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Association of *FGFR2* (rs2981579) Gene Polymorphism with the Risk of Mesial Occlusion

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Abstract—The molecular-genetic testing of the polymorphic rs2981579 (C>T) locus of the *FGFR2* gene as the marker of increased predisposition to the development of mesial occlusion was carried out in 110 patients with mesial occlusion and 103 general-population control subjects from Ukraine. It was shown that polymorphism rs2981579 in gene *FGFR2* is associated with mesial occlusion (OR = 1.67, 95% CI = 1.14–2.45, $p = 0.009$). Compared to CC carriers, TT+CT carriers had a 3.21-fold higher risk of mesial occlusion (95% CI = 1.57–6.57, $p = 0.001$). We found the protective effect of the homozygous allele C on mesial occlusion development (OR = 0.31, $p = 0.001$). This is the first published data on *FGFR2* polymorphisms rs2981579 (C>T) in patients with mesial occlusion.

Keywords: mesial occlusion, gene polymorfism, *FGFR2*
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INTRODUCTION

Hereditary predisposition to many multifactorial orthodontic pathologies, depending on the contribution of the genetic component, determines the magnitude of the total risk of developing this type of disease. Influence of the environment is considered as one of the factors of the formation of maxillofacial morphology in the process of postnatal growth. However, particularly the genetic mechanisms dominate during embryonic cranial morphogenesis and can cause the formation of pathologies not only during the antenatal but also early postnatal ontogenesis [1–3]. Mesial occlusion is one of the common occlusion anomalies, which is characterized by a forward position of the lower jaw in relation to the upper one. In this occlusion anomaly, either the lower jaw is over-developed, or, on the contrary, the upper one is under-developed, or both. The range of polymorphisms of candidate genes that can play a role in the formation of mesial occlusion is broad and includes groups of genes controlling the metabolic and homeostatic systems [4–6].

Recently, more and more attention is drawn to the study of the influence of the *FGFR2* gene polymorphism on the formation of orthodontic pathologies [5–8]. The functional significance of the products of this gene is evidenced by a wide range of pathologies in the development of which *FGFR2* mutations play a significant role. The most commonly known mutations in the *FGFR2* gene are associated with Crouzon, Pfeiffer, and Apert syndromes (cited by [9]). Disturbances in the FGF-FGFR system are also observed in Alzheimer's disease, Duchen's muscular dystrophy, diabetic retinopathy, and atherosclerosis (cited by [10]). *FGFR2* mutations play an important role in the processes of carcinogenesis [11]. In orthodontics, cases of developmental defects caused by *FGFR2* mutations are accompanied by maxillofacial hypoplasia, relative mandibular prognathism, and associated problems in the formation of pathological occlusions [12]. The importance of mutations in this gene is shown in the development of nonsyndromal craniosynthoses, which are accompanied by mesial forms of occlusion [13]. There are only a few studies of the role of *FGFR2* in the formation of mesial occlusion not associated with syndromes and craniosynthoses.

The range of *FGFR2* mutations detected up to the present is characterized by a wide range of changes in the activity of FGF-FGFR signaling pathways, which, in turn, leads to a wide range of pathological changes in the presence of different mutations in *FGFR2*. This determines the relevance of the search for new polymorphic variants of the *FGFR2* gene, which may cause the formation of mesial occlusion that is not related to syndromic forms. There are currently no data on the role of polymorphism rs2981579 of gene *FGFR2* in the onset of this pathology. Research of genetic mechanisms that control the development of mesial occlusion is important both in terms of the development of prevention and treatment measures and the prognosis of orthodontic correction [3, 4].

Table 1. Distribution of specific polymorphisms rs2981579 (C/T) of gene *FGFR2* among mesial occlusion subjects and the compliance of the distribution of genotypes to the Hardy–Weinberg equilibrium

Genotype	Genotype frequency, *n* (share)		χ^2	*p*
	experimental	expected		
Mesial occlusion, *n* = 110				
CC	13 (0.118)	21.78 (0.198)		
CT	72 (0.655)	54.34 (0.494)	11.61	<0.001
TT	25 (0.227)	33.88 (0.308)		
Control group, *n* = 103				
CC	31 (0.301)	33.78 (0.328)		
CT	56 (0.544)	50.37 (0.489)	1.27	0.26
TT	16 (0.155)	18.75 (0.182)		

The purpose of this work was to investigate associations of the *FGFR2* gene polymorphism (rs2981579, C>T) with the risk of development of mesial occlusion.

