Assignment of the Gene for a New Hereditary Nail Disorder, Isolated Congenital Nail Dysplasia, to Chromosome 17p13

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Isolated congenital nail dysplasia is an autosomal dominant disorder recently observed in a large family from southern Germany. The disorder is characterized by longitudinal streaks, thinning, and impaired formation of the nail plates leading to increased vulnerability of the free nail margins. In most cases, all fingernails and toenails are similarly involved with some accentuation of the thumb and great toenails. Histologic changes include hypergranulosis of the nail matrix and epithelial outgrowths from the nail bed. Patients do not show any alterations of hair growth and dentition, no malfunction of sweat glands and sensory organs, and no skeletal abnormalities. Isolated congenital nail dysplasia manifests from the first year of life with variable expressivity. In order to localize chromosomally the gene underlying isolated congenital nail dysplasia, linkage to the known keratin gene cluster regions on chromosomes 12q12 and 17q21 was ruled out first. The analysis of 150 microsatellite markers on various chromosomes mapped the isolated congenital nail dysplasia gene to the 6 cM interval between markers at D17S926 and D17S1528 on chromosome 17p13. Markers at D17S849, D17S1840, and D17S1529 co-segregated completely with the isolated congenital nail dysplasia locus. The maximum two-point LOD score was found for the marker at D17S1840 ($Z_{max} = 6.72$ at $\Theta_{max} = 0.00$). The identified region harbors no currently known genes involved in skin or nail abnormalities. Isolated congenital nail dysplasia probably represents a novel isolated defect of nail development. The localization of this gene is, therefore, the first step towards the identification of a new factor in nail formation. ***Key words: chromosome 17p13/isolated congenital nail dysplasia. J Invest Dermatol 115:664–667, 2000***

Various forms of nail dystrophy are part of a complex clinical picture in many congenital disorders, including multisystem diseases, chromosomal aberrations, and ectodermal dysplasias. Ectodermal dysplasias are an extensive nosologic group of more than 150 entities featuring variable combinations of defects in skin, hair, tooth, nail, and sweat gland development. They may or may not be accompanied by other defects and malformations such as mental retardation, syndactyly, deafness, cleft lip/palate, ptosis, ear duct anomalies, etc. (Pinheiro and Freie-Maia, 1994).

Other nail dystrophies are closely associated with skeletal abnormalities. Nail–patella syndrome, the best known example of this group, is characterized by various types of nail changes, hypoplastic patellae, iliac horns, elbow abnormalities, and nephropathy (Sabnis *et al*, 1980). In Iso-Kikushi syndrome, dysplasia predominantly of the index fingernails is often combined with skeletal anomalies of the corresponding phalanges (Samman, 1995). Furthermore, there is a number of genodermatoses with characteristic nail involvement, such as dyskeratosis congenita, pachyonychia congenita, Darier's disease, and Hailey–Hailey disease.

More rarely, genetic conditions exclusively involve the nails, such as in hereditary variants of congenital malalignment of the great toenails, absence and hypoplasia of the nails, koilonychia, trachyonychia, leukonychia, brachyonychia, and others (reviewed by Samman, 1995; Shwayder, 1995). Only one nail, a pair, or a minority of nails are usually affected in these disorders. Besides, there are few congenital anomalies with involvement of all fingernails and toenails, such as the congenital nail deformity reported by Friederich (1950), the "soft nail disease" (Prandi and Caccialanza, 1977), the "hereditary twenty-nail dystrophy" observed by Pavone *et al* (1982), and the "familial severe twenty-nail dystrophy" of Arias *et al* (1982).

Here we report the linkage study and assignment of a gene for a novel genetic entity, isolated congenital nail dysplasia (ICND). ICND is transmitted in an autosomal dominant manner documented through five generations in a large family from southern Germany (Hamm *et al*, in press). Affected individuals present with longitudinal streaks, thinning, and variously disturbed formation of the nail plates leading to increased vulnerability of the free nail margins. In most of the 22 affected family members examined, all fingernails and all toenails are involved with some accentuation of thumbnails and great toenails. Single nails may show longitudinal angular ridges that occasionally start at a conspicuous red prominence near the proximal nail fold, poorly developed lunulae, platonychia/koilonychia with the nail plate sometimes overgrowing the lateral nail fold, and notches and fissures of the free nail margins (**Fig 1**). Histologic examination of nail biopsies revealed an abnormal keratinization of the matrix epithelium with a broad granular layer, which is lacking in normal nails, and epithelial strands and buds extending from the nail bed. Both of these histopathologic features point to an epithelial origin of the disorder.

