

Assignment of the locus for ichthyosis prematurity syndrome to chromosome 9q33.3–34.13

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J Med Genet 2004;41:208–212. doi: 10.1136/jmg.2003.012567

Autosomal recessive congenital ichthyosis (ARCI) is a clinically and genetically heterogeneous group of inherited disorders of keratinisation, with an estimated incidence of one per 200 000 newborns.¹ In Scandinavia, the prevalence is closer to one in 50 000.^{2–3} By electron microscopy, ARCI can be classified into four subgroups—ichthyosis congenita I–IV—and one so far undefined group. Six loci have been associated with ARCI: on chromosomes 2q34 (LI2 (MIM 601277)), 3p21 (NCIE2 (MIM 604780)), 14q11.2 (LI1 (MIM 242300) and NCIE1 (MIM 242100)), 17p13.1 (LI5 (MIM 606545)), 19p12–q12 (LI3 (MIM 190195)), and 19p13.1–p13.2 (NNCI (MIM 604781)).^{4–9}

Genes that correspond to four of these have been identified: the transglutaminase 1 gene (*TGM1* (MIM 190195)) on chromosome 14q11, the comparative gene identification 58 (*CGI-58* (MIM 604780)) on chromosome 3p21, two genes from the lipoxygenase (LOX) family—lipoxygenase-3 (*ALOX3*) and 12(R)-lipoxygenase (*ALOX12B* (MIM 603741))—on chromosome 17p13.1, and, most recently, the adenosine triphosphate binding cassette 12A (*ABCA12A* (MIM 607800)) on chromosome 2q34.^{10–14}

The transglutaminase 1 protein takes part in the formation of the lipid envelope on the surface of epidermal keratinocytes. Cells with a disrupted *TGM1* gene have a defective epidermal barrier function. *TGM1* is altered in one third of patients with ichthyosis congenita type I and all patients with ichthyosis congenita type II.^{2 12 15 16}

Mutations in the *CGI-58* gene on chromosome 3p21 cause Chanarin-Dorfman disease (CDS (MIM 275630)). The protein CGI-58 belongs to a large family of proteins characterised by an α/β hydrolase fold and contains three sequence motifs found in the esterase, lipase, and thioesterase subfamily. Affected patients have raised levels of triacylglycerol, and CGI-58 has been postulated to be involved in recycling of triacylglycerol derived monoacylglycerol or diacylglycerol to specific phospholipids or in the catabolism of long chain fatty acids.¹¹

Mutations in either of the two lipoxygenase genes (*ALOX3* and *ALOX12B*) on chromosome 17p13.1 result in a mild form of ARCI.¹⁴ The two genes are linked physically with a high sequence similarity, and they are related functionally. They are expressed mainly in epithelial cells, such as keratinocytes.¹⁷ LOX genes are involved in fatty acid metabolism and the maintenance of the cutaneous permeability barrier.

Ichthyosis prematurity syndrome (IPS) is a distinct form of ARCI that is reported almost exclusively in the Norwegian population.^{1 18 19} To date, the only exceptions are two Finnish families and one north Italian family.^{20 21} It was observed as a unique syndrome because of its ultrastructural features of the skin and was published as ichthyosis congenita type IV.^{1 22} The pattern on electron microscopy is characterised by membrane aggregations in the upper epidermal cells. Pregnancies with an affected foetus are complicated by polyhydramnion, and ultrasound shows opaque amniotic

