ORIGINAL ARTICLE

CONTENTS

SNP rs2981579 in the FGFR2 gene as a risk factor for class II malocclusion in the Ukranian population

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ABSTRACT

Aim: The aim of the study was to analyze the association of SNPrs2981579 FGFR2 gene as a risk factor for skeletal class II malocclusion in a Ukrainian population.
Materials and Methods: Genotyping of 103 patients from different regions of Ukraine diagnosed with of class II malocclusion was performed. The control group included 106 residents of Kyiv City without orthodontic pathologies and chronic diseases. The association of the T rs2981579 allele of the FGFR2 gene with the risk of developing of class II malocclusion was investigated (OR = 1.58, p = 0.02).
Results: The risk class II malocclusion in the control group did not depend on the allelic status of SNP rs2981579; the additive inheritance pattern showed that for heterozygotes (C/T) OR was 1.54, p = 0.01, for homozygotes for the variant allele (T/T) OR was 1.53, p = 0.01.
Conclusions: The SNP rs2981579 (C>T) of FGFR2 gene locus is a factor of increased susceptibility to the development of class II malocclusion in the Ukrainian population.

KEY WORDS: FGFR2 gene, class II maloclusion, Ukranian population

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INTRODUCTION

The Angle's class II accounts for more than a third of all malocclusions in the world, and is more common in Caucasians than in other races [1]. The phenotype of skeletal abnormalities of this type of occlusion is not homogeneous and is usually characterized by retrusion of the lower jaw (mandibular retrognathism), protrusion of the upper jaw (maxillary prognathism) or a combination of both. It can manifest as an isolated malocclusion or as part of a number of syndromes [1-3].

The etiology of skeletal class II malocclusion appears to have multifactorial nature; it has been shown that the influence of environmental, genetic, developmental factors and their combination play a significant role in the development of skeletal anomalies of this type. The discovery of a significant hereditary component in the formation of the skeletal class II malocclusion has naturally led to a growing interest in uncovering the genetic mechanisms that contribute to the development of these anomalies. Studies of twins have shown that class II is inherited as a variable autosomal dominant trait or as a polygenic expression of critical morphological features [4]. Molecular studies have identified specific genes [5-8] and signaling pathways involved in jaw growth and the development of dental occlusion that may contribute to the formation of pathological forms of malocclusion [8,9].

FGFR2 affects the proliferation, differentiation, and apoptosis of osteoblasts, participating in bone growth [9]. FGFR2 is a highly conserved tyrosine kinase receptor that is involved in a number of signal transduction pathways that are crucial for osteogenic differentiation [9-11]. Autosomal dominant mutations in *FGFR2* are associated with the development of a number of skeletal diseases, including Behar-Stevensen syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome, Cruson syndrome, and Apert syndrome, which can be accompanied by malocclusion [5,12]. In orthodontics, cases of malformations caused by FGFR2 mutations are accompanied by maxillary hypoplasia, relative mandibular prognathism, and related problems of pathological occlusion formation [13]. Mutations of this gene have been shown to be important in the development of non-syndromic craniosynostosis accompanied by the malocclusions [14].

FGFR2 is known to be involved in 19 of the 20 major skeletal signaling pathways associated with class III malocclusion. At the same time, it was also found that FGFR2 is also associated with class II skeletal maloc-

clusion and is present in 17 of the 20 major signaling pathways associated with class II maloclusion [8]. The few genetic studies have detected associations of a number of SNP *FGFR2* gene with the risk of development of malocclusion, most of which are located in the 2nd intron of the gene [6,8,9,15,16]. Studies on the significance of *SNP rs2981579 FGFR2* for the formation of class II malocclusion have not yet been conducted.

AIM

The aim of the study was to analyze the association of SNP rs2981579 *FGFR2* gene as a risk factor for class II malocclusion in a Ukrainian population.

