Original Research
Behavioral Sciences

Association of MTR and MTRR genes and oral health-related quality of life in children with dental caries

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Abstract: This study aimed to assess whether genetic polymorphisms in MTR and MTRR are potential biomarkers of oral health-related quality of life (OHRQoL) in children with caries. A cross-sectional study was designed wherein pairs of parents/caregivers and children (aged two–five years) were selected. Clinical examination was used to detect dental caries, which were classified as low-severity and high-severity caries. The Early Childhood Oral Health Impact Scale (ECOHIS) questionnaire was used to assess OHRQoL. Genomic DNA extracted from the saliva was used to analyze two missense genetic polymorphisms: MTR (rs1805087) and MTRR (rs1801394). Mann–Whitney non-parametric test was used to analyze candidate genes with OHRQoL scale and domain, with a significance level of $p \leq 0.05$. MTR (rs1805087) was found associated ($p = 0.05$) with children's OHRQoL subscale scores in the dominant model (GG + AG). Genetic polymorphisms in MTR may increase the risk of poor OHRQoL in children with caries. Further studies are needed to investigate genetics, molecular factors, and OHRQoL.

Keywords: Genetics; Dental caries; Oral health; Quality of life, children

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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https://doi.org/10.1590/1807-3107bor-2023.vol37.0076

Submitted: April 18, 2022
Accepted for publication: March 17, 2023
Last revision: April 17, 2023

Introduction

Untreated dental caries is a public health challenge that affects many individuals in most countries.[1] Dental caries affects oral health-related quality of life (OHRQoL), not only because of oral symptoms but also because of the functional limitations that untreated dental caries can affect family, social, and psychological relationships.[2-7]

Evidence regarding a genetic basis of health-related quality of life (HRQol) in medicine is emerging.[8-11] The need to incorporate new insights on the role of biological and physiological variables has led to the creation of an international and interdisciplinary consortium of research in HRQoL, the "Consortium for Genetics and Quality of Life Research (GeneQoL)".[12] This consortium raised biological and genetic mechanisms possible involved in HRQol, suggesting genes associated with HRQoL-related symptoms, such as pain, mood, and fatigue.[13-15] Genes affect fundamental cellular processes and how the patient feels in general.[16]

MTHFR (methylenetetrahydrofolate reductase) was identified in GeneQoL as one of the possible biomarkers for HRQoL.[12] This gene

was associated with depressive disorder in a meta-analysis of 20 genetic polymorphisms and 18 genes.[17] Depressive disorders have been associated with folate and vitamin B12 deficiency, as well as hyperhomocysteinemia. The T allele of the *MTHFR* gene causes impaired methylation reactions in the central nervous system.[18] Incident depression has been related to lower levels of folate and vitamin B12 and higher homocysteine levels.[19]Similar to *MTHFR*, the *MTR* and *MTRR* genes are also involved in the metabolism that regulates folate/homocysteine.[20]

Supported by the literature mentioned above, the objective of the present study was to investigate the genetic background of OHRQoL in a population that has already demonstrated an impact on OHRQoL (in children with caries experience). We investigated whether genetic polymorphisms of genes previously identified as possible biomarkers for HRQoL (*MTR* and *MTRR*) are associated with OHRQoL in children with caries.

Methodology

This cross-sectional study was approved by the local Human Ethics Committee of the Universidade Federal Fluminense (Protocol nº 3.939.452), and has been described in accordance the Strengthening the Reporting of Genetic Association (STREGA) statement checklist.[21] The parents/caregivers of all children signed written informed consent allowing the child to participate in the study.

Sample selection

The study participants were invited to participate according to the following inclusion criteria: pairs of parents/caregivers and children aged 2–5 years, of both sexes, and whose parents/legal guardians had signed and returned the informed consent and questionnaire. The sample was recruited by convenience over 18 months from 33 public schools in Nova Friburgo, Rio de Janeiro, Southeastern Brazil. The sample size was determined based on the global minor allele frequencies, that was established as ≤0.20 due to the sample size of the groups. As this is the first study to evaluate polymorphisms in MTR and MTRR genes as potential biomarkers for OHRQoL, an exact sample size calculation was not possible.

