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ARTIGO CIENTÍFICO

EVALUATION OF THE ASSOCIATION BETWEEN DELAYED SKELETAL MATURATION AND A SINGLE NUCLEOTIDE POLYMORPHIM IN THE GENE ENCODING ESTROGEN RECEPTOR ALPHA

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ABSTRACT

Background: Cervical vertebrae are important biological indicators of child and adolescent developmental stages. Single nucleotide polymorphism (SNP) can unbalance the functions of important hormones that can consequently impact the individual's skeletal maturation. **Objective:** To evaluate the association between delayed skeletal maturation (DSM) and a SNP in the gene encoding estrogen receptor alpha (*ESR1*) in girls. **Methods:** This cross-sectional study screened 74 girls with age ranging from 10 to 11 years undergoing to orthodontic treatment. Girls with systemic disease or syndrome were excluded. Skeletal maturation analysis was performed using the method by Baccetti et al. (2005). Girls who were at CS3 and CS4 stages were considered controls and girls who were at earlier stages were considered with delayed in skeletal maturation (DSM). Genomic DNA was used to evaluate the SNP PvuII/rs2234693 in *ESR1* using Real-Time PCR. Fisher's test was applied to compare alleles between groups. **Results:** Twenty-eight girls were finally included in the analysis. There was no association between DSM and PvuII (rs2234693) ($p=0.404$). **Conclusion:** DSM was not associated with the SNP PvuII (rs2234693) in *ESR1* in girls.

Key-words: Age Determination by Skeleton, estrogen receptors, genes.

1 Introduction

Skeletal discrepancy under child development can be a decisive biological indicator for complex childhood disorders (FORLINO and MARINI, 2016; ZEMEL, 2017; THOMPSON, 2021). These patients require a broad understanding of skeletal growth, maturation, and development. Biological indicators of individual skeletal maturity are three: increase in height (BJÖRK and HELM, 1967), skeletal maturation of the hand and wrist (FLORES-MIR et al.,

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2014) and changes in the morphology of the cervical vertebrae (CERICATO et al., 2015). Several presentations of cervical vertebrae have gained increasing interest over the past decades as a biological indicator of individual skeletal maturity (MCNAMARA JR and FRANCHI, 2018; GHALEB et al., 2019; CARELLI et al., 2020; CARELLI et al., 2021), since analysis of cervical vertebrae is performed on the lateral cephalograms, a type of radiography routinely available for orthopedic/orthodontic diagnosis (MCNAMARA JR and FRANCHI, 2018).

It is worth mention that skeletal maturation is directly related to sex (ERHAMZA et al., 2020), ethnicity (KLÜNDER et al., 2020), environmental factors (XU et al., 2021), systemic conditions (KANG et al., 2020) and genetics factors (TEVES et al., 2015; KÜCHLER et al., 2021; XU et al., 2021). Advances in lifestyle and nutrition around the world gave popularity to accelerated puberty, especially in girls (PARENT et al., 2003). Skeletal maturation, in turn, was accelerated in response to early exposure to sex hormones, resulting in differences between bone age and chronological age (KANG et al., 2020). Estrogen is a sex hormone present and active throughout an individual's life (PATEL et al., 2018). Although it is primarily responsible for female characteristics, it also plays an important role in the neuroendocrine, vascular, skeletal, and immune systems of both sexes (ALMEIDA et al., 2017; CHRIST et al., 2018; PATEL et al., 2018). Estrogen's mechanism of action is mediated mainly by intracellular ERα receptor, encoded by the *ERS1* gene (HAMILTON et al., 2017).

Many single nucleotide polymorphisms (SNPs) in *ESR1* have been widely described with an impact on bone phenotype (AKILZHANOVA et al., 2014; ZHU et al., 2018; OMORI et al., 2020). SNPs are changes that occur naturally due to changes in DNA with more than one variation, its population frequency being greater than 1%. Although most SNPs are functionally neutral, a part of them may exert an allele-specific effect on the regulation of gene expression and/or on the function of the protein encoded by it, which leads to different characteristics between individuals (SHERRY et al., 1999; YE, 2000; NIELSEN, 2004). This change in phenotype can directly influence health promotion, disease prevention and therapy (AKILZHANOVA et al., 2014; ZHU et al., 2018; OMORI et al., 2020). Thus, the present study proposed to evaluate the association between delayed skeletal maturation (DSM) and a well-known SNP in *ESR1* in girls.

