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Authors: Jin Zhang, Dong Han, Shujuan Song, Ying Wang, Hongshan Zhao, Shaoxia Pan, Baojing Bai, Hailan Feng

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Correlation between the phenotypes and genotypes of X-linked hypohidrotic ectodermal dysplasia and non-syndromic hypodontia caused by ectodysplasin-A mutations

Jin Zhang¹#, Dong Han¹#, Shujuan Song²#, Ying Wang¹, Hongshan Zhao²,³, Shaoxia Pan¹, Baojing Bai⁴, Hailan Feng¹*

1. Department of Prosthodontics, Peking University School and Hospital of Stomatology, Beijing, China
2. Department of Medical Genetics, Peking University Health Science Center, Beijing, China
3. Peking University Center for Human Disease Genomics, Peking University Health Science Center, Beijing, China
4. Department of Prosthodontics, Capital University School of Stomatology, Beijing, China

#: These authors contributed equally to this work
*: To whom correspondence should be addressed

Hailan Feng, M.D.
Department of Prosthodontics, Peking University School and Hospital of Stomatology
22 Zhongguancun Nandajie, Haidian District, Beijing 100081, China

Email: kqfenghl@bjmu.edu.cn

Tel: 0086-10-62179977-5232

Shujuan Song, Ph.D.

Department of Medical Genetics, Peking University Health Science Center,
38# Xueyuan Road, Haidian District, Beijing 100191, China

Email: shujuansong@bjmu.edu.cn

Tel: 0086-10-82802975
Abstract:

Mutations in the ectodysplasin-A (EDA) gene can cause both X-linked hypohidrotic ectodermal dysplasia (XLHED) and non-syndromic hypodontia (NSH). The correlation between the phenotypes and genotypes of these two conditions has yet to be described. In the present study, 27 non-consanguineous Chinese XLHED subjects were screened and 17 EDA mutations were identified. In order to investigate the correlation between genotype and phenotype, we also reviewed related studies on NSH subjects with confirmed EDA mutations and compared the differences in the clinical manifestations and EDA mutations of the two conditions. Tooth agenesis was observed in addition to abnormalities of other ectodermal organs. Tooth agenesis was more severe in XLHED subjects than in NSH subjects, and there were statistically significant differences in 10 tooth positions in the XLHED and NSH subjects, including canines, premolars, and molars. With the exception of one splicing mutation, all mutations in the NSH subjects were missense mutations, and these were most likely to be located in the tumor necrosis factor (TNF) domain. Further, more than half of the mutations in the XLHED subjects were speculated to be loss-of-function mutations, such as nonsense, insertion, and deletion mutations, and these mutations were distributed across all EDA domains. Our results show that there exists a correlation between the phenotypes and genotypes of XLHED and NSH subjects harboring EDA mutations. Further, our findings suggest that NSH is probably a variable expression of XLHED. This finding might be useful for clinical diagnosis...
and genetic counseling in clinical practice, and provides some insight into the
different manifestations of *EDA* mutations in different ectodermal organs.

**Keywords:** *EDA*; mutation; tooth agenesis
1. Introduction:

Hypohidrotic ectodermal dysplasia (HED) is a hereditary condition characterized by aplasia or hypoplasia of the skin and associated structures (hair, nails, teeth, and sweat glands). The affected subjects usually present with hypohidrosis, alopecia, hypodontia, or anodontia. Two forms of HED have been described according to the inheritance pattern and molecular etiology, namely, X-linked HED (XLHED) and the autosomal dominant and recessive forms. XLHED is the most frequent form of HED. Most subjects with XLHED harbor mutations in the EDA gene, which has a detection rate of 63%–95% [1–5]. The EDA gene contains nine exons and occupies a 425-kb segment in the long arm of the X-chromosome (Xq12-13.1). Extensive alternative splicing of this gene produces different isoforms, the longest of which encodes a 391-amino acid protein containing a transmembrane domain, a furin cleavage site, a collagen-type region, and a C-terminal tumor necrosis factor (TNF) homology domain.