MATERIALS AND METHODS

Genotyping of polymorphic markers of gene *FGFR2* (rs2981579, C>T) was carried out in 110 patients from different regions of Ukraine diagnosed with mesial occlusion, which were treated at the Dental Medical Center of the Bogomolets NMU during 2014–2015. The control group included 103 inhabitants of Kyiv without orthodontic pathologies and chronic diseases. All individuals gave their informed consent for the study. The work complied with the requirements of the medical ethics commission developed in accordance with the provisions of the Council of Europe Convention on the Protection of Human Dignity in Biomedical Aspects (1997) and the Helsinki Declaration of the World Medical Association (2008).

Genomic DNA was extracted from samples of buccal epithelium by sorption purification using the Sorbo-AM kit (AmpliSense, RF) kit according to the manufacturer's instructions. For genotyping, restriction analysis of polymerase chain reaction products in agarose gel was used. For amplification of the *FGFR2* gene section (rs2981579, C>T), specific primers for the studied polymorphism were used: direct (5'-GTGACTCCCTTCATCGTG-3') and reverse (5'-GGCTCCTGGTCTATTTCTC-3'). Amplification was carried out on a GeneAmp PCR System 2400 thermocycler (Perkin Elmer, Singapore). The amplification mode consisted of initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, DNA hybridization with primers and synthesis of the sequence complementary to the matrix DNA at 55°C for 30 s, elongation at 72°C for 30 s, and final elongation at 72°C for 10 min.

For identification of alleles of gene *FGFR2* (rs2981579, C>T), hydrolysis of the amplified DNA fragments was carried out by restriction enzyme PstI (10 units) (Fermentas, United States) according to the manufacturer's instructions. Restriction products were visualized by electrophoresis in 3% agarose gel with ethidium bromide. Three genotypes were identified, CC, CT, and TT, where the minor allele T indicated the absence of a restriction site for endonuclease PstI. When T was present in both alleles, a PCR product of 437 bp remained unsplit. In the absence of T, the product was split into two fragments of 350 and 87 bp. The heterozygous variant was identified by the presence of three lanes, 437, 350, and 87 bp.

The genotype frequencies were checked for compliance with the Hardy–Weinberg equilibrium using the chi-squared test. The method of binary logistic regression was used to evaluate the multiplicative, dominant, and recessive as well as additive (Cochran-Armitage test for linear trends) models of inheritance. The degree of association was determined by calculating the odds ratio (OR) and its confidence interval (CI).

RESULTS AND DISCUSSION

The results of the analysis of the genotyping of the subjects for polymorphism rs2981579 (C/T) of gene *FGFR2* and the compliance of the distribution of the genotypes to the Hardy–Weinberg equilibrium are presented in Table 1.

The distribution of the genotypes for polymorphisms rs2981579 (C/T) of gene *FGFR2* in the control group did not differ from the theoretically expected; however, in the group of patients with mesial occlusion, it significantly differed from the Hardy–Weinberg equilibrium. This discrepancy is due to a lower frequency of homozygotes of both the types and a higher number of heterozygotes compared to the theoretically expected frequencies of these genotypes.

The results of determining the frequencies of allele rs2981579 (C/T) of gene *FGFR2* among the subjects of

Table 2. Multiplicative model of inheritance of polymorphism rs2981579 (C/T) of gene *FGFR2* in individuals with mesial occlusion

Alleles	Mesial occlusion, $n = 110$	Control group, $n = 103$	χ^2	p	OR	
					value	95% CI
C	0.445	0.573	6.90	0.009	0.60	0.41–0.88
T	0.555	0.427			1.67	1.14–2.45

Table 3. Additive model of inheritance of polymorphism rs2981579 (C/T) of gene *FGFR2*

Genotypes	Mesial occlusion, $n = 110$	Control group, $n = 103$	χ^2	p	OR	
					value	95% CI
C/C	0.118	0.301	8.65	0.003	0.31	0.15–0.64
C/T	0.655	0.544			1.59	0.92–2.76
T/T	0.227	0.155			1.60	0.80–3.20

Table 4. Dominant model of inheritance of polymorphism rs2981579 (C/T) of gene *FGFR2* in individuals with mesial occlusion

Genotypes	Mesial occlusion, $n = 110$	Control group, $n = 103$	χ^2	value	value	
					value	95% CI
C/C	0.118	0.301	10.84	0.001	0.31	0.15–0.64
C/T + T/T	0.882	0.699			3.21	1.57–6.57

the groups studied are presented in Table 2. The frequency of the minor allele T in the group of patients with mesial occlusion is significantly greater than in healthy subjects, 0.56 vs. 0.43, respectively ($p = 0.009$). The risk of pathological occlusion of this type when carrying the T allele is significantly increased, OR = 1.67 (95% CI = 1.14–2.45).

