Palmoplantar keratoderma of the Gamborg-Nielsen type (PPK-GN) or PPK of Norrbotten recessive type (OMIM 244850) was described as a diffuse PPK with autosomal recessive inheritance in patients from the northernmost county of Sweden by Gamborg Nielsen in 1985 (1). PPK-GN manifests with pronounced, often transgressive hyperkeratosis of palms and soles and an erythematous border next to the hyperkeratotic skin, tapered fingers are also observed. Mal de Meleda (MDM; OMIM 248300) or keratosis palmoplantaris transgradiens of Siemens is an autosomal recessive skin disorder first described in 1898 by Neumann (2) in patients from the island of Mljet (Meleda) in Dalmatia, Croatia. MDM is clinically characterised by symmetric transgressive PPK, and sometimes associated with lichenoid or keratotic plaques over joints, redness in the palms and soles, brachyactyly, cone-shaped fingers, pseudoainhum and nail abnormalities with pachyonychia. The progressive lesions can lead to reduced mobility of hands and feet because of contractions (2–8).

PPK-GN shows a similar, but less severe phenotype than most often seen in MDM, i.e. milder hyperkeratosis and no nail dystrophies or lichenoid plaques and no pachydermia or distant keratosis except for knuckle pads in some affected individuals (9). Histological features of PPK-GN are prominent hyperkeratosis and a transit region with a broadened granular layer. However, morphologically this transformation delay is less pronounced in PPK-GN than in MDM. Ultrastructural analyses also showed differences between MDM and PPK-GN in the affected epidermis suggesting the 2 disorders are not identical (9).

Linkage of MDM to the distal long arm of chromosome 8 was found in 1998 (3) and in 2001 it was established that individuals affected by MDM were found to carry mutations in the \textit{SLURP1} gene (10) that encodes secreted lymphocyte antigen 6/urokinase-type plasminogen activator receptor related protein-1. A Dutch patient with a MDM phenotype but lacking mutations in the \textit{SLURP1} gene was described in 2002 (11) and an individual with PPK of the Nagashima-type was also found to be devoid of mutations in the \textit{SLURP1} gene in 2008 (12). The Nagashima-type of keratoderma is characterised by a transgressive and non-progressive PPK inherited in an autosomal recessive manner but with a milder phenotype than seen in classical MDM (12).

The aim of this study was to investigate if 15 Swedish patients affected by PPK-GN carry mutations in the \textit{SLURP1} gene.

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The aim of this study was to investigate if 15 Swedish patients affected by PPK-GN carry mutations in the \textit{SLURP1} gene.
METHODS

Patients and material

Fifteen individuals with PPK from 9 families were included in this study. Fourteen were born in Norrbotten or Västerbotten, the 2 northernmost counties of Sweden, and one person was born in the south of Sweden. The presentation of PPK was very similar in all affected individuals and no keratoderma was present in any of the parents. These observations fit an autosomal recessive mode of inheritance that was verified by the observation of identical homozygous haplotypes in the 8qter region in affected individuals from 7 of the families (data not shown).

Clinical and ultrastructural characteristics of the patients from the northernmost counties of Sweden were previously described by Kasil et al. in 1990 (9).

Venous blood samples were obtained from the patients, their siblings and parents.

This study was approved by the ethics committees of Uppsala and Umeå University. Informed consent was obtained from all individuals involved.

Mutation screening and sequence alignments

Genomic DNA was extracted from peripheral blood using standard procedures. PCR primers to amplify the coding regions and flanking exon-intron sequences of SLURP1 (NM_020427.2) were designed by the use of Primer3 (13). PCR products were purified using EXOSAPIT (Fermentas, ThermoFisher). Bidirectional sequencing was performed using ABI Prism Big Dye termination V3.1 cycle sequencing kit and an ABI3130XL machine (Applied Biosystems). Sequences were analyzed with SeqScape V2.5 (Applied Biosystems). Exon 3 of SLURP1 was sequenced in 50 control samples (100 chromosomes) to evaluate the novel mutation found.

Multiple sequence alignments of the SLURP-1 protein were performed using the alignment programme ClustalW2 (European Bioinformatics Institute) and the Alamut software 2.0 (Interactive Biosoftware, France).

RESULTS

Mutation analyses and clinical characteristics

Fourteen of the 15 individuals affected by PPK-GN were homozygous for a previously described mutation in exon 1 of SLURP1, c.43T>C, that results in a change of tryptophan to arginine, p.Trp15Arg (14). The parents of the affected individuals were all heterozygous carriers of this mutation (data not shown). The 14 affected individuals showed a marked, sharply demarcated, and waxy, usually yellowish PPK. Hyperkeratosis was also present on the back of fingers and toes, especially distally and around the joints. The hyperkeratosis was surrounded by erythema and patches of thinner erythematous skin were often interspersed in the hyperkeratotic skin. Fingers were often tapered towards the tips and occasionally constricting bands were present on the fingers. Maceration between toes was frequent and in some cases interdigital maceration was also observed.

Complaints of painful fissures and foul-smelling hyperhidrosis among the patients were common. Most affected individuals had on-going or past histories of complicating fungal infections. Generally, the initial manifestation of PPK appeared during the first year of life, the skin changes then progressed until adulthood, sometimes with continuing progression during adult life but rare occasions of regression were also noted (Fig. 1).

