

## Evidence of Association between *MTRR* and *TNF-α* Gene Polymorphisms and Oral Health-Related Quality of Life in Children with Anterior Open Bite

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*Genetic polymorphisms could explain the inter-individual differences in the oral health-related quality of life (OHRQoL) of children with anterior open bite (AOB). Objective: To assess the impact of AOB on OHRQoL in children and to evaluate whether MTR (rs1805087), MTRR (rs1801394), TGFβ1 (rs1800469) and TNF-α (rs1799964, rs1799724 and rs1800629) genes are potential biomarkers for OHRQoL in children with AOB. Study design: A cross-sectional study was performed with 173 children aged between 2-6 years. The Brazilian version of Early Childhood Oral Health Impact Scale (ECOHIS) was applied. Genetic polymorphisms were analyzed using real-time PCR. Mann-Whitney U-test and Chi-square were used. Results: The overall mean ECOHIS scores were 5.49 (SD= 5.72) and 3.45 (SD = 4.49) (p < 0.01) in the AOB and control groups, respectively. Children with the CC genotype of TNF-α (rs1799724) had a significantly higher psychological QoL level. The MTRR AA genotype group showed a lower QoL level in the child subscale (p = 0.006), function (p = 0.017), and psychological (p = 0.006) domains. There was no significant difference between OHRQoL and the genetic polymorphisms in MTR and TGFβ1. Conclusions: Genetic polymorphisms in TNF-α and MTRR are associated with the impact on the OHRQoL in children with AOB.*

**Keywords:** Open bite, malocclusion, quality of life, genetic polymorphism

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## INTRODUCTION

Anterior open bite (AOB) is a common dentoalveolar or skeletal condition characterized as a vertical malocclusion.<sup>1</sup> AOB is the absence of vertical overlap of the mandibular incisors by the maxillary incisors, when the posterior teeth are in full occlusion.<sup>2</sup> The prevalence of AOB ranges from 1.5% to 11% depending on the ethnic group.<sup>3</sup> AOB is a multifactorial condition involving several components, including dental, skeletal, neurological, respiratory, and parafunctional habits such as sucking habits.<sup>4-6</sup> Sucking habits include the use of pacifiers and fingers/thumb sucking, which are non-nutritive sucking habits<sup>7,8</sup>, and nutritive sucking habits such as bottle feeding and breastfeeding.<sup>9,10</sup> Some studies also suggested that genetic factors play a role in the AOB etiology.<sup>11,12</sup>

Traditional clinical indicators are not instruments that provide complete information to determine the oral health-related quality of life (OHRQoL); therefore, socio-dental indicators are used as a complement to obtain the psychological and social range affected by the oral health.<sup>13</sup> In order to obtain more reliable analyses and results, these clinical and socio-dental indicators should be evaluated together<sup>14</sup>, because only then can they provide information regarding the disease's impact on the patient's life<sup>15</sup>. Previous studies demonstrated that malocclusion affects OHRQoL, especially the emotional and social well-being.<sup>16</sup> Specifically, AOB had a negative impact on the quality of life (QoL) of preschool children and their families.<sup>17</sup>

Regarding the influence of genetic polymorphisms on QoL, initial approaches and investigations were made by the North Central Cancer Treatment to evaluate the relationship between the patient's genetic history and health-related quality of life (HRQoL). Genetic polymorphisms have influenced the QoL, and this has been demonstrated by some studies<sup>18-20</sup> mainly in the medical field. The gene encoding tumor necrosis factor- $\alpha$  (*TNF- $\alpha$* ) was previous related to HRQoL, specifically fatigue, which is a commonly reported symptom in cancer patients.<sup>21</sup> The genes methionine synthase (*MTR*) and *MTRR* (methionine synthase reductase) are important for the metabolism of homocysteine,<sup>22</sup> which is associated with depression and anxiety.<sup>23</sup> The gene transforming growth factor beta 1 (*TGF $\beta$ 1*) is also associated with depression.<sup>24</sup> Therefore, these genes could potentially affect the individual's QoL.