The results of analysis according to the additive model of inheritance of rs2981579 (C/T) polymorphisms of gene *FGFR2* are shown in Table 3, which shows that the equally increased risk of development of mesial occlusion in this model is observed both in the homozygotes for the minor allele (T/T) and in the heterozygotes (G/T), 1.60 and 1.59, respectively.

Table 4 shows the results of analysis according to the dominant model of inheritance of the rs2981579 (C/T) variant allele of the *FGFR2* gene, suggesting that the group of individuals, both heterozygous and homozygous for allele T, is characterized by a significantly increased risk of developing mesial occlusion (OR = 3.21, $p = 0.001$). Homozygous C/C carriers have a significantly lower risk of developing mesial occlusion, that is, the presence of this genotype has a protective effect on the development of pathology.

Attention is drawn to the fact that the equally increased risk of mesial occlusion development is found both in homozygous and heterozygous carriers of the variant allele (Table 3), where, in the general group of carriers of the variant allele, in accordance with the dominant model of its inheritance, it is twice as high as for individual genotypes (Table 4). Thus, the presence of an expression product of even one variant allele rs2981579 (C/T) of gene *FGFR2* is sufficient to increase the risk of developing this pathology.

In determining the functional significance of allelic variants of gene *FGFR2* in the development of orthodontic pathologies, it is advisable to consider basic molecular mechanisms of their influence. A feature of the growth factors of fibroblasts is their multifunctionality, which determines their important role both in embryogenesis and during the life of an adult organism. They participate in the processes of differentiation and proliferation of cells of various types and in the regulation of cell migration and survival, tissue regeneration, and processes of angiogenesis and neurogenesis. FGF2 is involved in the regulation of the basic processes of cell existence, such as proliferation, differentiation, survival, cell adhesion, migration, mobility, and apoptosis. In vivo, FGF2 regulates the processes of limb formation, wound healing, angiogenesis, vasculogenesis, and blood vessel remodeling as well as participates in carcinogenesis processes [14]. Factors of fibroblast growth affect the cells through the receptor group (FGFR). The binding of the ligand to the receptor results in dimerization of *FGFR2* and subsequent activation of its tyrosine kinase domain, serving as a signal for binding to the adapter proteins. In the end, this results in activation of the MAPK-kinase cascade RAS-RAF-MEK1/2-ERK1/2. In most cases, the

now-known mutations in gene *FGFR2* result in excessive activation of its expression, which causes the development of a number of pathologies.

In the norm, *FGFR2* is responsible for the development of the bone and articular system participating in the regulation of differentiation as well as the proliferation of osteoblasts and chondrocytes. Increased activity of the FGF signaling pathway in the embryo and children leads to the development of skeletal abnormalities, including dwarfism, craniosynthesis syndromes, and achondroplasia. In an adult organism, FGFs are involved in the processes of physiological and pathological angiogenesis. There are only a few studies about the role of *FGFR2* in the formation of mesial occlusion not associated with syndromes and craniosynthosis. Mesial occlusion may be the result of upper jawbone retrognatism and/or excessively rapid growth of the mandible. In some studies, it is noted that mutations in *FGFR2* may be associated with upper jawbone retrognatism and the formation of mesial occlusion [15]. Studies of individuals in the Brazilian population showed that mutations in gene *FGFR2* can be associated with the development of mesial occlusion [16]. It was found that polymorphisms rs2162540 and rs11200014 of gene *FGFR2* are associated with the development of this pathology, and the presence of minor alleles increased the risk of development of mesial occlusion by 1.68 and 1.84 times [7]. Our results suggest that the presence of the variant T allele rs2981579 of the *FGFR2* gene is a risk factor for the development of mesial occlusion in the Ukrainian population, where both heterozygotes and homozygotes for the minor allele of this polymorphism have an increased risk of developing this pathology. The mechanism of manifestation of the revealed patterns remains unclear. It can be assumed that, in both the cases—the presence of one or two minor alleles—they more or less regulate activation of the fibroblast growth factor receptor and FGF-FGFR2 signaling pathways. The consequence of such activation may be the tissue-specific increase in the stimulation of processes that lead to disproportionate development of the dental-maxillofacial system.

Thus, for the first time, the presented work shows the association of polymorphism rs2981579 of gene *FGFR2* with the development of mesial occlusion. The obtained results are relevant in terms of more precise identification of mechanisms of the formation of the pathology of the dental-maxillofacial system, which will provide an opportunity to improve the method of orthodontic treatment and will open prospects for the development of methods of possible pharmacological prophylaxis and treatment at an early age associated, in particular, with the regulation of FGF-FGFR2 signaling pathways.

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