A novel missense mutation in the *EDA* gene associated with X-linked recessive isolated hypodontia

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Abstract Isolated hypodontia, or congenital absence of one to six permanent teeth (OMIM 300606), is a common condition that affects about 20% of individuals worldwide. We identified two extended Pakistani pedigrees segregating X-linked hypodontia with variable expressivity. Affected males show no other associated anomalies, and obligate carrier females have normal dentition. We analyzed the families with polymorphic markers in the ectodysplasin A (*EDA*) gene region and obtained significant linkage to the phenotype in each pedigree ($Z_{\max}$ 3.29 and 2.65, respectively, at $\Theta = 0.00$). Sequence analysis of the coding regions of *EDA* revealed a novel missense mutation c.1091T>C resulting in a methionine to threonine substitution (p.M364T) in the tumor necrosis factor (TNF) homology domain. Met364 is a highly conserved residue located on the outer surface of the EDA protein. From our findings, we suggest that the mutation disturbs but does not destroy the EDA structure, resulting in the partial and unusually mild ED phenotype restricted to hypodontia.

Keywords X-linked recessive hypodontia · *EDA* gene · Missense mutation · EDA model

Introduction

Hypodontia is defined as the congenital lack of between one and six teeth, which is a common anomaly throughout the world. It is estimated that about 20% of humans are affected by the isolated absence of one or several permanent teeth (Vastardis 2000). Hypodontia may be inherited in an autosomal recessive, autosomal dominant, X-linked dominant, or X-linked recessive mode. The most commonly missing permanent teeth are the third molars (20%), second premolars (3.4%), and maxillary lateral incisors (2.2%) (Simons et al. 1993). Four genes underlying isolated or nonsyndromic hypodontia have been identified so far: *PAX9* (Stockton et al. 2000), *MSX1* (Vastardis et al. 1996), *AXIN2* (Mostowska et al. 2006), and *EDA* (Tao et al. 2006). Mutations in the *EDA* gene are usually associated with syndromic hypodontia involving other ectodermal tissues, but two recent reports indicate that certain *EDA* mutations may cause isolated hypodontia (Tao et al. 2006; Tarpey et al. 2007).

We studied two unrelated extended families with an X-linked recessive form of hypodontia. Affected individuals from both families presented with otherwise normal ectodermal structures, including nails, body hair, and facial features, and they reported normal sweating. Hypodontia affects mainly incisors and to some extent canines and premolars with a variable expression. All female carriers investigated are nonpenetrant.

Materials and methods

Subjects

Two unrelated large consanguineous families from geographically distinct areas of Punjab, Pakistan, were

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ascertained to have hypodontia after clinical examination (Fig. 1a). Analysis of the pedigrees suggested an X-linked recessive mode of inheritance in both families. Altogether, nine affected males were identified in both families, whereas five obligate carrier females appeared asymptomatic. All living normal and affected individuals underwent medical and oral examination at the Nishtar Hospital, Multan, Pakistan. The most striking feature in affected individuals of both families was the congenital absence of at least one mandibular and/or one maxillary incisor in both primary and permanent dentition. In two cases, all eight incisors were missing. There were no visible teeth malformations in affected males. The body and scalp hair, skin, and nails were normal, and the males reported normal sweating and heat tolerance.

Genetic analysis and EDA modelling

DNA was extracted from peripheral blood samples obtained from nine affected and 17 healthy family members. Genetic linkage analysis was performed with the polymorphic markers DXS8040, DXS453, DXS8107, and DXS8052 spanning the *EDA* gene locus. Two-point log of odds (LOD) score was calculated using the MLINK program of FASTLINK computer package (Cottingham et al. 1993). Sequencing was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Invitrogen, San Diego, CA, USA) on an ABI PRISM 3700 sequencer (Applied Biosystems). Chromatograms were analyzed using Sequencher v.4.1.2 (Gene Codes Corporation). Primers and sequencing conditions have been described previously (Visinoni et al. 2003). Informed consent was obtained from all individuals who participated in this study. Modeling of the EDA 3D structure was performed using software ViewerLite v5.0 and PDB file 1RJ7 (Hymowitz et al. 2003).

