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Genetic Association between the Risk of Dental Caries and *MTR* Gene Polymorphism in Chinese Children

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Early childhood caries (ECC) is common in children. Little is known about the genetic association of the methionine synthesis reductase (*MTRR)* gene rs1801394 and methionine synthetase (*MTR*) gene rs1805087 polymorphisms with ECC, which was examined in the Chinese Han population. Genotyping was performed using the buccal mucosa from 150 normal and 150 ECC children. For genotype and allele distribution comparison, Chi-square test and multiple logistic regression analysis were performed. The odd ratio (OR) and 95% confidence interval (CI) were calculated. *MTR* gene rs1805087 AG genotype distribution in the ECC group was clearly different from the control group (P = 0.029), and the ECC risk in cases with AG genotype was 0.525 times lower than those carrying AA genotype (95% CI = 0.292-0.942). Logistic regression analysis after adjustment for other clinical indicators determined that the *MTR* gene rs1805087 AG genotype was still strongly associated with susceptibility to ECC (OR = 0.499, 95% CI = 0.273-0.913, P = 0.024). Significant association was also seen for sugary food intakes (OR = 1.965, 95% CI = 1.162-3.321, P = 0.012), tooth brushing (OR = 0.569, 95% CI = 0.356-0.924, P = 0.023) and sex (OR = 0.562, 95% CI = 0.349-0.907, P = 0.018) with ECC risk. No notable genetic association was found between *MTRR* gene rs1801394 polymorphism and ECC risk. *MTR* gene rs1805087 polymorphism may aggrandize the susceptibility to ECC, and AA genotype appeared to be a dangerous element for the development of ECC.

Keywords: Chinese Han population; early childhood caries; genetic predisposition; *MTR* gene
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Introduction

Early childhood caries (ECC) is one of the most common diseases in children and adolescents worldwide (Seow 2018). Pain induced by ECC may affect the ability to chew, thus negatively affecting the patient's growth and immunity (Chen et al. 2021). Moreover, the occurrence of decayed baby teeth may increase the probability of decayed permanent teeth (Al-Zahrani et al. 2014). In China, the incidence of ECC is as high as 50-70% (Du et al. 2018). Poor diet and environmental factors are important causes of ECC (Alazmah 2017). Notably, the importance of heredity in ECC susceptibility has been widely presented (Soares et al. 2021). Up to now, multiple genes that are related to enamel formation, immune responses, saliva, and taste have been identified to be related to the ECC onset, such as *MBL2*, *ENAM*, and *DLX3* (Ohta et al. 2015; Borilova Linhartova et al. 2018; Mokhtari et al. 2019).

Genetic polymorphism refers to the variation or allele of inherited genes in the same population living in the same region at the molecular level of genes (Ellegren and Galtier 2016). In genetics, genetic polymorphisms make various hosts susceptible to different diseases and are associated with the effectiveness of treatment (Penes et al. 2017). Single nucleotide polymorphisms (SNPs) are DNA sequence polymorphisms caused by variation of a single nucleotide at the genomic level. It is one of the most common heritable variants in humans. Many phenotypic differences in the human body, and susceptibility to drugs or diseases are related to SNPs (Joob and Wiwanitkit 2018). In

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response to dental caries risk, a number of related SNPs have been identified. Methionine synthesis reductase (MTRR) and methionine synthetase (MTR or ETH) are key enzymes in the metabolic pathways of folic acid, homocysteine and vitamin B12 (Elizabeth et al. 2017). As previous studies reported, vitamin B12 levels are related to the onset onset of caries (Hugar et al. 2017). Besides, cases with reduced concentrations of folic acid are more prone to carriers (MacKeown et al. 2003). All evidence illustrates the eminent role of MTRR and MTR in caries. It is known that the gene polymorphisms can medicate the expression of MTRR and MTR, demonstrating the potential role of *MTRR* and *MTR* gene polymorphisms in caries (He et al. 2020). Given that the *MTRR* gene rs1801394 and *MTR* gene rs1805087 polymorphisms were introduced common polymorphisms in previous studies (Qu et al. 2016; Han et al. 2017), this study was conducted on these two polymorphisms. Interestingly, Antunes et al. (2017) have reported the genetic association of *MTRR* gene rs1801394 polymorphism with ECC susceptibility in Rio de Janeiro. Particularly, there was no evidence for the genetic association between enhanced risk of ECC and *MTRR* and *MTR* gene polymorphisms in the Chinese population.

The purpose of this study was to examine the correlation between *MTRR* gene rs1801394 and *MTR* gene rs1805087 polymorphisms and ECC susceptibility in the Chinese Han population, in order to provide a basis for the identification and prevention of ECC genetic susceptibility genes.

