Association between EDAR Polymorphisms and Non-Syndromic Tooth Agenesis in the Chinese Han Population

Yi Ting CHEN¹#, Hao Chen LIU¹#, Dong HAN¹, Yang LIU¹, Hai Lan FENG¹

Objective: To explore the relationship between single nuclear polymorphisms (SNPs) in ectodysplasin A receptor (EDAR) and EDAR-associated death domain (EDARADD) genes and non-syndromic tooth agenesis.

Methods: Ten putative SNPs in EDAR and EDARADD were selected, and a case-control study was conducted in 112 subjects with non-syndromic tooth agenesis and 112 normal control subjects. DNA was obtained from peripheral blood samples. Genotyping was performed by Sanger sequencing.

Results: Three SNPs (rs3749098, rs3749099, and rs10432616) in EDAR exhibited significant differences in the alleles and/or genotype frequencies between the case group (individuals with non-syndromic tooth agenesis) and control group (normal individuals). The T allele was identified in the SNP rs3749098 in 99.1% of the case group and in 96.0% of the control group (P = 0.0326). Regarding the SNP rs3749099, the C allele was identified in 99.1% of the case group and in 96.0% of the control group (P = 0.0326). Regarding the SNP rs10432616, the C allele was identified in 97.8% of the case group and in 100.0% of the control group (P = 0.0245).

Conclusion: Our results suggested that SNPs in EDAR could be a pathogenic factor for non-syndromic tooth agenesis. Furthermore, EDAR can be regarded as a marker gene for the risk of tooth agenesis.

Key words: case-control study, ectodysplasin A receptor, non-syndromic tooth agenesis, single nucleotide polymorphism


Tooth agenesis refers to the congenital absence of one or more teeth, and is the most common developmental anomaly in human dentition¹. The prevalence of dental agenesis of permanent teeth was estimated to be approximately 5.89% of the Chinese population² and 1.6% to 9.6% of Caucasians³. Although many potential and determinative factors affect tooth development, genetic factors are the most important risk factor for tooth agenesis⁴. Tooth agenesis can occur either in association with other genetic diseases as part of a recognised clinical syndrome, or as an isolated form. To date, mutations in at least nine genes, including WNT10A, WNT10B, PAX9, EDA, MSX1, AXIN2, EDARADD, NEMO, and KRT17 have been identified in patients with non-syndromic tooth agenesis⁵-⁸. However, there are still many individuals with non-syndromic tooth agenesis that could not be identified as carrying mutations in these nine genes.

One of the most studied pathogenic genes of tooth agenesis is Ectodysplasin A (EDA)⁴. EDA controls the induction, morphogenesis and maintenance of ectodermal structures such as teeth, hair and sweat glands. EDA, a part of the TNF family, binds itself to the EDA receptor (EDAR). EDAR sends a signal downstream via a cytosolic adaptor protein known as the EDAR-associated death domain (EDARADD). Many studies
have shown the association between EDA mutations and non-syndromic tooth agenesis. To date, however, no EDAR mutations and only one EDARADD mutation has been reported in association with non-syndromic tooth agenesis.

Tooth development is a highly complicated process involving many genes and signalling pathways. Any changes along the signalling pathway may change the outcome of tooth development and, on occasion, may even cause tooth development arrest. Single nucleotide changes, which occur at a high frequency in the human genome, are the most common polymorphisms and may affect the function of genes. Many studies suggest that gene polymorphisms may be a risk factor for tooth agenesis. Therefore, we speculated that gene polymorphisms in the EDAR and EDARADD genes could be a risk factor for non-syndromic tooth agenesis. In this study we focus on the relationship between SNPs in the EDAR and EDARADD genes and non-syndromic tooth agenesis. Our results show that single nucleotide polymorphism markers rs10432616, rs3749099, and rs10432616 are associated with tooth agenesis in the Chinese Han population.

### Materials and methods

#### Participants

The study involved 112 non-consanguineous patients with non-syndromic tooth agenesis (excluding third molar), and 112 non-consanguineous normal controls, who were referred to the Department of Prosthodontics, Peking University School and Hospital of Stomatology. All participants were examined by prosthodontics specialists to determine the status of dentition. Oral examination and dental treatment history were required and panoramic radiographs were taken to confirm the congenital absence of teeth. The size and shape of the tooth was also noted. Details of the study population are presented in Table 1. Written informed consent was obtained for DNA analysis from all participants or parents of child participants. This experiment was conducted under the approval of the Ethics Committee of Peking University Health Science Center.