Results

Hypodontia and *EDA* missense mutation

An X-linked recessive inheritance for a phenotype restricted to the absence of three to 12 teeth was evident in both pedigrees. Obligate carrier females had normal dentition and normally shaped teeth. Most strikingly, all affected males showed a reduced number or a total absence of incisors (Fig. 1b, c). Dentition of premolars and molars was less affected. Linkage to the *EDA* gene region was confirmed using microsatellite markers. A significant LOD score was obtained for marker locus DXS8052 in the two families ($Z_{\max}$ 3.29 and 2.65, respectively, at $\Theta = 0.00$; Table 1). An identical allele size for DXS8052 segregated

hypodontia in both families, which suggested a shared ancestral mutation for a single *EDA* mutation. Sequencing of the eight coding exons and exon–intron boundaries of *EDA* on genomic DNA from all affected individuals revealed a novel c.1091T>C transition in exon 9 (Fig. 1d). This mutation results in p.M364T located in the highly conserved tumor necrosis factor (TNF) domain of EDA (Fig. 2a). The five women with normal dentition and affected male offspring were all carriers of this mutation. In addition, three healthy female family members without affected offspring were identified as carriers. The C allele in exon 9 was excluded on 200 unrelated Pakistani control chromosomes.

Position of Met364 in EDA 3D model

Modeling of the EDA 3D structure using ViewerLite v5.0 and PDB file 1RJ7 suggested the Met364 residue is localized on the outer surface of the crystal structure (Fig. 2b, c). The position excludes Met364 from being part of the interacting surface between EDA monomers. The residue occupies a space in immediate proximity to the side chains Ile260, Leu266, and Leu271 and relatively close to the Tyr320 residue (Fig. 2d). Thus, the hydrophobic interactions between Met364 and the nonpolar and hydrophobic side chains of Ile260, Leu266, and Leu271 suggest they are important to maintain the integrity and structure of the EDA monomer.

Discussion

Our results confirm that certain *EDA* mutations may be associated with isolated hypodontia. Mutations in *EDA* are known to cause ectodermal dysplasia (OMIM 305100), a clinical condition characterized by hypotrichosis (sparseness of scalp and body hair), hypohidrosis (reduced ability to sweat), and hypodontia (congenital absence of teeth). More than 64 mutations in the *EDA* gene have been identified in association with ED (Visinoni et al. 2003), but *EDA* is rarely reported as being implicated in isolated tooth agenesis (Tao et al. 2006; Tarpey et al. 2007). The EDA is a type II transmembrane protein containing a small N-terminal intracellular part and a larger C-terminal extracellular part. The extracellular part consists of a collagen-like domain and a TNF-like domain. The TNF domain has been shown to form homotrimers that are important for interaction with EDA receptors (EDAR; Hymowitz et al. 2003).

The Met364 residue is a highly conserved amino acid found in a series of known EDA proteins (Fig. 2a). M364 localizes to the outer surface of the crystal structure at the bottom of β -strand G (Hymowitz et al. 2003; Fig. 2b). The

Fig. 1 **a** Pedigrees of family A and family B segregating X-linked recessive incisor hypodontia. Affected males are indicated by *filled squares*, and carrier females are identified by *circles with a dot in the center*. **b** Schematic representation of congenitally missing teeth in available affected individuals showing the predominant incisor hypodontia phenotype. **c** Panoramic X-ray of a 14-year-old affected

male (family B, ind. IV:9). *Arrows* indicate positions of missing teeth. **d** Deoxyribonucleic acid (DNA) sequence chromatograms from *EDA* exon 9 showing the different genotypes at position c.1091 (*arrows*) in three individuals from family B. A healthy male (ind. IV:3; *upper panel*), a female carrier (ind. IV:8; *mid panel*), and an affected male (ind. IV:9; *lower panel*)

residue does not directly interfere with the surface between monomers, and it is therefore not localized within the receptor-binding site (Fig. 2c). However, the side chain occupies a space in close proximity to the side chains Ile260, Leu266, and Leu271 (Fig. 2d) and points toward amino acids Glu308 and Val309, both of which have several interactions in the EDA structure (Hymowitz et al.

