Association Between Tooth Agenesis and Polymorphisms of FGFR1, IRF6, MSX1 and PAX9 Genes in Patients from Tîrgu Mureș

Kerekes-Máthé Bernadette[1], Jobbágy-Óvári Gabriella[2], Székely Melinda[1], Cămărășan Alina[1], Borș Andreea[1], Mucenic Simona[1], Varga G[2]

[1] Department of Morphology of Teeth and Dental Arches; Technology of Dental Prosthesis and Dental Materials, Faculty of Dental Medicine, University of Medicine and Pharmacy, Tîrgu Mureș, Romania
[2] Department of Oral Biology, Semmelweis University, Budapest, Hungary

Aim: Tooth agenesis is the most prevalent congenital malformation in humans. Many studies showed the importance of genetic factors in the emergence of tooth agenesis. MSX1, PAX9, PVRL1, IRF6, FGFR1, AXIN2 are genes involved in tooth agenesis. In this study we attempted to determine genetic traits data of patients from Tîrgu Mureș regarding tooth agenesis.
Material and method: Thirtyfour patients with tooth agenesis and 51 healthy volunteers were examined. Oral mucosal scrapings were collected from all the subjects. DNA was isolated and a genotyping experiment was performed. The procedures included four single nucleotide polymorphisms (SNPs): PAX9 -912 C/T, MSX1 3755 A/G, FGFR1 T/C, and IRF6 A/G.
Results: Besides the dominant allele, we observed the presence of the rare allele as well in each investigated polymorphism. There was a statistically significant difference in the distribution of the FGFR1 T/C gene polymorphisms between the two groups (p=0.02). Differences in the distribution of the IRF6, MSX1 and PAX9 gene polymorphisms were not significant statistically (p>0.4).
Conclusions: Our study showed, that FGFR1 T/C (26190464) polymorphism is a significant risk factor for non-syndromic tooth agenesis, preferential premolar agenesis. PAX9 and MSX1 gene may be associated with syndromes that include tooth agenesis. Further investigations are needed.

Keywords: tooth agenesis, gene polymorphism, FGFR1, IRF6, PAX9, MSX1

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Introduction

Tooth agenesis is the most prevalent congenital malformation in humans. Agenesis of permanent teeth ranges from 2.6 to 14.6%, excluding third molars and it is population-dependent. Hypodontia defines a situation where the patient is missing one to six teeth; if more than six teeth are missing it is called oligodontia. The most severe case of agenesis is anodontia, when all the teeth are missing. These anomalies can be non-syndromic or associated with different syndromes. Both environmental and genetic factors can lead to failure in tooth development. Many studies showed the importance of genetic factors in the emergence of tooth agenesis. Several mutations of the genes MSX1 and PAX9, which are transcription factors, appear to be critical in the early tooth development and leads to agenesis [1,2]. PVRL1 variants contribute to non-syndromic cleft lip and palate [3]. Interferon Regulatory Factor 6 (IRF6) and Fibroblast Growth Factor Receptor 1 (FGFR1) are associated with premolar agenesis [4]. The axis inhibition protein 2 (AXIN2) polymorphisms are also associated with tooth agenesis. An AXIN2 mutation has been associated with both tooth agenesis and colon neoplasia [5]. Tooth agenesis studies reveals differences in the pattern of involved genes in different populations, using genotyping experiments, where a single nucleotide polymorphism (SNP) can be differentiate. SNPs are DNA sequence variations that occur when a single nucleotide (A, T, C or G) in the genome sequence is altered. In this study we intended to determine genetic traits regarding tooth agenesis of patients from Târgu Mureş.

Material and method

Our study involved 85 subjects, divided in two groups, living in Tîrgu Mureş. The case group comprised 34 patients with tooth agenesis and the control group involved 51 volunteers. The inclusion criteria in the case group were at least one missing adult tooth germ, excluding third molars, the absence of craniofacial malformation and absence of systemic diseases. Tooth agenesis was diagnosed based on anamnestic data and dental panoramic radiographs in each case. The inclusion criteria in the control group were normal dentition, without any dental or craniofacial abnormalities. All patients have signed an informed consent and the study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Tîrgu Mureş. Oral mucosal scrapings were collected using cytological brushes, from all the patients.

DNA was isolated using the NucleoSpin Tissue kit (Macherey-Nagel GmbH & Co. KG, Düren), according to the manufacturers' protocols. The integrity of DNA was controlled by electrophoresis on 1% agarose gel. The concentration of DNA was measured by a fluorometer (Quibit, Invitrogen, Carlsbad, CA).

The genes we selected for SNP genotyping were associated previously with tooth agenesis [4,6,7] (Table I).

