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Localization of a novel locus for hereditary nail dysplasia to chromosome 17q25.1-17q25.3

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We report on a six-generation Pakistani consanguineous family with autosomal recessive transmission of a form of hereditary nail dysplasia. Affected individuals presented with onycholysis of fingernails and anonychia of toenails. Associated abnormalities of ectodermal appendages were not observed in any of the affected individuals. Linkage has been established to chromosome 17q. A maximum multipoint analysis logarithm of the odds ratio score of 4.85 was obtained at marker D17S1301. Due to the consanguineous nature of this kindred, the gene for nail dysplasia is probably contained within a 5.0-cM (3 MB on the sequence-based physical map) region of homozygosity flanked by markers D17S1807 and D17S937.

In the recent past, investigations of a number of developmental disorders affecting the tissue of ectodermal origin have revealed the involvement of several genes in normal development of ectodermal tissue. Developmental abnormalities of the nails constitute a large and extremely heterogeneous group of disorders. They range from slight, hardly noticeable change to the complete absence of nails. They may be noticeable at the time of birth or make an appearance at a later age. They may be inherited or acquired in fetal life; and they may appear as an isolated abnormality or may be a component of complex syndromes with associated abnormalities of other ectodermal appendages.

Isolated congenital nail dysplasia (NDIC, MIM 605779) is a rare autosomal dominant condition characterized by thinning and impaired formation of nail plates, mostly of all fingernails and toenails (1). A locus for the NDIC mapped to a 6-cM interval on 17p13 (2). This condition is different from the hereditary 20-nail dystrophy (3) that involves slowly progressive nail dystrophy with nail loss at 10–20 years of age.

In nail–patella syndrome (MIM 161200), dysplasia of the nails and absent or hypoplastic patellae are the cardinal features but others are iliac horns, abnormality of the elbows interfering with pronation and supination, and in some cases nephropathy. Mutations in *LMX1B* gene (MIM 602575), which maps to 9q, are responsible for causing nail–patella syndrome (4, 5).

A number of genodermatoses like pachyonychia congenita (PC, MIM 167200), Darier’s disease (MIM 12400), and X-linked dyskeratosis congenita (DC, MIM 305000) are associated with characteristic nail changes. PC represents a group of inherited ectodermal disorders characterized by dystrophic nails, palmar and plantar hyperkeratosis, hyperhidrosis, and blistering of feet. The two main subtypes of PC, Jadassohn–Lewandowsky syndrome (PC-1, MIM 167200) and Jackson–Lawler syndrome (PC-2, MIM 167210), are inherited in an autosomal dominant fashion. PC-1 has been shown to be due to mutations in either the keratin 16 gene (MIM 148067), which maps to chromosome 17, or the keratin 6 A gene (MIM 148041), which maps to chromosome 12. PC-2 had been described only in patients with mutations in keratin 17 gene (MIM 148069), which maps to chromosome 12. Darier–White disease (MIM 124200), also known as keratosis follicularis, is an autosomal dominant skin disorder characterized by warty papules and plaques in seborrheic areas (central trunk, flexures, scalp, and forehead), palmoplantar pits, and distinctive nail abnormalities. A causative gene for Darier’s disease, which resides at 12q23–q24.1 (6) and encodes the sarco/endoplasmic reticulum Ca^{2+} -ATPase type 2 isoform (MIM 108740). The features of DC are cutaneous pigmentation, dystrophy of the nails, leukoplakia of the oral mucosa, continuous lacrimation due to atresia of the lacrimal ducts, often thrombocytopenia, anemia, and in most cases testicular atrophy. Mutations in *XAP101* gene (MIM 305000), which maps to Xq28, are responsible for DC (7).

In Iso–Kikushi syndrome, dysplasia predominately of the index fingernails is often combined with skeletal abnormalities of corresponding phalanges (8). A kindred with seven individuals in two generations had a disorder characterized by onychodystrophy, anonychia, brachydactyly of the fifth finger, and digitalization of the thumbs, with absence or hypoplasia of the distal phalanges of the hands and feet (9). In autosomal dominant brachydactyly with the absence of middle phalanges and hypoplastic nails (MIM 112900), the changes in the middle phalanges are distinctive. Relatively bizarre, asymmetric digital anomalies, including absence of one or more digits, distinguish anonychia with ectrodactyly (MIM 106900). Bilateral corneal opacities and marked nail hypoplasia affecting all digits of the hands and feet were reported in a newborn son of a first-cousin Pakistani family (10).