Key points

- Autosomal recessive congenital ichthyosis (ARCI) is a clinically and genetically heterogeneous group of inherited disorders of keratinisation. To date, five genes have been identified that underlie ARCI, and two additional gene loci for ARCI have been assigned.
- Ichthyosis congenita IV is a rare form of ARCI that clinically is known as ichthyosis prematurity syndrome (IPS). Key features are complications in the mid-trimester of pregnancy, with premature birth of a child with thick caseous desquamating epidermis, respiratory complications, and eosinophilia that recovers into a lifelong non-scaly ichthyosis with atopic manifestations. The prevalence is high in a region across mid-Scandinavia.
- Thirteen families with 1–2 members affected by IPS and at least one affected and one healthy or affected sibling were recruited for linkage analysis. Three families with one affected child but no siblings were included for haplotype analysis. Overall, 14 families originated from a defined region of middle Norway and two from the adjacent region of middle Sweden.
- Genomewide linkage analysis was performed in the 13 informative families affected by IPS. Significant linkage was found with the short tandem repeat D9S778 on chromosome 9q34, with a maximum logarithm of odds (LOD) score of 3.73 ($\theta_m = 0.00$).
- Haplotype analysis and meiotic recombinants restricted the genetic interval to a 12 cM region between D9S250 and D9S63. Within this interval, a critical region was identified by allelic association. The combined results restrict the locus for IPS to a 1 Mb region at 9q33.3–34.13.
- The gene locus for IPS was mapped to a 1 Mb region with no indication of genetic heterogeneity. Haplotype analysis suggests one or two founder chromosomes in the population studied. Characterisation of the mutant gene may clarify the mechanisms behind normal skin formation.

fluid. The birth is premature, and delivery usually takes place in weeks 30–32 of pregnancy. The child becomes severely asphyctic after delivery, probably because of aspiration of amnion debris. At birth, the skin, particularly on the head and peripheral extremities, is covered by a thick, caseous, desquamating epidermis, which, within two weeks, improves to a benign dryness of the skin. Later, the phenotype is mild, with persisting dryness of the skin and white scaling of the capilli. The skin shows a cobblestone like surface, particularly

on distal extremities, and a leathery like thickening of the skin on the lower back and rima. In addition, patients have hyper eosinophilia. (²¹ and Gedde-Dahl Jr T, personal communication, 2003).

Ichthyosis prematurity syndrome segregates independently of the known ARCI loci and is more prevalent in a defined region in the adjacent middle parts of Norway and Sweden, with an estimated local heterozygote carrier frequency of one in 50, which suggests a prehistoric mutation.¹⁹ We report the chromosomal mapping of the IPS locus in 13 families informative for linkage analysis.

METHODS

Participants

We recruited 13 families with 1–2 members affected by IPS and at least one affected and one healthy or affected sibling. We also included three families with one affected child but no siblings for haplotype analysis. Altogether, 20 affected members and 13 healthy siblings were available for DNA studies (fig 1). Overall, 14 families originated from a defined region of middle Norway and two from the adjacent region of middle Sweden. None of the families were known to be related, and we ascertained no consanguinity. TG-D clinically examined the Norwegian families, who were all verified ultrastructurally by skin biopsies in Heidelberg. AV clinically examined the Swedish patients. Affected members of all families displayed the typical IPS phenotype, including premature birth with variable perinatal complications. Each patient's skin was affected severely at birth, but this turned gradually into a milder phenotype, with characteristic flat follicular hyperkeratosis. Hyper eosinophilia was verified in all patients at varying ages. In two of the informative families, an affected sibling died from neonatal complications (not shown in fig 1). The ultrastructural findings were consistent with ichthyosis congenita type IV in all patients and showed characteristic aggregations of bending membranes of the horn cells of epidermis. (¹ ²⁰ and Gedde-Dahl Jr T personal communication, 2003).

Genotyping of families

We extracted genomic DNA from whole blood and genotyped it with radioactively or fluorescently labelled microsatellites. The initial genome scan consisted of 364 microsatellite markers selected with an average distance of 10–15 cM throughout the genome. For radioactive labelling, we used forward primers labelled with ³²P-adenosine triphosphate. We amplified the markers by polymerase chain reaction (PCR) in a 10 µl reaction volume that contained 10 ng genomic DNA, 1 µl 10X PCR buffer (200 mM Tris (pH 8.4), 500 mM potassium chloride, and 15 mM magnesium chloride; Invitrogen, San Diego, CA, USA), 0.3 µl nucleotides (dNTPs) (25 mM), 0.2 µl of each primer (10 µM), and 0.03 µl Taq DNA Polymerase (5 U/µl) (Invitrogen, San Diego, CA, USA).