Correspondence to Bernadette Kerekes-Máthé
Email: mdetti3@yahoo.com

Table I. The analyzed SNP (single nucleotide polymorphism) assay informations

Gene	SNP	TaqMan SNP assay
Fgfr1	T/C (26190464)	C__8844845_10
Irf6	A/G (1149)	C__2500195_10
Msx1	A/G (3755)	C__26933394_10
Pax9	C/T (-912)	C__11921290_10

For the genotyping experiment we used the Applied Biosystems StepOne Real Time PCR System (Warrington, Cheshire, UK) and TaqMan SNP assays (Table I). The reactions consisted of 6.25 µl of 2X TaqMan Universal PCR Master Mix, 0.625 µl assay and 3 µl genomic DNA. The StepOne software automatically identified genotypes dependent on fluorescence intensities.

The experiment took place in cooperation with the Department of Oral Biology, Semmelweis University, Budapest, Hungary.

Statistical analysis was performed with GraphPad InStat 3.10 software, using Fisher's Exact Test with 95% Confidence Interval, and p values <0.05 were defined as statistically significant.

Results

Demographic data for both groups are presented in Table II. Data regarding the number and type of missing teeth in the case group are summarized in Table III.

The genotyping experiment showed both allele variations (the dominant and the rare one) in all the SNPs examined.

There was a statistically significant difference in the distribution of the FGFR1 T/C gene polymorphisms between the two groups (p=0.02, OR=0.5). The homozygous C/C genotype was present in 16.7% of the patients from the case group and in 27.5% of the control group. The homozygous T/T genotype was detected in 53.3% of the patients with agenesis and in 31.4% of the healthy persons. Heterozygous C/T state occured in 30% of case group and in 41.2% of control group. The rare C allele was present in 31.7% of the case group and in 48% of the controls. This allele showed a distribution of 26.3% in patients with only missing front teeth and 45.8% in patients with only missing lateral teeth. The T allele was present in 68.3% of the case group and in 52% of the control group.

Table III. Distribution of missing teeth in the case group

Number of teeth missing	
1 tooth missing	11 (32.3%)
2 to 6 teeth missing	20 (58.8%)
6 or more teeth missing	3 (8.8%)
Type of teeth more often missing	
Total teeth missing	75
Lateral incisor	31 (41.3%)
Second premolar	28 (37.3%)
First premolar	6 (8%)
Central incisor	5 (6.6%)
First molar	2 (2.6%)
Second molar	2 (2.6%)
Canine	1 (1.3%)

Table II. Demographic data of investigated subjects (n=85)

	Case group	Control group
Gender		
Male	11 (32.4%)	13 (25.5%)
Female	23 (67.6%)	38 (74.5%)
Mean age	24 years	30.5 years

The rare T allele of the gene PAX9 was present in 60.5% of patients with missing front teeth, in 42.3% of patients with missing lateral teeth and in 41.1% of healthy volunteers.

The distribution of the rare allele G of gene MSX1 showes 34.4% in the case group and 42.1% in the control group. The rare G allele of IRF6 gene is present in 11.3% of patients in the case group and in 20.5% of the controls.

We found no statistically significant difference in the distribution of the IRF6 (p=0.47), MSX1 (p=0.5) and PAX9 (p=0.48) gene polymorphisms between the groups studied.

Discussions

Many studies showed progresses in the understanding of molecular mechanisms that lead to tooth agenesis, but we are still far from knowing the complete genetic background of this anomaly [7,8]. Moreover, studies on different populations showed different genetic traits. This is the reason why every study on this subject is important, even performed on smaller groups of patients.

Vieira and his coworkers (2007) reported that genetic variation in the IRF6 locus, which has been implicated in the rare Van der Woude syndrome and cleft lip and palate, is associated with human tooth agenesis. Also, a marker in FGFR1, in which mutations cause Kallmann syndrome, was associated with premolar agenesis. Their findings provide further evidence that tooth agenesis is probably caused by several independent defective genes, acting alone or in combination with other genes [4]. In the present study we also found a significant association between a marker in the FGFR1 gene and tooth agenesis. The association of the IRF6 gene marker and tooth agenesis was not statistically significant in our study. This may be an indication that much larger study groups are needed to find genetic evidences.

According to several studies, mutations of the MSX1 and PAX9 gene may lead to agenesis. PAX9 plays a role both in palate and tooth formation, and a mutation of the coding region of this gene induces conditions with missing molars [6]. The mutations of the coding region of MSX1 primarily lead to missing premolars [7]. We observed the presence of the rare alleles as well as the presence of the dominant allele, in each polymorphism, but the differences between the two groups studied were not statistically significant.

There are many persons with tooth agenesis but having no identified mutation in the PAX9 or MSX1 gene, suggesting that these genes are associated with syndromes that include tooth agenesis. IRF6, FGFR1 and TGFβ3 have been shown to be associated with non-syndromic hypodontia [8]. Our results agree with these findings, however further investigations are needed to provide more information about these genetic traits.

This study was part of a long-term pending research at the Semmelweis University, Budapest, Hungary [9].

Conclusions

The present study showed that FGFR1 T/C (26190464) polymorphism is a significant risk factor for non-syndromic tooth agenesis, preferential premolar agenesis.

Our results suggest that PAX9 and MSX1 gene may be associated with syndromes that include tooth agenesis, but to provide more information about these genetic traits, further investigations are needed.

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