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ORIGINAL ARTICLE

Genetic Polymorphisms in the *DR2D*, *ANKK1*, *COMT*, *5HTT* Genes and Dental Fluorosis: Is There Any Interplay?

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ABSTRACT

Objective: To investigate the association between genetic polymorphisms in *DRD2*, *ANKK1*, *COMT*, and *5HTT* genes and dental fluorosis (DF). **Material and Methods:** 256 adolescents were examined, and dental fluorosis was diagnosed using the modified Dean index. Genomic DNA was collected, and seven single nucleotide polymorphisms (SNPs), two in the *DRD2* (rs6275 and rs6276), one in the *ANKK1* (rs1800497), two in the *COMT* (rs6269 and rs4818), and two in the *5HTT* (rs3813034 and rs1042173) were selected. Allele, haplotype, and diplotype frequency comparisons were performed. Multifactorial Dimensionality Reduction investigated SNP-SNP interactions. Allele and haplotype frequency comparisons were performed by PLINK version 1.06. The Fisher exact test performed genotypic analysis, and Poisson Regression was adjusted by gender. **Results:** In the allelic frequency analysis, rs6275 was associated with DF ($p=0.040$), and rs6276 was borderline ($p=0.07$), being confirmed in the haplotype ($p<0.05$) and diplotype ($p=0.007$) analysis. **Conclusion:** The data suggest that *DRD2*, *ANKK1*, *COMT*, and *5HTT* genes synergistically interact to increase the dental fluorosis risk.

Keywords: Fluorosis, Dental; Polymorphism, Genetic; Genetics.

Introduction

Dental fluorosis (DF) is a multifactorial condition resulting from continuous exposure of mineralized tissues, especially tooth enamel, to large amounts of fluoride during its formation, leading to hypomineralized, functionally and aesthetically compromised enamel [1]. However, studies on DF led to a paradigm shift when an inverse association between this condition and dental caries was discovered [2]. Despite the evident reduction in the rate of dental caries after water fluoridation, fluorosis in various forms has risen. Fluorosis is considered a systemic toxicity to fluoride, classified as skeletal, non-skeletal, and dental [3].

Although the molecular mechanisms responsible for fluorosis are still unclear, it is well-accepted that fluoride has an extremely high affinity for mineralized tissues. At elevated concentrations, fluoride disturbs the mineralization process of bones and teeth [4]. Skeletal fluorosis affects bones and joints and is a more severe problem than DF as it can cause permanent deformity [5]. The non-skeletal form of fluorosis can affect all soft tissues and organs of the body, causing cognitive and neurological alterations [6,7] and nephrological, cardiac, and thyroid disorders [5].

DF is a widespread condition among children and young people and is caused by fluoride consumption at a slightly higher level than optimal [8]. Clinically, the affected teeth could appear to have white spots, yellow to brownish discoloration, and/or pitting or mottling of enamel, depending on severity, which could be classified as very mild, mild, moderate, and severe [9]. Enamel mineralization is highly sensitive to free fluoride ions, so when fluoride gets incorporated into the enamel crystals, fluoride forms fluorapatite, which affects the subsequent mineralization by reducing the solubility of the mineral, thus modulating the ionic composition of the fluid surrounding the mineral [10]. Despite DF being closely linked to fluoride exposure, there is evidence that inter-individual genetic variations may contribute to the susceptibility or resistance to DF [11]. It is hypothesized that DF could result from a complex interplay between miscellaneous genes involved in bone/enamel formation exposed to environmental changes, including dietary patterns of fluoride intake and other nutrients [12-14].

More recently, it was found that Single Nucleotide Polymorphisms (SNPs) in genes that regulate the endogenous mechanisms of pain, stress, and anxiety, like Dopamine Receptors (*DRD2*), Ankyrin Repeat and Kinase Domain Containing 1 (*ANKK1*), Catechol-O-Methyl Transferase (*COMT*), and Serotonin Transporter gene (*5HTT*) are related with fluoride intake [15], bone calcification [16,17] and amelogenesis [18]. Interestingly, despite studies linking these genes with fluoride intake, bone calcification, and amelogenesis, there are still no studies on their correlation with DF. At this point, a central question emerges: Can genetic polymorphisms in genes associated with anxiety, stress, or emotional disorders, i.e., unusual candidate genes for DF, be associated with DF? To answer this question, this case-control study aims to evaluate if SNPs in *DRD2* (rs6275, rs6276), *ANKK1* (rs1800497), *COMT* (rs6269, rs4818), and *5HTT* (rs38133034 and rs1042173) genes alone or through synergistic interactions are associated with DF in a population of Brazilian adolescents.