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Abbreviations: ICND, isolated congenital nail dysplasia.

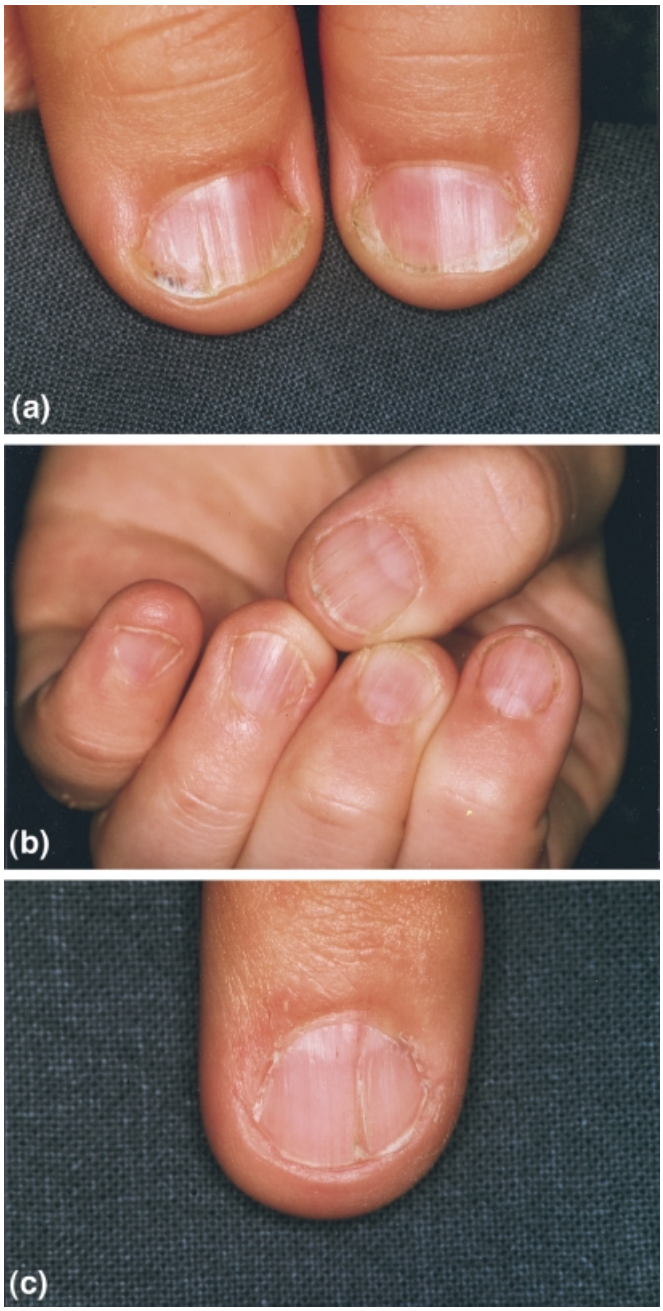

Figure 1. Clinical features of ICND. (*a*) ICND in a 9 y old girl. All fingernails of the left hand show thinning and longitudinal streaks. Some lunulae are poorly developed. (*b*) ICND in a 7 y old boy. The nail plates of the thumbs are striated and seem to broaden distally by overgrowing the lateral nail folds. (*c*) ICND in a 15 y old male. A marked longitudinal ridge arises from a small reddish dome-shaped prominence adjacent to the proximal nail fold and turns in a notch of the free nail margin.

ICND manifests from the first year of life and soon reaches an individually variable degree of severity with little change during life. Affected family members showed no associated skin, hair, or tooth alterations, as well as no malfunction of sweat glands or sensory organs. Skeletal malformations, including hypoplasia of the patellae, were excluded by radiographs. ICND is thus confined to the nails and represents a novel isolated defect of nail development and not a further form of ectodermal dysplasia.

PATIENTS AND METHODS

Analyzed individuals The linkage analysis was performed in 41 individuals from three generations of the affected family (**Fig 2*a***). The high number of affected females and males in all generations well documents the autosomal dominant mode of inheritance and the full penetrance of ICND. Studied family members included 22 affected and 19 unaffected persons. All of them were examined by two dermatologists (H.H. and S.K.), who established the ICND diagnosis and collected the clinical data. A detailed clinical and histopathologic description of the family is published elsewhere (Hamm *et al*, in press).

Genotyping DNA was extracted using the Nucleon II Kit (Scotlab), according to the manufacturer's instructions. Analyzed microsatellite markers were chosen from our microsatellite mapping panels, which are based on the Généthon final linkage map (Dib *et al*, 1996). The markers are evenly distributed over the entire genome with an average distance of 11 cM. Genetic maps for fine mappings were taken from the Généthon linkage map (Dib *et al*, 1996).

