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ORIGINAL ARTICLE

Single nucleotide polymorphism rs4284505 in microRNA17 and risk of dental fluorosis

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ABSTRACT

Objectives: The aim of this study is to evaluate the association between the single nucleotide polymorphism (SNP) rs4284505 within the gene that codifies microRNA17 (miRNA17) and dental fluorosis (DF) in a group of children.

Methods: Children living in a city with fluoridation of public water supplies were included. DF was assessed in erupted permanent teeth by Dean's modified index. The miR-SNP rs4284505 was selected in miRNA17 and genotyping was carried out by real-time PCR. Genotype and allelic distributions between DF and control, and between DF phenotypes (mild, moderate and severe) and control were analysed.

Results: Among a total of 527 children enrolled for the study, 383 were DF free and 144 presented DF. In the dominant model analysis (AA + AG *vs.* GG) the miR-SNP rs4284505 was associated with moderate DF, with carriers of the GG genotype having an increased risk of more than two times for DF ($p = 0.031$; Odds Ratio = 2.26, Confidence Interval 95%= 1.04-4.73). Allelic distribution showed borderline statistical significance for moderate DF with the carriers of G allele having an increased risk for DF ($p = .050$; Odds Ratio = 1.75, Confidence Interval 95%= 1.00–3.12).

Conclusion: The miR-SNP rs4284505 in miRNA17 was associated with an increased risk of DF.

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Introduction

Fluoride prevents dental caries development by having a significant effect on dental tissue and plaque formation before and after teeth eruption [1]; however, excess fluoride intake during enamel development may lead to dental fluorosis (DF) depending on time and duration of intake [2]. DF leads to permanent, white or yellow-brownish discolouring of permanent teeth and enamel hypomineralization phenotype. DF was described by Dean who proposed the Dean's Index, which ranges from 0 (normal) to 5 (severe) [3] to classify this condition. The severity of DF has been related to the amount of fluoride intake during enamel formation.

Despite, the pathogenesis of DF is intimately linked to the exposure to fluoride, which is considered the main environmental factor. Some studies have shown that a significant association between DF susceptibility and various single nucleotide polymorphisms (SNP) exist in genes expressed during enamel development [4–10], including the SNPs rs1256049, rs2234693 [5] and rs12154178 [11] in Oestrogen Receptor Alpha (ERα). ERα is codified by Oestrogen Receptor-1 (*ESR1)* gene, which is located on the sixth (6q25) chromosome [12]. ERα is a nuclear receptor that mediates the effect of oestrogen on cells, however, many other aspects are involved in the oestrogen function, including genomic and non-genomic factors, regulation of gene transcription and control of messenger ribonucleic acid (mRNA) stability and translation efficiency [13].

MicroRNAs (miRNAs) can regulate gene activity in a post-transcriptional manner. They are small non-coding RNAs referred to as 'micromanagers of gene expression' [14]. miRNAs are critical structures for cell and organism growth and development [15] and have been offering a new dimension of research towards understanding multifactorial diseases, such as DF. In this study, we explored the association between SNP in miRNA (miR-SNP) and DF, through the identification of a common miR-SNP in a miRNA that potentially binds in *ESR1*. Therefore, we evaluate the association between the miR-SNP rs4284505 in *miRNA17*and susceptibility to DF in children.

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Material and methods

The study was reviewed and approved by the local Ethical Committee from Pontifical Catholic University of Paraná (register number 487) according to Resolution 196/96 of the Health National Council. Legal guardians and children were informed with details about the research and both signed an Informed Consent/assent form before any intervention.

Studied population

For this cross-sectional study, children aged between 10 and 14 years old (mean ± SD = 12.0 ± 0.47) were recruited at public and private schools of Curitiba, Paraná, Brazil, a city with fluoridation of public water supplies. The publication of the government control of the public water measurements reported that in Curitiba the fluoride level ranged from 0.72 to 0.77 parts per million of fluoride (ppm F), in accordance with Paraná State regulation for water fluoridation and the prevalence of DF in this population was previously reported as 27.3% [11]. Children were not included if, using orthodontic appliances, syndromic or presented any other enamel alteration.

Determination of the dental fluorosis phenotype

Dental examinations were conducted in each child following international standards established by the World Health Organization [16] by trained examiners. To assess the consistency of each examiner (inter and intra-examiner reproducibility), duplicate examinations were conducted on 10% of the sample and the Kappa test was used to measure reliability and the value of 0.93, which indicated acceptable reproducibility of the data.