Non-syndromic hypodontia (NSH), unlike XLHED, exhibits no clinical features other than tooth agenesis. NSH is known to be associated with mutations in the PAX9, MSX1, and AXIN2 genes. Mutation of the EDA gene was first identified in an X-linked inherited NSH family in 2006 [6]. Subsequently, other studies have reported EDA mutations underlying NSH [7–12]. A review of previously published studies suggests that all mutations identified in the NSH cases are novel and there is a noticeable relationship between the genotype (EDA mutation site and inheritance
pattern) and the clinical phenotype (XLHED or NSH). Other studies [4, 13] have also confirmed that the mutated EDA proteins identified in XLHED and NSH subjects interact with the EDA receptor (EDAR) to varying extents. However, the correlation between the phenotypes and genotypes of these two conditions has yet to be described. In order to investigate this correlation, we identified 17 EDA mutations in 27 non-consanguineous XLHED subjects, and reviewed previously published data on NSH subjects, including detailed data on the subjects’ dentition.
2. Materials and methods:

2.1. Patient recruitment and phenotype evaluation

The patients enrolled in this study were recruited from outpatients of the Department of Prosthodontics, Peking University School and Hospital of Stomatodontology. We screened 27 non-consanguineous male XLHED subjects for EDA gene mutations. The subjects had a similar phenotype. They all had typical triad of the disorder, namely, hypohidrosis, hypotrichosis, and oligodontia, sometimes even anodontia. Mental development was normal. The scalp hair, eyelashes and eyebrows were sparse, thin, and dry. The subjects also had a characteristic facial appearance, including a prominent forehead, saddle-back nose, and protruding lips (Fig. 1).

Further, we collected blood samples from the parents of 18 subjects. Patient 27# had a family history of XLHED, with five of his family members also being affected. Patient 9# and his brother had a similar phenotype. Blood samples were also collected from the affected family members of subjects 27# and 9#. Congenital absence of teeth was confirmed by panoramic radiography. All XLHED subjects denied a history of tooth extraction. After informed consent was obtained, whole blood was collected from each participant. This study was conducted with the approval of the Ethics Committee of Peking University Health Science Center.

In addition, 120 unaffected unrelated individuals from the Chinese population were used as a control group.

2.2. DNA extraction and mutation identification

Genomic DNA was extracted from peripheral blood lymphocytes using a standard high-salt method. Polymerase chain reaction (PCR) primers were designed to cover
the entire cDNA containing eight exons and intron-exon junctions containing more than 100 base pairs. Screening of EDA gene mutations was performed by direct sequencing of the eight PCR fragments. If an EDA mutation was identified in a proband, samples from the patient’s family members were also screened for the mutation.

2.3. Genotype and phenotype analyses

Genotype and phenotype analyses were based on the present study and a review of the related studies. We used the data of 59 NSH subjects collected during our previous study [7,12] and other published studies on the positions of tooth agenesis [6, 8–11]. The affected individuals had normal hair, skin, and sweat production, but permanent teeth were missing. Among the 59 NSH subjects, four were sporadic and the remainders were familial. Of the four sporadic subjects, the condition in two was of maternal origin. In the NSH familial subjects, excluding the three subjects for whom the researchers did not have a maternal sample, the condition in 50 of the remaining 52 subjects was inherited from the mother. Because the available previous reports on XLHED caused by EDA mutations do not contain detailed clinical data on the oral manifestations, the XLHED data we used were obtained solely from our own previous study. For phenotype analysis, tooth agenesis of all XLHED participants with an EDA mutation was taken into consideration, including 20 subjects from among 27 non-consanguineous subjects and six affected relatives. All XLHED and NSH subjects and their relatives had permanent dentition.
2.4. Statistical analysis