Material and Methods

Study Design and Ethical Clearance

This case-control study was approved by the Health Sciences Research Ethics Committee of the Federal University of Paraná (Process no. 2.006.086). Before the data collection, adolescents and their parents/guardians received written information about the study and signed the informed consent form.

Sample

To estimate the sample calculation, we use data from the Curitiba Department of Education and Paraná State Secretary of Education regarding adolescents between 10 to 14 years old who are attending public and private schools from Curitiba, and the representative sample should be around 800 adolescents [19].

For DNA genomic analysis, the sample calculation was based on DF prevalence previously described in the 2010 edition of the SBBrasil project for the South region of Brazil (details on the project are available at http://www.sbbrasil2010.org) [20], so we used a random number generator website to randomly select 256 adolescents that were distributed in DF-affected and DF-unaffected groups. This work was conducted according to Strengthening the Reporting of Genetic Association Studies (STREGA) [21] and the Declaration of Helsinki guidelines.

Determination of DF Phenotype

Calibrated examiners conducted the dental examinations, and all were calibrated for DF clinical examination by an experienced specialist. Clinical examination was performed with the adolescents seated in a dental chair, and the examiner used a probe and a dental mirror. Thirty individuals were examined twice at two distinct points in time. The inter and intra-examiner coefficient of agreement (kappa) was higher than 0.80, indicating good data reproducibility.

DF was diagnosed using the modified Dean index [22], and the scores were registered in an appropriate formulary. This index allows the classification of DF into 3 degrees: mild (very mild and mild), moderate, and severe. The current analysis used data from early-erupting permanent teeth, and for the mixed dentition, only erupted permanent teeth were evaluated. All questionable cases were excluded. Adolescents with systemic health conditions, using orthodontic appliances, or syndromic were excluded from this study.

DNA Samples and Genotyping

After performing the clinical examination, buccal cells were individually collected in a private environment so as not to cause any embarrassment to the adolescent. Each individual was given two drops of mouthwash with 5 mL of autoclaved 3% glucose solution for 1 minute, with an interval of 5 minutes each. After each mouthwash, oral mucosa was scraped with a wooden spatula [23]. The solutions were deposited in collecting tubes and posteriorly stored in a recipient containing ice until they arrived at the molecular laboratory, where genomic DNA was extracted according to the previously established protocol [24]. Each tube was centrifuged at 2000 rpm for 10 min to separate the pellet of cells that detached from the oral mucosa of the supernatant (saliva + 3% glucose). This cell pellet was transferred to a buffer solution (10 mM Tris-HCl, 0.1 M EDTA, 0.5% SDS - pH 8.0, Sigma-Aldrich Corp., Saint Louis, MO, USA) and stored in a freezer at –20°C until used. In the following days, DNA was extracted by adding 10 µL of proteinase K (20 mg/mL) to the solution, left for 08 hours at 65oC. The DNA was purified by adding 10 M ammonium acetate, precipitated with isopropanol and ethanol, then resuspended in 50 µl of 10 mM Tris (pH 7.8) and 1 mM EDTA. After resuspension of the DNA in Tris-EDTA buffer, the DNA in each sample was quantified in a spectrophotometer (Biophotometer, Eppendorf AG, Hamburg, Germany).

The SNPs were selected by consulting the website of the International HapMap Project (www.hapmap.org), a union of several countries, to develop a map with patterns of DNA sequence variations. In this database, information about the SNPs in the genes of interest is available. Two SNPs in *the DRD2 gene (rs6275 and rs6276), one in the ANKK1 gene (rs1800497), two in the COMT gene (rs6269 and rs4818), and two in the 5HTT* gene (rs3813034 and rs1042173) were selected. Table 1 describes the characteristics of the studied genes and SNPs.

Table 1. Candidate genes description and selected SNPs.

