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Report

Assignment of a Novel Locus for Autosomal Recessive Congenital Ichthyosis to Chromosome 19p13.1-p13.2

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Autosomal recessive congenital ichthyosis (ARCI) is a rare, clinically and genetically heterogeneous genodermatosis. One gene (transglutaminase 1, on 14q11) and one additional locus (on 2q33-35, with an unidentified gene) have been shown to be associated with a lamellar, nonerythrodermic type of ARCI. We performed a genomewide scan, with 370 highly polymorphic microsatellite markers, on five affected individuals from one large Finnish family with nonerythrodermic, nonlamellar ARCI. The only evidence for linkage emerged from markers in a 6.0-cM region on chromosome 19p13.1-2. The maximum two-point LOD score of 7.33 was obtained with the locus D19S252, and multipoint likelihood calculations gave a maximum location score of 5.2. The affected individuals share two common core haplotypes, which makes compound heterozygosity possible. The novel disease locus is the third locus linked to ARCI, supporting previous evidence for genetic heterogeneity of ARCI. This is also the first locus for a nonlamellar, nonerythrodermic phenotype of ARCI.

Autosomal recessive congenital ichthyoses (ARCI) represent a clinically and genetically heterogeneous group of relatively rare, inherited disorders of cornification. Traditionally, ARCI patients are classified as having either lamellar ichthyosis (LI [MIM 242300]) or congenital ichthyosiformis erythroderma (CIE [MIM 242100]) (Williams and Elias 1985). Classically, patients with LI are born as collodion babies encased in a plasticlike membrane that, within a few weeks, changes into scaling skin. In adulthood, the scales are large and brownish and there is ectropion of the eyes and, possibly, alopecia. In CIE, scales are thinner and erythrodermia is prominent. There is a continuum between these two clinical pictures, and precise diagnosis is sometimes hard to make.

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Two chromosomal loci, 14q11 and 2q33-q35, are known to be associated with LI (Russell et al. 1994; Parmentier et al. 1996). The transglutaminase 1 gene (TGM1; keratinocyte transglutaminase) on 14q11, whose product catalyzes the formation of cornified cell envelope, has been demonstrated to be defective in some patients with LI (Huber et al. 1995; Russell et al. 1995). However, only a fraction of patients with LI have TGM1 mutations, confirming the genetic heterogeneity of the disease, (Parmentier et al. 1995; Laiho et al. 1997). The altered gene, on locus 2q33-q35, is still unknown.

Finland has been a representative example of the power of a genetic isolate in the cloning of inherited disease genes. Religious and linguistic barriers, geopolitical position, and, most importantly, geography have ensured that the Finns remained in local and national isolation. This isolation has caused the presence of several genetically inherited diseases, grouped under the label "Finnish disease heritage," including ~30 mostly recessive genetic traits that are exceptionally common in Finland and rare among other populations. Internal isolates within the country have proven to be beneficial for

mapping of disease loci as well as traits that are not overrepresented in the country (Norio et al. 1973; Peltonen et al. 1999).

We have previously reported a TGM1 mutation screen of 38 Finnish families with ARCI (Laiho et al. 1997). In 13 families, a TGM1 mutation was found, but in 25 families, no TGM1 mutation could be identified. Here we present results from a genomewide scan in one family with ARCI, from an internal isolate in Finland (without a TGM1 mutation), with only five affected family members. We assigned a third ARCI locus on chromosome 19p13.1-p13.2. It is the first locus to be associated with nonlamellar, nonerythrodermic ARCI.

The diagnosis of ARCI was made at the Department of Dermatology, Helsinki University Central Hospital, and was based on electron microscopy. X-linked ichthyosis was excluded by measurement of steroid sulfate activity (from patient 6; fig. 1), and the clinical picture did not indicate X-linked ichthyosis. In histologic examination, the granular layer was present in all patients, excluding clear-cut ichthyosis vulgaris. The family analyzed in the linkage study (fig. 1) originates from a known genetic subisolate in Finland, the archipelago of Larsmo at the coast of Ostrobothnia. None of the other 37 families originate from Larsmo. The study protocol was accepted by the Ethical Board of the Helsinki University Central Hospital.