Markers were amplified individually in a final reaction volume of 10 µl containing 10 mM Tris, 1.5 mM $MgCl_2$, 100 µM each dNTP, 0.4 U polymerase (Perkin Elmer), 7.0 pmol of each primer, and 25 ng of genomic DNA. One of the primers was end-labeled with fluorescent dye. DNA amplification was carried out in an MJ Research PTC-225 thermal cycler. Polymerase chain reactions were then pooled and electrophoresed on an ABI 377 automatic sequencer. Data were analyzed using the computer programs Genescan v2.1 and Genotyper v2.0 (Perkin Elmer).

Linkage analysis Two-point LOD score calculation was performed with the LINKAGE v5.2 program package (Lathrop and Lalouel, 1984) with the help of the newly developed LINKRUN computer program (T.F. Wienker, unpublished data), using an autosomal dominant, fully penetrant model. Most likely haplotypes were constructed either manually or with CRI-MAP v2.41 option Chrompic (Lander and Green, 1989).

Gene mapping data were obtained from the NCBI Human Gene Map 99 (Deloukas *et al*, 1998). The search for candidate genes in the determined chromosome 17 region was performed by comparative analysis of human expressed sequence tag sequence data with the help of the computer program BLAST and the NCBI UniGene collection (http://www.ncbi.nlm.nih.gov/).

RESULTS AND DISCUSSION

Based on the clinical appearance of ICND, a genetic defect in one of the known keratin genes was initially considered (Lane, 1994; Corden and McLean, 1996; Irvine and McLean, 1999). Therefore, linkage to the keratin gene clusters on chromosomes 12q12 and 17q21 (Yoon *et al*, 1994; Milisavljevic *et al*, 1996; Ceratto *et al*, 1997) was first tested and ruled out (data not shown). Subsequently, a whole genome scan was started and, after testing 150 markers, linkage was obtained for the loci D17S1866 and D17S1845 (**Table I**). In order to determine the size of the interval containing the ICND gene, further microsatellite markers from this region were analyzed. The construction of likely haplotypes identified key recombination events in proband 10 at the locus D17S1528 and in probands 34 and 37 at D17S926 (**Fig 2*b***) and delineated a 6 cM ICND gene region on chromosome 17p13 (**Fig 3**). Markers at D17S849, D17S1840, and D17S1529 co-segregated completely with the disease gene. The maximum two-point LOD score was found for the marker at D17S1840 ($Z_{max} = 6.72$ at $\Theta_{max} = 0.00$; **Table I**). A sequence change involving 2 bp at the locus D17S1529 was detected in affected proband 38 and his son, proband 41. As the short distance to the neighboring markers (3.2 cM to D17S1528, 2.1 cM to D17S1840) rules out the possibility of double recombination events at this locus, we considered that change was a mutation in the microsatellite. Therefore, the marker at D17S1529 does not determine the ICND region in this study (**Fig 3**).

To our knowledge, there is no mouse model for this kind of nail dysplasia, which could help to identify and characterize the disease gene. The mapped ICND gene region harbors no currently known genes involved in skin or nail development. As associated clinical signs are lacking in ICND, we assume that the underlying gene codes for a factor exclusively controlling nail formation and growth. The histologic changes confined to the nail matrix and nail bed epithelium support the assumption that expression of the ICND gene might be restricted to nail keratinocytes. The NCBI Human Gene Map 99 (Deloukas *et al*, 1998), however, lists no