Children without caries were excluded. The exclusion criteria were parents/caregivers who did not write or speak fluent Brazilian Portuguese, parents/caregivers who did not sign or return informed consent, or did not fill out the questionnaires appropriately. Children with mixed dentition, who did not fully cooperate with the dental examinations, or with other potential confounding factors affecting the OHRQoL (*i.e.*, malocclusions such as increased overjet, anterior open bite, posterior crossbite, and anterior crossbite; dental trauma such as fractures, avulsion, and tooth discoloration; children undergoing orthodontic or prosthetic treatment; syndromic or with other special needs) were also excluded.

Data collection

This study was performed in four steps: a) Administration of a questionnaire (sociodemographic data and dental indicator instrument); b) Oral examination to determine caries experience; c) Collection of biological material and selection of genes/genetic polymorphisms; and 4) DNA extraction and genotype analysis.

All parents or caregivers answered a questionnaire regarding their children's characteristics (age and sex), ethnicity (obtained and ascertained based on self-reported information), and dental indicator instruments. All parents/caregivers were requested to complete the questionnaire at home, return it, and provide informed consent to the school.

Questionnaire application

The Early Childhood Oral Health Impact Scale (ECOHIS), validated in a Brazilian study,[22] was used as the socio-dental indicator. ECOHIS is composed of 13 items that assess the perception of parents about the OHRQoL of their children (nine items) and the impact on the family's quality of life (four items). The questions were divided into four descriptive domains in the child subscale: symptom domain (one item), child function domain (four items), child psychological domain (two items), and child self-limitation domain/social interaction (two items), and two domains for the family subscale:

parents' domain of suffering (two items) and family function domain (two items). The ECOHIS response categories were coded on a five-point scale: 0 = never, 1 = almost never, 2 = occasionally, 3 = often, and 4 = very often. The ECOHIS total scores and scores for individual domains were calculated as a simple sum of the response codes. This instrument ranges from 0 to 52 (0–32 for the child subscale and 0–16 for the family subscale). A higher ECOHIS score indicates a greater impact and/or more problems, i.e., worse OHRQoL. For analysis, only the child subscale was considered, as it was intended to detect the genetic relationship between the child and OHRQoL.

A pilot study was conducted to assess the reliability of OHRQoL. Parents and caregivers who were not part of the study population were recruited. The test-retest reliability analysis requires individuals' conditions to remain the same between the two administrations of the questionnaire. Therefore, the second questionnaire was administered two weeks later. The intra-class correlation coefficient (ICC= 0.98) was excellent.

Oral examination

A pediatric dentistry specialist trained and calibrated two examiners to perform oral examinations on schoolchildren. The training exercise (total of 24 hours realized over one week) for dental caries diagnosis was performed using images of different clinical situations. The reliability was assessed using weighted (dental caries) kappa statistics for the two separate dental examinations. This was performed in children aged 2–5 years (not part of the study population), with a two-week interval between sessions. Inter- and intra-examiner reliability presented substantial to near-perfect agreement (κ = 0.80 and 1.00, respectively).[23]

Dental caries were diagnosed using a modified World Health Organization protocol recommended for oral health research criteria for the diagnosis of decayed, missing, and filled teeth (dmft) in deciduous teeth because the evaluated sample presented only primary dentition.[24] Carious lesions were recorded as present when a break in the enamel was apparent on visual inspection. Teeth lost owing to trauma or exfoliated deciduous teeth were excluded from the final scores. White spot lesion was also evaluated according to '*the first sign of caries lesion on enamel that can be detected with the naked eye*' and used alongside with terms '*initial*' or '*incipient*' lesions.[25] Caries severity was classified into two groups, as follows: low caries experience, dmft Score = 1–5 or presence of white spot lesion; or high-caries experience, dmft Score ≥ 6.[26]

Biological material collection and molecular analysis

A CytoSoft™ CP-5B brush (Medical Packaging Corp., Camarillo, USA) was used to collect duplicate cheek smear samples from each participant. All collected materials were stored, processed, and analyzed.