Gene	Position	SNP	MAF	Base Change
DRD2	11q23.2	rs6275	0.473	A/G
	11q23.2	rs6276	0.466	C/T
ANKK1	11q23.2	rs1800497	0.325	G/A
COMT	22q11.1-q11.2	rs6269	0.356	A/G
	22q11.1-q11.2	rs4818	0.296	C/G/T
5HTT	17q11.2	rs3813034	0.483	A/C
	17q11.2	rs1042173	0.485	G/T

MAF stands for minor allele frequency. Retrieved from the database: ncbi.nlm.nih.gov.

Statistical Analysis

Allele and haplotype frequency comparisons were performed by PLINK version 1.06. The Fisher exact test performed genotypic analysis, and Poisson Regression was adjusted by gender. Prevalence Ratio (PR) and 95% Confidence Interval (95% CI) were obtained by Poisson Regression. These analyses were performed by IBM SPSS version 25.0 (IBM Corp. Armonk, USA), and values of $p<0.05$ indicate statistical difference. Multifactor Dimensionality Reduction (MDR) was done to identify SNP-SNP interactions using gender as co-variables. MDR performs a 10-fold cross-validation consistency (CVC), testing balancing accuracy (TBA), and the 1000 permutation test to determine the statistical significance of the models. Models with the cross-validation consistency of 9/10 or 10/10 and the TBA > 0.55 and $p \leq 0.05$ were considered the best models. Entropy values were calculated, and MDR created an interaction graph.

Results

The sample was composed of 45 (17.6%) affected by DF and 211 (82.4%) unaffected individuals; 145 (56.6%) were female, and 111 (43.4%) were male. Regarding the DF type, most affected individuals presented mild, very mild, or moderate conditions. Only three girls presented DF in the severe condition. Table 2 summarizes the collected data.

Table 2. Characteristic of the studied population according to DF distribution.

Dental Fluorosis	Male	Female	Total
	N (%)	N (%)	N (%)
Unaffected	92 (82.9)	119 (82.1)	211 (82.4)
Very mild	11 (9.9)	9 (6.2)	20 (7.8)
Mild	3 (2.7)	6 (4.1)	9 (3.5)
Moderated	5 (4.5)	8 (5.5)	13 (5.1)
Severe	0 (0.0)	3 (2.1)	3 (1.2)

Table 3 shows the allelic and haplotype analysis in the affected and unaffected groups. There is no association between DF and studied SNP in *ANKK1*, *COMT*, and *5HTT* genes in allelic or haplotype models, both models ($p>0.05$). Still, the DRD2 gene presents an interesting result: the minor allele in rs6275 suggests an association with DF ($p=0.040$), while the minor allele in rs6276 was next to a significant association with DF ($p=0.07$). When the analysis was performed to investigate the interaction SNP x SNP, haplotypes T-G and C-A in rs6275 and rs6276 again were associated with DF ($p=0.04$ and $p=0.05$, respectively).

Table 3. Comparisons of allele and haplotype frequencies.

	SNPs	Minor Allele	Control	DF	p-value#
DRD2	rs6275	C	35.7	47.6	0.040
	rs6276	A	37.4	47.6	0.077

ANKK1	rs1800497	C	22.1	26.7	0.352
COMT	rs6269	G	39.8	34.7	0.420
	rs4818	G	34.4	38.4	0.365
5HTT	rs38133034	C	43.5	40.5	0.638
	rs1042173	C	39.8	34.7	0.583
		Haplotypes			
DRD2	rs6275 and rs6276	T - G	62.8	50.6	0.042
		T – A	1.5	2.5	0.556
		C – A	35.5	46.8	0.058
COMT	rs6269 and rs4818	A – C	60.1	67.2	0.314
		A – G	1.5	0	0.347
		G – C	6.0	5.1	0.804
		G – G	32.3	27.5	0.480
5HTT	rs38133034 and rs1042173	A – A	54.9	58.1	0.626
		A – C	1.3	1.3	0.985
		C – A	0.09	1.3	0.787
		C – C	42.7	29.1	0.581

#PLINK compares the frequencies between the major alleles by chi-square test; *PLINK compares the frequencies between the expected number of haplotypes by the Fisher test; Statistically significant.