We compared the missing teeth of XLHED and NSH subjects with confirmed EDA mutations, and for all subjects’ compiled data on the number of missing teeth at each position in the four quadrants of the mouth. The number of left and right side teeth at the same site was summed, and the data for maxillae and mandibles were combined separately. The data were analyzed using a chi-square test. The entire dentition was divided into three sections, namely, anterior teeth, premolars, and molars, to examine the features of tooth agenesis in XLHED and NSH subjects. The chi-square test was performed separately for anterior teeth vs. premolars, anterior teeth vs. molars, and premolars vs. molars.
3. Results

3.1. *EDA* mutations identified in the XLHED group

We analyzed the *EDA* gene mutations in 27 unrelated male XLHED subjects. Seventeen mutations, including nine novel mutations, were identified in 20 non-consanguineous subjects (Table 1). The mutation detection rate was 74%. Among these mutations, four deletions (882-885 Del4, 252DelT, 614DelT, and 106-118 Del13), one insertion (435 Ins4), and two nonsense mutations (G215X and Q358X) were predicted to lead to premature termination of protein translation. A 648-665 Del18 mutation resulted in the formation of a truncated protein that lacked six amino acids. The other nine mutations were missense mutations.

Among the family members of all subjects, the mothers of 13 subjects were *EDA* mutation carriers. The brother of patient 9# and five family members of patient 27# harbored an *EDA* mutation.

3.2. Differences in phenotype

We compared the number of missing teeth in 26 XLHED and 59 NSH subjects. We examined 20 XLHED subjects and six affected family members of subjects 9# and 27# who were carrying an *EDA* mutation. These family members were also diagnosed as having XLHED. The latter group comprised those NSH subjects we reviewed from previous reports containing data on oral manifestations.

Twelve of the 20 XLHED subjects had records of primary dentition. Among these, five children had lost all of their primary teeth and others had lost at least 13 teeth.
The remaining teeth were small in size. There were five maxillary central incisors remaining and three were peg-shaped. In addition to tooth agenesis, hypohidrosis and hypotrichosis were observed in all XLHED subjects.

NSH subjects exhibited no clinical features other than tooth agenesis. The average number of missing teeth (excluding the third molar) was 21.35 in XLHED subjects and 10.64 in NSH subjects (Fig. 2B). A statistically significant difference (P < 0.01) was observed between the percentage of missing teeth in XLHED (76%) and NSH (38%) subjects. Furthermore, with the exception of the incisors, statistically significant differences were found for 10 tooth positions (Fig. 2A).

Among the tooth positions that did not show statistically significant differences between XLHED and NSH subjects, the incisors of XLHED subjects were likely to exhibit morphologic abnormalities, notably, peg-shaped incisors or incisors with a smaller mesiodistal dimension. Among the XLHED subjects, there were 29 maxillary central incisors and three mandibular lateral incisors. Whereas most of these abnormal incisors were severely peg-shaped, five of the maxillary central incisors had a smaller mesiodistal dimension.

Among the NSH subjects, the percentages of lateral incisor agenesis (76%) and lower central incisor agenesis (75%) were statistically significantly higher than those for agenesis at other positions. A statistically significant difference (P < 0.01) was observed between these positions and the others.

If the whole dentition is divided into three sections (anterior teeth, premolars, and
molars), the features of XLHED and NSH become clearer (Fig. 3). In subject with XLHED, the percentage of missing premolars or molars was significantly higher than that of missing anterior teeth. No statistically significant difference was observed between the premolars and molars. In the NSH subjects, it was the anterior teeth that were more likely to be missing, whereas the molars were the least affected.

