A new mutation in EDA gene in X-linked hypohidrotic ectodermal dysplasia associated with keratoconus

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Hypohidrotic ectodermal dysplasia (HED) was first described in 1848 by Thurnam. HED belongs to ectodermal dysplasias (EDs), which are developmental impairments of ectodermal-derived tissues. X-linked hypohidrotic ectodermal dysplasia (XLHED) is the most common form of the EDs and consists in abnormal development of teeth, hair, and eccrine sweat glands. XLHED is determined by mutations in the ED1 gene, which is responsible for the coding of ectodysplasin-A(EDA-A), a protein that regulates ectodermal appendage formation. In the present study we found both in our proband and in the mother the same missense mutation in exon 9 (c.957 C>A), which resulted in an aminoacid change at position 319 (Ser319Arg). This latter anomaly might alter the charges in the TNF domain of EDA-A, affecting the stability of the protein and therefore the interaction with its receptor. The male propositus presented classical manifestations of HED except for keratoconus (KC) and, to the best of our knowledge, this association has not been previously described. The identification of this new mutation may contribute to evaluating the genotype/phenotype correlations. Finally, this report can give useful information about the genetic basis of KC and HED. Future studies will allow us to understand if a genetic bond exists between them.

Key words: **Ectodermal dysplasia - Mutation, missense - Keratoconus.**

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Hypohidrotic ectodermal dysplasia (HED), or Christ-Siemens-Touraine syndrome, was first described in 1848 by Thurnam.[1] In 1921, Thadani [2] assigned HED to the X chromosome and linkage studies have since mapped this disorder to Xq12.2-13.1.[3]

HED is found world-wide with an estimated incidence of 1 per 100 000 births.[4] HED belongs to ectodermal dysplasias (EDs), which are developmental impairments of ectodermal-derived tissues. EDs form a large and complex nosologic group with over 200 different clinical conditions.[5] It has been proposed to classify all these forms according to clinical findings,[6] molecular genetic data and corresponding clinical features [7] or according to the function of the protein encoded by the mutated gene.[8] X-linked hypohidrotic ectodermal dysplasia (XLHED) is the commonest form of the ectodermal dysplasias and consists in abnormal development of teeth, hair, and eccrine sweat glands, resulting in either absent or malformed structures.[9] Kere

et al.[10] identified the gene whose mutations are responsible for XLHED. These authors demonstrated that XLHED is determined by mutations in the ED1 gene, constituted by 12 exons, 8 of which are responsible for the coding of ectodysplasin-A (EDA-A). Affected males usually present most or all features of the typical phenotype, including distinctive facies, frontal bossing, brittle and sparse hair, linear wrinkles around eyes, maxillary hypoplasia, "saddle" nose, and prominent lips. Teeth are often missing or misshapen and the skin is smooth and dry with hypohidrosis.[11] In female carriers the severity of the disorder varies considerably, but most of them have mild to moderate manifestations of these typical features, ranging from none to some degree of hypodontia, hypotrichosis and hypohidrosis.[12] In these heterozygous individuals the clinical symptoms are influenced by random X-chromosome inactivation, which makes an accurate diagnosis difficult.[4] The disorder, if unrecognized, is one of the causes of repeated bronchitis, fever of unknown origin and sudden death during infancy and early childohood [13] as a consequence of the neurological sequelae of hyperthermia.[14] Treatment is supportive and includes protection from heat exposure, early denture fittings, skin, hair, ear, nose and nail care, and genetic counselling for family planning.[15, 16] In the present study the *ED1* gene of three members of a family has been analyzed by DNA sequencing. We identified both in the mother and the son the same new missense mutation in exon 9 (c.957 C>A), which resulted in an aminoacid change at position 319 (Ser319Arg). The male proband presented classical manifestations of HED except for keratoconus (KC) and, to the best of our knowledge, this association has not been previously described. Although the etiology of KC is still unknown, genetic factors may play an important role. Our study may provide useful information about the genetic basis of KC and HED. Combined with previous mutation reports, this study allowed us to evaluate genotype/phenotype correlations and to study the potential effect of mutations on EDA functional structure.

