GENETIC POLYMORPHISM IN *ESR2* AND RISK OF TOOTH AGENESIS

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Palavras-chave: Polimorfismo de Nucleotídeo Único. Receptor Alpha de Estrogênio. Receptor Beta de Estrogênio. Anodontia. Agenesia Dentária.

RESUMO

Introdução: A agenesia dentária (AD) é a ausência congênita de um ou mais dentes. Vários estudos vêm sugerindo o forte componente genético para essa condição. **Objetivo:** O presente estudo teve como objetivo avaliar se os polimorfismos genéticos nos genes que codificam os receptores de estrógeno (*ESR1* e *ESR2*) estão associados à ocorrrência de AD isolada em uma amostra brasileira. **Métodos:** Radiografias panorâmicas de 142 pacientes ortodônticos foram avaliadas para determinar AD de dentes permanentes (excluindo terceiros molares). O DNA dos pacientes foi extraído das células da mucosa bucal contidas na saliva para avaliar polimorfismos genéticos em *ESR1* (*rs2234693* e *rs9340799*) e *ESR2* (*rs1256049* e *rs4986938*) por genotipagem usando a técnica de PCR em tempo real. Para análises estatísticas, associações entre as distribuições dos alelos e genótipos e a ocorrrência de AD foram avaliadas para cada polimorfismo genético, com um alfa estabelecido de 5%. **Resultados:** Treze pacientes tiveram pelo menos 1 dente congenitamente ausente. O número de dentes congenitamente ausentes variou de 1 a 11. Os polimorfismos genéticos *rs2234693* e *rs9340799* no *ESR1* e *rs1256049* no *ESR2* não foram associados à AD ($p > 0,05$). Para o polimorfismo genético *rs4986938* no *ESR2*, as distribuições dos genótipos e dos alelos foram estatisticamente diferentes entre os pacientes com e sem AD ($p < 0,05$). O genótipo CC e o alelo C estavam super-representados nos pacientes com AD. **Conclusão:** Houve associação entre o polimorfismo genético *rs4986938* no *ESR2* e a ocorrrência de AD.

Keywords: Polymorphism, Single Nucleotide. Estrogen Receptor Alpha. Estrogen Receptor Beta. Anodontia. Tooth Agenesis.

ABSTRACT

Introduction: Tooth agenesis (TA) is the congenital absence of teeth. Several studies have proposed a strong genetic background for this condition. **Aim:** The present cross-sectional study aimed to evaluate whether genetic polymorphisms in the genes that code for estrogen receptors (*ESR1* and *ESR2*) are associated with the presence of isolated TA in a Brazilian sample. **Methods:** Panoramic radiographs of 142 orthodontic patients were assessed to determine TA of permanent teeth (excluding third molars). DNA of patients was extracted from buccal cells from saliva to evaluate genetic polymorphisms in *ESR1* (*rs2234693* and *rs9340799*) and *ESR2* (*rs1256049* and *rs4986938*) by genotyping using the real-time PCR technique. For statistical analyses, associations between the distributions of the alleles and genotypes, and the ocurrence of TA were assessed for each genetic polymorphism, with an established alpha of 5%. **Results:** Thirteen patients had at least 1 congenital missing tooth. The number of congenitally missing teeth ranged from 1 to 11. The genetic polymorphisms *rs2234693* and *rs9340799* in *ESR1* and *rs1256049* in *ESR2* were not associated with TA ($p > 0.05$). For the genetic polymorphism *rs4986938* in *ESR2,* the genotype and allele distributions were significantly different between the patients with and without TA ($p < 0.05$). The CC genotype and the C allele were overrepresented in the TA patients. **Conclusion:** The genetic polymorphism *rs4986938* in *ESR2* was associated with the ocurrence of TA.

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INTRODUCTION

Tooth agenesis (TA) is the congenital absence of one or more primary or permanent teeth. This trait is one of the most common congenital anomalies in humans. Permanent teeth are more usually affected than primary ones.[1] The most common absent teeth are third molars, followed by premolars and upper lateral incisors.[2,3] Reports on the overall prevalence of TA in the permanent dentition vary substantially according to the studied population.[4]

TA can be classified into syndromic and non-syndromic TA. Syndromic TA refers to complex developmental syndromes associated with congenitally missing teeth.[5] More than 60 syndromes catalogued in Online Mendelian Inheritance in Man (OMIM) are associated with TA. Non-syndromic TA includes a congenitally missing tooth (or teeth) in an isolated form, without an association with any other major birth defect.[6] However, several studies have proposed a strong genetic background for isolated TA.[6-12]

Estrogen regulates cell growth, differentiation, and development. Cellular signaling of estrogen is mediated by estrogens receptors (ER), which have two forms: α and ß. The ER α is codified by *ESR1* and ER ß is codified by *ESR2.*[13] Expression of estrogen receptors have been identified in dental tissues.[14-16] Due to the role of estrogen in the differentiation of tooth-forming cells,[17,18] it is possible to hypothesize that genetic polymorphisms in *ESR1* and *ESR2* are involved in the occurrence of TA. The present study aimed to evaluate whether genetic polymorphisms in *ESR1* and *ESR2* genes are associated with isolated TA.