Thus, from the aforementioned studies, it is observed that there is emerging evidence regarding the genetic basis of health-related quality of life (HRQoL), as well as the genetic component of AOB.<sup>5,17,25</sup> Furthermore, as it is necessary to incorporate new knowledge regarding genetic biomarkers for OHRQoL. In this study, the investigators hypothesize that AOB impact OHRQoL and genetic polymorphisms modulate the impact on OHRQoL. The purposes of this study were 1) to assess the impact of AOB on OHRQoL in children 2) to evaluate whether *MTR*, *MTRR*, *TGF $\beta$ 1* and *TNF- $\alpha$*  genes are potential biomarkers for OHRQoL in children with AOB.

## MATERIAL AND METHOD

### Ethical Approval, Type of Study, and Sampling

This cross-sectional study was performed according to the principles of the Helsinki Declaration, and after being reviewed and approved by the Local Human Ethics Committee (3.939.452). All parents/caregivers of the children involved in this study signed

a free and informed consent form authorizing their participation allowing the use of the answered questionnaire. The study followed the guidelines of the Strengthening the Reporting of Genetic Association study statement checklist.<sup>26</sup>

The sample comprised of children aged between 2-6 years with AOB, recruited during 18 months from the public schools in the city of Nova Friburgo, Rio de Janeiro, in the southeastern region of Brazil. Exclusion criteria were parents/caregivers who did not sign the informed consent form, did not return the informed consent form, or did not fill out the forms properly; lack of participation of the child for medical reasons; child's absence from preschool on the days scheduled for clinical examination; and lack of cooperation during the examination. Children in mixed dentition stage, as well as those with potential confounders for OHRQoL (dental caries, dental trauma, prosthetic or orthodontic treatment, and syndromes or special needs) were also excluded during clinical examination.

## Data Collection

### Non-clinical data

For data compilation, a questionnaire about their children's characteristics, including sex, age, and ethnicity, was answered by the parents/caregivers.

All parents were requested to self-complete a questionnaire validated in the Brazilian Portuguese language.<sup>27</sup> The socio-dental indicator used was the Early Childhood Oral Health Impact Scale (ECOHIS) that evaluated the OHRQoL of the children involved in the study through their parents' perception. The ECOHIS presents 13 items in the child subscale corresponding to four descriptive domains: child symptoms domain (1 item), child function domain (4 items), child psychological domain (2 items), and child self-image/social interaction domain (2 items); and two domains for the family subscale: parent distress domain (2 items) and family function domain (2 items). For statistical analysis, only the child subscale was considered to detect the genetic relation with OHRQoL. The response categories of ECOHIS were determined on a 5-point scale: 0 = never; 1 = hardly ever; 2 = occasionally; 3 = often; and 4 = very often. After completion of the questionnaire by the parents, in addition to the total ECOHIS scores, the scores for the individual domains were calculated by summing the scores of the answers. The scoring in this assessment method ranges from 0-52 (0-36 for child subscale and 0-16 for family subscale). Higher ECOHIS values correspond to more problems and/or higher impact on the OHRQoL.

In order to detect the reliability of the target population, a pre-test study was performed before applying the OHRQoL questionnaire. A new convenience sample (n = 34, 10% of the minimum required sample) comprising of parents/caregivers and children was recruited. A second questionnaire was administered two weeks later, as the test-re-test reliability analysis requires that the conditions of the participants remain stable over time. The intraclass correlation coefficient of 0.98 was considered good.

### Clinical data

Initially, for the evaluation of the clinical data, two specialists in pediatric dentistry were previously calibrated and trained for AOB diagnosis through oral examination in children. Images of different AOB-related clinical situations were used for 24-hour diagnostic training. To confirm the reliability, the kappa statistical test was

used in two different exams with a one-week interval between them. This reliability test was performed on 30 children aged 2-6 years, who were not included in the sample. At the end of the tests, inter- and intra-examiner results provided near perfect agreement ( $\kappa = 1.00$  and  $1.00$  respectively).

