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Ectodermal dysplasia of hair and nail type: mapping of a novel locus to chromosome 17p12-q21.2

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Summary

Background—Ectodermal dysplasias (EDs) describe a large and complex group of disorders characterized by abnormal development of the skin and appendages (hair, nails, teeth and sweat glands). Of the approximately 200 different EDs, about 30 have been studied at the molecular level. In an effort to understand the molecular bases of ED of hair and nail type, we studied a Pakistani consanguineous family with multiple affected individuals.

Objectives—To localize the gene responsible for the autosomal recessive form of ED of hair and nail type.

Methods—Genotyping of nine members of the family, including five affected and four normal individuals was performed using microsatellite markers mapping to candidate regions, harbouring genes involved in related phenotypes. Five epithelial keratin genes located in the candidate region were sequenced to identify the pathogenic mutation.

Results—We mapped the disease locus to a 24.2-cM interval flanked by markers D17S839 and D17S1299 on chromosome 17p12-q21.2 ($Z_{\max} = 4.4$). DNA sequencing of five epithelial keratin candidate genes, present in the disease locus, did not reveal any pathogenic mutation in the affected individuals.

Conclusions—The gene for ED of hair and nail type has been mapped to chromosome 17p12-q21.2 in a Pakistani consanguineous family. Failure to detect mutations in epithelial keratin genes suggests that the mutation may lie either in regulatory regions of one of the epithelial keratin genes or in another unknown gene, located in the linkage interval, with a possible role in the development of ectodermal appendages.

Keywords

chromosome 17p12-q21.2; ectodermal dysplasia of hair and nail type; Pakistan

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Conflicts of interest

None declared.

Ectodermal dysplasia (ED) defines a family of congenital, developmental disorders with primary defects in at least two ectoderm-derived structures. The main tissues of ectodermal origin affected in EDs are hair, teeth, nails and eccrine glands. Approximately 200 distinct EDs have been described so far; however, only about 30 EDs have been explained at the molecular level with identification of the causative genes. Recently, Lamartine¹ proposed to classify EDs, according to the functions of the genes discovered, into four major subgroups: cell–cell signalling, cell adhesion, regulation of transcription and development.

ED of hair and nail type (MIM 602032) is a rare congenital disorder characterized by trichodysplasia (hypotrichosis, partial or total alopecia) and dystrophy of finger and toe nails with no other associated abnormality. Various clinical forms of pure hair and nail ED have been reported. Calzavara-Pinton *et al.*² reported an autosomal recessive ED family with clinical features of nail dystrophy and trichodysplasia. Scalp, beard, axillary and pubic hair were broken off leaving a stubble 1–10 mm in length. Eyebrows, eyelashes and body hair were completely absent. In a Brazilian family, pure hair–nail dysplasia was inherited in an autosomal dominant fashion with complete penetrance and variable expressivity.³ Affected individuals showed hypotrichosis of the whole body with varying degrees of severity. The nails were short, fragile and spoon shaped. Barbareschi *et al.*⁴ described an autosomal dominant pure hair–nail dysplasia in a mother and son. Both the affected individuals had hypotrichosis limited to the bilateral frontotemporal areas of the scalp. There was also hair loss in the outer third of the eyebrows. Micronychia and onychorrhexis were the main findings in the finger and toe nails. Harrison and Sinclair⁵ reported a hair–nail ED in a 3-year-old girl. Clinical features of this affected girl included generalized scalp hypotrichosis, absent eyebrows, short eyelashes and nail dystrophy in all digits. Both the child's parents were clinically unaffected, suggesting either autosomal recessive inheritance or a de novo autosomal dominant mutation. An autosomal recessive form of pure hair and nail ED was described in a Pakistani family of 13 affected individuals.⁶ The patients had thin scalp and body hair and fine eyebrows and eyelashes. Nail dystrophy was present in all digits. The disease locus in this family was mapped to a 3.92-cM region on chromosome 10q24.32–q25.1.