Candidate genes were chosen according to the Consortium for Genetics and Quality of Life Research guidelines.[12] We used the UCSC Genome Browser website to identify previously characterized single-nucleotide polymorphisms for each candidate gene according to their possible function regulation. Two missense genetic polymorphisms were selected and investigated in MTR (rs1805087) and MTRR (rs1801394) genes. The characteristics of the studied genetic polymorphisms are summarized in Table 1.

Genomic DNA for molecular analysis was extracted, as reported previously.[27] The amount and purity of

Table 1. Details on the genetic markers' studied.

Gene (polymorphism)	Position	Functional consequence	Ref SNP alleles	MAF
MTR (rs1805087)	Chr.1: 236885200 (GRCh38)	Missense variant, coding sequence variant: Asp919Gly	A/G	G = 0.2182
MTRR (rs1801394)	Chr.5: 7870860 (GRCh38)	Missense variant, coding sequence variant: Met22Ile	A/G	G = 0.3642

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

DNA were determined by the spectrophotometer instrument (Nanodrop® 1000, Thermo Scientific, Wilmington, USA). Only DNA samples with an A260 nm/A280 ratio greater than 1.8 were used. All the examiners were blinded to the assignment of the sample groups. Analysis of the presence of gene polymorphisms was performed by real-time polymerase chain reactions using the TaqMan assay (Agilent Technologies, Stratagene Mx3005P). Primers, probes, and the universal main mixture were procured from Applied Biosystems (Foster City, USA).[28]

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Macintosh, v.23.0 (IBM Corp., Armonk, USA), with a significance level of $p < 0.05$. The standard chi-square test was used to test the deviation of the Hardy–Weinberg balance. Variables were tested for normal distribution using parametric tests. Based on the Kolmogorov–Smirnov test, the Mann–Whitney non-parametric test was used to analyze candidate genes using the OHRQoL scale and domain. OHRQoL was compared between the low- and high-caries groups. The association between genotypes and OHRQoL was assessed in all children and stratified according to caries severity (low and high-caries).

Results

Initially, 622 children-caregiver pairs were invited to participate in this study. One hundred and forty-one patients were lost to follow-up. Participants did not agree to complete the OHRQoL questionnaire. After applying the eligibility criteria, 130 child–caregiver pairs were included. A flowchart summarizing the patient selection and the final sample is shown in Figure.

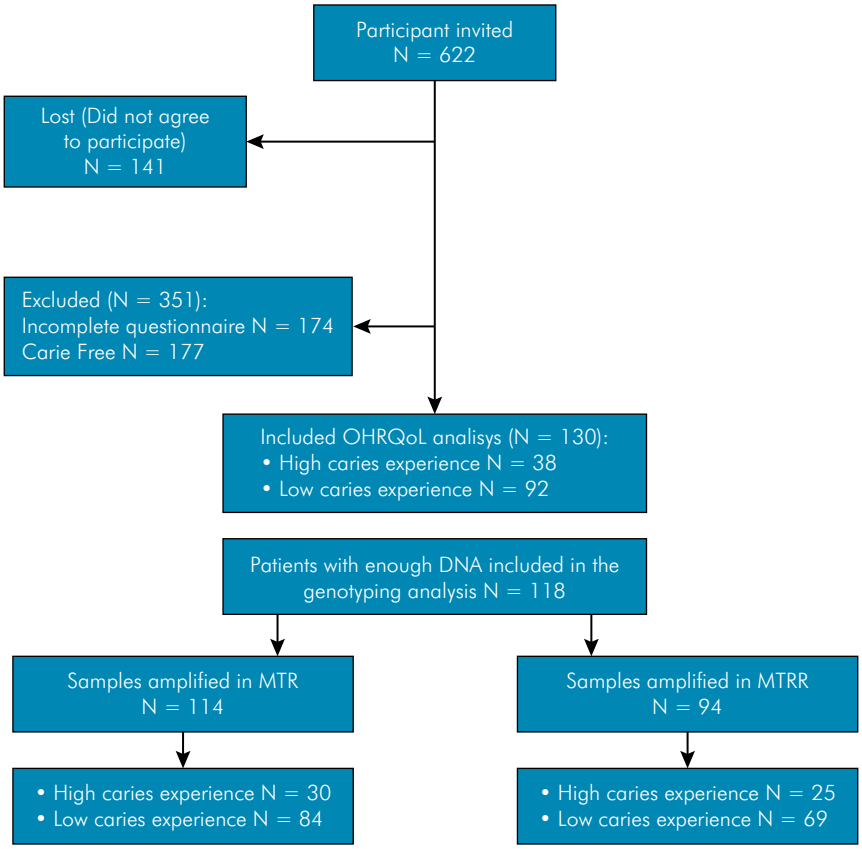

Figure. The flow chart summarizing patient selection and final sample.