MATERIALS AND METHODS

The genotyping of 103 patients (59 female and 44 male participants) with class II maloclusion from different regions of Ukraine, who were seeking orthodontic treatment at the Department of Prosthodontics and Orthodontics of the Kyiv Medical University, was carried out. The diagnosis of skeletal class II malocclusion was made based on of cephalometric examination with analysis of lateral cephalometric images by the method of A.M. Schwarz.

The following parameters were taken into account when making the diagnosis class II maloclusion: true length of the lower jaw (inclusion criterion – less than the required norm (N-Se+3 mm); true length of the upper jaw (inclusion criterion – meets the required norm: 2/3 of the true length of the lower jaw, or it may be more than the desired (Sol) value); ÐF angles (inclusion criterion – more than 85°), and ÐMM (inclusion criterion – more than 90°).

The control group consisted of 106 residents with an orthognathic occlusion without dental anomalies and chronic diseases (59 female and 47 male participants). All participants (or their parents in case of children younger than 18) gave written inform consent. The study was conducted in accordance with the requirements of the Medical Ethics Commission, developed in accordance with the provisions of the Council of Europe Convention for the Protection of Human Dignity with regard to Biomedicine (1997) and the World Medical Association's Helsinki Declaration (2008).

Genomic DNA was extracted from buccal epithelial samples using the Scientific GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA) according to the manufacturer's instructions. Genotyping was performed by PCR-RFLP (polymerase chain reaction, restriction fragment length polymorphism analysis). For amplification of the *FGFR2* gene region (rs 2981579, C>T), specific primers were used: forward (5'- GTGACTCCCTTCATCGTG-3') and reverse (5'-GGCTCCTGGTCTATTTCTC-3'). Amplification was performed on a GeneAmp PCR System 2400 thermal cycler (Perkin Elmer, Singapore). Amplification mode: initial denaturation – 94°C, 5 min; 35 cycles: denaturation – 94°C, 30 sec, hybridization – 55°C, 30 sec, elongation – 72°C, 30 sec; final elongation – 72°C, 10 min. After amplification, the PCR product was exposed to 10 U of PstI restriction enzyme (CTGCA↓G) for 16 hours at 37°C. The lengths of restriction fragments were visualized by electrophoresis using 2% agarose gel. The *TT* genotype corresponded to the presence of a fragment of 437 bp, *CC* – 350 and 87 bp, and *CT* – 437, 350 and 87 bp.

The distribution of genotype frequencies was checked for compliance with Hardy-Weinberg equilibrium (χ^2 test). The allelic, recessive, dominant, and additive inheritance models were evaluated using the logistic regression method. The degree of association was determined by calculating the odds ratio (OR) and its confidence interval (CI).

RESULTS

The frequency of the *rs2981579* genotypes of the *FGFR2* gene in the control group was: CC – 30.2%, CT – 54.7% TT – 15.1%; frequency of the minor allele (T) – 0.43. In the experimental group with a class II maloclusion: CC – 13.6%, CT – 65.0% TT – 21.4%; frequency of the minor allele (T) – 0.54. The frequency of subjects carrying homozygous of the major *C* allele in the control group was 2.22 times higher than in the experimental group of patients with a class II maloclusion ($p < 0.05$). The 1.42-fold increase in the frequency of homozygous carriers of the minor T allele in the experimental group of patients with class II maloclusion compared to the control group was nominal and did not have a sufficient level of statistical significance ($p = 0.24$). The distribution of genotypes in the control group did not differ from the population equilibrium ($p = 0.4$), however, in the group of patients with a class II maloclusion, the distribution of genotypes differed statistically significantly ($p = 0.02$) from the Hardy-Weinberg equilibrium. Deviations from the principle of population equilibrium can be caused by various reasons: randomness due to insufficient sample size; genotyping errors; heterogeneity of the sample – it may consist of representatives of different populations with different allele frequencies; heterogeneity of the sample may also be due to selective advantages of carrying certain genotypes in different forms of pathology. Considering the fact that the distribution of genotypes in the control

Table 1. Association of the SNP rs2981579 allele of the FGFR2 gene with the risk of developing class II malocclusion