Table 4 summarizes each gene's genotype distributions among affected and unaffected groups. Again, *DRD2* shows essential results. Individuals who carried the C allele in rs6275 and the A allele in rs6276 have more probability of developing DF. In fact, genotype CC for rs6275 (p^u=0.04; p^m=0.007; PR^m=5.37) and genotype AA for rs6276 (p^m=0.009; PR^m=5.10) increased significantly the change for DF. There was no significant association between DF and genotypes in other genes (p>0.05). To confirm the association between rs6275 and rs6276 with DF, the next step was to use diplotype analysis. Diplotypes CC+AA evidenced an increased probability of DF (p=0.007). Diplotype TC+GA was bordeline (p=0.095). When the analysis was adjusted by DF phenotype (very mild, mild, moderate, and severe), there was evidence that the *DRD2* gene, rs6275, was strongly associated with very mild DF in the genotype CC (p^m=0.003). The rs6276 also was associated with very mild and mild DF in the AA genotype (p^m=0.004) (Table 5).

Table 4. Genotypic distribution between individuals affected or unaffected by DF.

Genes	SNPs	Genotypes	DF		Control		p^u	p^m	PR^m (95% CI)
			N	%	N	%			
DRD2	rs6275	TT	90	44.3	15	35.7	Ref.	0.089	2.85 (0.85–9.58)
		TC	81	39.9	14	33.3	>0.999	0.007	5.37 (1.59–18.11)
		CC	32	15.8	13	31.0	0.041	0.089	2.85 (0.85–9.58)
	rs6276	GG	83	41.7	13	30.2	Ref.	0.107	2.70 (0.80–9.05)
		GA	83	41.7	19	44.2	0.342	0.009	5.10 (1.51–17.25)
		AA	33	16.6	11	25.6	0.145	0.107	2.70 (0.80–9.05)
ANKK1	rs1800497	TT	128	61.5	24	55.8	Ref.		
		TC	68	32.7	15	34.9	0.714	0.718	1.15 (0.53–2.48)
		CC	12	5.8	4	9.3	0.311	-	-
COMT	rs6269	AA	56	36.6	14	38.9	Ref.		
		AG	72	47.1	19	52.8	>0.999	0.704	0.86 (0.39–1.86)
		GG	25	16.3	3	8.3	0.380	0.268	0.45 (0.11–1.83)
	rs4818	CC	61	43.3	14	46.7	Ref.		
		CG	63	44.7	15	50.0	<0.999	0.555	0.79 (0.37–1.69)
		GG	17	12.1	1	3.3	0.287	0.198	0.27 (0.04–0.94)
5HTT	rs38133034	AA	48	31.0	12	32.4	Ref.		
		AC	79	51.0	20	54.1	<0.999	0.691	1.19 (0.50–2.83)
		CC	28	18.1	5	13.5	0.779	0.924	1.06 (0.29–3.86)
	rs1042173	AA	50	31.8	13	34.2	Ref.		
		AC	75	47.8	19	50.0	<0.999	0.920	1.04 (0.46–2.35)
		CC	32	20.4	6	15.8	0.608	0.866	0.89 (0.25–3.17)

p^u was obtained by Fisher Exact Test; p^m and Prevalence Ratio (PR) were obtained by Poisson regression adjusted by gender; A complete or quasi-complete data separation made analyzing some genotypes in Poisson regression impossible.

Table 5. Comparisons between the control group and categorized fluorosis groups.

Genes	SNPs	Genotypes	Control		DF (Very Mild and Mild)				DF (Moderate and Severe)			
			N	%	N	%	p^u	p^m	N	%	p^u	p^m
DRD2	rs6275	TT	90	44.3	11	27.8		Ref.	4	26.7		Ref.
		TC	81	39.9	7	33.3	0.621	0.274	7	46.7	0.359	0.317
		CC	32	15.8	9	38.9	0.110	0.003#	4	26.7	0.216	0.167
	rs6276	GG	83	41.7	9	22.2		Ref.	4	25.0		Ref.
		GA	83	41.7	10	44.4	>0.999	0.310	9	56.3	0.251	0.210
		AA	33	16.6	8	33.3	0.159	0.004†	3	18.8	0.416	0.426
ANKK1	rs1800497	TT	128	61.5	15	55.0		Ref.	9	60		Ref.
		TC	68	32.7	10	40.0	0.658	0.985	5	33.3	>0.999	0.929
		CC	12	5.8	3	5.0	0.382	>0.999	1	6.7	>0.999	0.868
COMT	rs6269	AA	56	36.6	10	41.2		Ref.	4	30.8		Ref.
		AG	72	47.1	11	47.1	0.814	0.798	8	61.5	0.555	0.519
		GG	25	16.3	2	11.8	0.498	0.156	1	7.7	>0.999	0.613
	rs4818	CC	61	43.3	10	47.1		Ref.	4	50.0		Ref.
		CG	63	44.7	11	47.1	>0.999	0.585	4	50.0	>0.999	0.970
		GG	17	12.1	1	5.9	0.450	0.222	0	0.0	0.575	-
5HTT	rs38133034	AA	48	31.0	6	22.2		Ref.	6	46.2		Ref.
		AC	79	51.0	13	50.0	0.799	0.097	7	53.8	0.563	0.549
		CC	28	18.1	5	27.8	0.741	0.113	0	0.0	0.090	-
		AA	50	31.8	7	21.1		Ref.	6	46.2		Ref.
		AC	75	47.8	13	52.6	0.806	0.273	6	46.2	0.548	0.498
		CC	32	20.4	5	26.3	>0.999	0.301	1	7.7	0.251	0.220