3.3. Differences in genotype

In this study, 17 different mutations were identified in the XLHED subjects: 12 missense mutations (60%), two nonsense mutations, five deletions, and one insertion. To date, 10 different mutations have been identified in NSH [6–12]. With the exception of one splicing mutation located in intron 5, the other nine mutations were all missense mutations (90%) (Fig. 4A): two missense mutations located in exon 1 affected the transmembrane domain, and seven missense mutations located in exons 7, 8, and 9 affected the TNF homology domain. EDA mutations causing NSH were mostly located in the TNF domain, whereas the mutations responsible for XLHED were distributed across all the EDA domains (Fig. 4B).
4. Discussion

Previous studies that have focused solely on XLHED [2,4,5,14–16] have found no genotype-phenotype correlations in those affected by this condition. However, from a comparison of XLHED and NSH caused by EDA mutations, we suggest that genotype-phenotype correlations exist in these two conditions.

The detection rates of EDA mutations ranges from 65%-94% reported in other studies [17]. The 74% detection rate in this study was very close to the combined detection rate in nine previous reports (159 mutations in 226 unrelated subjects) [1-5,18-20]. These studies analyzed all exons. Together with the present study, the overall detection rate was 70.8%.

On the basis of previous research, approximately 80% of the EDA mutations identified in XLHED subjects are small intragenic changes, including point mutations, small deletions, and insertions [21]. In contrast, large deletions, even loss of an entire exon and complete gene deletion, have also been reported [1,2,22].

The EDA mutations identified in NSH and XLHED are of different types. The mutations observed in NSH subject are mainly missense mutations, whereas this type of mutation generally occurs in only half of the XLHED subjects. Other mutations result in more severe protein impairment. Furthermore, a total of 143 different EDA mutations have been reported to be responsible for XLHED, including 68 missense mutations and other mutations such as splicing mutations, deletions, insertions, and nonsense mutations (Fig. 4C). The proportion of each pattern of EDA mutation observed in this study is approximately similar to that reported in previous studies.

Mutations in the transmembrane domain, encoded by exon 1, interfere with
transmembrane transport and probably change the polarity of amino acids [23,24]. Mutations in the furin protease recognition sequence, which is encoded by exon 3, prevent proteolytic cleavage of EDA leading to the formation of a disordered protein [25]. Mutations in the collagen-like domain encoded by exons 5 and 6 may prevent TNF domain multimerization [25]. The mutations in exons 7–9 in the TNF homology domain will impair the specific binding of both EDA splice variants to EDAR [23,24].

All of the mutations identified in the present study are located in exons 1, 3, 5, 8, and 9. On the basis of a review of all coding region mutations, some researchers have suggested that 98% of EDA mutations are located in these five exons [2,5]. Further, the CpG-containing arginine codon at position 156 in exon 3 is a notable mutation hot spot in the EDA gene [5], and three mutation patterns at this site have been detected: c.467G>A (Arg156His), c.466C>T (Arg156Cys) [2], and c.466C>A (Arg156Ser) [26]. The Arg156His mutation was also identified in our study, but not as frequently as c.1133C>T (Thr378Met), which was identified four times in 20 cases.

In addition to the differences in other ectodermal organs, the phenotypes of XLHED and NSH also differ from each other. Tooth agenesis in XLHED is considerably more severe than that observed in NSH. The residual incisors exhibit a peg-shape abnormality in XLHED, whereas this abnormal phenotype was not found in the NSH subjects in our work or in the subjects examined in other studies [6–12]. We supposed that was because that the EDA-EDAR signaling pathway plays a role in regulating cusp morphogenesis through the enamel knot during the development of tooth germ [27] and EDA mutations interfered the function of EDA-EDAR signaling pathway. Previous studies have shown that normal enamel function needs an adequate EDA-EDAR signal and the effect was dose dependent [28, 29]. Although some
mutations in \textit{EDA} gene alter the ability of \textit{EDA} to bind to receptor EDAR, most of the \textit{EDA} mutations underlying XLHED result in the loss of the binding ability, thus affecting crown morphology. Radiographic examination showed the maximum diameter of the teeth of XLHED patients was apically displaced [30]. Consequently, the incisors might display a quite different conical crown.