All affected individuals displayed a nonlamellar, nonerythrodermic phenotype not directly resembling either classic LI or classic CIE, although such patients can be

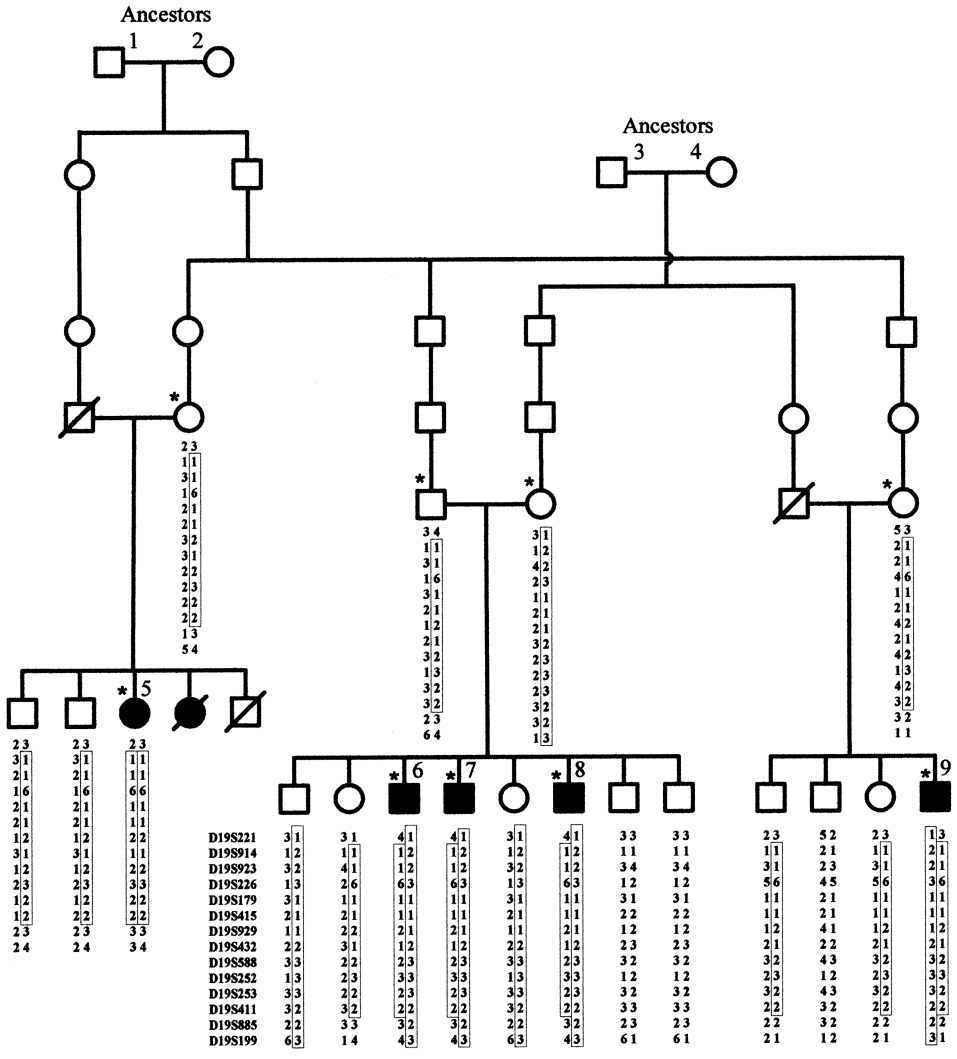

Figure 1 Pedigree and haplotypes of the family with nonlamellar, nonerythrodermic ARCI linked to chromosome 19. Persons marked with an asterisk (*) were included in the genome scan. Ages of the affecteds are as follows: patient 5: 54 years old, patient 6: 12 years old, patient 7: 11 years old, patient 8: 8 years old, and patient 9: 17 years old.