2003). It is also positioned in close proximity to Tyr320 that if mutated to Y320C, results in an overall disruption of the EDA structure (Hymowitz et al. 2003; Schneider et al. 2001; Fig. 2b, d). The M364T substitution replaces a long nonpolar methionine with a bulkier and shorter polar threonine. This substitution is expected to affect the hydrophobic interactions with the neighboring nonpolar

Table 1 Two-point log of odds (LOD) scores with microsatellite markers in the *EDA* gene region

Marker locus	Physical position ^a	Marshfield map (cM) ^b	LOD score at recombination fraction θ				
			0	0.05	0.1	0.2	0.3
Family A							
DXS8040	68163024	53.58	2.995	2.641	2.284	1.571	0.883
DXS453	69364800	56.29	0.837	0.686	0.546	0.306	0.132
DXS8107	69637956	55.75	2.995	2.621	2.246	1.511	0.824
DXS8052	69729590	55.75	3.289	2.88	2.472	1.673	0.934
Family B							
DXS8040	68163024	53.58	2.354	2.046	1.736	1.126	0.588
DXS453	69364800	56.29	2.224	1.917	1.609	1.005	0.479
DXS8107	69637956	55.75	1.020	0.846	0.674	0.351	0.092
DXS8052	69729590	55.75	2.650	2.326	1.995	1.336	0.742

^a Sequence-based physical map distances in bases according to Build36.2 of the human reference sequence (International Human Genome Sequence Consortium 2001)

^b Gender-averaged Kosambi map distance (cM) from the Marshfield genetic map (Broman et al. 1998). The *EDA* gene is located between markers DXS8040 and DXS453

Fig. 2 a Multiple species sequence alignment of known ectodysplasin (EDA)-A1 sequences showing a high evolutionary conservation around position M364. Sequences were obtained from ENSEMBL and analyzed using BioEdit v7.5.0.3. **b, c** Location of the Y320, Q364, and M364 residues within the tumor necrosis factor (TNF)-domain of EDA-A1 in the tertiary structure of the monomer (**b**, side view) and the quaternary structure of the homotrimer (**c**, view from top). A Y320C mutation was previously found implicated in the full ED syndrome, whereas a Q358E mutation was associated with isolated hypodontia. **d** Zoom-in of the TNF domain, indicating the M364 residue with neighboring amino acids. Pictures were generated using ViewerLite v5.0 and PDB file 1RJ7 (Hymowitz et al. 2003)

and hydrophobic side chains of Ile260, Leu266, and Leu271. This assumption is consistent with the homology structure modeling, which indicates a loose structure at one

end of the molecule (Arnold et al. 2006). Mutations in the EDA protein may cause impaired function by either altering the overall structure, decreased transmembrane

trafficking and proprotein cleavage, or by direct inhibition of the interaction with the cognate EDAR. According to the previous report of a Q358E mutation associated with hypodontia (Tarpey et al. 2007), we hypothesize that the M364T substitution partially interferes with the overall structure of EDA. This may lead to reduced stability for the EDA homotrimer and/or reduced affinity for their target receptors. A reduced but not disrupted homotrimerization could also explain the partial phenotype restricted to dentition observed in our families. Carrier females with one normal allele may then have the ability to compensate for the mutant EDA allele. Further functional characterization and crystal structure of mutant EDA protein is now required to clarify the effect of this and other mutations associated with hypodontia.

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