In the present study, we studied Pakistani kindred that segregate ED of hair and nail type in an autosomal recessive fashion. By performing parametric linkage analysis using microsatellite markers, we have localized the gene responsible for this autosomal recessive form of hair–nail disorder to chromosome 17p12-q21.2. Five epithelial keratin genes located in the candidate region were sequenced to identify the pathogenic mutation.

Materials and methods

Human subjects

A Pakistani consanguineous family was studied, in which three males and four females were affected with ED of hair and nail type (Fig. 1). Prior to starting the study, approval was obtained from the Quaid-I-Azam University Institutional Review Board. In addition, informed consent was obtained from all the family members who participated in the study. The family members rarely marry outside the community, 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. All of the affected individuals underwent examination at the Department of Dermatology, Pakistan Institute of Medical Sciences (PIMS), Islamabad, Pakistan.

Extraction of genomic DNA and genotyping

Venous blood samples were obtained from nine family members, including five affected individuals. Genomic DNA was extracted from whole blood following a standard protocol, quantified by spectrophotometric measurement of optical density at 260 nm and diluted to 40 ng μL^{-1} for amplification by polymerase chain reaction (PCR). PCR amplification of micro-satellite markers was performed according to standard procedure in a total volume of 25 μL , containing 40 ng genomic DNA, 20 pmol of each primer, 200 $\mu\text{mol L}^{-1}$ of each dNTP, 1 U of *Taq* DNA polymerase (MBI Fermentas, York, U.K.) and 1 $\times$ PCR buffer (MBI Fermentas). PCR was carried out for 35 cycles, with the following thermal cycling conditions: 95 °C for 1 min, 57 °C for 1 min, 72 °C for 1 min, followed by final extension at 72 °C for 7 min in a thermal cycler 9600 (Perkin-Elmer, Wellesley, MA, U.S.A.). PCR products were resolved on 8% nondenaturing polyacrylamide gel, and geno-types were assigned by visual inspection. The family was tested for linkage by using microsatellite markers tightly linked to candidate genes involved in related phenotypes. These included: type I keratin (*KRT*) genes at 17q21.2 (D17S1814, D17S1299, D17S800, D17S1787, D17S1860, D17S934); type II keratin (*KRT*) genes at 12q13.13 (D12S297, D12S368, D12S398, D12S90); ectodermal dysplasia type 2 (*ED2*) gene gap junction protein beta-6 (*GJB6*) at 13q12.11 (D13S1316, D13S175, D13S633, D13S250, D13S787); ectodermal dysplasia type 3 (*ED3*) gene ectodysplasin A1 anhidrotic receptor (*EDAR*) at 2q13 (D2S293, D2S1893, D2S1891); ectodermal dysplasia type 4 (*ED4*) gene poliovirus receptor-like 1 (*PVRL1*) at 11q23.3 (D11S1364, D11S924, D11S1299, D11S925, D11S4089); ED of hair and nail type locus on chromosome 10q24.32–q25.1 (D10S1239, D10S1267, D10S1264, D10S254); human hairless (*HR*) gene at 8p21.3 (D8S298, D8S1786, D8S1734); desmoglein (*DSG*) and desmocollin (*DSC*) genes at 18q12.1 (D18S478, D18S847, D18S36, D18S536); envoplakin (*EVPL*) gene at 17q25.1 (D17S1807, D17S1301, D17S785, D17S801, D17S802); loricrin (*LOR*) gene at 1q21.3 (D1S442, D1S498, D1S2715, D1S305, D1S1595); and plakophilin 1 (*PKP1*) gene at 1q32.1 (D1S1660, D1S373, D1S1726, D1S2622, D1S2686, D1S2655).