#PR = 4.28 (95% CI=1.64–11.17); †PR = 4.04 (95% CI=1.54–10.55); p^u was obtained by Fisher Exact Test; p^m and Prevalence Ratio (PR) were obtained by Poisson regression adjusted by gender; All comparisons were performed with the control group as a reference; A complete or quasi-complete data separation made analyzing some genotypes in Poisson regression impossible.

Next, we performed the between-gene multifactor dimensionality reduction (MDR) analysis step for the gene-gene interactions. Table 6 shows the best SNP *versus* SNP combinations. Among the combinations, the best SNP combination with the highest value of test of balanced accuracy was rs6276 (*DRD2*), rs1800497 (*ANKK1*), rs4818 (*COMT*), rs1042173 (*5HTT*) (p=0.035). So, this combination reveals a strong synergistic relation between these polymorphisms and predisposed to DF (Figure 1).

Table 6. Summary of MDR analysis results for DF.

Locus Number	Best Combination	CV*	TBA**	p-value***
2	rs6275 (*DRD2*),rs38133034(*5HTT*)	5/10	0.490	0.959
3	rs6275 (*DRD2*),rs4818 (*COMT*), rs38133034 (*5HTT*)	6/10	0.559	0.582
4	rs6276(DRD2), rs1800497 (*ANKK1*), rs4818 (*COMT*), rs1042173 (*5HTT*)	9/10	0.650	0.035
5	rs6276 (*DRD2*), rs1800497 (*ANKK1*), rs6269 (*COMT*), rs4818 (*COMT*), rs1042173 (*5HTT*)	4/10	0.542	0.703
6	rs6276 (*DRD2*), rs1800497 (*ANKK1*), rs6269 (*COMT*), rs4818 (*COMT*), rs38133034 (*5HTT*), rs1042173 (*5HTT*)	9/10	0.560	0.575
7	rs6275 (*DRD2*), rs6276 (*DRD2*), rs1800497 (*ANKK1*), rs6269 (*COMT*), rs4818 (*COMT*), rs38133034 (*5HTT*), rs1042173 (*5HTT*)	10/10	0.547	0.664

*Cross-validation consistency; **Testing Balanced Accuracy;***p-values were based on a 1000 permutations test.