Here is described the mapping of a locus for an autosomal recessive form of nail dysplasia in a family from a remote area of Pakistan. After excluding the regions for both type I (17q12–q21) and type II (12q13) keratin genes and few other epidermally expressed genes, linkage was established to a region on chromosome 17q25.1–q25.3.

Materials and methods

Family history

A large six-generation Pakistani kindred was ascertained and investigated for hereditary nail dystrophy as an isolated abnormality without any allied ectodermal imperfection. Prior to the start of study, approval was obtained from the Quaid-I-Azam University Institutional Review Board. Informed consent was obtained from all individuals who agreed to participate in the study. The family members rarely marry outside the family, and consequently consanguineous unions are common. The pedigree (Fig. 1) provided convincing evidence of autosomal recessive mode of inheritance, and consanguineous loops accounted for all the affected persons being homozygous for the mutant allele. Clinical information was obtained for all family members with particular attention paid to skin, dentition, sweating, nails, and scalp and body hair. The eyebrows, eyelashes, axillary, and scalp and pubic hair were normal. All the affected individuals were in good general health condition with no evidence of immune system dysfunction or unusual susceptibility to skin tumors. At birth, both finger and toenails were normal. Onychodystrophy of finger and toes starts at the age of 7–8 years. Dystrophy initiates from free margins of nails and progress gradually toward onychodermal band, nail plate, lunula, cuticle, and ultimately reaches to eponychium. Onychodystrophy found in our family differentially affects the finger and toenails. Dystrophy of both finger and toenails start at the same time but leads to anonychia (complete absence of nails) on toenail and onycholysis (wide separation of nail from nail bed and dystrophy of free margins) on fingernails (Fig. 2).

DNA extraction and genotyping

Venous blood samples, 10–15 ml, were collected from 12 members of the family, and high molecular DNA was extracted from leukocytes following the standard method (11).

Polymerase chain reaction (PCR) reactions were carried out in 25- μ l reaction volumes containing 40 ng of genomic DNA, 20 pmol of primers, 200 μ M of each dNTP, 1 U of *Taq* DNA polymerase (MBI Fermentas, UK), and 2.5- μ l reaction buffer (KCl 50 mM, Tris–Cl pH 8.3, and $MgCl_2$ 1.5 mM). The thermal cycling conditions used included 95°C for 5 min, followed by 40 cycle of 95°C for 1 min, 55–57°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min. PCR was performed by the use of thermal cycler 'Gene Amp PCR system 2700' obtained from Applied Biosystems (Foster City, CA). PCR products were resolved on 8% non-denaturing polyacrylamide gel, and geno-types were assigned by visual inspection.

The following microsatellite markers linked to several candidate genes/loci were genotyped in the 12 family members: D13S787, D13S175, D13S292 (ED2), D2S176, D2S410 (ED3), D11S1998, D11S4464 (ED4), D17S800, D17S806, D17S934 (keratin type I), D12S1290, D12S368, D12S297 (keratin type II), D18S56, D18S36, D18S536, D18S1149 (desmoglein and desmocollin cluster), D1S534, D1S442, D1S498 (epidermal differentiation complex), D14S1040, D14S50, D14S264 (transglutaminase I) and D20S107, D20S119 (transglutaminase II/III). After known loci were excluded, a genome scan was conducted by using highly informative polymorphic microsatellite markers from Linkage Mapping Set 10

(Invitrogen, Carlsbad, CA). An initial genome wide screen was carried out using markers spaced at 20-cM intervals by the use of DNA from four of the affected individuals (V-8, VI-5, VI-6, and VI-8).

Linkage analysis

Two-point linkage analysis was carried out using MLINK of the FASTLINK computer package (12). Multipoint linkage analysis was performed using ALLEGRO (13). For the analysis of an autosomal recessive mode of inheritance with complete penetrance and a disease allele, frequency of 0.001 was assumed. Equal allele frequencies were used in the analysis. However, because it is well known that using allele frequencies, which are too low can lead to false-positive results, a sensitivity analysis was performed. The marker allele that was segregating with the disease was varied between 0.2 and 0.8, and both two-point and multipoint linkage analysis were carried out. The order of the markers and their maps distances was based upon the Marshfield (14) (<http://www.marshmed.org/genetics/>) and deCode (15) genetic maps. Haplotypes were constructed using SIMWALK2 (16, 17).