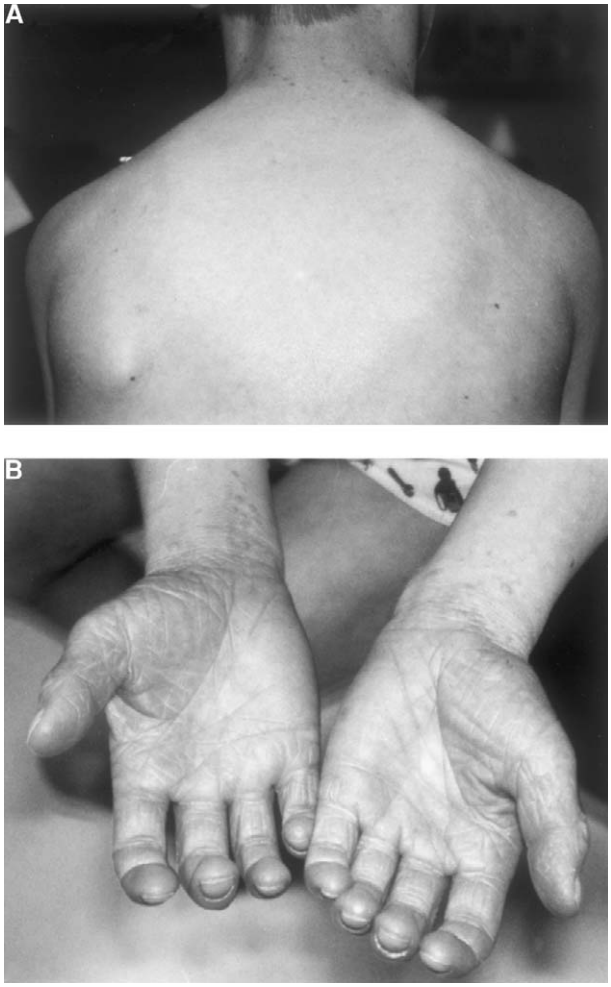

Figure 2 *A,* Skin of patient 6 (8 years old at time of photograph). On the dorsal aspects of the upper arms, fine, white, nonlamellar scaling is seen, demonstrating the mildness of scaling. Erythrodermia is not present. *B,* The palms of patient 6 show hyperkeratotic, hyperlinear skin. Papules on the volar surface of the wrist are a consequence of atopic dermatitis.

diagnosed as having CIE. Scales in the skin were fine and white (fig. 2*A*), and neither erythrodermia nor ectropion was present. Scaling was more prominent in the knees, ankles, and ears. Palms and soles appeared to be hyperlinear (fig. 2*B*). Patients 6–8 were born as collodion babies, whereas patients 5 and 9 were ichthyotic at birth. Patients 6–8 had atopic dermatitis and patients 7 and 8 had asthma, but these conditions were also present in unaffected family members. In addition, patient 5 had normotensive hydrocephalus that was interpreted not to be associated with ichthyosis. Only a few changes were seen in electron microscopic studies of the affected individuals, including minor abnormalities in keratinosomes, which suggest a functional rather than a structural alteration (fig. 3).

Genomic DNA for genotyping was extracted from leukocytes of peripheral venous EDTA blood (5 ml) by standard procedures (Sambrook et al. 1987). DNA was amplified by means of the touchdown-PCR procedure (Don et al. 1991). PCR products were then pooled so that, at most, four PCR products of different sizes were pooled together, and gel electrophoresis was run on an ALFexpress automated sequencer (Pharmacia Biotech). Genotypes were analyzed by the AlleleLinks 1.0 software package (Pharmacia Biotech).