Materials and Methods

Study population

A total of 300 children undergoing oral and dental examination or treatment at Changsha Stomatological Hospital were recruited in the current study. The oral and dental examination was done via using oral and dental examination by two experienced examiners. ECC was defined as one or more decayed (cavitated or non-cavitated), missing tooth caused by caries, or filling tooth in children under 6 years old (Plutzer and Keirse 2012). According to the caries experience, all cases were divided into the healthy control (HC) group and ECC group, with 150 cases in each group.

The inclusion criteria were as follows: (1) Age 3-5 years old; (2) Chinese Han population that had no blood relationship; (3) No orthodontic appliances in the mouth. Exclusion criteria: (1) Patients with periodontal disease, mucosal disease and other oral diseases; (2) Patients with other systemic diseases; (3) History of antibiotic use within one month before specimen collection. This study protocols were designed under the approval of the Ethics Committee of Changsha Stomatological Hospital. Each participant or their parent was informed of the experimental design and signed informed consent to participate in the genetic analysis.

Questionnaire Application

Detailed information of all children was obtained from their parents, including age, sex, frequency of tooth brushing and sugary food intake, and eating habits at school. In addition, the parental education level was also recorded.

Sample collection

Buccal epithelial cells were obtained by repeated scraping of the buccal mucosa in the mouth using buccal swabs. Patients were asked not to eat or drink for 30 min prior to sampling. The samples were air-dried at room temperature and stored at −20℃.

DNA extraction and genotyping

Buccal epithelial cells were applied for the extraction of the genomic DNA through using buccal epithelial cells in accordance with the manufacturer's instructions. Then the isolated DNA was stored in the refrigerator at −20°C for later use.

Genotyping of *MTRR* gene rs1801394 and *MTR* gene rs1805087 polymorphisms was performed by polymerase chain reaction (PCR) and Sanger sequencing. Targeted fragments were amplified from extracted genomic DNA by PCR, and the PCR profile comprised a 5-min initial denaturation at 95℃, 34 cycles of denaturation at 95℃ for 30 s, at 58℃/60℃ annealing for 30 s, extension for 60 s at 72℃, and the final extension for 5 min at 72℃. The details of each primer pair and annealing temperature are summarized in Table 1. Then directing sequencing of each PCR product was performed using a sequencer ABI Prism 3100 Genetic Analyzer (Applied Biosystems, International Equipment Trading Ltd., Vernon Hills, IL, USA) by Sangon Biological Engineering Technology (Shanghai, China).

Statistical analysis

Under the application of SPSS 22.0 (SPSS Inc., Chicago, IL, USA) software, the statistical analysis was accomplished. Quantitative variables were shown as mean and standard deviation (SD), which were analyzed using student's t test. The distributions of sex, tooth brushing and eating habits were compared by the Chi-squared test between groups. Deviations from Hardy-Weinberg equilibrium (HWE) were evaluated using Chi-square test, and a value of $P > 0.05$ was considered as conforming the HWE. A multiple logistic regression analysis was performed to identify factors independently related to ECC risk via calculating the odd ratio (OR) and 95% confidence interval (CI). A P value less than 0.05 indicated a significant difference.

Results

Characteristics of study participants

Table 2 presents the demographic characteristics of the study participants in the two groups. Sex, frequency of tooth brushing and sugary intake, and regulatory eating at school revealed significant differences between the ECC and the control groups ($P < 0.05$). By extension, a high

Table 1. The sequences of forward and backward primers of *MTRR* gene rs1801394 and *MTR* gene rs1805087 polymorphisms.

SNP	Primer sequence	Annealing temperature
MTRR		
rs1801394	5′-CAGCAGGGACAGGCAAAGG-3′	58°C
	5′-AGATCTGCAGAAAATCCATGTACCAC-3′	
MTR		
rs1805087	5′-TGGCTATCTTGCATTTTCAGTTCC-3′	60°C
	5′-TCC AAAGCCTTTTACACTCCTCAAA-3′	

SNP, single nucleotide polymorphism.

Table 2. Comparison of demographic characteristics between the two groups.

Characteristics	HC (n = 150)	ECC (n = 150)	χ^2	*P* value
Sex			4.839	0.028*
Male	90 (60.00)	71 (47.33)		
Female	60 (40.00)	79 (52.67)		
Parental education level			1.080	0.299
Primary school and below	81 (54.00)	72 (48.00)		
Secondary school and above	69 (46.00)	78 (52.00)		
Frequency of tooth brushing			4.476	0.034*
≤ once/day	80 (53.33)	98 (65.33)		
> once/day	70 (46.67)	52 (34.67)		
Frequency of sugary food intake			5.079	0.024*
≤ once/week	55 (36.67)	37 (24.67)		
> once/week	95 (63.33)	113 (75.33)		
Regularly eat at school			4.348	0.037*
Yes	90 (60.00)	72 (48.00)		
No	60 (40.00)	78 (52.00)		

Data were expressed as number and percentage. * means a significant difference.
HC, healthy control; ECC, early childhood caries.

proportion of females were found in ECC group compared to the control group (P = 0.028). Similarly, cases in the ECC group had a low frequency of tooth brushing (P = 0.034) and a high frequency of sugary food intake (P = 0.024). In addition, a significantly small percentage of patients were able to eat regularly at school in the ECC group (P = 0.037). But there was no significant difference in the distribution of parental education level (P > 0.05).