Inheritance model	Experimental group, n = 103	Control group, n = 106	OR (odds ratio)		p
Allelic	allele frequency		value	95 % CI	
C	0.461	0.575	0.63	0.43 – 0.93	0.02
T	0.539	0.425	1.58	1.08 – 2.33	
Additive	genotype frequency		value	95 % CI	
C/C	0.136	0.302	0.36	0.18 – 0.73	**0.01**
C/T	0.650	0.547	1.54	0.88 – 2.69	
T/T	0.214	0.151	1.53	0.75 – 3.11	
Dominant	genotype frequency		value	95 % CI	
C/C vs	0.136	0.302	0.36	0.18 – 0.73	0.004
C/T +T/T	0.864	0.698	2.75	1.37 – 5.53	
Recessive	genotype frequency		value	95 % CI	
C/C + C/T vs	0.786	0.849	0.65	0.32 – 1.33	0.24
T/T	0.214	0.151	1.53	0.75 – 3.11	

Source: compiled by the authors of this study

group corresponds to the Hardy-Weinberg law, and the number of individuals genotyped is comparable to the number of genotyped patients with a class II maloclusion (106 and 103, respectively), the most likely reason for the discrepancy in distribution may be the heterogeneity of the sample of patients with class II maloclusion. So that it is necessary to take these features into account when interpreting the results.

The results of the analysis of the association of SNP *rs2981579* of *FGFR2* gene with the risk of class II maloclusion in different inheritance patterns are presented in Table 1.

Table 1 shows that the frequency of the variant allele among patients with a class II maloclusion is statistically significantly higher than in the control group (0.54 and 0.43, respectively, p = 0.02). Variant allele T (SNP rs2981579) increases the risk of developing a class II maloclusion by 1.58 times (OR = 1.58, p = 0.02). The additive inheritance model revealed statistically significant differences in the distribution of genotypes between the experimental group of patients with class II maloclusion and the control group (p = 0.01). At the same time, the risk of developing class II maloclusion was not additive and did not depend on the allelic status of the minor allele T. Both heterozygous (CT) and homozygous (TT) carriers of the minor allele showed an increased risk of developing this pathology – the odds ratio was 1.54 and 1.53 (p = 0.01), respectively. The dominant model of inheritance also revealed statistically significant differences in the distribution of SNP *rs2981579* genotypes of the *FGFR2* gene between the control and experimental groups. In particular, in the group of carriers of the *C/T + T/T* genotypes, the risk of developing a class II maloclusion is 2.75 times higher than in homozygous carriers (CC) of the major allele (p = 0.004). Homozygous carriage of the major allele of SNP rs2981579 has a protective effect on the development of this orthodontic pathology, OR = 0.36 (p = 0.004). In the recessive inheritance model, there were no statistically significant differences in the distribution of the SNP rs2981579 genotypes of the *FGFR2* gene between the study groups. This is due to the fact that both heterozygous and homozygous carriers of the variant allele of T SNP rs2981579 have an equally increased risk of developing a class II malocclusion (additive model).

DISCUSSION

rs2981579 is localized in the second intron of the *FGFR2* gene and is a proxy for *rs1219648,* which is part of a haplotype of 8 closely linked SNPs, which, in turn, is a risk factor for breast cancer development by increasing *FGFR2* gene transcription [17]. It should be noted that the currently known SNPs of FGFR2 gene associated with skeletal abnormalities are also localized in the 2-nd intron and are closely linked. At the same time, more SNPs of *FGFR2* genes were detected in skeletal class III malocclusion than in skeletal class II malocclusion. In particular, *rs2981578* (OR, 1.708; *P* = 0.007) and *rs10736303* (OR, 1.717; *P*

= 0.007) were associated only with class III skeletal anomalies, but not with class II malocclusion [9]. In the same study, it was shown that *rs2162540* (OR, 1.717; *P* = 0.003) is associated only with the risk of developing a class II maloclusion. Thus, different polymorphisms of *FGFR2* gene may have different effects on the development of sagittal occlusion.