Children were seated in a chair, near a place with the presence of natural lighting and the examiner used a probe and dental mirror. DF (mild, moderate or severe) was assessed using the Dean's index modified [3] during the dental examination and a score was registered. The subjects were divided into groups according to the presence or absence of DF and according to the DF severity (mild, moderate and severe).

miR-SNP selection and laboratory analysis

Based on the previous studies associating *ESR1* with DF [5,11], a search was performed in order to identify miRNAs that potentially bind to the mRNA of the *ESR1*. The search and selection of the miRNA was performed through the miRanda software [17], which was used to scan miRNAs–*ESR1* mRNA interactions. This software uses a target prediction algorithm and identified the miRNA17 as one of the miRNAs that targets the mRNA of *ESR1*, with a mirSVR score of −0.36. Subsequently, the SNPs in the miRNAs were identified through the Genome Browser and the miR-SNP rs4284505 (A>G) in miRNA17 was selected due to the fact that it was the only SNP with a minor allele frequency higher than 10%.

Genomic DNA extracted from saliva samples was used for the genotyping analysis. The saliva samples collection and DNA extraction from buccal cells are described in Aidar et al. [18]. Blinded genotyping was performed by real-time polymerase chain reaction (PCR) using the TaqMan assay in the real-time PCR system ABI PRISM® 7500HT (Foster City, CA, USA). The probes and the master mix are from Applied Biosystems (Foster City, CA, USA).

Table 1. Phenotypes of subjects.

Gender, *N* (%)	
Male	254(48.2)
Female	273(51.8)
Phenotypes, *N* (%)	
DF free	383(72.7)
Mild DF	100(19.0)
Moderate DF	29(5.5)
Severe DF	15(2.8)

Statistical analysis

Data were analysed using Prism Graphpad 8. Chi-square test or Fisher exact test were used to compare alleles and genotype distributions between groups in the co-dominant and dominant model. The analysis was performed according to the presence or absence of the phenotype (DF *vs.* Control) and according to the severity of the phenotype (mild DF, moderate DF or severe DF *vs.* Control). All tests were performed with an established alpha of 0.05. Hardy–Weinberg equilibrium was evaluated using the chi-square test within a selected polymorphism in each population set.

Results

Five hundred twenty-seven children were included, among them, 144 presented DF and 383 were DF-free (control). The sample characteristics are presented in Table 1. There were no significant differences in sex distributions between the DF and control groups ($p = .368$).

The observed genotypes of the miR-SNP rs4284505 were in Hardy-Weinberg equilibrium (χ^2=0.01). Genotype distributions in the co-dominant and dominant models and allele distribution between DF and control groups are presented in Table 2. In the dominant model analysis (AA + AG *vs.* GG) the miR-SNP rs4284505 was associated with moderate DF, with carriers of the GG genotype having an increased risk of more than two times for DF ($p = .031$; Odds Ratio = 2.26, Confidence Interval 95%= 1.04–4.73). Allelic distribution showed borderline statistical significance for moderate DF with the carriers of G allele having an increased risk for DF ($p = .050$; Odds Ratio = 1.75, Confidence Interval 95%= 1.00–3.12). The other comparisons were not statistically significantly different among groups ($p > .05$).

Discussion

The emerging picture that small RNA molecules can regulate gene expressions was an important advance in molecular biology. In fact, miRNAs emerged as posttranscriptional regulators, involved in many biological processes including, cell cycle, differentiation, proliferation, migration, secretion, and

Table 2. Genotype (in the co-dominant and dominant models) and allelic distribution between dental fluorosis (DF) and control groups.