For the clinical examinations, the children were seated in a chair under natural light, and gauze, tongue depressors, and millimeter plastic ruler were used. It was determined that AOB malocclusion would be considered present if there was a minimum vertical gap of 0.5 mm between the maxillary and mandibular incisors in centric occlusion. Children with history of previous orthodontic treatment or speech therapy, any malocclusion other than AOB, or presence of syndromes, systemic disorders, and oral clefts were not considered in the sample.

*DNA sample collection and Genotyping*

Genomic DNA for molecular analysis was extracted from the buccal cells. Buccal cells were collected by rinsing the mouth for 60 s with 15 mL of saline and expectorating the rinse in a 50 mL propylene tube. The samples were stored at -20 oC until the beginning of the DNA extraction step (Kuchler et al. 2012). Genomic DNA was extracted from buccal epithelial cells following a modified protocol reported by Aidar & Line (2007). Shortly after incubation, the tubes were centrifuged for 10 min at 550 xg at room temperature to pellet the buccal cells. The supernatant was discarded and 1 mL of extraction solution (Tris-HCl 10 mM, pH 7.8; EDTA 5 mM; SDS 0.5 %) containing proteinase K (100 ng/ mL) (Invitrogen, Grand Island, NY, USA) was added to cause lysis of the cells and proteins. After overnight incubation, the non-digested proteins were removed by adding 400 μL of 10 M ammonium acetate. The solutions were mixed by gently reversing the tube 20 times and centrifuging at 12,000 xg for 15 min. The supernatant was separated into two clean 1.5 mL microtubes (700 μL), and the DNA was precipitated with 700 μL of isopropanol at -20 oC for 30 min. After centrifugation for 20 min at 12,000 xg at 4°C, the supernatant was poured off and the pellet was washed with 1 mL of 70 % ethanol. Ethanol was decanted carefully after centrifugation at 12,000 xg for 15 min at 4°C, and the tubes were reversed and allowed to air-dry for 45 to 60 min on an absorbent paper. The DNA was resuspended in 100 L of TE buffer (10 mM Tris [pH 7.8] and 1 mM EDTA) and stored at -20°C. <sup>28-29</sup>

The amount and purity of the DNA were determined by the spectrophotometer instrument (Nanodrop ® 1000, Thermo Scientific, Wilmington, NC, USA).

Candidate genes were chosen according to the Consortium for Genetics and Quality of Life Research.<sup>30</sup> We used the UCSC Genome Browser website to identify previously characterized single-nucleotide polymorphisms (SNPs) for each candidate gene according to their possible function regulation. A total of six SNPs in *TNF-α* (rs1799964, rs1799724 and rs1800629), *MTR* (rs1805087), *MTRR* (rs1801394), and *TGFβ1* (rs1800469) genes were selected and investigated. The characteristics of the studied SNPs are presented in Table 1.

Selected genes were genotyped by real-time polymerase chain reactions using the TaqMann method<sup>31</sup> by Agilent Technologies (Stratagene Mx3005P). The real-time PCR was performed in a total volume of 3 mL (4 ng DNA/reaction, 1.5 mL Taqman PCR master mix, and 0.075 SNP assay; Applied Biosystems). Thermal cycling was performed starting with a hold cycle of 95°C for 10 min, followed by 40 amplification cycles of 92°C for 15 s and 60°C for 1 min. (Ranade et al. 2001). The cycle threshold (Ct) is defined as the number of cycles required for the fluorescent signal to exceed the threshold. In a real-time PCR assay, a positive reaction is detected by the accumulation of fluorescent signals, and Ct levels are inversely proportional to the amount of the target nucleic acid in the sample. The primers were pre-designed by Applied Biosystems (Foster City, CA, USA). All examiners at the laboratory were blinded to the samples' group assignment.