2 Material and Methods

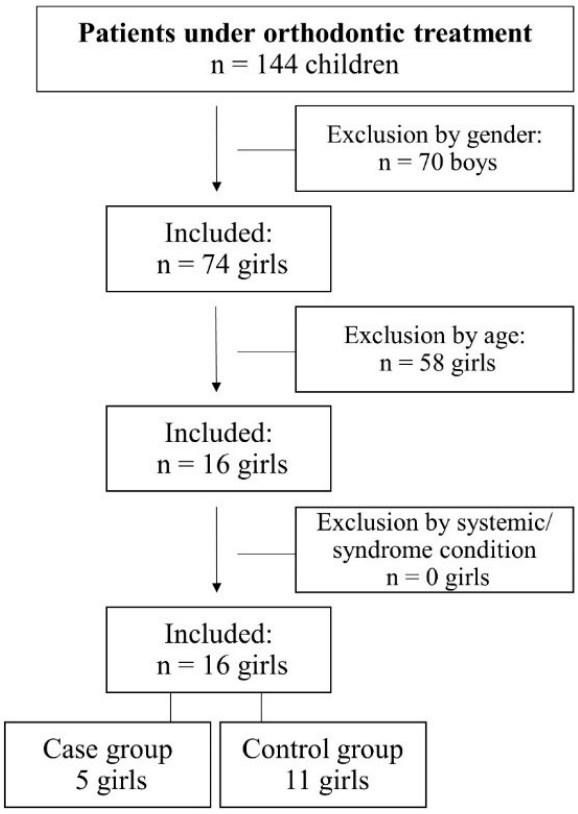
Ethical aspects

This study was approved by the Ethics Committee of the School of Dentistry of Ribeirão Preto, University of São Paulo, Brazil (CAAE: 35323314.7.0000.5419). All the parents/legal guardians and children who agreed to participate in this study signed the consent and assent form, respectively.

Sample Characterization

This cross-sectional study screened 144 orthodontic patients (70 boys and 74 girls) looking for girls with age ranging from 10 to 11 years who was undergoing to orthodontic treatment at School of Dentistry of Ribeirão Preto, University of São Paulo (FORP/USP) and had a complete pre orthodontic record with anamnesis and cephalometric radiograph. The sample was obtained for convenience. Children with systemic disease or syndrome were excluded. This screened is presented in the flow chart below (Figure 1).

Figure 1. Sample characterization



To evaluate skeletal maturation, the method proposed by Baccetti et al. (2005) was used. Girls who were at CS3 and CS4 stages were considered controls and girls who were at earlier stages were considered delayed in skeletal maturation (DSM). The evaluation was performed by a single examiner previously trained and calibrated.

Genotyping analysis

Genomic DNA for molecular analysis was extracted from saliva cells based on the method reported by Küchler et al. (2012). One intronic SNP was selected due to the fact that the minor allele frequency was higher than 20% and this SNP is well investigated in the literature in other conditions. The SNP PvuII (rs2234693) is located in the intron region of the *ESR*1. The SNP was genotyped by real-time polymerase chain reactions (Real-Time PCR) using TaqMan assay step OnePlus Real-Time PCR System (Applied Biosystems, Foster City, California, USA).

Statistical Analysis

Chi-square test and Fisher exact test was used to compare genotypes and alleles distribution, respectively, among the groups using GraphPad Prism 5.0 (Graph-Pad, San Diego, CA, USA). A significance level of 5% was adopted ($p<0.05$).

Results

A total of 16 girls were included in this study. Among them, 11 (68.75%) were classified as controls and 5 (31.25%) were classified as cases (DSM). Among the 11 control girls, 5 carried the homozygous genotype TT, 5 carried the heterozygous genotype CT and only 1

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carried the uncommon homozygous genotype CC. While in the DSM group, 2 carried the homozygous genotype TT, 2 carried the heterozygous genotype CT and 1 carried the uncommon homozygous genotype CC (*p=0.829*). Table 1 presents the description of each included girl.

Table 1. Description of each included girl

Patient #	Cervical stage	DSM	Age	rs2234693
1	CS4	No	11	CC
2	CS3	No	10	CT
3	CS2	Yes	11	TT
4	CS3	No	11	CT
5	CS1	Yes	11	TT
6	CS3	No	11	TT
7	CS4	No	10	TT
8	CS2	Yes	11	CT
9	CS3	No	11	TT
10	CS3	No	11	CT
11	CS2	Yes	11	CC
12	CS2	Yes	11	CT
13	CS3	No	11	TT
14	CS3	No	10	CT
15	CS3	No	11	TT
16	CS3	No	11	CT

Table 2 presents SNP PvuII (rs2234693) alleles distributions among DSM and control groups. The T (major) allele was detected in 31.8% of cases (n=7) and 40.0% of controls (n=4), while the C (minor) allele was detected in 68.2% of cases (n=15) and 60.0% of controls (n=6). The data show that there was no association between DSM and the SNP PvuII (rs2234693).

