>
<td>Yes</td>
<td>NT</td>
</tr>
</tbody>
</table>

*aNo such mutation found in 50 controls.
*bAnalysed at the Department of Clinical Genetics, Aarhus University Hospital, Denmark.
*cAnalysed by Dr Paul Bowden, UWCM, Cardiff, UK.

EHK: epidermolytic hyperkeratosis; LM: light microscopy; EM: electron microscopy; LEI: linear epidermolytic ichthyosis; IBS: ichthyosis bullosa of Siemens; PPK: palmoplantar keratoderma; ND: not diagnostic; NT: not tested.
leukocytes (17). There seems to be a correlation between widespread LEI and the risk of germ-line transmission (22). The patient with LEI (VIII) had rippled and hyperkeratotic streaks on his right-side extremities, as well as thickened macerated skin in his right axilla (Fig. S1). Blood leukocytes, but no keratinocytes from affected skin, were available for molecular genetic analysis; no mutations in K1 or K10 could be detected.

We could not identify any mutation in patient XVI. The patient was adopted from Korea, and had clinical features of IBS (Fig. S2). Mutations outside the hotspot region of KRT2 are possible.

The treatment options for EI are less than satisfactory. Retinoids are used in more severe cases of EI, but are usually moderately effective and carry a risk of side-effects when given systemically (24). Nine of our patients had been treated with systemic retinoids (etretinate, acitretin, isotretinoin or alitretinoin). Of these, 3 patients (patient I with K1 mutation and patients XIV and XV with K10 mutations) had acceptable treatment responses and are continuously on retinoid therapy. However, in another 2 patients (II and III) with K1 mutations skin symptoms such as blistering and erosions worsened during treatment; presumably they are more vulnerable to a retinoid down-regulation of K2, which may otherwise, to some extent, compensate for a mutated or missing K1 protein (14).

ACKNOWLEDGEMENTS

We wish to thank Johannes Kristensen for his help in arranging patient consultations at Bispebjerg Hospital. This study was supported by grants from Kgl. Hofbuntmager Aage Bangs Fond and from the Welander and Finsen Foundations.

REFERENCES