Clinical report

The proband was born at term after an uneventful pregnancy and delivery. Family history was not relevant. He had had episodes of hyperthermia since he was 8 months old. Diagnosis of HED, based on clinical manifestations, was made when he was 1 year old. We evaluated the boy at the age of 11 years and 2 months. On physical examination, weight was 28.5 kg (5^{th}-10^{th} centile), height 131.5 cm ($<5^{th}$ centile) and head circumference 50.8 cm ($<3^{rd}$ centile). He had linear wrinkles around eyes, prominent lips, sparse and fine hair (Figures 1, 2), dry skin and dental abnormalities. The baseline panoramic radiograph revealed bilateral agenesia of the central and lateral incisors in the upper dental arch. The

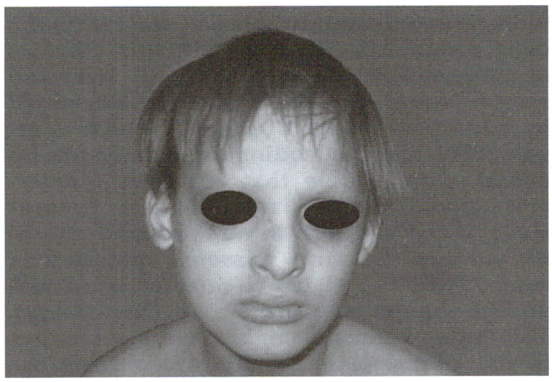

Figure 1.—Frontal view of the patient. Typical X-linked hypohidrotic ectodermal dysplasia facial appearance in the proband (age 11 years). Note linear wrinkles around eyes, prominent lips, sparse and fine hair.

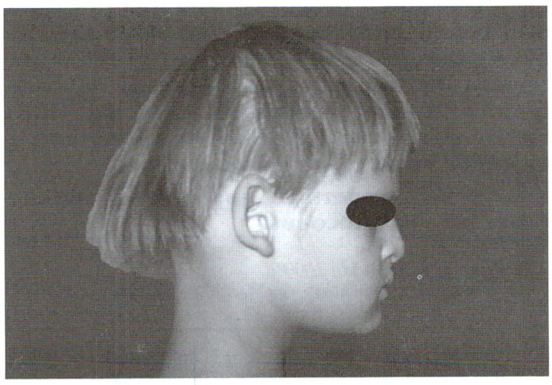

Figure 2.—The proband in lateral view.

right maxillary mesial premolar was infra-occluded, retained in the alveolar process and mesially oriented, its crown overlapped the root of the right maxillary canine. There were morphological and structural anomalies of the crown of the right maxillary first molar, which appeared enlarged in size. In the lower dental arch agenesia of the left central mandibular and lateral incisors and of the right central mandibular incisor was noted. The left mandibular canine was laterally oriented. The right mandibular lateral incisor was partially infra-occluded in the alveolar process. The right mandibular canine was laterally oriented. Finally, ophtalmological examination showed KC (grade III), very low secretion rate of tears, more pronounced in the left eye, and visual impairment only in the left eye. Genomic DNA of the patient and his parents was extracted from peripheral blood leukocytes using QIA amp DNA Minikit (Qiagen). Genomic DNA fragments corresponding to exons 1 and 3-9, intron-exon boundaries and flanking intronic sequences of the EDA-A1 (GenBank NM_001399.4, starting at the ATG translation initiation codon) gene were amplified by PCR. Amplified PCR products were checked on 1.5% agarose gels, purified with ExoSAP-IT (GE), then sequenced bidirectionally using Big DYE terminator v1.1 cycle sequencing kit (Applied Biosystems) and analyzed on an ABI 3100 Genetic Analyzer (Applied Biosystems). Each mutation was confirmed by re-amplification of a second DNA product and re-sequencing.