Table 2: Allele distribution between groups

Gene	***ESR1***	
SNPs	**Pvull (rs2234693)**	
Alleles	**T (Major)**	**C (Minor)**
DSM group	7 (31.8%)	15 (68.2%)
Control group	4 (40.0%)	6 (60.0%)
p-value*	*0.702*	
Odds Ratio *	1.43	
Confidence Interval 95%*	0.35 – 6.88	

Notes: Fisher exact test was performed. * in comparison with control group.

3 Discussion

In the present study, the null hypothesis was confirmed, in which the SNP in *ESR1* (PvuII, rs2234693) was not associated with DSM. Skeletal maturity can help in therapeutic management (BACCETTI et al., 2005; CERICATO et al., 2015; CARELLI et al., 2020) as well

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as, serving as a clinical parameter for complex childhood disorders (FORLINO and MARINI, 2016; ZEMEL, 2017; THOMPSON, 2021). DSM are increasingly frequent in clinical practice and widely associated with sexual dimorphism, ethnicity, environmental, systemic and genetic factors as mentioned above (TEVES et al., 2015; ERHAMZA et al., 2020; KANG et al., 2020; KLÜNDER et al., 2020; KÜCHLER et al., 2021; XU et al., 2021). However, in relation to genetic factors, gaps still remain in the specific related literature given the wide involvement of *genes* in the physiological and/or pathological process of bone development, maturation and growth (MAROZIK et al., 2019; MARAÑÓN-VÁSQUEZ et al., 2020; KÜCHLER et al., 2021).

The physiological relationship of estrogen and the development and maintenance of bone tissue is currently well elucidated. Nevertheless, conditions of deficiency or even overdose are also evidenced with permanent adverse effects on bone tissue (ALMEIDA et al., 2017). Given the molecular activity of estrogen mediated mainly by the *ESR1 gene*, it is plausible to suppose that SNPs in *ESR1* would be associated with bone development disorders, in special, DSM. *ESR1* receptor act in bone resorption (BINDER et al., 2009) and regulate the odonto/osteogenic differentiation (WANG et al., 2013). SNP in *ERS1* have previously been associated with bone development and maturation phenotypes (ZHU et al., 2018; SCALCO et al., 2019; FARIAS-CISNERO et al., 2020; OMORI et al., 2020). Therefore, it is possible that our result is a false-negative due to the small sample size.

The SNP PvuII (rs2234693) was selected due by their frequency in the population, location in the gene and previous demonstration of association with phenotypes related to bone. Previous studies demonstrate that SNP in PvuII (rs2234693) can influence bone mineral density in postmenopausal women (SHU et al., 2020) as well as significantly interfering with bone mineral density and effectiveness of hormone replacement therapy in women with Turner syndrome (SCALCO et al., 2019); syndrome characterized by the total or partial absence of the second sex chromosome in phenotypic women with a drastic decrease in estrogen production as the main clinical manifestations (GRAVHOLT et al., 2017). Furthermore, the SNP PvuII (rs2234693) have already been described in association with idiopathic scoliosis in young people (TANG et al., 2006) and with severe degree of curvature (INOUE et al., 2002). Nevertheless, the influence of the SNP PvuII (rs2234693) and the DSM becomes questionable, given the direct relationship between density, development, bone maturation and the processes of bone remodeling and formation.

However, our results showed no association between the SNP PvuII (rs2234693) and DSM. Such results can be justified since the expression of polymorphic genes can occur in different ways according to tissues and cells, since the expression profiles of estrogen receptors are also quite different (HAMILTON et al., 2017). It is also possible to hypothesize that other SNPs in *ESR1* could be involved in DSM in both, girls and boys. Furthermore, estrogen signaling may be due to an interaction both at the nuclear level and in the plasma membrane (HAMILTON et al., 2017). At the nuclear level, the main signaling pathway is described by the direct binding of estrogen to the regulatory elements of DNA (HAMILTON et al., 2017). It is also worth mention that the small sample size obtained by a convenience sample, compared to other studies (SCALCO et al., 2019; SHU et al., 2020), may also have influenced the results. It is suggested that further studies be carried out. In conclusion, the studied SNP known as PvuII (rs2234693) located in estrogen receptor encoding gene (*ESR1*) was not associated with DSM in girls.

4 Conclusion

DSM was not associated with the SNP PvuII (rs2234693) in *ESR1* in girls.

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