*Statistical analysis*

The data were analyzed using the Statistical Package for Social Science (IBM SPSS version 23.0, USA). The level of significance was set at 5% (p < 0.05). Age differences between the groups were calculated using t-test. Fisher's exact and chi-square tests were used to compare the demographic differences between the groups. The scores of the ECOHIS indexes were calculated by summing up the numeric responses for each item. The variables were tested for normal distribution by the Kolmogorov-Smirnov test. Means and medians were obtained for overall items and subscale scores for the case and control groups. The Mann-Whitney U-test was used to compare the mean and median scores between the AOB and control

**Table 1. Details on the genetic markers' studied.**

Gene (SNP)	Position	Functional Consequence	Ref SNP Alleles	MAF
<i>TNF-α</i> (rs1799964)	chr6:31574531 (GRCh38.p12)	Downstream transcript variant, upstream transcript variant	C/T	C=0.2190
<i>TNF-α</i> (rs1799724)	chr6:31574705 (GRCh38.p12)	Downstream transcript variant, upstream transcript variant	C/T	T=0.0990
<i>TNF-α</i> (rs1800629)	chr6:31575254 (GRCh38.p12)	Upstream transcript variant	A/G	A=0.0903
<i>MTR</i> (rs1805087)	Chr.1: 236885200 (GRCh38)	Missense variant, coding sequence variant	A/G	G=0.2182
<i>MTRR</i> (rs1801394)	Chr.5: 7870860 (GRCh38)	missense_variant, coding sequence variant, non coding transcript variant, upstream transcript variant	A/G	G=0.3642
<i>TGFβ1</i> (rs1800469)	Chr.19: 41354391 (GRCh38)	Downstream transcript variant, upstream transcript variant	A/G	A=0.3680

Note: Obtained from databases: <http://www.thermofisher.com>, <http://www.ncbi.nlm.nih.gov> and <http://genome.ucsc.edu>

groups, as well as in children with AOB, to compare the genotype distributions between OHRQoL domains in a dominant model. The standard chi-square test was used to test for deviation from the Hardy-Weinberg equilibrium.

**RESULTS**

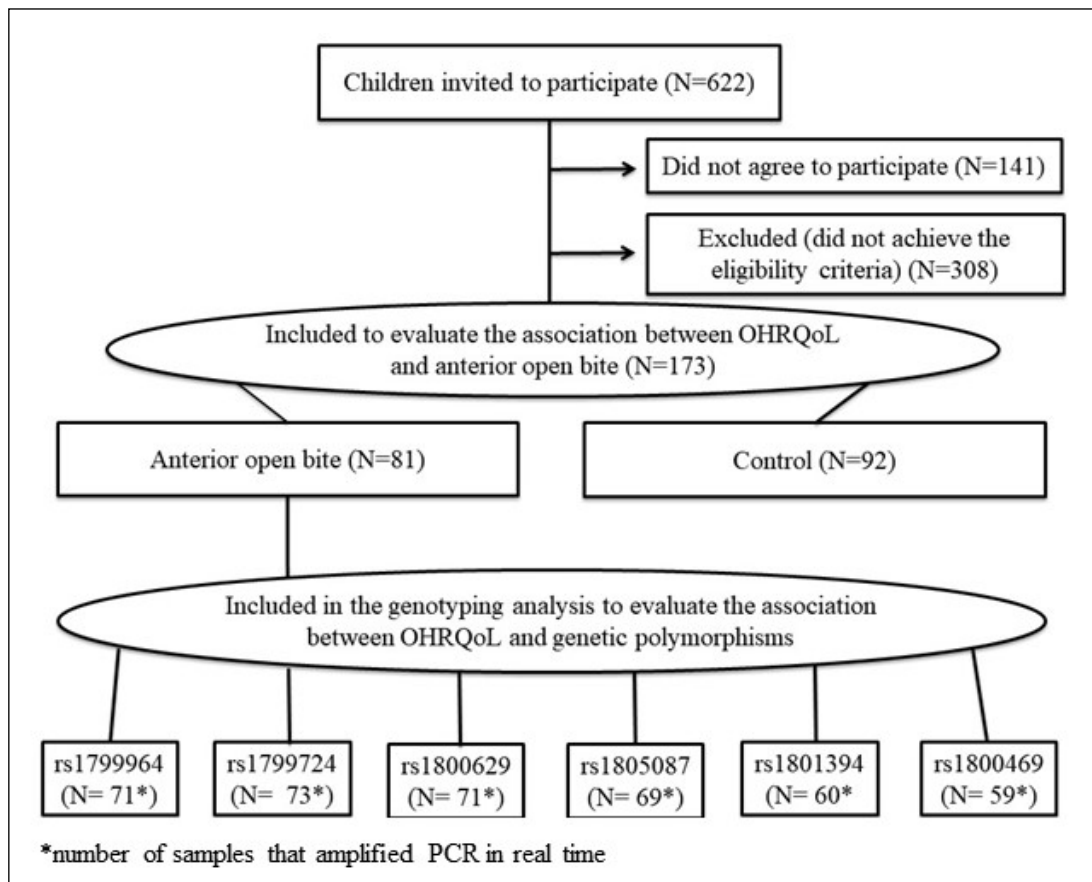
A total of 622 pairs of children and their parents/caregivers were invited for the study. The response rate was 77.3% (481/622). Of these, 141 children were excluded due to lack of participation of the child for medical reasons; incomplete questionnaires; child's absence from preschool on the days scheduled for the clinical examination; and a lack of cooperation during the examination. During the clinical examination, 308 children with potential confounders for OHRQoL (dental caries, dental trauma, prosthetic or orthodontic treatment, and syndromes or special needs) were excluded. The final sample consisted of 173 children (81 with AOB and 92 with normal occlusion). The flowchart of the study is presented in Figure 1.

