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ORIGINAL RESEARCH

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Association of third molar maturity with estrogen receptors-encoding genes

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Abstract

Background: Single nucleotide polymorphisms (SNPs) have been linked to variations in dental development, influencing traits such as eruption timing. This study examined the association between third molar maturity and SNPs in genes encoding estrogen receptors alpha (*ESR1*) and beta (*ESR2*). **Methods**: This was a cross-sectional study design using a convenience sample of genomic DNA and orthodontic records of Brazilian children. Third molar maturity was calculated in panoramic radiographs using a dental age estimation method previously developed. A delta value (dental age-chronological age, DA-CA) was calculated in order to show whether the patient has a tendency toward normal third molar maturity, delayed third molar maturity (negative values), or advanced third molar maturity (positive values). DNA isolated from cells in saliva was used for genotyping four SNPs: 2234693 and rs9340799 in *ESR1*; and rs1256049 and rs4986938 in *ESR2*. Statistical analysis was made with p-values < 0.05 indicating statistical significance. **Results**: Eighty-nine children were included in the study, 49 (55.1%) were girls and 40 (44.9%) were boys. There was no statistical difference between sexes and third molar maturity ($p > 0.05$). For the SNP rs9340799 in the maxilla, patients carrying AA genotype exhibited significantly delayed maxillary third molars maturity ($p < 0.05$). **Conclusions**: Third molar maturity is associated with the SNP rs9340799 in *ESR1*. Our study supports that third molars development time is genetically influenced.

Keywords

Dental development; Estrogen; Genes

1. Introduction

Dental development is a complex, lengthy and progressive process orchestrated by various local, systemic and genetic factors [1, 2]. This assertion underscores the variability of dental development among specific populations [3–7]. The dental maturation can be used as indicators of age in clinical practice [5, 6, 8], forensic odontology age estimation [9], and archaeological investigations [10]. In 1973, Demirjian *et al.* [11] introduced a method to estimate dental age based on dental development, scoring the tooth stages of calcification from A to H. This method is widely used for estimating dental age in children and adolescents due to its reliance on the developmental stages of permanent teeth. In 2017, Hofmann *et al.* [12] adapted Demirjian's original scoring method for assessing age based on the maturity of the third molar. The method focused on the formation stages of the third molars (also known as wisdom teeth), as they are the last teeth to develop and can provide information about age during late adolescence and early adulthood [12], different from Demirjian *et al.* [11] that exclude third molars from the analysis. Hofmann's method [12], like other dental age estimation techniques, considers the potential influence of gender differences; females may show slightly earlier dental development than males.

Sex hormones, such as estrogen, have an important role on differences observed between boys and girls development. Estrogen is a critical hormone for female and male development, it has an important role in children and teenagers development and is also expressed in oral and dental tissues. Scientific evidence from animal models demonstrated that alterations in Seric levels of estrogen can affect the maxilla and mandible growth and development [13], dental eruption [14], gene expression in the dental germ, and alterations in the dental structures morphology [14, 15]. The estrogen action mechanism is primarily mediated by the intracellular estrogen receptors alpha and beta (ERα and ERβ), which are encoding

by the genes estrogen receptor alpha (*ESR1*) and estrogen receptor beta (*ESR2*). They belong to the nuclear receptor family and act as transcription factors when they are induced by the ligands [16].

Single Nucleotide Polymorphisms (SNPs) are the most common type of genetic variation among individuals. They represent changes in a single nucleotide of the DNA. SNPs contribute to individual differences in traits, such as disease susceptibility and developmental phenotype variations. SNPs in *ESR1* and *ESR2* have been suggested to influence dental development/dental maturity [6]. Therefore, this study aims to evaluate if SNPs in *ESR1* and *ESR2* are associated with third molar maturity.

2. Materials and methods

2.1 Study design

The study adhered to the Strengthening the Reporting of Genetic Association (STREGA) guidelines [17].

2.2 Sample characterization

This study has a cross-sectional designed, which means that the data was collected at a single point in time and researchers gathered information from participants simultaneously, without following them over a period. The study used a convenience sample obtained from the orthodontic records of Brazilian children who sought orthodontic treatment between 2015 and 2019 at the Orthodontic Clinic of the School of Dentistry of Ribeirão Preto. The radiographic evaluation of these patients was performed between 2021 and 2022 using the pre-treatment radiograph.