For fluorescent labelling, we modified the forward primers with the fluorescent labels carboxyfluorescein (FAM), hexachloro-6-carboxy-fluorescein (HEX), or tetrachloro-6-carboxy-fluorescein (TET). We amplified the markers by PCR in a 10 µl reaction volume that contained 20 ng of genomic DNA, 1 µl 10X Platinum PCR buffer without magnesium chloride (200 mM Tris (pH 8.4) and 500 mM potassium chloride) (Invitrogen, San Diego, CA, USA), 0.3 µl magnesium chloride (50 mM), 0.3 µl dNTPs (25 mM), 0.05 µl of each primer (100 µM), 0.03 µl Platinum Taq DNA Polymerase (5 U/µl) (Invitrogen, San Diego, CA, USA). We subjected the reactions to an initial denaturation for 5 minutes at 96°C followed by 35 cycles at 96°C for 30 seconds, 55°C for 45 seconds, and 72°C for 45 seconds, before a final elongation at 72°C for 7 minutes. We separated

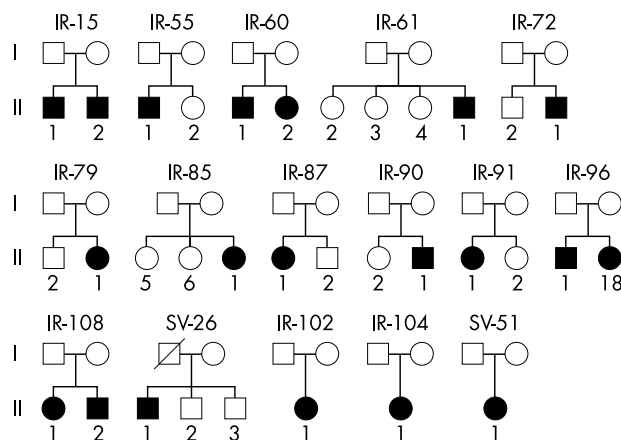


Figure 1 Pedigrees affected by IPS analysed in a study to assign the locus for IPS. All parents were obligate carriers and are numbered I-1 and I-2, respectively. A deceased father was not available for analysis, and deceased and unavailable children are not shown.

the radioactively labelled PCR products on a 4.5% polyacrylamide gel with 7 M urea in 0.5 × TEB buffer. We developed radioactively labelled fragments on Kodak BioMax MS films (Amersham Biosciences, Piscataway, NJ, USA). We separated the pooled fluorescently labelled PCR products on a 4% polyacrylamide gel on an ABI 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). We analysed fragments with Genescan software (version 3.1.3) and Genotyper software (version 3.7) (Applied Biosystems, Foster City, CA, USA).

Linkage and haplotype studies

We drew haplotypes with the Cyrillic family drawing program (version 2.1.3) (Cherwell Scientific Publishing, Oxford, UK). Two point LOD score calculations were made with the MLINK program of the LINKAGE package (version 5.1).²³ We performed linkage analysis assuming an autosomal recessive mode of inheritance, an equal male and female recombination rate, and full penetrance. We set the disease allele frequency to one per 150 000, as some obligate carriers were born outside the high risk area, and we gave the marker alleles equal frequencies in the population. As all parents were genotypically known but phase unknown, however, this material concerns “certain” families in which gene frequencies are irrelevant to the linkage analysis outcome.²⁴

Association studies

For the association study, we used the allele sizes of genetically linked markers in the affected children and their parents to construct the parental “affected” and “unaffected” (control) haplotypes. The regional aggregation of families made the parental control haplotypes safer than non-regionally weighted control families. This corresponds to the transmission disequilibrium test (TDT).

We calculated the association of allele sizes to the disease loci with the ψ^2 distribution.