#### DNA extraction

Genomic DNA samples from participants were extracted from peripheral blood lymphocytes using the TIANamp Blood DNA kit (Tiangen, Beijing, China). The extracted DNA samples were stored at -20°C prior to analysis.
SNP Selection

SNP sites in EDAR and EDARADD were selected based on their location according to the dbSNP (www.ncbi.nlm.nih.gov/SNP). SNPs chosen were located in the coding and regulatory regions. Details of the SNP sites are presented in Table 2.

Polymorphism genotyping

Genotyping experiments were performed by TsingKe Biological Technology (Beijing, China; www.tsingke.net). PCR primers and single base extensions were designed using Assay Designer software package (Sequenom, San Diego, CA, USA). All 12 exons of EDAR and 6 exons of EDARADD, as well as their exon–intron boundaries, were amplified by polymerase chain reaction. The PCR products were sequenced by Sangon Biotech Company (Beijing, China) using BigDye Terminator v 3.1 (Applied Biosystems, Foster City, CA, USA) and a 3730 DNA sequencer (Applied Biosystems). The sequencing results were analysed with SEQMAN PRO genetic analysis software (DNASTAR, Madison, WI, USA).

Statistical analysis

The goodness-of-fit chi-square test was performed to check Hardy-Weinberg equilibrium of the observed genotype frequencies, compared with control subjects. The associations between genotypes and the risk of tooth agenesis were estimated by computing the odds ratio (OR) and their 95% confidence intervals (95% CI) from logistic regression analyses. All statistical tests for this analysis were performed using SPSS 22.0 software.

Results

Three SNPs (rs3749098, rs3749099, and rs10432616) in EDAR exhibited significant differences in the alleles and/or genotype frequencies between the case group (individuals with non-syndromic tooth agenesis) and control group (normal individuals) (Table 3). The T allele was identified in the SNP rs3749098 (MA = 0.01) in 99.1% of the case group and in 96.0% of the control group (P = 0.0326). The TT and CT genotype frequencies were 98.2% and 1.8% respectively in the case group and 92.0% and 8.0% respectively in the control group (P = 0.0304). Regarding the SNP rs10432616 (MAF < 0.01), the C allele was identified in 97.8% of the case group and in 100% of the control group (P = 0.0245). The CC and CT genotype frequencies were 95.5% and 4.5% respectively in the case group and 100% and 0% respectively in the control group (P = 0.0237).

We then investigated the distribution of genotype and allele in different gender groups. For males, one SNP rs10432616 exhibited significant differences in the alleles and genotype frequencies between the male case group (male individuals with non-syndromic tooth agenesis) and male control group (normal male
The C allele was identified in 96% of the male case group and in 100% of the male control group ($P = 0.0239$). The CC and CT genotype frequencies were 92.1% and 7.9% respectively in the male case group and 100% and 0% respectively in the male control group ($P = 0.0225$). For females, no SNP exhibited significant differences in the alleles and genotype frequencies between the female case group (female individuals with non-syndromic tooth agenesis) and female control group (normal female individuals) (Table 5).

### Discussion

Even though the exact mechanism of tooth agenesis has not yet been determined, genetic factors, as well as environmental, radiotherapy and chemotherapy, are some of the factors that may trigger tooth agenesis.

Despite the statistical analysis, the results are not conclusive due to the small sample size. Further studies with a larger sample population are required to confirm the association between the identified SNPs and tooth agenesis.

### Table 3

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP site</th>
<th>Sample</th>
<th>N</th>
<th>Allele</th>
<th>$P$</th>
<th>Odds Ratio (95% CI)</th>
<th>Genotype</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDAR</td>
<td>rs3749098</td>
<td>Case</td>
<td>112</td>
<td>T</td>
<td>C</td>
<td>0.0326</td>
<td>TT</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Control</td>
<td>112</td>
<td>2</td>
<td>2</td>
<td>0.0304</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDAR</td>
<td>rs3749099</td>
<td>Case</td>
<td>112</td>
<td>C</td>
<td>T</td>
<td>0.0326</td>
<td>CC</td>
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<tr>
<td></td>
<td></td>
<td>Control</td>
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<td>2</td>
<td>2</td>
<td>0.0304</td>
<td>CT</td>
<td></td>
</tr>
<tr>
<td>EDAR</td>
<td>rs10432616</td>
<td>Case</td>
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<td>C</td>
<td>T</td>
<td>0.0245</td>
<td>CC</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Control</td>
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<td>5</td>
<td>5</td>
<td>0.0237</td>
<td>CT</td>
<td></td>
</tr>
</tbody>
</table>

CI: confidence intervals. *P* - values lower than 0.05 written in bold.