Serious agenesis of the posterior teeth and peg-shaped incisors are the two main oral manifestations in XLHED. In NSH subjects, the percentage of missing teeth in the anterior area is significantly higher than that in the posterior section. Further, among the anterior teeth, the lower incisors and upper lateral incisors are the positions at which teeth are most frequently missing. This feature can contribute to clinical diagnosis and genetic counseling in clinical practice. According to the patient’s phenotype, researchers can design a molecular diagnosis plan, which involves a series of steps for detecting the potential mutant gene. We recommend that, initially, the \textit{EDA} gene should be screened for mutations in NSH subjects who exhibit congenital absence of lower and upper lateral incisors.

The reason for different \textit{EDA} mutations resulting in varying phenotypes is not clear. Nearly half of the \textit{EDA} mutations identified in XLHED subjects, including deletions, insertions, and nonsense mutations, which are likely to lead to severe impairment of protein function, were not found in NSH subjects. Although missense mutations were identified in both XLHED and NSH, these mutations were mostly located in the TNF homology domain. In our previous study [12], we analyzed the three-dimensional protein structure of EDA and found that the position of amino acids in the TNF homology domain was crucial to the structure and function of the protein. Most
mutations identified in XLHED resulted in the loss of EDA's ability to interact with EDAR; however, in the case of NSH-associated mutations, EDA shows weak binding activity [4,13]. The EDA signaling pathway plays a more significant role in tooth development than in the development of other EDA-dependent structures, such as skin and its appendages [31,32]. Therefore, the EDA mutations associated with NSH lead only to tooth agenesis, whereas the other organs of ectodermal origin remain normal.

We identified a missense mutation [Asp316Gly (D316G)] in one of our XLHED subjects. This patient presented with typical ectodermal dysplasia, including sparse hair, dry skin, XLHED facial features, and entirely missing deciduous and permanent dentition. Interestingly, the same mutation was also detected in a reported NSH family in which the subjects had oligodontia and the missing tooth number varied from 11 to the entire dentition (excluding the third molars). A further study has also shown that a family with selective tooth agenesis, which was associated with the D316G EDA mutation, presented with anodontia or severe oligodontia, suggesting that this mutation reduced the ability of EDA to bind to receptor EDAR [13]. A mutation in Gly directly affects the stability of the EDA trimer in the following three ways: by decreasing the inter-subunit interactions, by increasing the flexibility of this region, and by affecting the fluctuation of the adjacent loop [12]. This may account for the severe tooth agenesis observed in both XLHED and NSH subjects. Since the same mutation was detected in subjects with different phenotypes, NSH is probably a
variable expression of XLHED. An alternative explanation might be that modification of other genes in the NFκB pathway, which interact differently with mutated EDA proteins, leads to variation and different phenotypes.

In conclusion, our findings may provide evidence that there is a correlation between the genotypes and phenotypes of XLHED and NSH caused by EDA mutations. There are still differences of opinion as to whether NSH is an isolated condition or whether it is merely a variable expression of XLHED. We propose that there is no clear distinction between XLHED and NSH. The correlation between the phenotypes and genotypes of these two conditions can be useful for disease analysis, clinical diagnosis, and genetic counseling. Further protein functional studies on the relationship between the phenotype and genotype of the EDA gene are required to clarify the role of EDA in the development of teeth and other ectodermal organs, and also to elucidate the pathogenesis of hypodontia.
Acknowledgements

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Table 1. The EDA mutations identified in this study

<table>
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<th>Mutation NO.</th>
<th>Subject NO.</th>
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<td>6</td>
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<td>c.947A&gt;G</td>
<td>Asp316Gly</td>
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Novel mutations identified in the present study are marked with an asterisk
Figure legends

Fig. 1. Clinical phenotype of Chinese X-linked hypohidrotic ectodermal dysplasia patient.

(A) Facial features of subject 3#. (B) Oral manifestation of subject 3# shows most deciduous teeth missing and the residual central incisors are peg-shaped. (C) Panoramic radiograph of subject 3# shows congenital absence of most permanent teeth except for maxillary incisors of both sides, left lateral maxillary incisor, and maxillary and mandibular first molars of both sides.