It was found that the SNP of *FGFR2* in the 2nd intron, associated with a predisposition to abnormal bite patterns, are binding sites for RUNX2 and SMAD4, which are the main regulators of osteoblast differentiation and proliferation. [9, 18, 19]. Binding of RUNX2 and SMAD4 promotes *FGFR2* gene expression. RUNX2 directly regulates *FGFR2* gene, increasing the proliferation of osteoblast precursors, and SMAD4 is involved in signaling pathways (*BMP* and *TGF-β*) that play a fundamental role in the development of the embryonic skeleton and postnatal bone homeostasis [9, 20, 21]. The study by Jiang Q. et al. [9], which revealed the association of the SNPs rs2981578 and rs10736303 of the *FGFR2* gene only with class III abnormalities, but not with class II abnormalities, showed that minor alleles of these SNPs reduced the binding affinity and enhancing effect of RUNX2 and SMAD4 and decreased the expression level of *FGFR2* gene.

Craniofacial syndromes caused by *FGFR2* gene mutations often include multiple premature fusions of cranial suture; however, the mechanisms of their effect on skeletal class III malocclusion remain unclear. One possible mechanism may be the early closure of the midline of the upper jaw and other facial sutures between the upper jaw and zygomatic bone, the edge of the eye socket, and the nasal bones [22]. The mandible is craniofacial bone that does not have a suture connection, can be affected by changes in *FGFR2* gene expression and grow to normal size, resulting in class III skeletal malocclusion. Jiang Q. et al. [9] believe that, thus, the loss of binding of pro-osteogenic transcription factors may correlate with hypoplasia of the maxilla, but not with mandibular prognathism. One of the possible explanations for the association of *rs2981579* with the risk of class II maloclusion found in our study could be that minor alleles of *rs2981579,* on the contrary, cause an increase in *FGFR2* gene expression [17]. The mechanism of their effect will be the opposite of the one described by Meyer K. B. et al. [17], which, accordingly, could lead to the development of class II skeletal anomalies.

It should be noted that in the above-mentioned study by Jiang Q. et al. [9], no regulatory effects similar to *rs2981578* and *rs10736303* were found in the SNP rs2162540, which showed an association with the risk of developing class II skeletal anomalies. However, we suggest, the effect of the SNP *rs2981579* of the *FGFR2* gene is more complex and may cause the development disorder of a normal sagittal relationship between maxilla and mandible in general at early stages, and further dental maloclusions in the mesial or distal planes may involve additional factors and various types of their interactions. This assumption is due to the fact that previous studies have also found an association of *rs2981579* with the risk of developing a skeletal class III malocclusion in a Ukrainian population [16]. The situation is complicated by the fact that mutations in SNPs *of FGFR2* can directly affect the growth of both the upper and lower jaw, since *FGFR2* gene is expressed not only in the sutures but also in significant amounts in the periphery of the membranous bones. Indirect confirmation of the assumptions about the complex nature of the impact of the *FGFR2* gene on the formation of sagittal plane malocclusions may be confirmed by studies that show that different SNPs of a number of the same genes and their interactions may be involved in the etiology of sagittal and vertical malocclusions [23].

CONCLUSIONS

We investigated the association between the T rs2981579 allele of the *FGFR2* gene and the risk of developing a class II maloclusion (OR = 1.58, p = 0.02). The risk of developing a skeletal class II malocclusion in the study group did not depend on the allelic status of SNP rs2981579; the additive inheritance model showed that for heterozygotes (C/T) OR was 1.54, p = 0.01, for homozygotes for the variant allele (T/T) OR was 1.53, p = 0.01. The SNP rs2981579 (C>T) of FGFR2 gene locus is a factor of increased susceptibility to the development of skeletal class II malocclusion in a Ukrainian population. However, the mechanisms that determine the effect of minor alleles of rs2981579 on the formation of skeletal class II malocclusion require further research.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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