Association of MTRR gene rs1801394 and MTR gene rs1805087 polymorphisms with ECC susceptibility

As displayed in Table 3, the results determined that the genotype frequencies of both *MTRR* gene rs1801394 and *MTR* gene rs1805087 polymorphisms conform to the HWE. Three genotypes of *MTRR* gene rs1801394 polymorphism were detected in both cases and control groups, in which the AA genotype was found most frequently, followed by the AG genotype and the GG genotype. However, there was no significant difference in the distribution of the three genotypes between the ECC and the control groups (P > 0.05). Similarly, the allele frequencies were also counted, and no significant differences were observed between the HC and ECC groups (P > 0.05). Thus, no notable genetic association was found between *MTRR* gene rs1801394 polymorphism and ECC risk.

For *MTR* gene rs1805087 polymorphism, only two genotypes were found in the study population, in which the AA genotype was the most prevalent, while the AG genotype was less common. The frequency distribution of *MTR* gene rs1805087 AG genotype in the ECC group was clearly different from the HC group (P = 0.029), and the ECC risk in cases with AG genotype was 0.525 times lower than those carrying AA genotype (95% CI = 0.292-0.942). Similar difference was also found in terms of allele distribution, and the G allele was found at a lower frequency in ECC patients compared to the HC group (P = 0.040). Taken together, *MTR* gene rs1805087 AA genotype appeared to be a dangerous element for the development of ECC.

Table 3. The genotype and allele frequencies of *MTRR* and *MTR* gene polymorphisms.

Genotype/Allele	HC (n = 150)	ECC (n = 150)	χ^2	OR (95% CI)	*P*
MTRR- rs1801394					
AA	87 (58.00)	97 (64.67)	-	1	-
AG	54 (36.00)	48 (32.00)	0.841	0.797 (0.491-1.294)	0.359
GG	9 (6.00)	5 (3.33)	1.506	0.498 (0.161-1.544)	0.220
A	228 (76.00)	242 (80.67)	-	1	-
G	72 (24.00)	58 (19.33)	1.925	0.759 (0.514-1.121)	0.165
P^{HWE}	0.872	0.750			
MTR-rs1805087					
AA	113 (75.33)	128 (85.33)	-	1	-
AG	37 (24.67)	22 (14.67)	4.747	0.525 (0.292-0.942)	0.029*
GG	-	-	-	-	-
A	263 (87.67)	278 (92.67)	-	1	-
G	37 (12.33)	22 (14.67)	4.229	0.563 (0.323-0.979)	0.040*
P^{HWE}	0.085	0.332			

Data were expressed as number and percentage. * means a significant difference.
HC, healthy controls; ECC, early childhood caries; OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

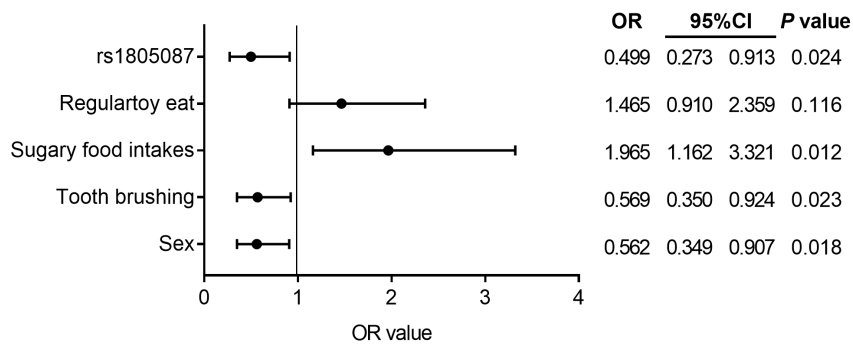

Fig. 1. The forest map of logistic regression analysis results.
Identification of markers independently related to early childhood caries (ECC) risk. OR, odds ratio; CI, confidence interval.