Fig. 2. Comparison of the positions of missing teeth in X-linked hypohidrotic ectodermal dysplasia and non-syndromic hypodontia.

(A) The percentages of missing teeth at each tooth position in X-linked hypohidrotic ectodermal dysplasia and non-syndromic hypodontia are compared. Key: x-axis = tooth position; y-axis = percentage missing. Maxillary teeth are aligned above the x-axis and mandibular teeth are aligned below the x-axis: (1) central incisor, (2) lateral incisor, (3) canine, (4) first premolar, (5) second premolar, (6) first molar, and (7) second molar. The statistical differences are marked with an asterisk: *, 0.01 < P < 0.05; **, P < 0.01.

(B) The number of missing teeth is compiled for each position (excluding the third molar) based on the data from this study and previous reports on non-syndromic
hypodontia. The numerators are the number of missing teeth and the denominators are the sum of the teeth that the subjects should have. The combined number of missing teeth on both the left and right sides is indicated at the bottom. The number enclosed in parentheses indicates the percentage of missing teeth.

Fig. 3. Comparison of tooth agenesis in anterior, premolar, and molar regions in X-linked hypohidrotic ectodermal dysplasia and non-syndromic hypodontia.

The entire dentition is divided into three sections, i.e., anterior teeth, premolars, and molars. The values indicate the percentage of missing teeth in the three sections in X-linked hypohidrotic ectodermal dysplasia or non-syndromic hypodontia. The pairs of sections with statistically significant differences are linked and marked with asterisks; *, 0.01 < P < 0.05; **, P < 0.01.

Fig. 4. Structure and distribution of EDA mutations underlying X-linked hypohidrotic ectodermal dysplasia and non-syndromic hypodontia.

(A) Construction of EDA mutations underlying X-linked hypohidrotic ectodermal dysplasia and non-syndromic hypodontia: (a) EDA mutations underlying X-linked hypohidrotic ectodermal dysplasia identified in the present study. (b) EDA mutations underlying non-syndromic hypodontia based on a review of the literature. (c) All reported EDA mutations underlying hypohidrotic ectodermal dysplasia.

(B) Distribution of EDA gene mutations identified in X-linked hypohidrotic
ectodermal dysplasia and non-syndromic hypodontia. The diagram of the *EDA* gene contains domains (above) and exons (below). Mutations are marked at the corresponding positions of the gene. (a) *EDA* mutations underlying X-linked hypohidrotic ectodermal dysplasia identified in the present study. (b) *EDA* mutations underlying non-syndromic hypodontia based on a review of the literature. Key: TM, transmembrane domain; TNF, tumor necrosis factor. Missense mutations are indicated in red, frame shift mutations in black, nonsense mutations in blue, the deletion mutation in yellow, and the splicing mutation in green. Novel mutations are marked with an asterisk.
Fig 1. Clinical phenotype of Chinese X-linked hypohidrotic ectodermal dysplasia patient.

Fig 2. Comparison of teeth missing position between hypohidrotic ectodermal dysplasia and non-syndromic hypodontia.

<table>
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<td>Max L</td>
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Tu et al., 2006; Turley et al., 2007; Wang et al., 2008; Xu et al., 2008; Li et al., 2009; Man et al., 2008; Song et al., 2009
Fig 3. Comparison of tooth agenesis in anterior, premolars and molars regions between hypohidrotic ectodermal dysplasia and non-syndromic hypodontia.

Fig 4. Construction and distribution of EDA mutations underlying hypohidrotic ectodermal dysplasia and non-syndromic hypodontia.
Fig 2. Comparison of teeth missing position between hypohidrotic ectodermal dysplasia and non-syndromic hypodontia.

Fig 3. Comparison of tooth agenesis in anterior, premolars and molars regions between hypohidrotic ectodermal dysplasia and non-syndromic hypodontia.