Table 2. Association between level of caries experience and OHRQoL.

Variables	Caries experience groups				
Oral Health-related Quality of life (OHRQoL)	Low caries experience (n = 92)		High caries experience (n = 38)		p-value
	Median (Q1-Q3)	Mean (SD)	Median (Q1-Q3)	Mean (SD)	
Child Subscale	3.00 (1.00-6-00)	3.90 (3.79)	4.00 (2.00-7.00)	4.55 (3.15)	0.12
D1 – Symptoms	0.00 (0.00-0.00)	0.39 (0.79)	0.00 (0.00-1.00)	0.55 (0.89)	0.28
D2 – Function	2.00 (0.00-3.00)	1.89 (1.92)	2.50 (1.00-4.00)	2.86 (2.20)	0.01
D3 – Psychological	0.00 (0.00-2.00)	1.23 (1.80)	0.00 (0.00-2.00)	0.97 (1.30)	0.78
D4-Self-image/social interaction	0.00 (0.00-0.00)	0.38 (1.05)	0.00 (0.00-0.00)	0.15 (0.54)	0.30

Oral Health-Related Quality of Life (OHRQoL); Domain (D); low severity caries- active experience (white spot lesion ≥ 1 and/or dmf-t < 5); high severity of caries experience (component dmft ≥ 5); Q1 = percentile 25, Q3 = percentile 75; Mann-Whitney test, with significance level of 0.05; Bold indicates statistical significance.

The overall mean ECOHIS score recorded was 3.90 (standard deviation = 3.79) in the high-caries experience group and 4.55 (standard deviation = 3.15) in the low caries experience group, and the median scores were 3.00 (1–6) in the high-caries experience group and 4.00 (2–7) in the low caries experience group with no statistical significance difference (p = 0.129). Children with high-caries experience were found to have a significantly negative impact on OHRQoL in the functional domain (p=0.01). There was no significant difference between symptoms, psychological, self-image/social interaction domains, and dental caries experience. These results are presented in Table 2.

The genotypic distribution of each genotype was consistent with Hardy–Weinberg equilibrium proportions (data not shown). The associations between genotypes (in the dominant and recessive models) and domains (child subscale, symptoms, function, psychological, and self-image/social interaction) of QoL are summarized in Table 3. The *MTR* gene (rs1805087) was associated (p = 0.05) with the child subscale of the ECOHIS in the dominant model in the high-caries experience group. The *MTRR* gene (rs1801394) showed a borderline association (p = 0.08) with the functional domain of the ECOHIS in the recessive model in the high-caries experience group. There was no statistically significant association between each polymorphism in *MTR* and *MTRR* genes (dominant and recessive models) and the domains (function, psychological, self-image/social interaction) in children with caries experience (total group, low- and high-severity caries activities) (p > 0.05) (Table 3).

Discussion

The OHRQoL indicates a sense of well–being related to dissatisfaction with or satisfaction with life, which can be affected by health problems and individual purposes. Another factor is the capacity to socialize and have good relationships with peers.[29] Dental caries are widely associated with a negative impact on OHRQoL.[2–7,30] In this study, we assessed genetic polymorphisms in a population that had already demonstrated that dental caries affected OHRQoL in our previous study.[1–7] Severe untreated dental caries with clinical consequences negatively impacted the children's OHRQoL, regardless of toothache and socioeconomic factors.[3] The reasons for patients seeking dental treatment involve the symptoms domain and function domain such as difficulty in eating certain food items, drinking hot or cold beverages, and pronouncing some words.[7] In the present study, these conditions were also confirmed, and an impact was observed in the function domain, which is related to the child's ability to drink, eat, pronounce words, and even attend school. We believe that this domain is more easily affected in children with high–caries experience, as demonstrated in the literature.[1–7] We observed that

Table 3. Association between *MTR (rs1805087)*, *MTRR (rs1801394)* and OHRQoL in the group of children with low severity caries-active and high severity caries-active.