RESULTS

Linkage and haplotype analysis

Genomewide scan

Genotype analysis excluded linkage to the known ARCI loci with markers that covered the six loci at chromosomes 2q34, 3p21, 14q11, 17p13, 19p12–q12, and 19p13.1–p13.2. An initial genomewide linkage analysis with 364 radioactively labelled markers was performed with four families affected by IPS. The markers were selected with an average distance

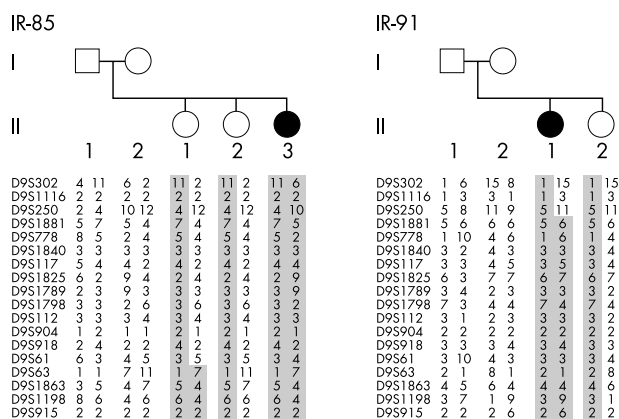


Figure 2 Recombination events that narrowed down the IPS locus. The upper limit was defined by a recombination event in family IR-91 and the lower limit by a recombination event in family IR-85. The region that segregated with the phenotype is shaded.

of 10–15 cM throughout the genome. The results gave a first indication for linkage on five different chromosomal loci. The family material was expanded to include a total of nine families to investigate these loci. This gave a maximal two-point LOD score >1.0 ($\theta_{\max} = 0$) on chromosome 8 (LOD 1.70 with marker D8S277), chromosome 9 (LOD 1.70 with marker D9S60), chromosome 10 (LOD 1.17 with marker D10S215), chromosome 15 (LOD 1.24 with marker D15S125) and chromosome 20 (LOD 1.70 with marker D20S120). Investigation of flanking markers and haplotypes excluded linkage for the loci except for chromosome 9.

Refinement of the interval

The family material was extended to 13 families affected by IPS from Norway and Sweden. We tested the power of our study by simulation analysis with all 13 families; this provided a maximum expected LOD score of 3.73. To refine the region on chromosome 9, adjacent markers were selected from public databases (National Center for Biotechnology Information, Ensembl, and deCODE). We determined the markers' order and positions by comparing information from all three databases. Further analysis resulted in a maximum

combined LOD score of 3.73 with marker D9S778 at $\theta_{\max} = 0$. All families affected by IPS that we investigated supported linkage to chromosome 9q, yet with individually insignificant scores. Haplotype analysis showed key recombination events in two families. The IPS locus was restricted towards the centromere by a recombination event between markers D9S250 and D9S778. The telomeric border was defined by a recombination event between markers D9S61 and D9S63 (fig 2). This localised the IPS locus between the markers D9S250 and D9S63: a region of about 11 cM. A region consisting of 18 marker loci in the interval D9S250–D9S63 segregated completely with the phenotype in all families (table 1). This interval corresponded to a physical distance of approximately 9 Mb at 9q33.3–34.13 (table 2).

We performed association studies with the markers to identify a commonly shared haplotype in the families. This gave a positive ψ^2 score ($p < 0.05$) for three adjacent markers: D9S1798 ($p = 0.0012$), D9S1821 ($p = 0.0286$), and D9S112 ($p = 1.1 \times 10^{-7}$) for allele sizes 240 bp (allele 3), 171 bp (allele 2), and 138 bp (allele 3), respectively. The results showed a possible common haplotype that consisted of these three markers. This region, however, was not shared by all individuals (loss of homozygosity). Investigation of the haplotypes that surrounded the three markers showed two possible core haplotypes, A and B, in the families. The central marker D9S904, which did not show association ($p = 0.2142$ for allele 1 and $p = 0.4126$ for allele 2), separated the two possible core haplotypes. The D9S904 marker allele was the only one shared by all individuals, which was illustrated in families IR61 and IR90 (fig 3). The two haplotypes were expanded with the centromeric markers D9S1789 and D9S1825 and the telomeric markers D9S918, D9S61, and D9S1795.