The age of the included children ranged from 2-6 years, with a mean (standard deviation [SD]) age of 3.18 (1.35) years. Age, sex and ethnicity did not differ between the groups ( $p > 0.05$ ).

The overall mean ECOHIS scores observed were 5.49 (SD 5.72) and 3.45 (SD 4.49) and median scores were 4.00 and 2.00 ( $p < 0.01$ ) in the AOB and control groups, respectively (Table 2). Regarding the subscale domain, children with AOB also presented lower OHRQoL in the total scale ( $p < 0.01$ ), child subscale ( $p < 0.01$ ), function domain ( $p < 0.01$ ), and physiological domain ( $p < 0.01$ ).

The results of the comparison between the genetic polymorphisms and OHRQoL in children with AOB ( $n = 81$ ) are summarized in Table 3. Children with the CC genotype of *TNF-α* (rs1799724) had a significantly higher psychological QoL level than those with the other genotype. The *MTRR* AA genotype group showed a lower QoL level in the child subscale ( $p = 0.006$ ), function ( $p = 0.017$ ), and psychological ( $p = 0.006$ ) domains as compared to the AG/GG genotype group. There was no significant difference between the genetic polymorphisms in *MTR* and *TGFβ1* and the OHRQoL (Table 3).

**Fig. 1 Study design flowchart**



**Table 2: Comparison of the mean and median between AOB and control group**

	AOB (n=81)		Control (n=92)		P value
	Mean (SD)	Median (Q1-Q3)	Mean (SD)	Median (Q1-Q3)	
<b>Total Scale</b>	5.49 (5.72)	4 (0-9)	3.45 (4.49)	2 (0-5)	0.01
<b>Child Subscale</b>	3.78 (3.76)	3 (0-6)	2.33 (3.01)	1 (0-4)	0.01
Symptoms domain (1 item, range 0-4)	0.47 (0.82)	0 (0-1)	0.33 (0.73)	0 (0-0)	0.22
Function domain (4 items, range 0-16)	2.11 (2.27)	0 (0-4)	1.22 (1.60)	0 (0-2)	0.01
Physiological domain (2 items, range 0-8)	1.19 (1.49)	0 (0-2)	0.80 (1.62)	0 (0-1)	0.01
Self-image and social interaction domain (2 items, range 0-8)	0.23 (0.69)	0 (0-0)	0.14 (0.76)	0 (0-0)	0.11
<b>Parental Subscale</b>	1.72 (2.86)	0 (0-3)	1.12 (2.06)	0 (0-2)	0.18
Parental distress (2 items, range 0-8)	0.90 (1.69)	0 (0-1)	0.68 (1.29)	0 (0-1)	0.48
Family function (2 items, range 0-8)	0.81 (1.56)	0 (0-1,5)	0.43 (1,00)	0 (0-0)	0.18