In our proband we identified a missense mutation (c.957 C>A) in exon 9, which resulted in an aminoacid change at position 319 (Ser319Arg), the same mutation in heterozygosis was found in the mother. This variant has not been previously described in literature. Analysis with SIFT and Polyphen softwares points out how this variant can be considered "probably damaging": the change of a Serine with an Arginine alters the charges in that domain of the protein and affects an aminoacidic residue highly conserved between species. From a functional point of view the variant we describe falls in a TNF-family domain, which interacts with the EDAR receptor activating the signaling pathway; the majority of the mutations accumulate in the TNF-homologous regions.

Discussion and conclusions

The *ED1* gene is responsible for the coding of ectodysplasin-A (EDA-A). EDA-A, a 391 amino acid type-II transmembrane protein, is a new member of the TNF ligand superfamily, involved in the early epithelial-mesenchymal interaction, that regulates ectodermal appendage formation[17] with a role in embryonic morphogenesis.[18-20] The protein has a characteristic structure, which may be associated with its function.[21] It is composed of an N-terminal intracytoplasmic domain, a transmembrane domain and an extracellular domain containing a furin recognition domain, a small collagenous domain and a C-terminal homology TNF domain.[13] This domain is essential for the function of the protein, as missense mutations localized in it lead to a full ED1 phenotype.[8] Ectodysplasin, like other collagenous proteins, forms homotrimers and is released by proteolytic shedding.[17-22] The *ED1* gene encodes two isoforms of EDA-A, EDA-A1 and EDA-A2. They bind to two different receptors: EDA-A1 binds to a protein called EDAR, encoded by the human homologue of the mouse downless gene;[22] EDA-A2 binds to another X-linked receptor (XEDAR).[23] Once the trimers are formed at the membrane, the protein is released and interacts with its receptor, inducing the activation of the nuclear factor (NF)-κB pathway [24] through the adapter protein EDARADD.[25] In addition, JNK/c-fos/c-jun is a second major EDA-dependent pathway, and additional regulatory signals, particularly from the epidermal growth factor (EGF) receptor, are also known to take part in this process.[26] Therefore ectodysplasin acts as a soluble ligand mediating a positive signal for cell survival, growth and differentiation.[8] This signaling pathway is intimately associated with interactions between the epithelial and mesenchymal tissues and also regulates the morphogenesis of hair follicles.[27] To date,