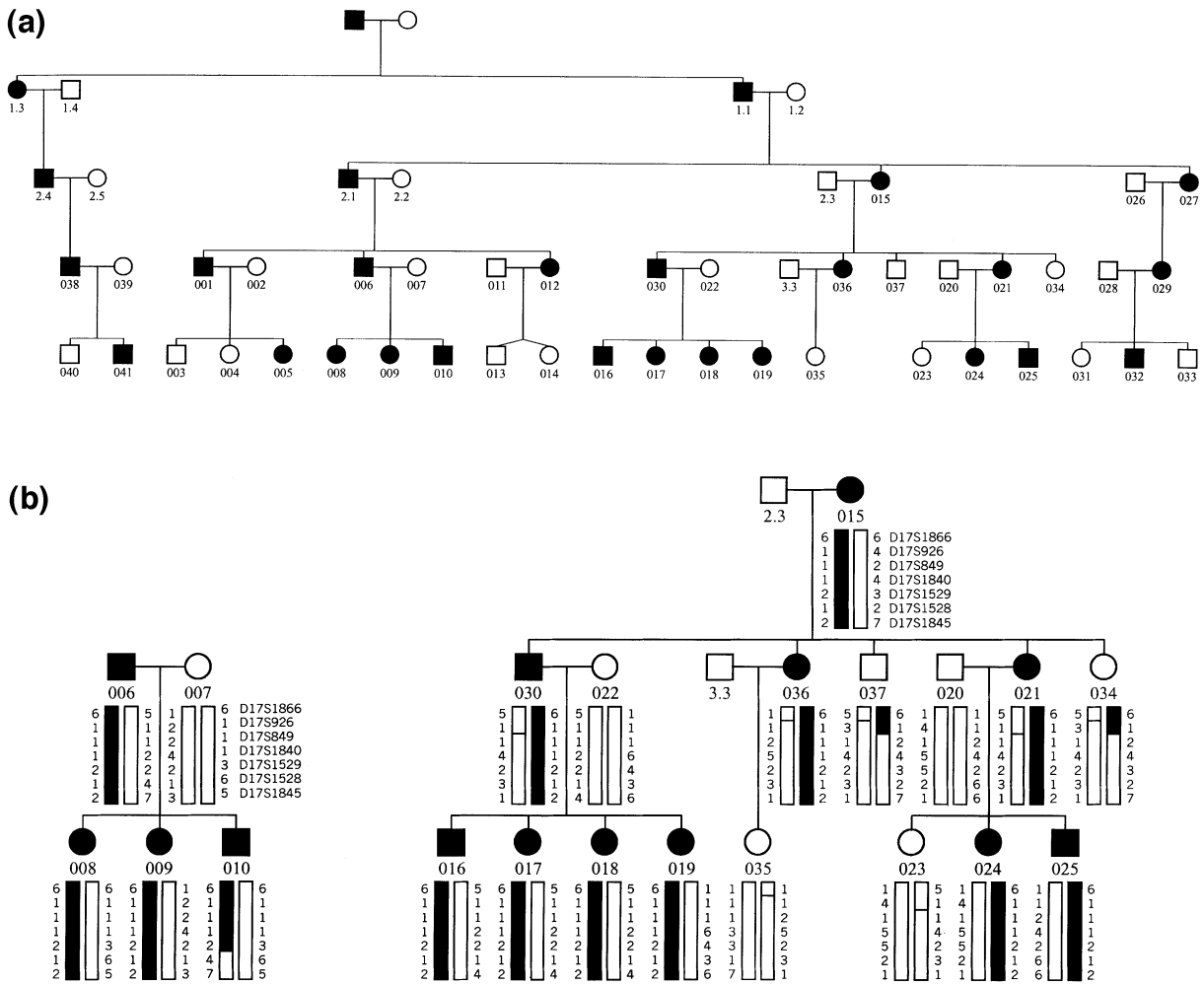

Figure 2. Pedigree of the investigated ICND family and construction of haplotypes. (*a*) Complete pedigree. The filled symbols indicate individuals affected by ICND. The high number of affected females and males in all generations shows an autosomal dominant mode of inheritance with full penetrance. (*b*) Excerpts from the pedigree with corresponding haplotypes showing the decisive recombination events. The black bar represents the affected haplotype that was found in all affected family members. A change of filled bar to open bar indicates a recombination event. Recombination events determining the ICND interval were identified in individuals 10, 34, and 37.

expressed sequence tags expressed exclusively in human keratinocytes in the ICND gene region. The interval contains the *DOC2B* gene coding for a double C2-like domain-containing protein, which is expressed ubiquitously and supposed to be involved in Ca^{2+}-dependent intracellular vesicle trafficking in various cell types (Sakaguchi *et al*, 1995). It has been shown that an altered cytosolic concentration of Ca^{2+} in epithelial cells impairs the transport of desmosomal proteins to the plasma membrane. This results in disrupted desmosome assembly and decreases the epidermal integrity (Stuart *et al*, 1996; Sakuntabhai *et al*, 1999). Therefore, it could be speculated that a defect in *DOC2B* might alter the cellular response to changes of the Ca^{2+} concentration and affect the transport of certain molecules involved in differentiation of keratinocytes; however, as *DOC2B* is expressed ubiquitously, it is only considered a minor candidate gene for ICND. Further characterization of expressed sequence tags and identification of other transcripts are necessary in order to identify more candidate genes for ICND.