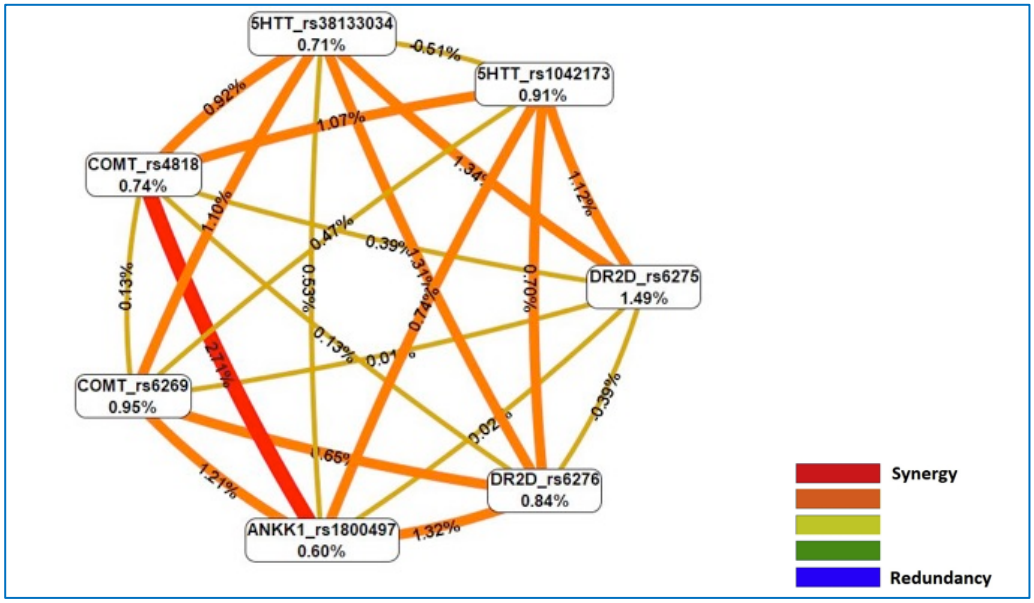

Figure 1. Entropy graph. This graph shows the entropy value of each SNP individually by percentages inside the nodes. The percentages on the lines indicate the entropy values resulting from the combination between SNPs. The different colors of the lines indicate the entropy kind, like red and orange lines, a synergic entropy, and blue and green, a redundancy entropy.

Discussion

Could SNPs commonly associated with anxiety, stress, or emotional disorders contribute to the increased risk for DF? This is the key question of this case-control study, and to answer this question, we designed a study that used genetic analysis tools that allowed for some interesting observations. DNA samples from 256 Brazilian adolescents were collected, and allelic, genotypic, haplotype, and diplotype analyses in *DRD2* (rs6275 and rs6276), *ANKK1* (rs1800497), *COMT* (rs6269 and rs4818) and *5HTT* (rs38133034 and rs1042173) genes were performed. Our first observation was that in the DRD2 gene, both SNPs showed an association with DF in the allelic and genotypic model, which was also evident in the haplotype and diplotype analysis. Surprisingly, the between-gene multifactor dimensionality reduction analysis step, used to investigate the gene-gene interactions, reveals that the combination rs6276 (*DRD2*), rs1800497 (*ANKK1*), rs4818 (*COMT*), and rs1042173 (*5HTT*) acts synergist and predisposed to DF.

Dopamine is the predominant neurotransmitter in the central nervous system, involved in various physiological processes [25], and its receptor is a family of protein-coupled receptors called Dopamine Receptor 1 through 5. Dopamine Receptors D2 are codified by *DRD2* and Ankyrin Repeat Domain Containing One (*ANKK1*) genes, which have similar patterns of association [26-28]. It is known that the D2 receptor is related to motor activity, reinforcement mechanisms, learning, and memory, plus susceptibility to post-traumatic stress disorder [29]. In addition, *DRD2-ANKK1* rs1800497 has been linked to body weight, pathological eating behavior, risk of alcoholism [29], and lower bone density [30].

COMT gene encodes the Catechol-O-methyltransferase enzyme, a key enzyme that recaptures dopamine in the synaptic cleft [7] and exhibits high activity in the prefrontal cortex [31]. It acts, supposedly linked with reward mechanism processing [32], affecting the neurobiological process of recognition and processing of emotional information [33]. *5HTT* gene is implicated in bone metabolism [16,17], modulation of development and growth, promoting the regulation of epithelial-mesenchymal interactions, stimulating cell migration and differentiation during the formation of the neural tube and branchial arches, and encodes a particular plasma membrane receptor for the uptake of serotonin from the synaptic cleft [34].

According to what was previously described, in principle, there is no known biological plausibility to investigate these four gene-gene interactions and their association with DF; meanwhile, there are pieces of evidence that bring these genes from fluorosis. Concerning the *COMT* gene, it was observed that some polymorphisms are common in children who live in areas with endemic fluorosis. Specific polymorphisms were investigated and associated with lower cognitive levels in the studied population [35,36]. Our study found no association with DF when we examined the *COMT* SNPs, rs6269 and rs4818, alone or in combination.