Mutation detection

To screen mutation in envoplakin (EVPL) gene, all 22 exons and splice junctions were first optimized for PCR conditions with genomic DNA, and then, two affected individuals and an unaffected control were amplified. The PCR products were purified and sequenced on an ABI Prism 310 automated sequencer, using the ABI Prism Big Dye terminator Cycle Sequencing Ready Reaction sequencer Kit (PE Applied Biosystems).

Results

Based on the clinical appearance of nail dysplasia in our family, linkage to markers linked to candidate genes/loci ED2, ED3, ED4, keratin type I and 2, desmoglein and desmocollin cluster, epidermal differentiation complex, and transglutaminase I/II/III) was tested first. Examination of the marker haplotypes (data not shown) revealed that the affected pedigree members were heterozygous for different combinations of the parental alleles, and thus, linkage based upon homozygosity to the candidate genes was excluded.

After excluding the candidate genes/loci, a genome scan was carried out using homozygosity mapping. DNA samples from four affected individuals (V-8, VI-5, VI-6, and VI-8) were genotyped with 220 microsatellite markers spaced approximately every 20 cM. A marker on chromosome 17q25 (D17S840) was found to be homozygous in all the four affected individuals. All family members were genotyped for marker D17S840 and four additional markers D17S1301, D17S1839, D17S785, and D17S1817 located within this region. These markers displayed logarithm of the odds ratio (LOD) scores >2.2 (Table 1) and were all homozygous in the affected individuals. Additional markers D17S1874, D17S942, D17S1821, D17S789, D17S2059, D17S1351, D17S515, D17S1807, and D17S937 were then genotyped. A maximum two-point LOD score of 3.0 was obtained at $\theta=0$ with marker D17S1351. Multi-point linkage analysis produced a maximum LOD score of 4.8 at marker D17S1301. The three-unit support for the nail dysplasia locus is flanked by markers D17S1821 and D17S937. This interval is 19.7 and 20.8 cM according to the

Marshfield (14) and deCode (15) genetic maps, respectively. Due to the number of consanguineous matings within this kindred, the gene for nail dysplasia is probably contained within the region of homozygosity observed in affected pedigree members. The region of homozygosity is flanked by markers D17S1807 and D17S937. This interval is 5.0 and 6.5 cM in length according to the Marshfield and deCode genetic maps, respectively. According to the sequence-based physical map Human Genome Project Santa-Cruz (18), the region of homo-zygosity is 3 MB.

Discussion

It was not possible to estimate allele frequencies from the founders of this pedigree due to its small size. Therefore, equal allele frequencies were used in the linkage analysis. Only two alleles were observed to segregate for the markers in the studied chromosomal 17 region except for marker D17S1351. Thus, the lowest allele frequency, which was used in the analysis was 0.33 for this marker. For linkage analyses, if the allele frequencies, which are used, are much lower than the true allele frequencies, a false-positive result may be observed (19). Therefore, a sensitivity analysis was carried out. The frequency of the allele that was segregating with the disease allele was varied between 0.2 and 0.8. For this range of allele frequencies, the multipoint maximum LOD score varied between 4.9 and 3.1. Given that the average heterozygosity of these markers is 0.77 (SD 0.07), it is highly unlikely that all of the marker alleles, which are segregating with the disease allele have a frequency of greater than 0.8, and therefore, linkage has falsely established.

After excluding linkage with the keratin and other epidermally expressed candidate genes, we performed a genome-wide search to localize the gene for autosomal recessive nail dysplasia to the long arm of chromosome 17. This locus is distinct from the locus for another primary nail dysplasia namely autosomal dominant NDIC, which is located on the short arm of chromosome 17 (2). This is not surprising, because affected individuals with NDIC display longitudinal streaks and thinning of nail plates, features which are not observed in our family.