	Control	Total DF			Mild DF			Moderate DF			Severe DF		
	N (%)	N (%)	p-value	OR (95%CI)	N (%)	p-value	OR (95%CI)	N (%)	p-value	OR (95%CI)	N (%)	p-value	OR (95%CI)
Genotype distribution in co-dominant model													
AA	63(16.4)	27(14.7)	Reference		22(22.0)	Reference		3(10.3)	Reference		2(13.4)	Reference	
AG	197(51.4)	59(40.9)	0.199	0.69 (0.40–1.22)	40(40.0)	0.070	0.58(0.32–1.04)	11(37.9)	0.998	1.17(0.34–4.03)	8(53.3)	0.999	1.27(0.30–6.11)
GG	123(32.2)	58(40.3)	0.91	0.97 (0.55–1.68)	38(38.0)	0.782	1.01(0.64–1.87)	15(51.7)	0.188	2.56(0.73–8.56)	5(33.3)	0.999	1.28(0.26–6.57
Genotype distribution dominant model													
AA + AG	260(67.8)	86(59.7)	Reference		62(62.0)	Reference		14(48.7)	Reference		10(66.4)	Reference	
GG	123(32.2)	58(40.3)	0.072	1.42 (0.95–2.13)	38(38.0)	0.266	1.29(0.82–2.03)	15(51.7)	0.031*	2.26(1.04–4.73)	5(33.3)	0.999	1.05(0.39–2.88)
Allelic distribution													
A	323(42.1)	113(39.2)	Reference		84(42.0)	Reference		17(29.3)	Reference		12(40.0)	Reference	
G	443(57.9)	175(60.8)	0.389	1.12 (0.85–1.48)	116(58.0)	0.966	1.00(0.76–1.38)	41(70.7)	0.050*	1.75(1.00–3.12)	18(60.0)	0.852	1.09(0.54–2.26)

OR: Odds Ratio; CI: Confidence interval. All comparisons were performed with the control group.
*Indicates statistical significance difference.

apoptosis, essential for the development of various organs, including the enamel [19]. Additionally, distinct expression patterns of miRNAs in subjects suffering from fluorosis were recently observed [20,21]. However, to the best of our knowledge, this is the first study to investigate miRNA-SNP in the aetiology of DF. miRNAs are non-coding single-chain RNAs required for gene sequencing following translation and transcription [22].

Previous studies have demonstrated that several SNPs in genes expressed during amelogenesis are associated with enamel conditions [8,10,23–25] these SNPs could be responsible for changes of tooth enamel leading to different phenotypes. In the present study, we decide to evaluate a miR-SNP, which may play functional roles through affecting the transcription of the target gene, altering pri-miRNA/pre-miRNA processing, or exerting effects on miRNA-mRNA interactions [26]. The target gene selected here was *ESR1*, which was previously associated with DF in a Chinese population [5] and in our population [11], both populations have a high prevalence of DF. Curitiba, the studied city here, was one of the first Brazilian cities to promote the fluoridation of water supply [27]. Historically, Curitiba has a higher prevalence of fluorosis than other Brazilian cities [28], which allowed the genetic studies to understand SNPs associated with individual risk of DF. Following a similar pattern, Mexican [10,29] and Chinese [5–7] populations have also been extensively studied due to their high prevalence of DF and allowed the identification of many molecular mechanisms involved in DF.

The miRNA17 was identified as potentially binds *ESR1* through a bioinformatic prediction. It is possible that miRNA17 affects the final expression of this gene during amelogenesis. Sehic et al. [19] identified many miRNAs during mouse incisor development, although the authors used microarrays containing a higher number of probes, miRNA17 was not in this panel. However, it is possible that miRNA17 is expressed in mouse and human enamel during amelogenesis. We were able to observe that the G allele, as well as the GG genotype in the dominant model, were associated with an increased risk of moderate DF phenotype, therefore, this provides the theoretical basis which suggests that the miR-SNP rs4284505 are involved only in severe phenotypes.

Although we were not able to observe a statistical difference for severe DF, it is important to emphasise that the sample size of severe DF cases was only 15 and it is possible that limited statistical power explains the failure of association. However, it is also possible that the association observed for moderate DF cases is a type one error. Therefore, we suggested that future studies should replicate our research with a larger sample of severe cases to confirm the results.

It is known that oestrogen has a significant impact on osteoclast activity stimulation as well as on calcium phosphate deposition [30]. Likewise, oestrogen can play a role in the enamel mineralisation process [31,32]. Therefore, it is possible that miR-SNP rs4284505 affects *ESR1* and consequently promotes alterations in the oestrogen pathway affecting the capacity of the enamel mineralise in the presence of fluoride leading some individuals with a higher risk of DF. It is important to mention that even susceptible individuals will develop DF only if they were exposed to fluoride. In conclusion, the miR-SNP rs4284505 within the gene that codifies miRNA17 was associated with an increased risk of moderate DF.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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