Association of sequence variants in frizzled-6 with autosomal recessive nail dysplasia (NDNC-10) in Pashtun families

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Abstract

Primitive epidermis develops the nail apparatus. Nails have a strong and inflexible nail plate at the end of each digit. Very few genes responsible for causing non-syndromic form of nail dysplasia have been reported. In the current study, peripheral blood samples were collected from three unaffected individuals and four affected individuals of Family A, while blood from two affected and three unaffected individuals were taken of Family B. Genotyping in both the families was performed using highly polymorphic short tandem repeat microsatellite markers. Sanger sequence of the FZD6 gene was performed and analysed for segregation analysis. A comparative modelling approach was used to predict the three-dimensional structures of FZD-6 protein using Modeller 4. Linkage analysis mapped a disease locus on chromosome 8q22.3, harbouring FZD6. Targeted Sanger sequencing of all the coding exons of FZD6 revealed a nonsense sequence variant in pedigree A, whereas a missense sequence variant in pedigree B. Finding and literature indicates the disease spectrum of Pakistani population with claw-shaped nail dysplasia, particularly in families of Pashtun origin.

Keywords: Non-syndromic nail dysplasia, Chromosome 8q22.3, FZD6, Missense and nonsense sequence variants.

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Introduction

At the 9th week of gestation, initiation of the development of the human nail occurs and is completed during the 5th month of pregnancy, with the development of toenails lagging approximately a month behind the fingernails.¹ Matrix produces the nail, and its growth occurs over the nail-bed. The mature nail plate grows continuously throughout life as a result of matrix epithelial cell differentiation and consists of a number of hard and soft keratin molecules embedded in an amorphous matrix.¹² Most inherited nail disorders manifest either with nail hypoplasia or nail hypertrophy.³ Non-syndromic congenital nail disorder (NDNC) may be inherited in either an autosomal-dominant or autosomal-recessive fashion. It has been classified into 10 different types (NDNC1-10), which are described in Online Mendelian Inheritance in Man (OMIM).⁴ NDNC1 and NDNC2 are termed 20-nail dystrophy and koilonychia, respectively,⁵,⁶ and the genes for either condition remain unknown. NDNC3, or leuconychia, has genetic address to chromosome 3p21.3-p22 with pathogenic variants reported in the PLCD1 gene.⁷ Complete absence of nail (NDNC4) was reported to result from sequence variants in the RSPO4.⁸ Hereditary distal onycholysis and partial absence of nails were termed NDNC5 and NDNC6, respectively.⁹,¹⁰ NDNC7, characterised by longitudinal streaks in nails, nail plate thinning, and poorly developed lunula, was mapped at chromosome 17p13.¹¹,¹² The genes for NDNC5-7 have not been identified yet. Sequence variants in the COL7A1 gene were reported for NDNC8 which consists of pure toenail dystrophy with autosomal dominant inheritance.¹³ Onychodystrophy (NDNC9) was previously mapped to chromosome 17q25.1-17q25.3 for which the gene remains to be identified.¹⁴ Thick, shiny, hard and pigmented nails (NDNC10) are due to sequence variants in FZD6 on chromosome 8q22.3.¹⁵ The current study was planned to see the association of sequence variants in FZD6 with NDNC10 in Pashtun families.

Materials and Methods

The study was conducted from March to September 2017 after approval from the institutional review board of Kohat University of Science and Technology (KUST), Kohat, Khyber Pakhtunkhwa (KP), Pakistan. Informed written consent for clinical and molecular investigation was obtained from all participants involved in the study. Blood samples from 3 unaffected individuals and 4 affected individuals of Family A were collected. Blood from 2 affected and 3 unaffected individuals were taken of Family B. Genotyping for Family A and B was performed using highly polymorphic short tandem repeat microsatellite markers. Sanger sequence of the FZD6 gene was performed and analysed for segregation analysis. A comparative modelling approach was used to predict the three-dimensional structures of FZD-6 protein using Modeller 4. Linkage analysis mapped a disease locus on chromosome 8q22.3, harbouring FZD6. Targeted Sanger sequencing of all the coding exons of FZD6 revealed a nonsense sequence variant in pedigree A, whereas a missense sequence variant in pedigree B. Finding and literature indicates the disease spectrum of Pakistani population with claw-shaped nail dysplasia, particularly in families of Pashtun origin.

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obtained from all the subjects, and the study was conducted in accordance with the Declaration of Helsinki Principles. Two families with nail dystrophy were recruited from Pashto-speaking region of the KP province (Figure 1A-B). No evidence of any other abnormality of ectodermal appendages, including sweat glands, hair and teeth, was noted in any of the affected individuals in both families. The affected siblings in both families were born to healthy parents. Clinical examinations were performed at the respective local government hospitals where the families resided.