Identification of markers independently related to ECC risk via logistic regression analysis

A multiple logistic regression analysis was performed to identify factors independently related to ECC risk. Clinical factors that showed significant differences between ECC and HC groups in Table 2 were introduced into the multiple logistic regression model, which serves as independent variables. As displayed in Fig. 1, logistic regression analysis after adjustment for other clinical indicators determined that the *MTR* gene rs1805087 AG genotype was still strongly associated with susceptibility to ECC (OR = 0.499, 95% CI = 0.273-0.913, $P = 0.024$). In addition, significant association was also seen for sugary food intakes (OR = 1.965, 95% CI = 1.162-3.321, $P = 0.012$), tooth brushing (OR = 0.569, 95% CI = 0.350-0.924, $P = 0.023$) and sex (OR = 0.562, 95% CI = 0.349-0.907, $P = 0.018$) with ECC risk.

Discussion

Dental caries is a common disease in children and has been listed by the World Health Organization as one of the three priority infectious diseases (Mathur and Dhillon 2018). If not treated promptly, caries will cause persistent damage to the crown surface, leading to pulpitis, apicitis and other diseases, damaging the integrity of the masticatory organs, and then affecting the development of jaw bones and permanent teeth (Keels 2019). Therefore, the early prevention and treatment of caries is very important, which is of great significance for the subsequent bite and the normal development of the facial area. The etiology of ECC is complex. Molecular genetic studies have determined that ECC is probably caused by genetic variants such as gene SNPs (Alyousef et al. 2017). Previously, a number of SNPs have been identified to be related to ECC susceptibility, such as *KLK4* rs2235091, *MBL2* gene, *VDR* TaqI

(Alyousef et al. 2017; Sadeghi et al. 2021; Li et al. 2023). The present findings provide the first evidence that *MTR* gene rs1805087 polymorphism was significantly associated with susceptibility to ECC in the Chinese Han population. In terms of this polymorphism, AG genotype was closely associated with a decreased risk for the development of ECC.

As reported, poor oral hygiene behaviors and frequent consumption of sugary foods are the main causes related to the development of ECC (Bhatti et al. 2021). In the current study, 150 cases were enrolled in each group. It was observed that cases with frequent intake of sugary foods and tooth brushing less once every day were found in a large proportion of ECC patients. The findings were consistent with the previous evidence about the important role of oral hygiene behaviors and sugary food intake in the progress of ECC. Thus limiting the intake of sugary foods and developing good brushing habits are necessary to prevent ECC. Interestingly, AG genotype of *MTR* gene rs1805087 polymorphism was found only in a small proportion of ECC patients, indicating the important role of its genetic association with susceptibility to ECC. Moreover, logistic regression analysis after adjustment for other clinical indicators determined that the *MTR* gene rs1805087 AG genotype was still strongly associated with susceptibility to ECC. The findings provide strong evidence supporting the contribution of *MTR* gene rs1805087 polymorphism in the development of ECC. However, no notable genetic association was found between *MTRR* gene rs1801394 polymorphism and ECC risk. Notably, as Antunes et al. (2017) reported, *MTRR* gene rs1801394 polymorphism is identified to be a genetic risk factor for ECC susceptibility in Rio de Janeiro while *MTR* gene rs1805087 was not, which was inconsistent with our present results. The cause of this discrepancy is unknown, but we believe that *MTR* gene rs1805087 is worthy of further examination.

It is not clear how *MTR* gene rs1805087 polymorphism participates in the development of ECC. It is known that vitamin B12 (cobalamin) and folate can mediate the methylation cycle and the biosynthesis of DNA and RNA (Froese et al. 2019). The metabolic disturbance of Vitamin B12 and folate is associated with the incidence of caries (Hugar et al. 2017). MTR is responsible for the production of tetrahydrofolate and methionine, using for the ucleic acid synthesis and methylation reactions (Li et al. 2017). MTRR takes charge of the intracellular flow of folate via converting 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate (Antunes et al. 2023). SNPs that correlate to the metabolism of vitamin B12 and folate have been identified, including *MTRR* gene rs1801394 and *MTR* gene rs1805087 polymorphisms (Antunes et al. 2017). Rs1805087 is a common polymorphism in *MTR* gene, which can promote the enzymatic activity of MTR through facilitating the synthesis of homocysteine and the methylation of DNA (Goode et al. 2004). Based on the current results, rs1805087 was determined to be a valuable SNP in relation to ECC risk, and may take part in DNA methylation and the folate metabolic pathway indirectly through enhancing MTR expression. Nonetheless, this is a single-center study with small sample size, limiting statistical analysis, which limits the generalizability of the results. Therefore, the reported conclusions require further validation in a larger cohort.

In conclusion, the present case-control study determined that *MTR* gene rs1805087 polymorphism may aggrandize the susceptibility to ECC. Further studies with larger sample sizes or from diverse ethnicities are needed to validate the present findings.

Author Contributions

F.L. made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data, and draft of the manuscript. W.L.D., L.Z.L. and S. S.X. revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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