The candidate-gene approach was used first. The only known gene for ichthyotic diseases—TGM1, on chromosome 14q11—was excluded as the disease gene by SSCP analysis (Orita et al. 1989; Laiho et al. 1997) and by measurement of TGM1 activity as described by Huber et al. (1997). By genotyping and haplotype analysis, we also excluded five possible chromosomal loci: (1) the TGM2 and TGM3 locus in 20q11.2 (D20S107 and D20S119); keratin (KRT) loci (2) in 17q (D17S846 and D17S857) and (3) in 12q11-q13 (D12S368 and D12S137); (4) a second locus for LI in 2q33-q35 (D2S143, D2S128, and D2S157); and (5) the epidermal differentiation complex in 1q (D1S514 and D1S2345; data not shown).

After exclusion of the candidate genes, the power of our study samples targeting only one family with five affected members was tested by simulation analysis. This provided a maximum expected LOD score of 3.57 and a mean expected LOD score of 1.36 at recombination fraction (θ) 0.010, calculated with 1,000 replicants. A genomewide scan was performed with 370 highly polymorphic, fluorescently labeled microsatellite markers from the Cooperative Human Linkage Center Weber set

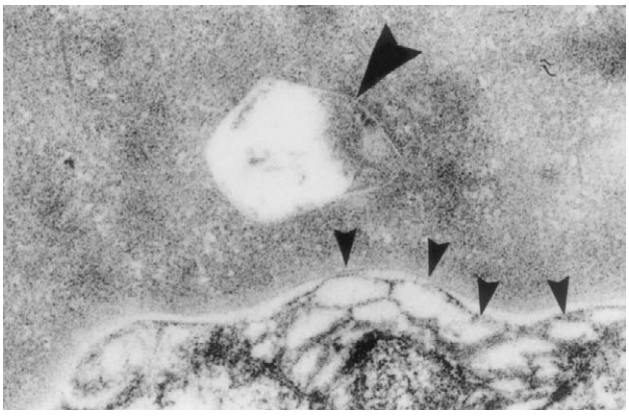

Figure 3 Electron micrograph of two cells of the epidermis of patient 6. Between the granular and keratin layer, in the keratinization line, there are pathological vacuolated keratinosomes without lamellar structures. Above the keratinization line, inside the horny cell, there is one lipid vacuole with some remnants of lamellar structures (original magnification × 25,000).

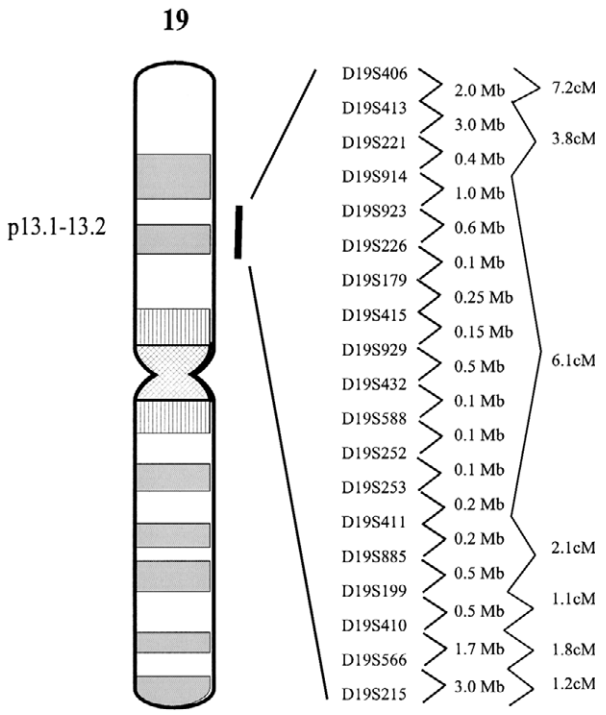

Figure 4 Order of loci and approximate distances between the loci on chromosome 19p13.1-p13.2. Markers were obtained from the Genome Database. The order and the distances of the physical map are based on metric physical map of chromosome 19 created by the Human Genome Center (Lawrence Livermore National Laboratory), whereas the genetic distances are based on a genetic map of chromosome 19 created by the Center for Medical Genetics (Marshfield Medical Research Foundation). The precise location of the marker D19S923 is unknown.