MATERIALS AND METHODS

Sample

The study protocol was reviewed and approved by the Ethics Committee of the School of Dentistry of Ribeirão Preto, University of São Paulo (CAAE 50765715.3.0000. 5419;01451418.3.0000.5419). Informed consent was obtained from all participant or parents/legal guardians during the orthodontic appointment.

Pre-treatment orthodontic records including panoramic radiographs from patients undergoing orthodontic treatment at the School of Dentistry of Ribeirão Preto, University of São Paulo were evaluated.

Patients younger than 6 years old, with craniofacial syndromes, oral clefts, chronic conditions, history of facial trauma or surgery, previous orthodontic treatment, records with missing radiographs, or radiographs with poor quality, were excluded for analyses. The sample consisted of 142 individuals aged 7 to 49 years old.

Determination of the tooth agenesis phenotype

All TA cases were clearly evidenced by assessing of the panoramic radiographs alone. All radiographs were examined following a standardized protocol.[2,3]

The inclusion criterion was having at least one permanent tooth affected, excluding third molars. TA was defined based on the age of subjects and the expected stage of tooth formation in the radiographs.[2,3] Second premolar agenesis was only considered on patients older than 8 years old.[3]

DNA extraction and genotyping

Patients were asked to rinse the mouth with 5 ml saline solution and expectorating the rinse in a propylene tube. DNA was extracted from buccal epithelial cells from this saliva sample.[19] The genetic polymorphisms in *ESR1* (*rs2234693* and *rs9340799*) and *ESR2* (*rs1256049* and *rs4986938*) were genotyping by real-time PCR technique using TaqMan technology (StepOne™ Real-time PCR System, Applied Biosystems, Foster City, USA). The characteristics of the polymorphisms assessed in *ESR1* and *ESR2* are presented in Table 1.

Statistical analysis

Absolute and relative frequencies were used to present the distributions of the genotypes and alleles for each polymorphism assessed. Fisher's exact test was performed to determine the association between these frequencies and the ocurrence of TA. Additionally, the Odds ratio was calculated for each association tested. A chi-square test was used to evaluate the Hardy-Weinberg equilibrium.

All analyses were performed using two-tailed tests on GraphPad Prism 5.0[a] package (Graph-Pad, San Diego, CA, USA) with a significance level of 5%.

RESULTS

Thirteen patients had at least 1 congenital missing tooth (mean age: 12.6 ± 1.56; 10 males, 3 females). The number of congenitally missing teeth ranged from 1 to 11. The characteristics of the TA patients are presented in Table 2. One hundred and twenty nine patients did not present TA (mean age: 15.4 ± 7.6; 58 males, 71 females).

The genotype and allele distributions for the studied genetic polymorphisms according to the ocurrence of TA are presented in Table 3. All the studied genetic polymorphisms were in Hardy-Weinberg equilibrium (Table 3).

The genetic polymorphisms *rs2234693* and *rs9340799* in *ESR1* and *rs1256049* in *ESR2* were not associated with TA ($p > 0.05$). The distribution of genotypes for all the studied genetic polymorphism *rs4986938* in *ESR2,* the genotype and allele distributions were significantly different between the patient with and without TA ($p < 0.05$). The CC genotype and the C allele were overrepresented in the TA patients.

Table 1: *Candidate genes and polymorphisms studied*

Gene	Locus	Genetic polymorphism	Type	MAF*	Base Change
ESR1	6q25.1	*rs2234693*	Intron variant	0.446	C/T
		rs9340799	Intron variant	0.281	A/G
ESR2	14q23.2	*rs1256049*	Intron variant	0.129	C/T
		rs4986938	Intron variant	0.259	C/T

*Note: *MAF: minor allele frequency*

Table 2: *Chraracteristics of the TA patients*

Patient	Age	Gender	Number of congenitally missing teeth	Congenitally missing teeth
1	13	Male	11	14,15,24,25,27,35,36,37,42,45,47
2	14	Female	2	35,37
3	13	Male	1	42
4	13	Male	1	22
5	11	Female	1	11
6	10	Male	9	31,32,33,34,35,36,37,41,42
7	11	Male	2	35,45
8	13	Male	1	45
9	16	Male	1	12
10	12	Male	4	31,41,42,43
11	14	Male	1	35
12	12	Female	1	32
13	12	Male	2	35,45

Table 3: *Distribution of genotypes and alleles according to the ocurrence of TA*