Variable		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
MTR (rs1805087)											
Total group caries-active (n = 114)											
Dominant model	GG + AG (n = 40)	2.00 (0.25–6.75)	0.33	0.00 (0.00–0.00)	0.13	2.00 (0.00–3.75)	0.62	0.00 (0.00–2.00)	0.312	0.00 (0.00–0.00)	0.58
	AA (n = 74)	4.00 (1.75–7.00)		0.00 (0.00–1.00)		2.00 (0.00–4.00)		0.00 (0.00–2.00)		0.00 (0.00–0.00)	
Recessive model	GG (n = 5)	1.00 (0.00–8.00)	0.39	0.00 (0.00–0.50)	0.66	0.00 (0.00–5.00)	0.66	0.00 (0.00–2.00)	0.458	0.00 (0.00–1.00)	0.67
	AA + AG (n = 109)	4.00 (1.00–7.00)		0.00 (0.00–1.00)		2.00 (0.00–4.00)		0.00 (0.00–2.00)		0.00 (0.00–0.00)	
Low severity caries–active (n = 84)											
Dominant model	GG + AG (n = 30)	2.50 (0.00–7.25)	0.82	0.00 (0.00–0.00)	0.35	2.00 (0.00–4.00)	0.98	0.00 (0.00–2.25)	0.702	0.00 (0.00–0.00)	0.55
	AA (n = 54)	3.00 (1.00–6.00)		0.00 (0.00–1.00)		2.00 (0.00–3.00)		0.00 (0.00–2.00)		0.00 (0.00–0.00)	
Recessive model	GG (n = 5)	1.00 (0.00–8.00)	0.50	0.00 (0.00–0.50)	0.77	0.00 (0.00–5.00)	0.80	0.00 (0.00–2.00)	0.477	0.00 (0.00–1.00)	0.74
	AA + AG (n = 79)	3.00 (1.00–6.00)		0.00 (0.00–0.00)		2.00 (0.00–3.00)		0.00 (0.00–2.00)		0.00 (0.00–0.00)	
High severity Caries–active (n = 30)											
Dominant model	GG + AG (n = 10)	2.00 (1.75–5.00)	0.05	0.00 (0.00–0.25)	0.17	2.00 (0.75–3.25)	0.31	0.00 (0.00–2.00)	0.152	0.00 (0.00–0.00)	1.00
	AA (n = 20)	4.50 (4.00–8.00)		0.00 (0.00–2.00)		4.00 (0.00–5.00)		1.50 (0.00–2.00)		0.00 (0.00–0.00)	
Recessive model*	GG (n = 0)	1.00 (0.00–8.00)	–	0.00 (0.00–0.50)	–	0.00 (0.00–5.00)	–	0.00 (0.00–2.00)	–	0.00 (0.00–1.00)	–
	AA + AG (n = 30)	4.00 (2.00–8.00)		0.00 (0.00–1.00)		2.50 (0.75–4.25)		0.00 (0.00–2.00)		0.00 (0.00–0.00)	
MTRR (rs1801394)											
Total group caries–active (n = 94)											
Dominant model	GG + AG (n = 61)	4.00 (2.00–7.00)	0.22	0.00 (0.00–0.00)	0.08	2.00 (0.00–4.00)	0.54	0.50 (0.00–2.00)	0.162	0.00 (0.00–0.00)	0.08
	AA (n = 33)	3.00 (1.00–5.50)		0.00 (0.00–1.00)		2.00 (0.00–3.50)		0.00 (0.00–2.00)		0.00 (0.00–0.00)	
Recessive model	GG (n = 12)	5.00 (2.00–7.00)	0.36	0.00 (0.00–0.00)	0.17	3.00 (1.00–4.00)	0.15	1.00 (0.00–3.00)	0.288	0.00 (0.00–0.00)	0.63
	AA + AG (n = 82)	4.00 (1.00–7.00)		0.00 (0.00–1.00)		2.00 (0.00–4.00)		0.00 (0.00–2.00)		0.00 (0.00–0.00)	