Note: Mann-Whitney test; p<0.05, bold font indicates statistical significance; Q1, Q3: 1<sup>st</sup> and 3<sup>rd</sup> quartile (25%,75%, respectively)

**Table 3. Association between genetic polymorphisms in MTR, MTRR, TGFβ1 and TNF-α genes and OHRQoL in the group with AOB.**

Gene	OHRQoL										
	Child Subscale		D1-Symptoms		D2-Function		D3-Psychological		D4- Self-image/ social interaction		
	Median (Q1-Q3)	P value	Median (Q1-Q3)	P value	Median (Q1-Q3)	P value	Median (Q1-Q3)	P value	Median (Q1-Q3)	P value	
<i>TNF-α</i> rs1799964	CC+CT	3 (1-6.5)	0.346	0 (0-2)	0.162	2 (0-4)	0.242	1 (0-2)	0.821	0 (0-0)	0.579
	TT	3 (0-6)		0 (0-0.25)		2 (0-4)		0.5 (0-2)		0 (0-0)	
<i>TNF-α</i> rs1799724	CT	6 (2.5-9.25)	0.061	0 (0-0.75)	0.797	3 (0.5-4.75)	0.190	2 (1.25-3.75)	<b>0.006</b>	0 (0-0)	0.941
	CC	2 (0-6)		0 (0-0.5)		2 (0-4)		0 (0-2)		0 (0-0)	
<i>TNF-α</i> rs1800629	AG	1 (0-7.5)	0.206	0 (0-1.25)	0.521	0 (0-2.25)	0.089	0 (0-2)	0.806	0 (0-0)	0.296
	GG	3 (1-6)		0 (0-0)		2 (0-4)		0 (0-2)		0 (0-0)	
<i>MTR</i> rs1805087	GG+AG	3 (0-6)	0.347	0 (0-1)	0.718	2 (0-4)	0.189	0 (0-2)	0.951	0 (0-0)	0.282
	AA	2 (0-6)		0 (0-0)		1 (0-3)		0 (0-2)		0 (0-0)	
<i>MTRR</i> rs1801394	GG+AG	2 (0-6)	<b>0.006</b>	0 (0-1)	0.476	1 (0-3)	<b>0.017</b>	0 (0-2)	<b>0.006</b>	0 (0-0)	0.287
	AA	6 (2.5-7.5)		0 (0-0)		3 (1.5-4)		2 (1.2.5)		0 (0-0.5)	
<i>TGFβ1</i> rs1800469	AA+AG	2 (0-6)	0.660	0 (0-0)	0.839	1 (0-4)	0.833	0 (0-2)	0.905	0 (0-0)	0.509
	GG	3 (0.25-5)		0 (0-0)		2 (0-3.75)		0 (0-2)		0 (0-0)	

Note: Mann-Whitney U test, with significance level of 0.05; Q1, Q3: 1st and 3rd quartile (25%,75%, respectively); Bold indicates statistical significance.

## DISCUSSION

The management of AOB is a challenge due to the difficulty associated with determining and addressing its etiological factors. This malocclusion found in preschoolers can affect the way children grow, talk, chew and taste food, and socialize. Therefore, socio-dental indicators should be used as a complement to a comprehensive treatment comprising of clinical and subjective aspects, which will allow the child to enjoy their health fully. To date, there are no studies reporting the association of clinical characteristics, genetic markers, and OHRQoL in preschoolers with AOB. The results of our study confirmed the hypothesis presented and observed that AOB affects OHRQoL.<sup>17,32</sup> In addition, our study showed that polymorphisms in the *TNF- $\alpha$*  and *MTRR* genes modulated the impact on OHRQoL.