Peripheral blood samples were collected from four affected (IV-2, IV-3, IV-4, IV-6) and three unaffected individuals (III-1, III-2, IV-1) of Family A, while blood from two affected (IV-1, IV-2) and three unaffected individuals (III-1, III-2, IV-3) were taken from Family B. From the blood lymphocytes, genomic deoxyribonucleic acid (DNA) was extracted using phenol-chloroform method and commercially available kit Nucleospin® Blood (Macherey-Nagel, Germany). Genotyping in both the families was performed using highly polymorphic short tandem repeat (STR) microsatellite markers, with an average heterozygosity of 0.70. The polymerase chain reaction (PCR) products were resolved in 8.0% non-denaturing polyacrylamide gel, and genotypes were assigned by visual inspection.

Haplotype analysis of the genotyped markers revealed a region of homozygosity among affected individuals of both the families on chromosome 8q22.3. FZD6, a cause of NDNC10, lies in this region, and thus appeared to be a strong candidate. Primers for PCR-amplification of FZD6 were designed using Primer3 software. Sanger sequencing was performed after standard purification, using a commercially available BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, CA, USA). Segregation analysis was carried out using the BioEdit Sequence Alignment Editor version 7.0.5.3. Pathological characteristics of the sequence variant were analysed using PolyPhen-2 algorithm.

Sequences of the primers were as follows:

- **FZD6-4F2**: GGTGACACTGTTGTTCCTAGG
- **FZD6-4R2**: CCTTACCTATGGCTCTTGTA
- **FZD6-6F**: AGTGACTAAGGATTTTTGGC
- **FZD6-6R**: GCTTTCCAAATGTGTTATGC

A comparative modelling approach was used to predict the three-dimensional (3D) structures of FZD-6 protein using Modeller 4. We used the X-ray crystal structure of the cysteine-rich domain of human FZD4 (Protein database [PDB] identity [ID]: 5BPB, sequence identity 39%) and human srGAP2 motif (PDB ID: 5I7D, sequence identity 36%), as a scaffold for the homology modelling of wild and mutant types of FZD-6 protein. The models were visualized using Pymol software (https://pymol.org/).

### Results

The age of the affected members ranged 10-30 years at the time of the study. All the affected members showed severe form of nail dysplasia. The affected individuals of both pedigrees displayed features of shiny, hyper-pigmented and hyperplastic nails. Few individuals had claw-shaped nails. Some of the affected individuals had hyponychia and onycholysis of both fingernails and toenails (Figure 1C).

The paternal and maternal family history in both the families did not reveal evidence for any type of nail or...
nail-associated dysplasia. Heterozygous carrier individuals had normal fingernails and were clinically indistinguishable from genotypically normal individuals. None of the affected members had any other associated abnormality of the teeth, hair, face and sweat glands.

Genotyping data and haplotype analysis showed a single homozygous stretch on chromosome 8q22.3, present only in affected individuals of both pedigrees. Subsequently, all the exons and exon-intron boundaries of FZD6 were sequenced in one affected and parents of both the families. Upon identifying the sequence variants, the same exon of the gene was sequenced in rest of the affected and unaffected individuals of the respective family.

Sequencing of the FZD6 revealed a single homozygous nonsense variant, changing glutamic acid to stop (c.1750G>T; Glu584*) in all the affected individuals of Family A that resulted in truncation. Sequencing analysis identified a missense variant, substituting glycine with aspartic acid (c.1266G>A; p.Gly422Asp) in all the affected individuals of Family B. Segregation analysis of FZD6 variants in both families was consistent with autosomal recessive inheritance. The sequence variants identified in Family A were: c.1750G>T; Glu584*. The sequence variants identified in Family B were: c.1266G>A; p.Gly422Asp.

Residue Gly422 is located in the helix region which does not involve any binding with nearby residues (Figure 2D). Among the nearby residues, the Ser421 made two hydrogen bonding with Ile417 and Trp300, while the other residues, Leu419, Leu423 and Leu663, had interactions that were hydrophobic. The substitution of glycine by glutamic acid at position 422 established a local conformation change in the nearby residues interactions as shown by the difference in bond lengths. In case of E584* sequence variant, the protein was truncated due to the premature stop codon and the loss subdomain was highlighted by magenta colour (Figure 2).

Discussion
In the present study two pedigrees collected from Khyber Pakhtunkhwa province were investigated. Affected individuals in both the families were showing pure autosomal recessive nail dysplasia phenotypes (NDNC10). The clinical features of all patients from these two families were in line with literature.15,25,26 According to Human Gene Mutation Database (HGMD) Professional 2018.1,27 there are 16 different sequence variants in the FZD6 reported as a cause of nail dystrophy, neural tubes defects and isolated nail dysplasia from different ethnicities around the world. The sequence variants (c.1750G>T; Glu584*) identified in Family A and (c.1266G>A; p.Gly422Asp) in Family B, have been described earlier.15,25,27,28 Also, claw development in mice has shown a regulatory role for FZD6-mediated Wnt signalling in the differentiation process of claw/nail formation.24 The extensive prevalence of the variant (c.1750G>T; Glu584*) as reported in separate studies,15,29 points towards a founder effect and implies that the variation is due to a single alteration event on an ancestral chromosome 8.

Conclusion
The findings confirm previously described sequence
variants in FZD6 and indicates the disease spectrum of Pakistani population with claw-shaped nail dysplasia, particularly in families of Pashtun origin.

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**Conflict of interest:** None.

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**References**


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