All orthodontics records of the patients of both sexes, aged 7 to 16 years and who had correctly completed records was included. Patients with a history of previous orthodontic treatment, patients with underlying syndromes, congenital abnormalities, craniofacial alterations, those with history of dento-facial trauma, bilateral dental agenesis or bilateral missing teeth and patients who presented third molars completely formed were excluded [6].

2.3 Third molars maturity evaluation

Evaluation of third molars maturity was performed using panoramic radiographs using Hofmann *et al.* [12]. Delta values (dental age-chronological age (DA-CA)) were calculated to achieve the maturity scores. The differences between the dental age and chronological age was performed to achieve the maxillary and mandibular maturity scores. The scores demonstrate whether the patient has a tendency towards normal dental maturity, delayed dental maturity (negative values) or advanced dental maturity (positive values) [5, 6]. Two examiners were calibrated and trained by a senior pediatric dentist. Weighted Cohen's Kappa test was performed and the intra-observer reliability (ranging from 0.82 to 1.00) and the inter-observer reliability (ranging from 0.79 to 1.00) were calculated and with presented good values.

2.4 Genotyping analysis

DNA was isolated from buccal cells from saliva based on the method previously reported [18]. SNPs with the minor allele frequency higher than 0.2 were screened. Four SNPs were selected based on previous studies results [6, 19, 20]. The selected SNPs rs2234693 and rs9340799 in *ESR1*; and rs1256049 and rs4986938 in *ESR2* were investigated. The laboratorial experiment was performed blinded by the patient's condition. The genotyping was made by Real-Time PCR (real-time polymerase chain reactions)—step OnePlus Real-Time PCR System, using TaqMan assay (Applied Biosystems, Foster City, CA, USA).

2.5 Statistical analysis

The third molar maturity (delta DA-CA) was assessed as a continuous variable. Chi-square was utilized to establish the Hardy-Weinberg equilibrium. Shapiro-Wilk test was used to test the data normality. One-way analysis of variance (ANOVA) and *t*-test were used to compare the mean deltas age according to the genotypes. Tukey's post-test was used for multiple comparisons. The statistical analysis was made using the software GraphPad Prism 9 (GraphPad, San Diego, CA, USA). The alpha used for all analyses of this study was 5% ($p < 0.05$).

3. Results

Of the initial 115 patients screened, 89 were included in this study. Forty-nine (55.1%) patients were females, and 40 (44.9%) patients were males. The Hofmann *et al.* [12] method overestimated the patients' age by 1.1 years. Sample details are presented in Table 1 (Ref. [12]).

Fig. 1 demonstrates the comparison of Delta (DA-CA) between sexes. The median delta for males was 13.37, while the median delta for females was 13.54 (Standard deviation = 1.56). There was no statistically significant difference ($p = 0.77$).

All the studied SNPs were in Hardy-Weinberg equilibrium ($p > 0.05$). The Delta DA-CA were compared among genotypes of each studied SNP (Table 2). In the dominant model for rs9340799 in the maxilla, patients with AA genotype exhibited significantly more delayed dental age ($p = 0.026$).

Table 3 demonstrates the association between SNPs in *ESR1* and *ESR2* in the dominant and recessive models. In the dominant model for the SNP rs9340799, individuals with the AA genotype exhibited a greater delay in the of maxillary third molars maturity compared to those with AG or GG genotypes ($p = 0.033$).

4. Discussion

Although genes *involved* in developmental dental anomalies have been widely explored in the past 2 decades, the study of genes *involved* in dental maturity has just emerged recently [6, 20–22]. Understanding the factors influencing dental maturity is important because dental development could be utilized to estimate chronological age, which is extensively used in forensic practices and archaeological studies. Knowing the

TABLE 1. Characteristics of the included sample.

Characteristic		n (%)
Gender, n (%)		
	Girls	49 (55.1%)
	Boys	40 (44.9%)
Chronological age in years		
Mean (standard deviation)		12.9 (2.39)
Third molars maturity according to the Hofmann *et al.* [12] (2017)		
Mean (standard deviation)		
	Total	13.7 (1.80)
	Maxilla	13.6 (1.75)
	Mandible	14.3 (2.30)
Delta DA-CA (yr)		
Mean (standard deviation)		
	Total	1.1 (1.67)
	Maxilla	0.89 (1.75)
	Mandible	1.4 (1.97)
Genotype, n (%)		
rs2234693		
	CC	13 (15.48)
	CT	37 (44.05)
	TT	34 (40.47)
rs9340799		
	AA	46 (53.49)
	AG	33 (38.37)
	GG	7 (8.14)
rs1256049		
	CC	83 (93.26)
	CT	6 (6.74)
	TT	0 (0)
rs4986938		
	CC	26 (31.71)
	CT	42 (51.22)
	TT	14 (17.07)