DISCUSSION

Autosomal recessive congenital ichthyosis is a heterogeneous group of disorders clinically and genetically. Ichthyosis prematurity syndrome seems to be a homogeneous subgroup clinically and from pathognomic ultrastructural features. A genomewide screen was initially performed on four families affected by IPS, and linkage to several chromosome regions was established. The material was extended to a total of 13 families, and linkage to all other chromosomes but

Table 1 Combined two point LOD scores at different recombination fractions for markers on chromosome 9q33.3–34.13 calculated from 13 families affected by IPS

| Marker | Two point LOD score at different values of θ | | | | | | | |
|---------|---|--------|--------|--------|-------|-------|-------|-------|
| | 0 | 0.001 | 0.010 | 0.050 | 0.100 | 0.200 | 0.300 | 0.400 |
| D9S302 | –∞ | –1.977 | –0.063 | 0.953 | 1.085 | 0.809 | 0.415 | 0.113 |
| D9S1116 | –∞ | –0.327 | 0.647 | 1.181 | 1.207 | 0.876 | 0.450 | 0.122 |
| D9S250 | –∞ | –0.777 | 1.106 | 1.981 | 1.942 | 1.341 | 0.672 | 0.181 |
| D9S1881 | 2.879 | 2.871 | 2.799 | 2.475 | 2.066 | 1.283 | 0.617 | 0.163 |
| D9S778 | 3.731 | 3.721 | 3.623 | 3.186 | 2.646 | 1.630 | 0.783 | 0.206 |
| D9S1840 | 1.492 | 1.488 | 1.444 | 1.252 | 1.023 | 0.612 | 0.287 | 0.075 |
| D9S117 | 3.056 | 3.046 | 2.956 | 2.560 | 2.083 | 1.231 | 0.570 | 0.147 |
| D9S1825 | 1.682 | 1.684 | 1.691 | 1.637 | 1.463 | 0.984 | 0.496 | 0.134 |
| D9S1789 | 1.800 | 1.796 | 1.760 | 1.583 | 1.340 | 0.843 | 0.408 | 0.108 |
| D9S1798 | 2.037 | 2.033 | 1.995 | 1.815 | 1.503 | 1.020 | 0.508 | 0.127 |
| D9S1821 | 1.755 | 1.750 | 1.704 | 1.501 | 1.247 | 0.766 | 0.365 | 0.096 |
| D9S112 | 3.004 | 2.995 | 2.906 | 2.520 | 2.060 | 1.236 | 0.583 | 0.152 |
| D9S904 | 0.859 | 0.859 | 0.851 | 0.792 | 0.684 | 0.431 | 0.206 | 0.054 |
| D9S918 | 2.402 | 2.396 | 2.337 | 2.064 | 1.709 | 1.032 | 0.484 | 0.126 |
| D9S61 | 3.004 | 2.995 | 2.906 | 2.520 | 2.060 | 1.236 | 0.583 | 0.152 |
| D9S1795 | 0.846 | 0.851 | 0.885 | 0.926 | 0.860 | 0.594 | 0.302 | 0.082 |
| D9S63 | 3.430 | 3.422 | 3.345 | 2.992 | 2.534 | 1.617 | 0.798 | 0.214 |
| D9S1863 | –∞ | –2.402 | –0.477 | 0.591 | 0.788 | 0.632 | 0.334 | 0.092 |
| D9S915 | –∞ | –3.373 | –1.400 | –0.136 | 0.266 | 0.399 | 0.259 | 0.079 |
| D9S1198 | –∞ | –1.625 | 0.284 | 1.279 | 1.378 | 1.021 | 0.535 | 0.149 |