6A (Dubovsky et al. 1995). Some additional markers from the Généthon map were also genotyped (Dib et al. 1996). The average intermarker distance was 9.3 cM; the largest gaps were 24 cM on chromosomes 1 and 20.

Linkage analysis was performed under the assumption of autosomal recessive inheritance for the ARCI gene, with an estimated gene frequency of 10^{-4} in the Finnish population, and equal recombination rates for males and females. Because of the early onset of the disease, complete penetrance was used. Two-point LOD score calculations were performed by means of the MLINK option of the LINKAGE software package (Lathrop et al. 1984; Cottingham et al. 1993). The SLINK option was used to estimate the informativeness of the family material used in the linkage analysis (Ott 1989; Weeks et al. 1990). The SIMWALK2 program was used for location score calculations as well as for determination of the most likely haplotypes. SIMWALK2 uses a Markov chain–Monte Carlo algorithm to traverse the space of legal genetic-descent graphs, or inheritance vectors, for each pedigree (Sobel and Lange 1996). Resulting location scores are directly comparable to multipoint LOD scores in log10 units. Allele frequencies were adopted both from the pedigree and from the unaffecteds in the Larsmo population.

The initial two-point linkage analysis of markers in the genomewide scan revealed two interesting regions, one on chromosome 9 (D9S158, LOD score 2.05) and another on chromosome 19 (D19S432, LOD score 2.28). The chromosome 9 area was excluded as a disease locus by analysis of the haplotypes formed by four additional microsatellite markers around the area of interest (data not shown). In contrast, when 11 additional microsatellite markers were analyzed from the chromosomal region 19p13.1-2, they revealed a region of ~6.0 cM in which all markers produced strong positive LOD scores (fig. 4 and table 1). The maximum two-point LOD score of 7.33 was obtained with locus D19S252 ($\theta = 0.00$), whereas a maximum multipoint location score of 5.2 was obtained in the close proximity of the locus D19S914. Somewhat surprisingly, two alleles were identified in the disease chromosomes with most markers.

The analysis of the 3-cM haplotype including the loci D19S914, D19S923, D19S226, D19S179, D19S415, D19S929, D19S432, D19S588, D19S252, D19S253, and D19S411 revealed two core haplotypes: haplotype 1-1-6-1-1-2-1-2-3-2-2, inherited from ancestors 1 and 2, and haplotype 2-2-3-1-1-1-2-3-3-3-2, inherited from ancestors 3 and 4 (fig. 1). Two adjacent loci in the middle of the haplotype, D19S415 and D19S179, revealed homozygosity in all affected individuals. However, the frequency of the D19S179 allele 1 was 68% in control chromosomes analyzed from unaffected inhabitants of the Larsmo archipelago, and the heterozygosity of the locus D19S179 in CEPH families is only 0.68. The frequency of allele 1 of locus D19S415 was 34%, and heterozygosity was 0.58. Thus, the significance of this finding of homozygosity remains to be studied further and

Table 1

Pairwise LOD Scores between ARCI and 11 Marker Loci on Chromosome 19p

	LOD Score at θ =					
Locus	.0	.01	.05	.1	.2	.3
D19S914	1.84	1.78	1.56	1.30	.82	.42
D19S923	2.84	4.45	4.64	4.30	3.28	2.13
D19S226	2.04	3.40	3.60	3.27	2.34	1.36
D19S179	1.67	1.62	1.43	1.20	.79	.44
D19S415	3.49	3.39	3.00	2.53	1.65	.89
D19S929	−1.27	.15	.59	.57	.29	.08
D19S432	.08	1.90	2.28	2.15	1.56	.89
D19S588	−.72	.70	1.12	1.08	.72	.36
D19S252	7.33	7.18	6.59	5.83	4.26	2.67
D19S253	−.65	.76	1.18	1.14	.76	.38
D19S411	3.41	3.33	2.98	2.55	1.69	.93