Continue

Continuation

Low severity caries–active (n = 69)										
Dominant model	GG + AG (n = 44)	0.36	0.00 (0.00–0.00)	0.23	2.00 (0.00–3.75)	0.82	1.00 (0.00–2.00)	0.167	0.00 (0.00–0.00)	0.14
	AA (n = 25)		0.00 (0.00–1.00)		2.00 (0.00–3.50)		0.00 (0.00–2.00)		0.00 (0.00–0.00)	
Recessive model	GG (n = 9)	0.81	0.00 (0.00–0.00)	0.32	2.00 (0.50–3.50)	0.86	2.00 (0.00–3.50)	0.231	0.00 (0.00–1.00)	0.55
	AA + AG (n = 60)		0.00 (0.00–0.75)		2.00 (0.00–3.75)		0.00 (0.00–2.00)		0.00 (0.00–0.00)	
High severity Caries–active (n = 25)										
Dominant model	GG + AG (n = 17)	0.39	0.00 (0.00–0.50)	0.25	3.00 (0.00–4.50)	0.51	0.00 (0.00–2.00)	0.790	0.00 (0.00–0.00)	0.32
	AA (n = 8)		0.50 (0.00–1.75)		2.00 (0.25–3.50)		0.00 (0.00–2.00)		0.00 (0.00–0.00)	
Recessive model	GG (n = 3)	0.33	0.00 (0.00–0.00)	0.83	4.00 (3.00–0.00)	0.08	0.00 (0.00–0.00)	0.503	0.00 (0.00–0.00)	0.59
	AA + AG (n = 22)		0.00 (0.00–1.25)		2.00 (0.00–4.00)		0.00 (0.00–2.00)		0.00 (0.00–0.00)	

Q1 = percentile 25, Q3 = percentile 75; Mann–Whitney test, with significance level of 0.05; Bold indicates statistical significance. *Mann–Whitney Test cannot be performed on recessive models' due empty groups

genetic polymorphisms in MTR presented results related to the impact of OHRQoL on the child and function domains.

In dentistry, some emerging studies have suggested that an individual's genetic background interferes with the perception of OHRQoL, providing evidence of the association between genetic polymorphisms and OHRQoL.[31–35] In individuals requiring orthognathic surgery, depression, temporomandibular disorders, and genetic polymorphisms in the *IL6* gene negatively impact OHRQoL.[32] Polymorphisms in the *ANKK1* gene are associated with a positive impact in women who undergo orthognathic surgery[31]. Individuals carrying the AA genotype for rs3800373 in *FKBP5* gene[34] reported more significant surgical discomfort associated with third molar surgery. Para–athletes who have experienced caries and possess the IL1A gene in a dominant model are significantly more likely to experience psychological discomfort than those with the alternate allele.[33] In this study, similar to a previous one that investigated the *TNF–α* gene as a biomarker for OHRQoL[35], we hypothesized that OHRQoL in patients with dental caries is influenced by the individual's genetic background. Only children with untreated dental caries were included since the main goal of the present study was to investigate the role of these genes in the OHRQoL of affected children. To the best of our knowledge, this is the first investigation of *MTR* (rs1805087) and *MTRR* (rs1801394) polymorphisms as biomarkers of OHRQoL. Our results support sample calculations for replicating these genes in new populations with different oral diseases.

The genes evaluated in the present study, *MTR* (methyltransferase 5 methyltetrahydrofolate–homocysteine) and *MTRR* (5–methyltetrahydrofolate–homocysteine methyltransferase reductase), are involved in the metabolism of vitamin B12 and folic acid at various stages of development.[20] The conversion of homocysteine into methionine is catalyzed by the MTR gene, which uses 5–methyltetrahydrofolate as a methyl and cobalamin (vitamin B 12) donor as a co–factor. During this reaction, methyl group transfer occasionally results in *MTR* inactivation. The *MTRR* gene catalyzes reductive methylation, which reactivates MTR. Thus,