Genotype/Allele	TA n (%)	No TA n (%)	OR (95% CI)	*p* value
ESR1 rs2234693				
CC	2 (15.4)	19 (15.4)	Reference	
CT	5 (38.5)	56 (45.5)	1.18 (0.22-6.40)	>0.999
TT	6 (46.2)	48 (39.0)	0.84 (0.16-3.87)	>0.999
C	9 (34.6)	94 (38.2)		
T	17 (65.4)	152 (61.8)	0.86 (0.35-1.94)	0.833
HWp		0.704		
ESR1 rs9340799				
AA	7 (53.8)	63 (51.2)	*Reference*	
AG	4 (30.8)	50 (40.7)	1.39 (0.41-4.42)	0.755
GG	2 (15.4)	10 (8.1)	0.56 (0.12-2.97)	0.613
A 1	8 (69.2)	176 (71.5)		
G	8 (30.8)	70 (28.5)	0.89 (0.39-2.06)	0.821
HWp	>0.999			
ESR2 rs1256049				
CC	11 (84.6)	121 (93.8)	*Reference*	
CT	2 (15.4)	8 (6.2)	0.36 (0.07-1.89)	0.229
TT	0 (0)	0 (0)	NA	>0.999
C	24 (92.3)	250 (96.9)		
T	2 (7.7)	8 (3.1)	0.38 (0.08-1.89)	0.230
HWp		>0.999		
ESR2 rs4986938				
CC	9 (69.2)	38 (32.2)	*Reference*	
CT	3 (23.1)	62 (52.5)	4.90 (1.38-17.39)	0.027*
TT	1 (7.7)	18 (15.3)	4.26 (0.56-49.11)	0.259
C	21 (80.8)	138 (58.5)		
T	5 (19.2)	98 (41.5)	2.98 (1.14-7.44)	0.034*
HWp		0.450		

*Note: HWp, Hardy-Weinberg p value. * Statistically significant association. NA=not applicable*

DISCUSSION

Some research groups that study dental development have been focused on the understanding of the etiology of TA and other complex conditions that affect the human dentition. In the past few years, much progress has been made in the identification of the developmental basis of odontogenesis and the genes involved in TA.[6-12]

In fact, genetic polymorphisms in many genes, including *MSX1* (msh homeobox 1),[20] *PAX9* (paired box 9),[20] *FGF3* (fibroblast growth factor 3),[7] *FGF10* (fibroblast growth factor 10),[7] *FGFR2* (fibroblast growth factor receptor 2),[7] *FGFR1* (fibroblast growth factor receptor 1),[21] *BMP2* (bone morphogenetic protein 2),[6] *BMP4* (bone morphogenetic protein 4),[10,12] *TGFß1* (transforming growth factor beta 1),[11] *TGFß3* (transforming growth factor beta 3),[10] *IRF6* (interferon regulatory factor 6),[9] *MMP1* (matrix metalloproteinase 1),[22] *MMP20* (matrix metalloproteinase 20),[22] *MMP9* (matrix metalloproteinase 9),[10] *MMP13* (matrix metalloproteinase 13),[10] and *AXIN2* (axin-related protein 2)[20] have been associated with non-syndromic TA. However, to the best of our knowledge, this is the first study evaluating genetic

polymorphisms in estrogen receptors genes - *ESR1* (*rs2234693* and *rs9340799*) and *ESR2* (*rs1256049* and *rs4986938*) – and their involvement in the etiology of TA.

Our results suggested that *ESR2 rs4986938* might be involved in the risk of TA. Analyses evidenced an association between the genotypes frequencies and the ocurrence of TA (p = 0.030) for this polymorphism. Most common homozygotes (CC) showed an increased risk (OR = 4.90; 95% CI: 1.38-17.39) of presenting TA when compared with heterozygotes (CT). Similarly, individuals carrying the C allele presented an increased risk (OR = 2.98; 95% CI: 1.14-7.44) of presenting this phenotype.

A previous report demonstrated the expression of an estrogen receptor-associated protein in tooth germs of human fetuses, suggesting the participation of these on early stages of odontogenesis.[23] Considering that estrogen deficiency can affect the development of teeth,[24] we assume that genetic variations in the gene that encodes the ERß could alter its function and, consequently, increase the risk of TA by altering the estrogen metabolism. With that point of view, a previous study showed an association between a polymorphism in the ER and dental fluorosis.[25] Despite our results being interesting, they should be evaluated with caution, because due to the small size of the sample, these could be a false positive (type I error).

Oligodontia is a rare genetic condition, which represents the congenital absence of six or more teeth in primary, permanent or both dentitions. In our sample, two patients presented oligodontia. It is possible that some genes are involved in the etiology only of severer TA cases (such as oligodontia), however, our sample size did not allow us to perform a stratified analysis, comparing only patients with oligodontia with control patients (without TA). In fact, mutations in *MSX1,*[26,27] *PAX9*[26] and *AXIN2*[28] were previously associated with oligodontia.

Odontogenesis is an extremely complex process involving the interplay between the oral ectoderm and the ectomesenchymal cells derived from the neural crest.[29] Odontogenesis is under strict molecular control,[30] in which an alteration in different genes/molecules could lead to TA. It is important to emphasize that although we observed a statistical association between *ESR2* and TA, the role of estrogen receptors must still be extensively elucidated. Further studies are necessary to evaluate the expression of ERß in the early stages of the odontogenesis. Similarly, new studies with a larger sample size are necessary to confirm the association observed in the present study.

CONCLUSION

The genetic polymorphism *rs4986938* in *ESR2* was associated with tooth agenesis in Brazilian individuals.

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