more than 100 mutations in the *ED1* gene have been described, most of them are missense mutations, but one fifth are insertions/deletions[21]. Missense mutations formed specific mutational hotspots.[28] The first hotspot is in the junction of the transmembrane and extracellular domains; the second hotspot is in a recognition sequence for the furin protease; the third hotspot is in the collagen-like domain. The last hotspot is in the TNF homologous region, which may affect the multimerization of EDA trimers.[11] The TNF domain has been shown to form homotrimers which are believed to be required for receptor interactions.[29] It has been suggested that the proteolytic release of the TNF domain is necessary for proper EDA-EDAR signaling as a paracrine mode of action during development.[11] Mutations occurring in this region may affect protein ability to interact with EDAR and to activate the nuclear factor-kB kinase signaling pathways, involved in epithelial-mesenchymal interactions. Several mutations in the TNF domain have been reported.[12, 28-30] In the present study we investigated a family with a new mutation. We found in our proband a missense mutation (c.957 C>A) in exon 9 of *ED1*, which resulted in an aminoacid change at position 319 (Ser319Arg), affecting the TNF homology domain. The same mutation in heterozygosis was detected in the mother, who appears to be completely normal with neither dental nor systemic abnormalities. It was not possible to investigate the pattern of X-inactivation in the mother, because she did not give her consent for further genetic studies. It is known that clinical symptoms in female carriers are influenced by the degree of inactivation of the normal allele on chromosome X.[31, 32] However, recent studies suggested that X-chromosome inactivation is different in skin and blood cells and, as a consequence, ectodermal and mesodermal tissues may differ with regard to factors related to X-chromosome inactivation.[30] The mutation we describe may alter the TNF domain, affecting the stability of EDA trimer and therefore the interaction of ectodysplasin A with its receptor. Few attempts have been made to correlate the genotype with the phenotype of the affected individuals. Patients harboring large deletions show only moderate features of HED, while the symptoms are more severe in patients carrying point mutation in highly conserved regions.[33] Mutations in exon 9 are rare and they occur with high frequency in Chinese patients.[21] Depending on where the mutation is localized in the *ED1* gene, it is possible to disrupt only the EDA-A function during tooth development and not the organ systems. To date only three mutations affecting the TNF domain have been described in association with tooth agenesis without causing other abnormalities,[12, 34] showing that these mutations only minimally affect the stability of EDA trimers. More frequently, mutations involving TNF domain are mostly responsible for the typical phenotype with abnormalities of teeth, hair and eccrine sweat glands. In accordance with previous data [11, 21, 33] our patient has sparse and fine hair, dry skin, dental abnormalities, heat intolerance and hyperpyrexia. Ophtalmological examination showed that lacrimation was nearly absent and KC of grade III was present. Dryness of the eyes is probably due to defective development of lachrymal glands, in accordance with the features of the disease. KC is a non-inflammatory corneal ectatic disorder characterized by progressive corneal steepening and stromal thinning, and it has never been associated with HEDs before. Its onset is usually at puberty and the estimated prevalence ranges from 50 to 230/100 000 in the general population.[35] The etiology of KC is still unknown, but the association with genetic syndromes (such as Leber's congenital amaurosis, trisomy 21, and Turner's syndrome) and genetic epidemiologic studies indicate that genetic factors may play an important role.[36, 37] Several attempts have been made to identify susceptibility gene loci for KC, but the lack of consistent chromosomal loci among different studies indicate genetic heterogeneity and illustrates the complex nature of the genetic contribution to the disease.[38] In conclusion, we have identified a novel missense mutation of the *ED1* gene resulting in a change in a single aminoacid

residue in the TNF domain of the protein. This mutation has not been described previously. Our findings give evidence for the presence of mutations of the *ED1* gene in XLHED. To the best of our knowledge, this is the first case report of HED with keratoconus. This association confirms the genetic heterogeneity of the disease. The identification of genes that contribute to non-Mendelian forms of keratoconus is still necessary, as its pathogenesis is poorly understood to date. Future elucidations of the molecular bases of HED and future linkage studies of KC will allow us to understand if a genetic bond exists between them.

Riassunto

Nuova mutazione nel gene EDA nella displasia ectodermica ipoidrotica X-linked associata a cheratocono

La displasia ectodermica ipoidrotica (HED) è stata descritta per la prima volta nel 1848 da Thurnam. La HED fa parte delle displasie ectodermiche (EDs), difetti di sviluppo dei tessuti di derivazione ectodermica. La displasia ectodermica ipoidrotica X-linked (XLHED) è la forma più frequente tra le EDs e consiste in un'anomalia di sviluppo di denti, capelli e ghiandole sudoripare eccrine. La XLHED è causata da mutazioni nel gene *ED1*, che codifica per la ectodisplasina-A (EDA-A), una proteina che regola la formazione degli annessi ectodermici. In questo studio è stata riscontrata nel nostro probando e nella madre la stessa mutazione missenso nell'esone 9 (c.957 C>A), che esitava in una mutazione aminoacidica in posizione 319 (Ser319Arg). Quest'ultima anomalia potrebbe alterare le cariche nel dominio TNF della EDA-A, modificando la stabilità della proteina e quindi l'interazione con il suo recettore. Il paziente presentava manifestazioni tipiche di HED ad eccezione di cheratocono (KC). Tale associazione non è stata descritta in studi precedenti. La nostra osservazione può contribuire a delineare le correlazioni genotipo/fenotipo. Questo report, infine, offre utili informazioni circa le basi genetiche di KC e HED. Studi futuri ci consentiranno di capire se tra loro esiste una correlazione genetica.

Parole chiave: Displasia ectodermica - Mutazione missense - Cheratocono.

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