It is difficult to hypothesize the nature of the defect underlying ICND as rather diverse mechanisms are as yet identified in disorders with nail dystrophies. Pachyonychia congenita is a group of genodermatoses that was attributed to mutations in keratins K6a, K6b, K16, and K17 (Bowden *et al*, 1995; McLean *et al*, 1995; Smith *et al*, 1998). Hallmarks of this condition are elevation and marked thickening of all nail plates and subungual hyperkeratosis. In patients

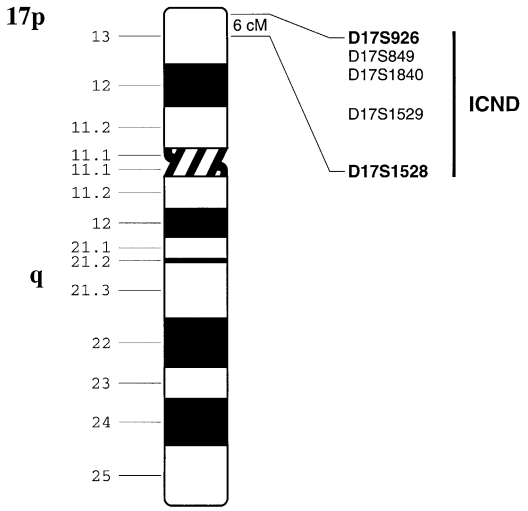

Figure 3. Localization of the ICND gene. The 6 cM interval containing the ICND gene on chromosome 17p13 is flanked by the markers at D17S926 and D17S1528.

suffering from monilethrix, a characteristic hair shaft abnormality often associated with platonychia and koilonychia, mutations in the basic hair keratins hHb1 and hHb6 have been identified (Winter *et al*, 1997a,b; Korge *et al*, 1999). Typical nail changes are an early sign of the often fatal X-linked dyskeratosis congenita, the underlying gene of which has recently been characterized as the human ortholog of the gene for the rat nucleolar protein NAP57 (Heiss *et al*, 1998). Deletions in the collagenous repeats of the ectodysplasin-A gene are responsible for anhidrotic ectodermal dysplasia, in which nail dysplasia is a facultative sign (Kere *et al*, 1996; Bayes *et al*, 1998). Intriguingly enough, the nail dystrophy in nail–patella syndrome is caused by a defect in the LIM-homeodomain protein Lmx1b (Dreyer *et al*, 1998). This protein plays a central part in the dorsal/ventral patterning of the limb, and its disruption results in hypoplastic nails, skeletal defects including absent patellae, and in a unique form of renal hypoplasia.

Typical nail changes in Darier's disease include red and/or white longitudinal streaks often ending in a V-shaped notch and thus share some features with ICND; however, in contrast to ICND, nail involvement in Darier's disease occurs considerably later in life, tends to be progressive, and is almost always associated with conspicuous skin lesions. Moreover, the two conditions differ markedly with regard to nail histology. Darier's disease and Hailey–Hailey disease, in which longitudinal white streaks of nail plates may also occur, are caused by mutations in the genes *ATP2A2* and *ATP2C1*, respectively, both encoding sarcoplasmic/endoplasmic reticulum Ca^{2+} pumps (Ruiz-Perez *et al*, 1999; Sakuntabhai *et al*, 1999; Hu *et al*, 2000; Sudbrak *et al*, 2000).

Nail lichen planus may be considered as another differential diagnosis to ICND. Lichen planus is a rather frequent inflammatory dermatosis of unknown origin that rarely affects more than one family member, probably in terms of polygenic predisposition, and may involve skin, mucosal membranes, hair follicles, and nails; however, exclusive involvement of the nails is unusual and even more exceptional in childhood (Peluso *et al*, 1993). To our knowledge, familial lichen planus limited to the nails has never been described. Moreover, ICND can easily be differentiated from nail lichen planus by the histologic lack of inflammatory signs.

Because of the rather specific combination of nail features ICND can be clearly distinguished from other hereditary conditions exclusively involving the nails. Only the ''familial severe twenty-nail dystrophy'' (Arias *et al*, 1992) resembles the clinical picture of ICND; however, affected individuals with that disorder show additional nail and tooth changes, including mild distal onycholysis, subungual hyperkeratosis, and malocclusion of the teeth. Although these clinical features of familial severe twenty-nail dystrophy were not observed in individuals affected by ICND, both disorders could be allelic and caused by different defects in the same gene. Only a linkage study in the family with familial severe twenty-nail dystrophy could finally resolve this differential diagnostic issue. To summarize, we describe here the mapping of the gene for a new autosomal dominant nail dysplasia, ICND. The localization of the ICND gene to a region on chromosome 17p13, which has not been connected with hereditary skin or nail disorders so far, represents the first step towards the identification of a new factor in nail formation. The characterization of this gene will then allow the study of genetic mechanisms specifically involved in nail development.

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