MTRR can act as a key regulator of the conversion of homocysteine into methionine. Homocysteine metabolism begins with the ingestion of folic acid through the diet. Folic acid is rapidly reduced to its active tetrahydrofolate form, which is then converted into 5,10–methyltetrahydrofolate. It is then converted to 5–methyl tetrahydrofolate by the methyl tetrahydrofolate reductase enzyme encoded by the *MTHFR* gene, which plays a crucial role in this metabolic pathway. This substrate is essential for the metabolism of nucleic acids and amino acids, including those necessary for nucleotide synthesis, and consequently, cell division, a fundamental process in development. The products of this reaction are the methyl groups used to synthesize methionine, which is necessary for DNA methylation.[20] *MTR* encodes the 5–methyl tetrahydrofolate–homocysteine enzyme methyltransferase. This enzyme, also known as cobalamin–dependent, catalyzes the final step in methionine biosynthesis.[36] In the second step of this metabolic pathway, the enzyme methionine–synthetase, encoded by the *MTR* gene, catalyzes the remethylation of homocysteine methionine, necessary for producing S–adenosyl–methionine, the universal methyl donor. Vitamin B12 is a co–factor for methylation. Vitamin B12, over time, becomes oxidized, and the methionine synthase enzyme is inactivated. Functional regeneration of methionine synthase requires the participation of another enzyme, methionine synthase reductase, encoded by the *MTRR* gene.[20]

Deregulation of this metabolic process in human studies showed that altered levels, such as low folate levels and high levels of homocysteine, increased reactive stress and may lead to vascular diseases and neuropsychiatric disorders that lead to behavioral changes. Some studies in medicine have supported this change in the social relations of behavior.[37,38] In this context, it has been observed that genes potentially play a role in the etiology of behavioral science. According to Bouchard and Loehlin,[40] much research in social sciences is seriously compromised if the genetic variation is not incorporated into exploratory models. There is abundant empirical evidence that genetic factors influence virtually all human psychological traits to a significant degree.[39] The *MTR* and *MTRR* genes were selected in this study as they participate in the metabolism that regulates folate, such as MTHFR, which has been suggested by the GENEQoL Consortium as a biomarker that influences HRQoL generating reactive stress, which can lead to vascular diseases as well as neuropsychiatric disorders that leads to behavioral alteration.[12] Therefore, the scientific basis and the justification of the investigation, as stated in items 2 and 3 of the STREGA statement, were applied in this study.[21]

We observed an association between OHRQoL and the two investigated missense polymorphisms in *MTR*. The findings of this study have some limitations. The 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. The present work was an initial exploratory study, and many factors are known to influence the variations in QoL. Another limitation of this study is that it is one of the first studies to search for genetic markers involved in OHRQoL. This study aimed to assess children with dental caries in a single population. To the best of our knowledge, this is the first study to evaluate the association of genetic polymorphisms in *MTR* and *MTRR* with OHRQoL. Further studies should be conducted to strengthen this evidence. This finding attracted our attention to a new field of investigation, which includes the aspects involved in individual OHRQoL at the molecular level. New prospective studies are necessary to evaluate the impact of genetics on patients' OHRQoL by evaluating other biomarkers after dental caries treatment. Knowledge of molecular biology can bring new modalities of complementary diagnosis and treatment for these patients to balance these molecular mechanisms, maximizing the patient's well–being and QoL after treatment.

According to Antunes et al.[35], dentistry originally had a predominantly restorative orientation. Gradually, with the incorporation of the basic ideas and principles of preventive dentistry, the area has increasingly become holistic and incorporates an expanded view of the patient. Therefore, it is

necessary to evaluate individuals as a whole. The lack of consideration of socio–dental aspects can compromise patient acceptance, perceptions, and expectations regarding dental treatments. A holistic and expanded view of the patient is also necessary.[40] Research on this topic can increase the level of information and quality of the treatment of dental caries, enabling health professionals to diagnose patients showing a more negative response to OHRQoL early and thus enabling appropriate care. Further studies are needed to investigate genetics, molecular factors, and OHRQoL.

Acknowledgments

All procedures performed in study were in accordance with the ethical standards of the institutional research committee (CAAE nº 28768820.5.0000.5626/ protocol nº 3.939.452) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The work was supported by individual scholarships: FAPERJ – Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (E–26/202.712/2018) and CNPq–Conselho Nacional de Desenvolvimento Científico e Tecnológico.

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