DA-CA: dental age-chronological age.

role of estrogen in the growth and development of both female and male organisms [23–25], including oral tissues [13–15], this study aimed to investigate the possible association between SNPs in *ESR1* and *ESR2* with third molar maturity using a method established for age estimation using third development. Interestingly, our results show that the rs9340799 SNP in *ESR1* influences third molar maturity.

To facilitate and expand the applicability of the method proposed by Demirjian *et al.* [11], Hofmann *et al.* [12] developed a dental age assessment system, also based on odontogenesis stages, specifically for third molars. The Hofmann *et al.* [12] method can be applied to children and young adults up to 24 years old. Besides topographical differences in the third molars maturity, the study also asserts the method's sensitivity to gender distinction. Particularly, the 15 to 16 age group showed significantly higher mineralization stages compared

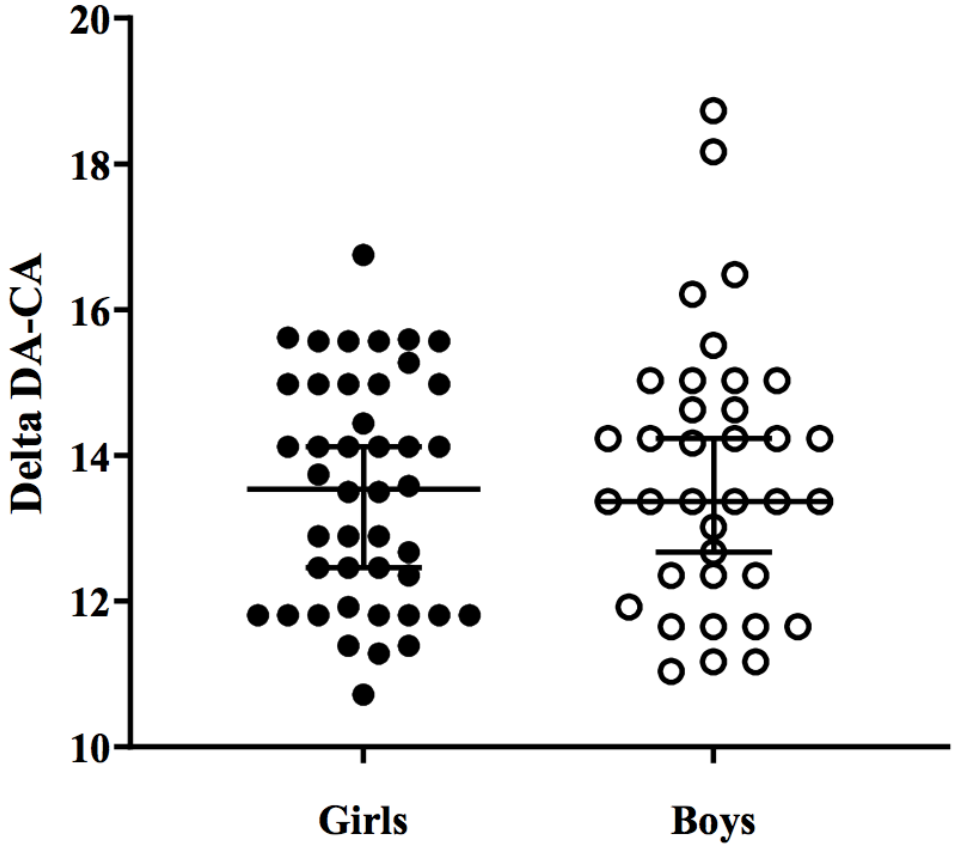

FIGURE 1. Third molars maturity Delta (DA-CA) distribution between sexes. Black dots mean girls and white dots means boys. DA-CA: dental age-chronological age.

TABLE 2. Comparison of the mean deltas according to the genotype.

