Identification of a Novel Locus Associated with Congenital Recessive Ichthyosis on 12p11.2–q13

Mordechai Mizrachi-Koren,*† Dan Geiger,‡ Margarita Indelman,* Ora Bitterman-Deutsch,§ Reuven Bergman,*¶ and Eli Sprecher*¶

*Laboratory of Molecular Dermatology, Department of Dermatology, Rambam Medical Center; †Biotechnology Interdisciplinary Unit and ‡Computer Science Department, Israel Institute of Technology, Haifa, Israel; §Dermatology Unit, Western Galile Hospital, Naharya, Israel; ¶Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel

Congenital recessive ichthyoses represent a vast and markedly heterogeneous group of diseases that have been mapped to at least seven distinct chromosomal loci. In this study, we ascertained two consanguineous families presenting with congenital ichthyosis. Using homozygosity mapping, we identified a 6.5 cM homozygous region on 12p11.2–q13 shared by all affected individuals. Multipoint logarithm of odds ratio (LOD) score analysis placed the new locus between markers D12S345 and D12S390 with a maximum LOD score of 4.79 at marker CH12SSR13. This region harbors *PPHLN1*, encoding periphilin 1, a protein involved in the cornification process. No deleterious mutations were identified within the coding region of this gene, suggesting the existence of another gene associated with epidermal differentiation on 12p11.2–q13.

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The recent years have witnessed spectacular advances in our understanding of disorders of cornification (Irvine and Paller, 2002). Mutations in genes encoding numerous proteins involved in the process of keratinization have been shown to cause a spectrum of inherited skin disorders, among which the ichthyoses, a clinically and genetically heterogeneous group of genodermatoses, rank first in severity and prevalence (Irvine and Paller, 2002). These disorders are often associated with defective skin barrier function, which can result in fluid and electrolyte imbalance, as well as life-threatening infections.

Ichthyoses are usually classified based on inheritance pattern, morphology and distribution of the scales, histological findings, and associated features. Congenital recessive ichthyoses (CRI) can be divided into non-syndromic and syndromic forms. The latter group includes numerous complex disorders such as Sjogren–Larsson syndrome (MIM270200) resulting from mutations in the fatty aldehyde dehydrogenase gene (De Laurenzi *et al*, 1996), Chanarin–Dorfman syndrome (MIM275630), a neutral lipid storage disease caused by mutations in CGI-58 (Lefevre *et al*, 2001), and Netherton syndrome (MIM256500) characterized by congenital ichthyosis, elevated levels of IgE, and hair structural anomalies, which results from mutations in SPINK5, encoding a serine protease inhibitor (Chavanas *et al*, 2000). The clinical spectrum of non-syndromic CRI includes severe, classic lamellar ichthyosis (LI1 MIM242300, LI2 MIM601277, LI3 MIM604777, LI5 MIM606545) characterized by collodion membrane presentation (neonates born with a shiny and transparent cover that dries to thick, hard skin, then cracks, and fissures), development of large, plate-like scales, ectropion (turning out of eyelids), eclabium (turning out of the lips) but no erythema (Williams and Elias, 1985). A second distinct subtype of CRI is congenital ichthyosiform erythroderma (CIE; MIM242100). The presence of an intense, generalized erythema and fine, white scales define this disease (Akiyama *et al*, 2003). Patients may also manifest as collodion baby, and may have decreased sweating with severe heat intolerance. About 30% of all patients with CRI have mutations in the gene TGM1 on chromosome 14q (Russel *et al*, 1995; Laiho *et al*, 1997). This gene encodes the enzyme transglutaminase-1, which catalyzes the transamidation of glutamine residues during the cross-linking of the cornified envelope proteins (Ishida-Yamamoto and Izuka, 2002). CRI has also been shown to be caused by mutations in genes encoding lipoxygenase-3 and 12(R)-lipoxygenase (ALOXE3, ALOX12B) on 17p13.1 (Jobard *et al*, 2002), the ATP-binding cassette (ABC)A12 transporter on 2q (Lefevre *et al*, 2003), and ichthyin on 5q33 (Lefevre, 2004). Other CRI cases have been mapped to 12q13 and 19p13.1–13.2 (Fischer *et al*, 2000; Virolainene *et al*; 2000; Hatsell *et al*, 2003), but the causative genes associated with these loci remain to be uncovered. Moreover, further, so far unknown loci for CRI must exist (Krebsova *et al*, 2001) demonstrating extensive genetic heterogeneity in CRI.

Although no formal epidemiological study of recessive ichthyoses has been performed in the Middle East, they are estimated to be extremely frequent in this region because of a high rate of consanguineous unions (Zlotogora, 1997). In

Abbreviations: ABC, ATP-binding cassette; CRI, congenital recessive ichthyoses; LOD, logarithm of odds ratio

this study, we mapped CRI to 12p11.2–q13 in two Israeli consanguineous families.

Results

Clinical findings We ascertained two Israeli families of Arab Moslem origin comprising 14 individuals, including five patients. In both families, parents were first-degree cousins. All five patients were born with normal-looking skin. No collodion membrane was noticed at birth. A few days after birth, fine whitish scales appeared.