Linkage analysis

PedCheck⁷ was used to identify Mendelian inconsistencies. Two-point linkage analysis was carried out using the MLINK programme of the FASTLINK computer package.⁸ The micro-satellite markers were ordered according to their sequence-based physical positions from build 35 and the genetic map distances were obtained from the Rutgers combined linkage–physical map of the human genome.⁹ Multipoint linkage analysis was performed using Allegro.¹⁰ It was not possible to estimate allele frequencies from the founders, because the markers were genotyped in only one family, so equal allele frequencies were initially applied. As false-positive results can be obtained when analysing the data using too low an allele frequency for an allele segregating with the disease locus,¹¹ a sensitivity analysis was carried out for the multi-point linkage analysis by varying the allele frequency of the alleles

that segregate with the disease locus from 0.2 to 0.6. Haplotypes were constructed using SIMWALK2.^{12, 13} For the analysis, an autosomal recessive mode of inheritance with complete penetrance and a disease allele frequency of 0.0001 were used.

Mutation analysis

The candidate region of the ED locus identified in the present study harbours six type I epithelial keratin genes. To screen for mutations in the keratin genes *KRT25A*, *KRT25B*, *KRT25C*, *KRT25D* and *KRT24* (MIM 607742), exons and splice junctions were PCR amplified from genomic DNA. PCR products were purified using Rapid PCR Purification System (Marligen Biosciences, Ijamsville, MD, U.S.A.) and were sequenced using the ABI Prism 310 Automated Sequencer, using the Big Dye Terminator Cycle Sequencing Kit (PE Applied Biosystems, Foster City, CA, U.S.A.) following purification in Centri-Sep Spin Columns (Applied Biosystems). Primer sequences to amplify exons and splice junctions of *KRT25A*, *KRT25B*, *KRT25C*, *KRT25D* and *KRT24* genes are available on request.

Results

Clinical findings

Clinical information was obtained for all family members with particular attention to skin, dentition, sweating, nails, scalp and body hair. All the affected individuals were born with total alopecia and dystrophic nails. In patients the hair was absent from the scalp, face, chest, arms and legs (Fig. 2a). At 5 years of age, the patients developed curly sparse thin hair on the scalp (Fig. 2b) that could be painlessly plucked without force. In the affected individuals, koilonychia was observed in both finger and toe nails (Fig. 3). The toe nails were thin and showed dystrophic appearance. The patients were in good general health, sweated normally and were of normal intelligence. Eye abnormalities, ichthyosis, oral leukokeratosis, dental anomalies, palmoplantar keratosis and pigmentation were absent. Results of routine laboratory tests, including white blood cell count and granulocyte function, were normal. Het erozygous carrier individuals had normal hair and nails, and were clinically indistinguishable from genotypically normal individuals.

Localization of hair–nail ectodermal dysplasia to chromosome 17

To identify the gene underlying ED of hair and nail pheno-type, a candidate gene approach was used. We performed parametric linkage analysis with microsatellite markers mapping to candidate regions, harbouring genes involved in related phenotypes. These genes included type I keratin genes at 17q21.2; type II keratin genes at 12q13.13; *GJB6* gene at 13q12.11; *EDAR* gene at 2q13; *PVRL1* gene at 11q23.3; ED of hair and nail type locus on chromosome 10q24.32–q25.1; *HR* gene at 8p12; *DSG* and *DSC* genes at 18q12.1; *EVPL* gene at 17q25.1; *LOR* gene at 1q21.3; and *PKP1* gene at 1q32.1.

Evidence suggestive of linkage was obtained initially with D17S1814, closely linked to type I keratin gene cluster on chromosome 17q21.1. In order to fine map the locus, 25 additional markers were selected from the Rutgers combined linkage–physical map of the human genome.⁹ After genotyping these markers, the data was analysed using two-point and multipoint linkage analysis. The maximum two-point LOD score ($Z_{\max}$) of 2.40 at zero

recombination was achieved with the marker D17S1824 and D17S1294 (Table 1). The maximum multipoint LOD score occurred at marker D17S1814 with a LOD score of 4.4. Additionally, a maximum multipoint LOD score exceeding 4.0 was obtained with several markers (D17S805, D17S1824, D17S1294, D17S1863, D17S1814; Fig. 4).