Gene	SNP	Delta	Genotype (mean delta (SD))			*p*-values
			CC (n = 13)	CT (n = 37)	TT (n = 34)	CC × CT × TT*
ESR1						
		Total	0.63 (1.19)	1.08 (1.53)	1.02 (2.01)	0.677
	rs2234693	Maxilla	0.56 (1.20)	1.10 (1.77)	0.74 (1.97)	0.654
		Mandible	0.61 (1.61)	1.49 (1.49)	1.52 (2.10)	0.246
			AA (n = 46)	AG (n = 33)	GG (n = 7)	AA × AG × GG*
		Total	0.88 (1.82)	1.48 (1.54)	0.31 (1.25)	0.175
	rs9340799	Maxilla	0.54 (1.88)	1.59 (1.54)	0.30 (1.23)	**0.026**[#]
		Mandible	1.56 (2.14)	1.62 (1.69)	0.14 (1.67)	0.220
ESR2						
			CC (n = 83)	CT (n = 6)	TT	CC × TT**
		Total	1.13 (1.67)	0.08 (1.54)	-	0.143
	rs1256049	Maxilla	1.02 (1.73)	−0.34 (1.59)	-	0.072
		Mandible	1.47 (1.72)	0.75 (1.66)		0.416
			CC (n = 26)	CT (n = 42)	TT (n = 14)	CC × CT × TT*
		Total	1.11 (1.15)	0.77 (2.05)	1.18 (1.09)	0.633
	rs4986938	Maxilla	1.00 (1.31)	0.61 (2.11)	0.97 (1.05)	0.592
		Mandible	1.42 (1.22)	1.21 (2.15)	1.26 (1.48)	0.961

*Note: Bold means statistically significant value. *ANOVA one-way with Tukey's post-test was used; **T-test was used; [#]difference between AA and AG. SNP: Single nucleotide polymorphisms; ESR: encoding estrogen receptors; SD: standard deviation.*

TABLE 3. Dominant and recessive model analysis.

Gene	SNP	Delta	Dominant model (mean delta (SD))			Recessive model (mean delta (SD))		
			TT	CT + CC	*p*-value	CC	CT + TT	*p*-value
ESR1								
		Total	1.02 (2.01)	1.03 (1.47)	0.982	0.63 (1.19)	1.09 (1.77)	0.410
	rs223469	Maxilla	0.74 (1.97)	0.91 (1.65)	0.659	0.56 (1.20)	0.90 (1.87)	0.547
		Mandible	1.52 (2.10)	1.20 (1.62)	0.230	0.61 (1.61)	1.56 (2.04)	0.130
			AA	AG + GG		GG	AG + AA	
		Total	0.88 (1.82)	1.28 (1.55)	0.291	0.31 (1.25)	1.12 (1.73)	0.262
	rs9340799	Maxilla	0.54 (1.88)	1.38 (1.56)	**0.033**	0.30 (1.23)	0.96 (1.81)	0.389
		Mandible	1.56 (2.14)	1.19 (1.84)	0.403	0.14 (1.67)	1.50 (2.00)	0.086
			CC	CT + TT		TT	CT + CC	
ESR2								
		Total	1.11 (1.15)	0.87 (1.86)	0.571	1.18 (1.09)	0.90 (1.76)	0.577
	rs4986938	Maxilla	1.00 (1.31)	0.68 (1.89)	0.452	0.97 (1.05)	0.75 (1.83)	0.665
		Mandible	1.42 (1.22)	1.29 (2.27)	0.785	1.26 (1.48)	1.35 (2.09)	0.877

Note: Bold means statistically significant value. T-test was used. SNP: Single nucleotide polymorphisms; ESR: encoding estrogen receptors; SD: standard deviation.

to chronological age in boys. Additionally, a difference was noted in the dental age of girls; boys demonstrated a more accelerated dental age than girls. This finding was justified by the likely faster pubertal development in girls compared to boys [12]. Therefore, in our present study, the method proposed by Hofmann *et al.* [12] was used to investigate variation in the third molar maturity. The method by Hofmann *et al.* [12], unlike the method by Demirjian *et al.* [11], focuses exclusively on third molars in the analysis. In our study, the method by Hofmann *et al.* [12] was therefore used to identify third molars with delayed or advanced stages of development.