CI: confidence intervals.
Table 4  Distribution of genotypes and alleles for 3 SNPs in the male case group and the male control group.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP site</th>
<th>Sample</th>
<th>N</th>
<th>Allele</th>
<th>P</th>
<th>Odds Ratio (95% CI)</th>
<th>Genotype</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDAR</td>
<td>rs3749098</td>
<td>Exon 10</td>
<td>Case 63</td>
<td>T</td>
<td>C</td>
<td>2</td>
<td>0.1507</td>
<td>3.100(0.614-15.662)</td>
</tr>
<tr>
<td>EDAR</td>
<td>rs3749099</td>
<td>Exon 10</td>
<td>Case 63</td>
<td>C</td>
<td>T</td>
<td>2</td>
<td>0.1507</td>
<td>3.100(0.614-15.662)</td>
</tr>
<tr>
<td>EDAR</td>
<td>rs10432616</td>
<td>Exon 10</td>
<td>Case 63</td>
<td>C</td>
<td>T</td>
<td>5</td>
<td>0.0239</td>
<td>0.960(0.927-0.995)</td>
</tr>
</tbody>
</table>

CI: confidence intervals. P-values lower than 0.05 written in bold

Table 5  Distribution of genotypes and alleles for 3 SNPs in the female case group and the female control group.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP site</th>
<th>Sample</th>
<th>N</th>
<th>Allele</th>
<th>P</th>
<th>Odds Ratio (95% CI)</th>
<th>Genotype</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
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<td>rs3749098</td>
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<td>Case 49</td>
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<td>C</td>
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<td>0.0814</td>
<td>1.024(0.997-1.053)</td>
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<td>EDAR</td>
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<td>Exon 10</td>
<td>Case 49</td>
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<td>0</td>
<td>0.0814</td>
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<tr>
<td>EDAR</td>
<td>rs10432616</td>
<td>Exon 10</td>
<td>Case 49</td>
<td>C</td>
<td>T</td>
<td>0</td>
<td>0.0814</td>
<td>1.024(0.997-1.053)</td>
</tr>
</tbody>
</table>

CI: confidence intervals.
three cysteine-rich domains, a trans-membrane domain and a death domain. Exon 3, 4, and 5 code ligand binding domain (LBD), exon 12 codes death domain (DD). To date, the function of exon 10 is not clear. EDAR interacts with another adaptor protein with a death domain named EDARADD. This is a direct signalling pathway from EDA and is involved in ectodermal tissue development, which includes tooth development. A number of studies suggested that EDA mutations cause non-syndromic tooth agenesis\(^1\)\(^-\)\(^2\)\(^-\)\(^20\), however, no EDAR mutations and only one EDARADD mutation\(^8\) was reported in association with non-syndromic tooth agenesis. Our results suggested that variation in EDAR could be a pathogenic factor for non-syndromic tooth agenesis.

Studies on polymorphisms and tooth agenesis are sporadic. Most of them focus on AXIN2, MSX1 and PAX9\(^{10,21-24}\). Furthermore there are only three studies that focused on the Chinese Han population\(^{10-12}\). While some of the tooth agenesis may be explained to be monogenic, there are many unique mutations that could not be explained via the same method\(^25\). The possibility of polymorphism in genes involved in tooth development may be risk factors for tooth deformation. Additionally, the interaction between polymorphism risk factors could lead to tooth agenesis, though larger sample size and multi-ethnic studies are required for confirmation of this hypothesis.

Dental development is a complex process involving many genes and signalling pathways. As more genes are confirmed as being involved in tooth agenesis, the networks and their interactions are gradually becoming clear. Studying the interaction of genes related with non-syndromic tooth agenesis will help us understand the development of teeth and lay the foundations for gene therapy.

In this study we confirmed an association between polymorphism of rs10432616, rs3749098, and rs3749099 in EDAR with tooth agenesis of the Chinese Han population. Future functional studies are necessary to illuminate the mechanism. More extensive EDAR screening for genetic variants is needed from larger sample sizes to confirm EDAR as a candidate genetic marker associated with tooth agenesis.

Acknowledgements

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Conflicts of interest

The authors reported no conflicts of interest related to this study.

Author contribution

Drs Yi Ting CHEN and Hao Chen LIU performed the experiments, collected and analysed the results and prepared the manuscript; Drs Dong HAN and Yang LIU prepared and revised the manuscript; Dr Hai Lan FENG designed and supervised the study and finally revised the manuscript.

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