The early diagnosis of malocclusion is hampered by the low percentage of preschoolers who visit the dentist.<sup>33</sup> Therefore, the assessment of the parents' perception of the child's oral health is particularly important in this population. International data on the impact of AOB on the HRQoL of preschoolers are scarce. It is important to mention that studies in Brazilian populations only assessed the association between AOB and HRQoL of preschoolers.<sup>17,32,34-36</sup> Five previous studies evaluated the impact of AOB on the HRQoL of preschoolers<sup>17,32,34-36</sup>, but only Ramos-Jorge *et al*<sup>17</sup> and Perazzo *et al*<sup>32</sup> found associations with OHRQoL. Ramos-Jorge *et al*<sup>17</sup> used the same questionnaire (ECOHIS) and the same age group (3-5 years) as our study, and they reported negative impacts on most ECOHIS domains, except for "symptoms." Perazzo *et al*<sup>32</sup> used the Scale of Oral Health Outcomes for Five-Year-Old Children (SOHO-5) and found higher scores in the perception of both children and their parents/caregivers, indicating worse HRQoL. These data are consistent with our study, which observed an impact on the total score on the child subscale and on the psychological and function domains. This can be explained by the fact that the AOB is more noticeable in this age group, in addition to being a condition often associated with sleep problems, difficulty in eating, and pronouncing some words. Two studies that also applied ECOHIS found contradictory results to what was presented above. Firmino *et al*.<sup>36</sup> reported that AOB was a protective factor for the OHRQoL of the family of preschoolers, while Abanto *et al*.<sup>35</sup> observed positive impact of AOB in the field of family function.

In medicine, emerging research has suggested that individual's genetic predisposition interferes with the perception of QoL. Several studies have provided evidence of the association between genetic polymorphisms and QoL.<sup>18,37-40</sup> Understanding the genetic aspects involved in QoL will allow the identification of patients susceptible to deficits in HRQoL and direct healthcare.<sup>41</sup> However, studies on OHRQoL and genetic polymorphisms are scarce. To the best of our knowledge, there are no studies in the dentistry field assessing the impact of genetic polymorphisms on the OHRQoL of preschoolers with AOB. Hence, this study evaluated whether the polymorphisms of the four genes, *TNF- $\alpha$* , *MTR*, *MTRR*, and *TGF $\beta$ 1*, could be biomarkers for OHRQoL.

*TNF- $\alpha$*  plays an important role in altering the interactions of the immune and neural systems, including levels of pro-inflammatory cytokines, increased pain and sensitivity, and increased inflammatory activity.<sup>42</sup> A meta-analytical study reinforced the evidence that

depression is accompanied by the activation of the inflammatory response system, and that the development of depression is related to significantly higher concentrations of the pro-inflammatory cytokines *TNF- $\alpha$* .<sup>43</sup> Kao *et al*.<sup>44</sup> systematically reviewed and integrated the available data on 5,055 candidate genes according to their evidence regarding depression. *TNF* was one of the top seven prioritized genes that could be used for individual replication. It is possible planning to explore their biological roles further in relation to depression. These data are in agreement with that of this study, which observed an association between the polymorphism in the *TNF- $\alpha$*  gene (rs1799724) with the psychological domain of OHRQoL. We suggest that this fact may occur due to the pleiotropic biological capabilities of *TNF- $\alpha$*  that may influence the function of almost all cell types.