cannot so far be considered to be conclusive evidence for a chromosomal region shared among all affected individuals. The recombinations observed in patient 9 restricted the ARCI locus between the loci D19S914 and D19S411. Patient 5 was homozygous for all the alleles in the region, which was expected, considering the consanguinity in the family. Yet her phenotype was similar to those of the other patients.

Somewhat surprisingly, the haplotype analysis suggests two different core haplotypes, which could predict two ancestral mutations segregating in the pedigree, resulting in the compound heterozygosity of patients. This must be confirmed, since two adjacent loci, D19S179 and D19S415, revealed homozygosity in patients. A finding suggestive of compound heterozygosity differs from the mutation spectrum of diseases enriched in the Finnish population, where one core haplotype and subsequently one mutation are typically found in most of the disease chromosomes (Ikonen et al. 1991; Höglund et al. 1996; Peltonen et al. 1999). However, our previous screening for TGM1 mutations in ARCI patients revealed 6 different mutations, and, for one of these mutations, two haplotypes were observed, suggesting two different origins of this mutation (Laiho et al. 1997). ARCI cannot be considered part of the Finnish disease heritage, and there is no evidence for the enrichment of ARCI in Finland. It may be that ichthyoses represent common inherited diseases in Finland and that a single founder effect is not to be expected. On the other hand, the archipelago of Larsmo is a well-known Finnish subisolate. First inhabitants have been estimated to have settled in the 13th century, but their origin is unknown. The inhabitants had little contact with the mainland, possibly because of difficulties in transportation and communication. Therefore, random inbreeding is common. Large family trees have been traced in another disease from Larsmo, tibial muscular dystrophy (Udd et al. 1991). In a community like Larsmo, where consanguinity has been present relatively recently, a mixing of two rare mutations of different origins into one consanguineous pedigree is possible (Haravuori et al. 1998). However, exclusion of the possibility that the homozygosity found with markers D19S179 and D19S415 in the pedigree is a chance event and does not reflect the presence of one old mutation in the pedigree remains to be proved in further studies.

In previous genetic studies of ARCI, mainly LI patients have been studied and have demonstrated association with the two known ichthyosis loci (Huber et al. 1995; Russell et al. 1995; Parmentier et al. 1996). Some groups have also linked patients with CIE to TGM1 mutations (Laiho et al. 1997; Hennies et al. 1998; Pigg et al. 1998). However, the new locus on 19p is associated with nonlamellar, nonerythrodermic CIE. The clinical picture of the patients at birth can be severe, but, later, the scaling is remarkably mild, resembling common dry skin, and clearly differs from that in patients described in previous linkage studies. Despite some common features (mild clinical course, atopic dermatitis) with another mild form of ichthyosis (ichthyosis vulgaris), patients in the family studied have features that are clearly distinct from ichthyosis vulgaris (Wells and Kerr 1966). Further studies will focus on identification of novel positional candidate genes with the chromosomal region 19p13.1-13.2 and will eventually expose the molecular defect in this phenotype.

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Electronic-Database Information

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

Center for Medical Genetics, Marshfield Medical Research Foundation, http://www.marshmed.org/genetics/ (for genetic maps)
Cooperative Human Linkage Center, http://lpg.nci.nih.gov/CHLC/ (for markers used)
Généthon, www.genethon.fr/genethon_en.html (for markers used)
Genome Database, http://gdbwww.gdb.org/ (for markers used)
Human Genome Center, Lawrence Livermore National Laboratory, http://www-bio.llnl.gov/bbrp/genome/genome.html (for metric physical maps of chromosome 19)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for LI [MIM 242300] and CIE [MIM 242100])

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