When the sensitivity analysis was carried out the maximum multipoint LOD score remained at marker D17S1814. As the marker allele frequencies which segregated with the disease locus were varied from 0.2 to 0.6, the maximum multipoint LOD score fluctuated between 4.6 and 3.4 at marker D17S1814 with the lowest LOD score being obtained when the allele frequencies were set to 0.6.

Haplotypes were then constructed to determine the critical recombination events (Fig. 1). The disease haplotype (region of homozygosity) is flanked by markers D17S839 and D17S1299 on chromosome 17p12-q21.2 and spans the 24.2-cM region according to the Rutgers combined linkage-physical human genome map.⁹ This region corresponds to 21.8 Mb on the sequence-based physical map.¹⁴ The critical recombinations defining the co-segregating interval occurred in affected individuals. A recombination event occurred in affected individuals V-6 and VI-2 between markers D17S839 and D17S1843 located on the short arm of chromosome 17. The haplotypes of affected individual VI-3 revealed that a recombination event has also occurred between markers D17S1814 and D17S1299 located on the long arm of chromosome 17.

Mutation analysis

The candidate region of homozygosity identified in the family on chromosome 17p12-q21.2 contains six genes for type I epithelial keratins *KRT25A*, *KRT25B*, *KRT25C*, *KRT25D*, *KRT24* and *KRT10* (MIM 148080), and a homeobox gene *WHN* (MIM 600838). To search for a pathogenic mutation in our family, all coding exons and splice sites of five of these genes (*KRT25A*, *KRT25B*, *KRT25C*, *KRT25D*, *KRT24*) were sequenced in two affected individuals of the family. Sequence variation was not observed in the affected individuals.

Discussion

The gene for ED of hair and nail type has been mapped to chromosome 17p12-q21.2 in a Pakistani consanguineous family. The candidate interval spans the 24.2-cM region according to the Rutgers combined linkage-physical map of the human genome,⁹ which corresponds to 21.8 Mb on the sequence-based physical map.¹⁴

Localization of the disease interval between markers D17S839 and D17S1299 excluded the type I hair keratin genes (*KRTHA1*, *KRTHA2*, *KRTHA3*, *KRTHA3B*, *KRTHA4*, *KRTHA5*, *KRTHA6*, *KRTHA7*, *KRTHA8*) from being considered as candidate genes. The type I hair keratin gene cluster spans a 970-kb region and is located distal to marker D17S1299. However, the University of California Santa Cruz genome browser identified six epithelial keratin genes (*KRT25A*, *KRT25B*, *KRT25C*, *KRT25D*, *KRT24*, *KRT10*), and a homeobox gene (*WHN*) in the candidate region of the ED family.¹⁴

The expression of four keratins KRT25A–KRT25D has been observed in skin (as a hair follicle-containing tissue), scalp, thymus and often tongue.¹⁵ Sprecher *et al.*¹⁶ has reported high level expression of another epithelial keratin, KRT24, in keratinocytes, placenta, colon and spleen. Considering the expression pattern of the five keratins, these were selected for mutation screening in the present family. However, sequence analysis of the coding exons and the splice junction sites of these epithelial keratin genes failed to reveal any sequence aberration in the affected individuals, thus excluding these keratins as causative genes for the hair and nail phenotype in the family.

An epithelial keratin (*KRT10*) gene and winged helix nude (*WHN*) gene, located in the candidate region, were not sequenced in the present study. Clinical features observed in patients with mutations in *KRT10* and *WHN* were not observed in the patients with ED of hair and nail type in the family described here. Dominant mutations in *KRT10* have been shown previously to cause epidermolytic hyperkeratosis in humans and mice.^{17–19} The *WHN* gene encodes a transcription factor regulating hair keratins. Mutations in this gene have been reported to cause T-cell immunodeficiency, alopecia and nail dystrophy.²⁰ These clinical features are dominated by severe immunodeficiency leading to death of the affected individuals at an early age.

