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

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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*

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Tooth agenesis refers to the congenital absence of one or more teeth, and is the most common developmental anomaly in human dentition[1]. The prevalence of dental agenesis of permanent teeth was estimated to be approximately 5.89% of the Chinese population[2] and 1.6% to 9.6% of Caucasians[3]. Although many potential and determinative factors affect tooth development, genetic factors are the most important risk factor for tooth agenesis[4]. 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 agenesis[5-8]. 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

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Table 1 Characteristics of subjects with non-syndromic tooth agenesis.

Characteristics	Number	Percentage
Gender Distribution		
Male	63	56.25%
Female	49	43.75%
Number of teeth missing per subject		
1 to 5 teeth missing	6	5.36%
6 or more teeth missing	106	94.64%
Frequency of teeth type missing		
Total teeth missing = 1766		
Maxillary central incisors	70	3.96%
Maxillary lateral incisors	158	8.95%
Maxillary canines	130	7.36%
Maxillary first premolars	154	8.72%
Maxillary second premolars	164	9.29%
Maxillary first molar	84	4.76%
Maxillary second molar	131	7.42%
Mandibular central incisors	145	8.21%
Mandibular lateral incisors	131	7.42%
Mandibular canines	96	5.44%
Mandibular first premolars	133	7.53%
Mandibular second premolars	165	9.34%
Mandibular first molar	75	4.25%
Mandibular second molar	130	7.36%
Number of patients with missing incisors	504	28.54%
Number of patients with missing canines	226	12.80%
Number of patients with missing premolars	616	34.88%
Number of patients with missing molars	420	23.78%

have shown the association between EDA mutations and non-syndromic tooth agenesis. To date, however, no EDAR mutations and only one EDARADD mutation[8] 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[9]. 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[10-12]. 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.

Table 2 Analysis of single nucleotide polymorphisms (SNPs)

Gene	SNP Site	Position	MAF
EDAR	rs151195196	Exon 02	< 0.01
	rs759735008	Exon 04	< 0.01
	rs61761321	Exon 04	0.02
	rs3749108	Exon 08	0.01
	rs3749098	Exon 10	0.01
	rs3749099	Exon 10	0.01
	rs200267845	Exon 10	< 0.01
	rs10432616	Exon 10	< 0.01
	rs3827760	Exon 12	0.24
	rs146567337	Exon 12	0.01
EDARADD	rs966365	Exon 01	0.22
	rs60808129	Exon 01	0.09
	rs200569815	Exon 02	< 0.01
	rs604070	Exon 06	0.13
	rs777172467	Exon 06	< 0.01
	rs753890063	Exon 06	< 0.01
	rs74942492	Exon 06	< 0.01
	rs753408117	Exon 06	< 0.01

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 rs3749099 (MAF = 0.01), the C allele was identified in 99.1% of the case group and in 96.0% of the control group ($P = 0.0326$). The CC 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

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

Gene	SNP site	Sample	N	Allele		P	Odds Ratio (95% CI)	Genotype			P
				T	C			TT	CT	CC	
EDAR	rs3749098										
	Exon 10	Case	112	222	2			110	2	0	
		Control	112	215	9	0.0326	4.646(0.993-21.752)	103	9	0	0.0304
				C	T			CC	CT	TT	
EDAR	rs3749099										
	Exon 10	Case	112	222	2			110	2	0	
		Control	112	215	9	0.0326	4.646(0.993-21.752)	103	9	0	0.0304
EDAR	rs10432616			C	T			CC	CT	TT	
	Exon 10	Case	112	219	5			107	5	0	
		Control	112	224	0	0.0245	0.977(0.958-0.997)	112	0	0	0.0237

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

individuals) (Table 4). 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).

None of the analysed SNPs in EDARADD 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).

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[4,13]. Studies have shown the incidence rate of tooth agenesis ranges from 1.6 to 9.6%[2,3], yet there are relatively few cases that can be tied to specific genetic mutation. These suggest tooth agenesis may be a polygenic disease. Numerous genes are found to be related to the tooth development process, involved in the coding of signalling molecules, transcription factors and factors that control differentiation and proliferation[14-17]. Being involved in so many of the tooth development processes, these genes can be presumed to have the potential to participate in causing tooth agenesis.

Different polymorphic alleles can exhibit different phenotypic variation in dental arrangements; therefore it is logical to speculate an association between tooth agenesis and gene polymorphisms. We thus focused on studying the association between tooth agenesis and SNPs.

In this study we identified three SNP markers in EDAR that are associated with tooth agenesis. Through statistical analysis rs10432616 was found to be a risk factor while rs3749098 and rs3749099 appear to be protective factors in tooth agenesis in the Chinese Han population. We noticed a strong association between two protective factors – rs3749098 and rs3749099 – that always seem to exist in pairs.

The three SNP markers are located on exon 10 of the EDAR gene. EDA, EDAR, and EDARADD belong to the same signalling pathway, which is very important in tooth development. EDAR consists of a signal peptide,

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

Gene	SNP site	Sample	N	Allele		*P*	Odds Ratio (95% CI)	Genotype			*P*
EDAR	rs3749098			T	C			TT	CT	CC	
	Exon 10	Case	63	124	2			61	2	0	
		Control	63	120	6	0.1507	3.100(0.614-15.662)	57	6	0	0.1439
EDAR	rs3749099			C	T			CC	CT	TT	
	Exon 10	Case	63	124	2			61	2	0	
		Control	63	120	6	0.1507	3.100(0.614-15.662)	57	6	0	0.1439
EDAR	rs10432616			C	T			CC	CT	TT	
	Exon 10	Case	63	121	5			58	5	0	
		Control	63	126	0	0.0239	0.960(0.927-0.995)	63	0	0	0.0225

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.

Gene	SNP site	Sample	N	Allele		*P*	Odds Ratio (95% CI)	Genotype			*P*
EDAR	rs3749098			T	C			TT	CT	CC	
	Exon 10	Case	49	98	0			49	0	0	
		Control	49	95	3	0.0814	1.024(0.997-1.053)	46	3	0	0.0796
EDAR	rs3749099			C	T			CC	CT	TT	
	Exon 10	Case	49	98	0			49	0	0	
		Control	49	95	3	0.0814	1.024(0.997-1.053)	46	3	0	0.0796
EDAR	rs10432616			C	T			CC	CT	TT	
	Exon 10	Case	49	98	0			49	0	0	
		Control	49	98	0			49	0	0	

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[18-20], 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 PAX910,[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.

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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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