Although several genes might be *involved* in dental development, we chose estrogen receptors due to its important role in the development and maturation of boys and girls, specially in adolescence, which is the age that third molars are actively developing. Although discrepancies in the development of boys and girls related to the interaction of sex hormones are widely described [26, 27], it is important to emphasize that variations were not observed in our study according to the sex. But it is important to emphasize that the method already takes the gender into consideration to calculate the dental age. Estrogen is strongly asserted to accelerate the development of girls earlier compared to boys [14]. Estrogen is a sex steroid hormone active during the subjects' life [25]. Though estrogen is mostly responsible for characteristics in females, it also plays a crucial role in the vascular, neuroendocrine, bone/skeletal, and immunological systems of both genders [23–25]. Our study did not identify significant differences between boys and girls, raising a question about the influence of estrogen pathways in dental development. However, previous studies support the role of estrogen and estrogen deficiency in dental development and tooth eruption process. In a study conducted on a murine model with continuously growing teeth (incisors), a significant reduction in eruption rate and dental development was observed when there was estrogen deficiency during the pubertal period [14]. Estrogen receptors are also widely described in dentin complex cells; ERα has been extensively described as playing a role in the proliferation and differentiation of ameloblasts [15]. Furthermore, in the murine model study, a significant difference was observed between estrogen-deficient and non-deficient groups, along with the ERβ's overexpression in the dental germ [14]. These findings suggest the influence of estrogen and its receptors on the development and consequent delay/acceleration of dental age and its potential outcomes.

SNPs and their associations help to understand mechanisms related to species evolution, genetic diseases, heredity, drug response and also variations among patients' development. SNPs are natural alterations occurring due to alterations in DNA material with one or more variations. Its frequency in the population is higher than 1% [28]. While most SNPs are functionally neutral, some SNPs could have an allele-specific effect on gene expression regulation, and (or) an effect on the function of the encoded protein, leading to different characteristics among individuals.

Briefly, our results demonstrated that in the dominant model for the rs9340799 SNPs in *ESR1*, patients with at least one G allele exhibited significantly more advanced third molar maturity than patients with one or two A alleles in the maxilla; in the recessive model, patients with G alleles showed accelerated third molar maturity compared to patients with A alleles in the mandible. Based on the present study's results, we could suggest that delayed third molar maturity might be *involved* with developmental alterations regulated by genetic components. Recent studies have reported that SNPs in estrogen receptors are *involved* in dental alterations. SNPs in rs9340799, rs2234693, rs1256049 and rs4986938 in *ESR1* were associated with changes in tooth size in both the maxilla and mandible [19, 29]; and the SNPs rs12154178 in *ESR1* were associated with various enamel defects [30, 31]. Furthermore,

the presence of rs1215417 SNPs in *ESR1*, in the dominant model, indicated a predisposition to enamel defects in patients with A alleles [31].

Our study has some important limitations that should be discussed. The convenience sampling may have limited the applicability of the findings to broader populations, limiting the generalizability of our findings. Additionally, the study's relatively small cohort could have reduced the statistical power to detect associations, especially for less frequent genotypes, leading to false negative results. Other important aspect that could be a limitation is the fact that only children that performed the radiograph exams before 2020 were included. There is some evidence that children's growth and maturity may have been affected by the COVID-19 pandemic due to various factors, including changes in lifestyle, nutrition, stress levels, and healthcare access. During COVID-19 pandemic, changes in children's physical and psychological health were recorded, and an interesting finding was an increase in precocious puberty cases and in pubertal progression rate [32], which could have affect third molar maturation.

Thus, our results show that genetics related to sex hormones might influence dental maturity, specifically third molars. However, further investigative studies are necessary to explore the functional impact of the rs9340799, investigating how this SNP affects estrogen receptor expression and function in dental tissues. Future studies should be performed including larger and more diverse populations to validate these findings and assess genetic influences across different ethnic groups.

5. Conclusions

The SNP rs9340799 in *ESR1* is associated with maxillary third molar maturity. Patients carrying AA genotype exhibited significantly delayed third molar maturity. In summary, our study supports that third molars development time is genetically influenced, and genes *involved* in sex hormones regulation may play an important role in the differences in third molars maturity observed among patients.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

ECK and CPL—conceived the idea; funding support. FBF, CK, ECK and CPL—designed the study. IRM, LAS and PFC—collected the data. IRM and GFS—analyzed the data. IRM, MANM, MAHMO, DCCB, LAA and ECK—writing–original draft preparation. IRM, ECK and CPL—writing–review and editing. All authors interpreted the data and revised the final version of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Human Ethics Committee of the of the School of Dentistry of Ribeirão Preto, University of São Paulo (CAAE #3.150.551). Informed consent or assent was obtained from all participants and/or their legal guardians.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. Livia Azeredo Antunes is serving as one of the Editorial Board members of this journal. We declare that Livia Azeredo Antunes had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to GS.

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