In one of the 15 investigated patients, a novel missense mutation, c.280 T>A in exon 3 of SLURP1 (Fig. S1) was found in one allele. This transversion causes a change of the amino acid cysteine in position 94 to serine, p.Cys94Ser. None of the 100 control alleles sequenced harboured the C.280T>A mutation. The other SLURP1 allele in the patient with the novel mutation carried the same recurrent missense mutation as the other patients, c.43T>C (Fig. S1'). One parent of the patient was shown to carry the mutation c.280T>A (Fig. S1') and the other parent the c.43T>C mutation (Fig. S1'). The patient is a 2-year-old boy, the only child of unrelated parents from the southern part of Sweden with no family history of skin disease. The boy was born at term after an uneventful pregnancy. Dry palms and soles were noted at birth, and at 4 months definite thickness, scaling and redness had developed and progressed. When immersed in water the skin became “spongy” and whitish. Pruritus often developed at night-time but there were no blisters and no skin lesions were observed elsewhere. He was referred to the Genodermatosis Clinic of Uppsala University Hospital under the diagnosis of epidermolysis bullosa simplex or keratoderma. He showed massive keratoderma, hyperhidrosis and focal peeling, but no blisters (Fig. 2) and had no specific treatment. A tentative diagnosis of recessive PPK-GN or epidermolysis keratoderma was made, but analysis of

1http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1840
SLURP1 mutations in Swedish keratoderma

Fourteen persons were found to be homozygous for a c.43T>C (p.Trp15Arg) mutation in the SLURP1 gene and as expected all parents of the affected individuals were heterozygous carriers. Since 13 affected individuals from 7 families originating from the 2 northernmost counties of Sweden have been found to carry identical homozygous haplotypes around the SLURP1 gene loci on chromosome 8qter (data not shown) a common founder for the mutation in this population is very probable. The c.43T>C mutation has been described previously in several different populations (8, 14, 16). The T to C transition introduces a positively charged arginine residue into the conserved non-polar signal sequence and the altered protein is predicted to have a shortened signal sequence and a weaker cleavage site at position 18 (14).

One of the 15 persons affected by PPK-GN was found to have compound heterozygous mutations in the SLURP1 gene, a novel c.280T>A mutation (p.Cys94Ser) in addition to the recurrent c.43T>C mutation; c.280T>A was not detected in 100 control alleles strengthening the assumption that this is a mutation causing disease and not a polymorphism. Moreover this mutation affects one of the conserved cysteine residues in the SLURP-1 protein that are essential in forming disulphide bridges within the protein, bridges that are critical for normal function of the protein.

So far, 16 distinct SLURP1 gene mutations have been reported in MDM (Table I). The PPK-GN patients in our study appears to have a milder phenotype than described in classical MDM and the mutations found are not identical with those described in the families from the Mljet island by Fischer et al. 2001 (10). However, our findings confirm that mutations in the SLURP1 gene are responsible also for the milder type of autosomal recessive PPK encountered in Sweden, and that PPK-GN and MDM indeed are allelic disorders.

Table I. Mutations in the SLURP1 gene known to cause Mal de Meleda or palmoplantar keratoderma of the Gamborg-Nielsen type

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Consequence of mutation</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>c.1A&gt;C</td>
<td>p.Met1Leu</td>
<td>Eckl et al. 2003 (14)</td>
</tr>
<tr>
<td>c.43T&gt;C</td>
<td>p.Trp15Arg</td>
<td>Eckl et al. 2003 (14)</td>
</tr>
<tr>
<td>IVS1 G-A+1</td>
<td>Altered splice site</td>
<td>Wajid et al. 2009 (19)</td>
</tr>
<tr>
<td>c.82delT</td>
<td>p.Cys82832Term</td>
<td>Fischer et al. 2001 (10)</td>
</tr>
<tr>
<td>c.129C&gt;A</td>
<td>p.Cys43Term</td>
<td>Muslumanoglu et al. 2006 (20)</td>
</tr>
<tr>
<td>c.212G&gt;A</td>
<td>p.Arg71His</td>
<td>Favre et al. 2007 (17)</td>
</tr>
<tr>
<td>IVS2 G-A+1</td>
<td>Altered splice site</td>
<td>Fischer et al. 2001 (10)</td>
</tr>
<tr>
<td>c.229T&gt;C</td>
<td>p.Cys77Arg</td>
<td>Charfeddine et al. 2003 (18)</td>
</tr>
<tr>
<td>c.244C&gt;T</td>
<td>p.Arg82Ser</td>
<td>Gruber et al. 2011 (21)</td>
</tr>
<tr>
<td>c.256G&gt;C</td>
<td>p.Gly86Arg</td>
<td>Eckl et al. 2003 (14)</td>
</tr>
<tr>
<td>c.280T&gt;A</td>
<td>p.Cys94Ser</td>
<td>Present study</td>
</tr>
<tr>
<td>c.286C&gt;T</td>
<td>p.Arg96Term</td>
<td>Fischer et al. 2001 (10)</td>
</tr>
<tr>
<td>c.297T&gt;C</td>
<td>p.Leu98Pro</td>
<td>Yerebakan et al. 2003 (22)</td>
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</tbody>
</table>
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REFERENCES