The identification of hair–nail ED locus in the family, close to the hair keratin gene cluster, suggests the involvement of a novel gene in the development of ectodermal appendages. The result obtained in the family is the first step towards positional cloning of a novel ED gene, whose identification is anticipated to provide new insights into epidermal differentiation.

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Fig 1.

Pedigree of the family segregating ectodermal dysplasia of hair and nail type. Affected males and females are indicated by filled squares and circles, respectively. Haplotypes for the most closely linked markers are shown below each symbol. The alleles are numbered 1–3 according to their size.

Fig 2.

Clinical findings in ectodermal dysplasia of hair and nail type. (a) Complete absence of hair on the scalp of an affected child (VI-2) at 4 years of age. (b) An affected male (V-3) at 25 years of age. The eyebrows and eyelashes are completely missing. (c) Sparse, short hair on the scalp of an affected male (V-3).

Fig 3. Clinical findings in ectodermal dysplasia of hair and nail type. (a) Finger nails of an affected male (V-3) showing severe koilonychia. (b) Toe nails of the same individual showing koilonychia and dystrophic appearance.

Fig 4. Multipoint linkage analysis of ectodermal dysplasia locus on chromosome 17p12–q21.31 using Allegro.¹⁰ Markers used are shown in Table 1.

Table 1

Two-point LOD score results between the ectodermal dysplasia locus and chromosome 17 markers

Marker	cM ^a	Physical position ^b	LOD score at recombination fraction $\theta=$						
			0.00	0.01	0.05	0.10	0.20	0.30	
D17S921	36.10	14 201 430	– ∞	– 0.69	– 0.11	0.04	0.08	0.05	
D17S839	37.80	14 425 972	– 3.09	– 0.82	– 0.23	– 0.05	0.03	0.03	
D17S1843	41.10	16 005 240	1.98	1.92	1.67	1.37	0.81	0.36	
D17S805	47.00	19 316 033	1.85	1.92	1.67	1.37	0.81	0.36	
D17S1824	49.60	23 684 196	2.40	2.40	2.10	1.80	1.10	0.50	
WHN	N/A	23 875 086							
D17S1294	50.70	25 406 301	2.40	2.40	2.10	1.80	1.10	0.50	
D17S1863	50.70	25 954 5 65	1.98	1.92	1.67	1.37	0.81	0.36	
D17S1540	54.10	28 395 303	– 2.60	– 0.10	0.40	0.50	0.30	0.10	
D17S1850	55.30	29 161 843	– 2.20	0.30	0.80	0.80	0.60	0.30	
D17S1293	56.40	29 584 279	2.10	2.10	1.80	1.50	1.00	0.50	
D17S1788	58.20	33 159 923	1.69	1.64	1.44	1.19	0.71	0.33	
D17S1818	60.40	34 416 633	– 2.00	1.00	1.00	1.00	1.00	1.00	
D17S1814	61.40	35 372 410	2.50	2.40	2.10	1.70	1.10	0.50	
KRT24	N/A	36 107 771							
KRT25A	N/A	36 157 800							
KRT25B	N/A	36 176 018							
KRT25C	N/A	36 186 587							
KRT25D	N/A	36 202 011							
KRT10	N/A	36 227 896							
D17S1299	62.00	36 247 937	– ∞	0.13	0.62	0.66	0.46	0.22	
D17S800	62.00	36 309 887	– ∞	– 1.00	0.10	0.40	0.40	0.20	
D17S1787	62.00	36 978 510	– ∞	– 0.67	– 0.10	0.05	0.08	0.04	
D17S1860	63.60	39 725 896	– ∞	– 1.00	0.10	0.40	0.30	0.20	
D17S934	63.60	40 413 206	– ∞	– 0.34	0.19	0.30	0.23	0.11	

^a Average-sex distance in cM according to the Rutgers combined linkage-physical human genome map⁹^b The physical position is based according to build 35 of the human genome¹⁴