Table 2 Positions for markers used on chromosome 9q33.3–34.13. Markers in the IPS locus are boxed. GenBank positions are from the Sequence Tagged Site map. The cM positions are sex averages

| Marker | Celera (bp) | GenBank (kb) | deCODE (cM) | Marshfield (cM) |
|---------|-------------|--------------|-------------|-----------------|
| D9S302 | 90354776 | 107879 | NA | 123.33 |
| D9S1116 | 96257364 | 114097 | 127.83 | 130.52 |
| D9S250 | 97441271 | 115280 | NA | 132.09 |
| D9S1881 | 100244349 | 118094 | 132.32 | 135.85 |
| D9S60 | 100244370 | 118094 | NA | NA |
| D9S778 | 100461315 | 118311 | NA | 135.85 |
| D9S1840 | 100553349 | 118402 | 132.50 | 136.47 |
| D9S117 | 100814751 | 118664 | NA | NA |
| D9S1825 | 101152424 | 119003 | 132.93 | 136.47 |
| D9S1789 | 101152872 | 119003 | 133.08 | 136.47 |
| D9S1798 | 102440915 | 120287 | 133.62 | 136.47 |
| D9S1821 | 102598065 | 120444 | NA | NA |
| D9S112 | 102641878 | 120488 | NA | NA |
| D9S904 | 103503982 | 121352 | 136.16 | 136.47 |
| D9S918 | 103724456 | 121572 | 135.38 | 136.47 |
| D9S61 | 104832243 | 122685 | NA | NA |
| D9S1795 | 105565410 | 123421 | 138.59 | 142.51 |
| D9S63 | 105914035 | 123769 | NA | NA |
| D9S1863 | 106640653 | 124515 | 140.35 | 144.13 |
| D9S915 | 109037297 | NA | 146.34 | 145.21 |
| D9S1198 | 109176073 | 127060 | NA | NA |

NA, information not available.

chromosome 9 could be excluded. Linkage to the six previously known ARCI loci was excluded with markers from each region. All families analysed in our study supported linkage to a single chromosome 9 region, and they proved linkage with a maximum cumulative LOD score of 3.73. The relatively high incidence of IPS at birth in the three counties of middle Norway with a population of 600 000 suggests a strong and ancient founder effect. Haplotype analysis of the 11 cM region on chromosome 9 showed two possible core haplotypes, A and B, in the 13 families. The central region of the two haplotypes, represented by marker D9S904, is shared in all affected individuals. We expected a single common ancestor haplotype for the mutation in all families, but the results showed two haplotypes that imply the presence of two founder mutations. One possible explanation is that historical recombination events have changed the original haplotype at a resolution beyond the markers used. Alternatively, a mutant allele may have shifted haplotype by a historic gene conversion that has resulted in two separate haplotypes for a single mutation. With the hypothesis of one or two common ancestral chromosomes, the region could be restricted by allelic association to a 1 Mb region between marker D9S112 and D9S918 (fig 3). This 9q region needs to be characterised with additional microsatellite and biallelic single nucleotide polymorphism markers in affected individuals to define and expand the possible core haplotypes.

The 11 cM interval identified on chromosome 9q by linkage analysis contains about 80 known or predicted genes and transcripts. The 1 cM region predicted by the haplotypes

and allelic association contains only 16 known or predicted genes and transcripts (GenBank).

Of these 16 genes, only eight has a known function, including the LIM homeobox transcription factor 1 β (LMX1B (MIM 602575)), zinc finger protein 297B (ZNF297B), Ral guanine nucleotide exchange factor RalGPS1A (RALGPS1A), angiopoietin like 2 (ANGPTL2 (MIM 605001)), solute carrier family 2 (facilitated glucose transporter) member 8 (SLC2A8 (MIM 605245)), zinc finger

Information on electronic databases

Accession numbers and URLs for data in this article are as follows:

- decode (<http://www.decodegenetics.com/>)
- Ensembl (<http://www.ensembl.org/>)
- GenBank (<http://www.ncbi.nlm.nih.gov/genbank>)
- Online Mendelian Inheritance in Man (OMIM) (<http://www.ncbi.nlm.nih.gov/omim/>) (for ABCA12A (MIM 607800), ALOX12B (MIM 603741), ANGPTL2 (MIM 605001), CGI-58 (MIM 604780), LI1 (MIM 242300), LI2 (MIM 601277), LI3 (MIM 190195), LI5 (MIM 606545), LMX1B (MIM 602575), NCIE1 (MIM 242100), NCIE2 (MIM 604780), NNCI (MIM 604781), RPL12 (MIM 180475), SLC2A8 (MIM 605245), STXBP1 (MIM 602926), TGM1 (MIM 190195), ZNF79 (MIM 194552))

| Family | IR-87 | IR-104 | IR-61 | IR-55 | IR-79 | IR-85 | IR-90 | IR-72 | IR-96 | IR-102 | IR-108 | SV-26 | IR-15 | IR-60 | IR-91 | SV-51 | A | B | | | | | | | | | | | | | | |
|---------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|---|---|
| Markers | 8 | 3 | 3 | 3 | 3 | 4 | 8 | 9 | 11 | 3 | 2 | 9 | 3 | 3 | 5 | 6 | 11 | 6 | 10 | 3 | 9 | 6 | 3 | 2 | 3 | 7 | 3 | 3 | 6 | 7 | 3 | 3 |
| D9S1825 | 2 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 3 | 3 | 2 | 3 | 3 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| D9S1789 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 5 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 5 | 1 | 3 | 3 | 5 | 3 | 3 | 2 | 3 | 4 | 7 | 4 | 3 | 3 | |
| D9S1798 | 1 | 3 | 3 | 3 | 2 | 1 | 1 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 3 | 2 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | |
| D9S1821 | 3 | 3 | 3 | 5 | 4 | 5 | 5 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | |
| D9S112 | 3 | 3 | 3 | 5 | 4 | 5 | 5 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | |
| D9S904 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | |
| D9S918 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 2 | 2 | 4 | 2 | 4 | 5 | 4 | 4 | 2 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| D9S61 | 3 | 3 | 3 | 3 | 8 | 4 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 4 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | |
| D9S1795 | 5 | 5 | 4 | 5 | 3 | 7 | 5 | 5 | 6 | 5 | 3 | 5 | 5 | 5 | 4 | 5 | 6 | 5 | 5 | 6 | 1 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

Figure 3 Patient haplotypes. The two common haplotypes are boxed and shaded. The markers shared in both haplotypes are in bold type.

protein 79 (pT7) (ZNF79 (MIM 194552)), ribosomal protein L12 (RPL12 (MIM 180475)), and syntaxin binding protein 1 (STXBP1 (MIM 602926)).

To further restrict the candidate region, samples from additional families affected by IPS are important for the identification of critical recombination events and common haplotypes. A future characterisation of the gene responsible for IPS may lead to a better understanding of the mechanisms behind normal skin formation and possibly to a clarification of factors behind premature birth and genetic predisposition to atopy.

ACKNOWLEDGEMENTS

We acknowledge the kind cooperation of the families and their doctors. We were indebted to I Anton-Lamprecht and I Hausser at the Institute of Ultrastructural Research of the Skin, University of Heidelberg, for the electron microscopic diagnostic verification of most of the patients. This work was supported by grants from Swedish Research Council, Children's Cancer Foundation of Sweden, Swedish Cancer Society, Swedish Medical Society, Torsten and Ragnar Söderbergs Fund, Borgström Foundation, and Welander-Finsen Foundation. The collection of the Norwegian families over two decades was supported initially by Nansenfondet og de dermed forbundne fonds, University of Oslo, and later by the Anders Jahres Fund's general support to basic research at Institute of Forensic Medicine, University of Oslo.

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Received: 18 July 2003

Accepted: 27 October 2003

Conflicts of interest: None declared.

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