Regarding the *5HTT* gene, Baudry et al. [18] found a serotonin concentration gradient from a vascular source of serotonin to the low-affinity uptake sites in the invaginated dental epithelium. The discovery of the presence of serotonin within the dental mesenchyme and dental epithelium was important for the hypothesis of the participation of serotonin in the development of the craniofacial structures [37], including teeth [38], which led us to analyze this gene in our work. In the present study, no associations were observed between the *5HTT* SNPs, rs3813034 and rs1042173, in any of the genetic models used. It is essential to mention that this lack of association between *COMT*, *5HTT*, and DF was observed when the analyses were performed for each SNP separately.

In the *DRD2* gene, the scenario was completely different: two polymorphisms were associated with DF in the allelic and genotypic models. In fact, genotype CC, allele C for rs6275, and genotype AA, allele A for rs6276, significantly increased the risk for DF, even in mild or very mild DF. Most cases of DF reported in our work were mild or very mild, corroborating the data reported in the current literature in Brazil, including the leading and largest survey on oral health carried out in Brazil [20,39,40]. Once results from genotypic and allelic models provided evidence that only *DRD2* was associated with DF, our next step was to analyze interactions between polymorphisms through haplotype and diplotype analysis. The first one, haplotype analysis, is important because it provides crucial information about evolutionary history and can help us identify genetic variants linked to various human traits [41]. In our study, 11 haplotypes were generated with the 7 SNPs of the studied genes. The result by T-G and C-A haplotype confirmed that the rs6275 and 6276 polymorphisms of the *DRD2* gene predispose to DF. For the diplotype analysis, the TT and GG genotypes of the unaffected individuals were combined and compared to the TC+GA and CC+AA in the affected individuals. The result was that the CC + AA diplotypes significantly increased the probability of DF. One by one, the genetic tools showed increased evidence for a specific genetic variation in *DRD2* associated with DF. In this sense, our study is the first to demonstrate this relation between SNPs in *DRD2* and DF.

How can we explain this significant association between dopamine and enamel damage? According to a previous study, pulpal stem cells have autoreceptors for dopamine and serotonin that are essential for pulpal stem cell functions, i.e., these cells produce, release, and respond biochemically to the effects triggered by these molecules [42]. In addition, serotonin and dopamine released from dental pulp stem cells are necessary to mobilize endogenous stem cells for tooth repair. Failures in the metabolic pathways mediated by these molecules can result in enamel damage [42]. Furthermore, serotonin-binding receptors are expressed in the early stages of the tooth germ and contribute to maintaining the adequate biochemical environment for tissue development. Indeed, serotonin uptake appears particularly important during mesenchymal interactions, and tooth development seems sensitive to even minor fluctuations in concentrations of this molecule [37].

Odontogenesis is a complex genetic phenomenon with a highly regulated process, resulting in dental and periodontal tissue formation [43]. The process involves many genes, and unraveling the genetic basis takes work. DF is also a complex condition; the biggest challenge is assessing the genetic molecular pathways through gene-gene interactions. In this sense, we performed the MDR technique. MDR allows for categorizing the

studied groups and identifying significant interactions between genes and SNPs [44]. The results are presented in an interaction map demonstrating which genes interact to promote the outcome. Our results showed a synergistic interaction that could affect individual susceptibility to DF. The best association was *DRD2* (rs6276; 0.84%), *ANKK1* (rs1800497; 0.60%), *COMT* (rs4818; 0.74%), and *5HTT* (rs1042173; 0.91%) gene polymorphisms which point to a synergist relation between these polymorphisms and predisposition to DF.

Finally, our study has some limitations, such as not considering other environmental factors like water consumption, alternative sources of fluoride, or socioeconomic aspects of the examined adolescents. So, the results presented here must be carefully interpreted. Despite that, as far as we know, this is the first study that evaluated the interaction between Dopamine, Cathecol-O-Methyltransferase, Ankyrin Repeat Domain Containing One, and Serotonin Transporter genes, which seem to show that they act synergistically with DF. Therefore, further studies should investigate the mechanisms of action of *DRD2* in dental mineralization once both studied polymorphisms are associated with DF in all genetic tests performed.

Conclusion

The SNPs in *DRD2* (rs6275 and 6276) are associated with mild and very mild dental fluorosis, and there is a synergism between the polymorphisms of the *DRD2* (rs6276), *ANKK1* (rs1800497), *COMT* (rs4818), and *5HTT* (rs1042173) genes in the manifestation of DF.

Authors' Contributions

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All authors declare that they contributed to a critical review of intellectual content and approval of the final version to be published.

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None.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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