The three patients in family 1 developed typical CIE features during childhood including severe erythroderma associated with generalized fine scaling. The erythroderma partly receded with age, but larger scales appeared and persisted through the years (Fig 1*a*). Palmoplantar keratoderma manifested in all three patients with skin thickening and fissuring (Fig 1*b*). A skin biopsy revealed epidermal compact hyperkeratosis, hypergranulosis, acanthosis, and papillomatosis. Over the years, the patients required repeated antifungal and antibiotic treatment courses because of tinea pedis, onychomycosis, and recalcitrant malodorous secondary bacterial infections. Patients 63 and 64 in family 1 received acitretin (50 mg per d) at the age of 35 and 30 y, respectively, for prolonged periods (1–2 y) with marked improvement.

In contrast with the early features of CIE manifested by family 1 patients, the two affected individuals of family 2 displayed only mild fine generalized scaling with minimal palmoplantar skin thickening (Fig 1*c*). They were treated satisfactorily with skin moisturizers and emollients only.

In both families, growth and psychomotor development had been normal. No other diseases or abnormal laboratory findings were reported.

Identification of a new locus associated with CRI In both families, we initially ruled out linkage to seven known CRI-associated loci on 2q33–p35, 3p21, 12q11–q13, 14q11.2, 17p13.2–13.1, 19p12–q12, 19p13.2–13.1 (not shown). Based upon the fact that the families were consanguineous, we assumed the existence in each family of a homozygous causative mutation. Using a panel of fluorescently labeled markers, we genotyped the three patients of family 1 at 304 microsatellite loci, and scrutinized our results for regions of homozygosity shared by all three affected individuals. We identified such a region between markers D12S85 and D12S345 (not shown).

Because of the very low numbers of informative microsatellite markers available in this region in public databases, we characterized 15 novel markers within the suspected disease interval, six of which were found to be informative in our two families (Table I). Using these and five additional markers derived from the NCBI Mapviewer database (http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9606) and spanning 19.9 cM on 12p11.2–q13, we genotyped all members of families 1 and 2. Multipoint logarithm of odds ratio (LOD) score analysis using the Superlink software for eight markers placed the new CRI-associated locus between markers D12S345 and D12S390 with a maximum LOD score of 4.79 at marker CH12SSR13 (Fig 2). Multipoint LOD score analysis performed separately for each of the two families revealed a positive LOD score of 2.88 for family 1 and 1.91 for family 2. Similar results were obtained using the HOMOZ software, which generated a maximum combined LOD score of 4.05 at marker CH12SSR13.

We established parsimonious haplotypes for each individual (Fig 3). Haplotype analysis revealed critical recombination events in individuals 68 and 65 at markers D12S1648 and D12S85, respectively, therefore defining the upper and lower boundaries of the disease interval in this family.

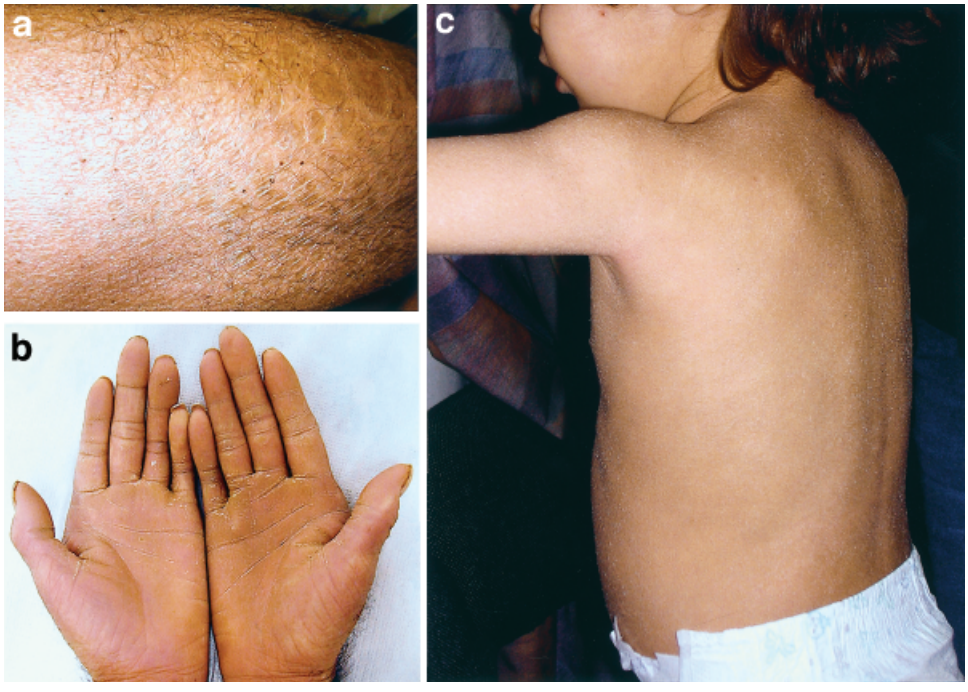

Figure 1
Clinical features. (*a*) Large lamellar scales over the left thigh of patient 64. (*b*) Thickening of the palmar skin in patient 63. (*c*) Fine whitish scales cover the skin of patient 93.