This study also evaluated polymorphisms in genes that encode enzymes involved in homocysteine metabolism, such as methionine synthase (encoded by *MTR*) and methionine synthase reductase (encoded by *MTRR*). *MTR* catalyzes the re-methylation of homocysteine to methionine in a reaction dependent on methyl cobalamin. Vitamin B12 acts as a co-factor for methylation.<sup>45</sup> Vitamin B12 oxidizes over time and the enzyme methionine synthase is inactivated. Functional regeneration of methionine synthase requires the participation of another enzyme, methionine synthase reductase, which is encoded by the *MTRR* gene.<sup>46</sup> The literature suggests an association between vitamin B12, folic acid, and homocysteine and depressive disorders.<sup>47</sup> Studies have shown that high levels of homocysteine have the potential to modulate neurotransmitters (dopamine, serotonin, and melatonin) and de-regulate neurons, thereby increasing the risk of depression.<sup>48</sup> Low plasma levels of folic acid were related to the diagnosis of depression.<sup>49</sup> Low levels of Vitamin B12 were associated with an increased risk of depressive symptoms.<sup>50</sup> These facts may justify the association between polymorphism in the *MTRR* gene and OHRQoL. The *MTRR* gene, according to the results of this sample, can be a biomarker for QoL.

Another candidate gene for the OHRQoL biomarker was *TGF $\beta$ 1* (rs1800469). The human *TGF $\beta$ 1* protein is considered one of the immunosuppressive cytokines, which plays a crucial role in the development of the central nervous system. It is responsible for functions, such as astrocyte differentiation, synaptogenesis, and neuronal migration.<sup>51</sup> Data suggest that carriers of the GG genotype of the *TGF $\beta$ 1* gene (rs1800469) were associated with increased severity of the depressive episode, and that it may be associated with increased TGF $\beta$ 1 concentrations.<sup>52</sup> Other polymorphisms of the *TGF $\beta$ 1* gene have also been associated with depression. In the case of rs1800470 (codon 10 position +869), patients with depression showed a higher frequency of the TT genotype than that of the control group.<sup>24</sup> In addition, another study showed that the CC genotype of the same genetic polymorphism was associated with a higher risk of developing depression and more severe depressive symptoms.<sup>53</sup> Although *TGF $\beta$ 1* has an important role in psychoneuroimmunology, there was no association between its genotypic distribution and OHRQoL in the present study. The absence of association may be due to the lower impact of the AOB on OHRQoL in relation to other pathologies, such as depressive processes. In addition, the impact of genetic polymorphisms on the response to OHRQoL may be limited.

Methodological limitations must be considered when interpreting our results. As this was a cross-sectional study, causality inference is not possible because exposures and results are evaluated simultaneously. In addition, data acquired through questionnaires may be subject to information bias. However, measures were taken to reduce possible bias, such as conducting a pilot study and using a validated assessment instrument. Another important methodological limitation is the small sample size, especially with regard to the subgroup of children with AOB, which must be interpreted carefully. Therefore, the present study can be considered the first step in examining the complex relationship between genetic polymorphisms and OHRQoL. Further studies are suggested in order to identify the genetic biomarkers for OHRQoL to assist health professionals in their daily activities as well as patients and mainly focusing on groups of children with malocclusion, which is one of the main public health problems. It would be also valuable for future research, the investigation of regular periodic monitoring in order to assess whether the possible biomarker could be influencing this child's perception of OHRQoL in the long term after treatment.

In general, dentists tend to focus on clinical aspects (functional and esthetic changes) and treatment planning. However, it is necessary to evaluate the individual as a whole. The lack of consideration of socio-dental aspects can compromise the patient's acceptance, perception, and expectations regarding dental treatment. A holistic and expanded view of the patient is necessary. Research on this topic can increase the level of information and quality of the treatment of AOB; consequently, this will enable health professionals to diagnose patients showing a more negative response of OHRQoL early, and thus be able to direct their healthcare appropriately. It is hoped that in the near future, the identification of genetic biomarkers and OHRQoL may become a standard practice in dental clinics and educational institutions.

## CONCLUSION

AOB affected the OHRQoL in children aged 2-6 years. *TNF-α* and *MTRR* genetic polymorphisms were the potential biomarkers for the OHRQoL in children with AOB.

## Conflict of Interest

The authors declare that they have no conflicts of interest.

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