A number of genes have been localized to the identified 3-MB region of homozygosity. The genes within this region include tylosis (MIM 148500), EVPL (MIM 601590), gelanin receptor-2 (MIM 603691), growth factor receptor-bound protein-2 (MIM 108355), integrin beta-4 (ITGB4, MIM 147557), cyclin-dependent kinase-3 (MIM 123828), sphingosine kinase-1 (MIM 603730), solute carrier family-9, isoform A3, regulatory factor-1 (MIM 604990), galactokinase-1 (MIM 604313), and SEC14-like 1 (MIM 601504). Of these genes, there are two of interest, ITGB4 and EVPL. Integrins (MIM 147557) are transmembrane glycoprotein receptors that mediate cell-cell adhesion and transduced signals that regulate gene expression and cell growth. Mutations in ITGB4 had been demonstrated earlier in patients with epidermolysis bullosa with congenital pyloric atresia (20). EVPL (MIM 601590) is a membrane-associated protein expressed in human stratified squamous epithelia in skin, oral mucosa, esophageal mucosa, and cervical mucosa (21). EVPL is homologous to the keratin-binding proteins desmoplakin I and II (MIM 125647), bullous pemphigoid antigen-1 (MIM 113810), and plectin (MIM 601282). It was suggested previously that EVPL might be a novel desmosomal component involved in anchoring keratin filaments to

desmosomes in stratified epithelia (21). All 22 exons of EVPL gene were sequenced in two affected individuals and an unaffected family member; however, no disease-causing mutation was detected. Further work is currently being carried out to identify the gene that is responsible for autosomal recessive hereditary nail dystrophy.

Electronic database information

The URLs for data presented herein are as follows: UCSC Human Genome Project, July 2003 assembly, <http://genome.cse.ucsc.edu/> (the physical positions of STRP loci); Marshfield Center for Medical genetics, <http://www.marshmed.org/genetics/> (for the order and genetic distances of STRP loci on 17q).

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Fig. 1.

Pedigree of the family with hereditary nail dystrophy. Filled symbols represent affected subjects. Clear symbols represent unaffected individuals. The disease-associated haplotype is shown in box beneath each symbol. Haplotypes were generated by SIMWALK2.

Fig. 2.
Clinical features of the nail dystrophy: Fingernails of individual VI-8 presenting onycholysis (top). Toenails of the same individual (VI-8) showing anonychia (bottom).

Table 1.

Two-point logarithm of odds ratio score results between the hereditary nail dysplasia locus and chromosome 17 markers

Marker name	deCode map position ^a	Marshfield map position ^b	Physical map position ^c	Recombination fraction					
				0.0	0.01	0.05	0.1	0.2	0.3
D17S1874	94.94	85.94	64614355	–Infinity	0.42	0.89	0.91	0.69	0.41
D17S942	95.49	85.94	64906671	–Infinity	–1.16	–0.00	0.32	0.39	0.25
D17S1821	97.29	85.94	65703011	–Infinity	–1.52	–0.30	0.09	0.25	0.17
D17S789	99.50	89.32	67093030	0.95	0.92	0.79	0.63	0.37	0.18
D17S2059	102.96	93.27	68965424	2.26	2.21	2.00	1.74	1.22	0.71
D17S840	103.29	93.27	69072593	2.48	2.41	2.17	1.87	1.29	0.76
D17S1351	106.23	95.99	71136562	3.03	2.96	2.66	2.29	1.56	0.90
D17S515	108.54	97.56	71771360	2.26	2.21	2.00	1.74	1.22	0.71
D17S1807	113.10	99.21	72824696	2.26	2.21	2.00	1.74	1.22	0.71
D17S1301	113.59	100.02	73144944	2.78	2.71	2.44	2.16	1.44	0.84
D17S1839	114.52	102.46	74265911	2.78	2.71	2.44	2.10	1.44	0.84
D17S785	115.34	103.53	74895412	2.21	2.16	1.94	1.67	1.14	0.66
D17S1817	115.44	103.53	74946179	2.78	2.71	2.44	2.10	1.14	0.84
D17S937	118.10	105.68	75807741	–Infinity	–1.04	–0.43	–0.22	–0.08	–0.0

^aSex-average Kosambi cM map position from the deCode (15) genetic map.

^bSex-average Kosambi cM map position from the Marshfield (14) genetic map.

^cSequence-based physical map distance in bases according to the Human Genome Project – Santa Cruz (16).

The genetic and sequence-based physical map distances are summarized. Markers displayed in bold flank the region of homozygosity.