ORIGINAL RESEARCH

Association between *rs144070672* and Early Childhood Caries: Case–Control Study

Aruna Sharma

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Abstract

Introduction: Genetic factors influence the susceptibility to early childhood caries. This study aims to evaluate the association between *rs144070672* and early childhood caries

Methods: Two hundred and forty-eight 3–6-year-old children were recruited into the study, of which 124 children were with early childhood caries and 124 children were without early childhood caries. DNA isolation was done from venous blood and polymerase chain reaction was done. These products were sequenced and the genotypes were analyzed.

Results: The samples did not reveal any mutant homozygous genotype differentiation. The odds ratio for the heterozygous genotype was 0.495 with 95% confidence intervals being (0.044–5.54) and a *p*-value of 0.561.

Conclusion: Single nucleotide polymorphism *rs144070672* does not increase susceptibility to caries in the studied population.

Keywords: Caries, Oral health, Pediatric dentistry.

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Introduction

Early childhood caries (ECC) is one of the most common, chronic conditions affecting children. ECC has an adverse effect on children, affecting their growth, development, behavior, and school performance.[1] Both environmental factors and genetic factors influence the susceptibility to ECC. Genes regulating enamel development, immune response, glucose metabolism, taste preferences, and salivary composition and flow have been reported to influence the susceptibility to ECC.[2]

Single nucleotide polymorphisms (SNPs) are the most common variations in the human genome and are characterized by change in a single nucleotide base at a specific position across the human genome. Single nucleotide polymorphisms of AMELX gene such as *rs17878486*, *rs946252*, and *rs4106416* were analyzed for increased susceptibility to ECC across various populations.[3–8] Variations of *AMBN* and *TUFT1* gene were evaluated for predisposition to caries, albeit with inconsistent results. Genetic polymorphism *rs1784418* of MMP20 gene and *rs2232091* of KLK4 gene was assessed for altered ECC susceptibility as these genes are known to play an important role in maturation of enamel.[9,10] Enamelin is one of the largest enamel matrix proteins encoded by ENAM gene located at 4q13.3. Chaussain et al.[11] suggested that variations *rs7671281* and *rs3796704* in the exon 10 region of ENAM gene were associated with increased susceptibility to acidic demineralization, in contrast to the results of Gerreth et al.[12] who did not report significant association between these SNPs and ECC. CT genotype of *rs3796703* increased the caries susceptibility in Chinese cohort[13] but did not display a significant association in Japanese cohort.[4] Systematic review conducted by Ganesh et al.[14] revealed that prevalence of ECC in India is approximately 49.6%. Hence, this study aimed to analyze the association between SNP*rs144070672* in the exon10 region of ENAM gene and ECC.

Department of Pedodontics and Preventive Dentistry, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India

Corresponding Author: Aruna Sharma, Department of Pedodontics and Preventive Dentistry, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India, Phone: +91 9444364251, e-mail: arunapatri@gmail.com

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Methods

Study Design: Case–Control Study

Sample Size

With the significance set at 5% and power at 80% and 2.3 as the estimated odds ratio, the calculated sample size in the cases and control groups was 124. The study was explained to the parents/guardians and 248 3–6-year-old healthy children without any systemic disease and whose parents gave a written informed consent were recruited into the study. A thorough intraoral examination was conducted and carious status was scored as per the ICDASII criteria. Children with ICDAS scores of 1–6 constituted the caries group and children with an ICDAS score of 0 constituted the control group. One milliliter of venous blood was collected for the purpose of DNA isolation from all the subjects recruited in to the study. The DNA isolation was done as per the manufacturer's instructions (Qiagen, Germany) and the extracted DNA was subjected to a polymerase chain reaction (PCR) as per the conditions described by Chaussain et al.[11] The PCR product was then sequenced to generate genotype data which were analyzed

Table 1: Demographic characteristics and genotype and allele frequencies of rs144070672

	Cases (n = 124)	*Controls (n = 124)*	*Odds ratio*	*95% CI*	*p-value*
Age (years)					
3	23	26	Ref.		
4	28	22	1.272	0.69–2.34	0.438
5	63	68	0.926	0.60–1.41	0.724
6	10	8	1.25	0.627–3.27	0.649
Genotype frequency					
CC	122	123	Ref		
CA	2	1	0.495	0.044–5.54	0.561
AA	0	0	–	–	–
Allele frequency					
C	246	247	Ref		
A	2	1	0.498	0.045–5.52	0.562

using the sequencing scanner software. These data were later subjected to statistical analysis.

Results

The demographic data of the participants are shown in Table 1. The number of 3-year-old children was 23 and 26, respectively, for cases and controls. The number of 4-year-old children was 28 and 22, respectively, for cases and controls. Sixty-three children in the cases group were aged 5 years whereas sixty- eight children in the control group were 5 years old; ten children in the cases group and eight children in the control group were 6 years old. The difference in the age between children with caries and children without caries was not significant ($p > 0.05$). Analysis of sequencing data did not reveal any statistically significant differences between the genotype frequencies in the cases and control groups. The homozygous CC genotype frequency was 122 in the cases group and 123 in the control group. The heterozygous CA was found in two children with ECC and in a single child without caries. The homozygous AA genotype was not differentiated in the analyzed population.

Discussion

Amelogenesis is under genetic control and polymorphisms of genes regulating enamel formation can influence the susceptibility to ECC. Association between genetic variations of ENAM gene and carious destruction of teeth was evaluated by various authors across populations with variable results.[3,4,6,8,11–13] These studies have suggested that ENAM is a candidate gene for dental caries.

ENAM gene encodes for the largest and the least abundant enamel matrix protein, enamelin. The enamelin protein when secreted is approximately about 186 KDa and is mainly found on the outer surface of enamel. This protein undergoes post-translational proteolysis resulting in the formation of 32KDa protein, which is generally found in the deeper layers of enamel.[15] The main functions of enamelin protein are it acts as a regulator for mineral deposition and also aids in the growth of hydroxyapatite crystallites.[16] Hence any variations of this ENAM gene can alter the enamelin protein and thus have a propensity to affect the mineral deposition of the secreted enamel, thereby increasing the susceptibility to ECC.[17] The exon10 of ENAM gene is one of the largest exons and as mentioned earlier, several polymorphisms in this region of ENAM gene were evaluated for association to carious destruction. However, none of the studies have analyzed the SNP*rs144070672* for association to ECC.

The polymorphism *rs144070672* is characterized by change in the nucleotide sequence from cytosine to adenine. This is a mis-sense mutation and can result in the change of amino acid from alanine to aspartic acid at the 772 position and the clinical significance of this mutation is uncertain. However, it has been postulated that this SNP may play a role in amelogenesis imperfecta. It is important to observe that the minor allele frequency is very less and there is no homozygous AA differentiation in the analyzed population. This could be because this is a highly conserved region of the ENAM gene and so changes rarely happen and if such a change or substitution occurs, then the quality of enamel formed is highly undermined.

The main strength of this study is that this is the first study to analyze the association between *rs144070672* and ECC. Though the study did not reveal any significant results lack of differentiation of the mutant homozygous genotype indicates that this is a highly conserved region across various mammals. Studies with larger sample sizes needed to understand the true association between this SNP and ECC. Hence, to conclude, it can be stated that ENAM is a candidate gene for dental caries and polymorphisms of this gene can affect the susceptibility to acidic demineralization. Studies with larger sample sizes across populations will aid in understanding the true association between SNPs of ENAM gene and ECC.

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