Original article

Association of polymorphisms in genes involved in enamel formation, taste preference and immune response with early childhood caries in Saudi pre-school children

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ABSTRACT

Dental caries is primarily elicited by modifiable factors such as inadequate oral hygiene, poor dietary practices and deficient fluoride exposure. However, there is a growing body of evidence suggesting the profound influence of genetic factors in dental caries susceptibility. The present study aimed to evaluate the association between single nucleotide polymorphisms (SNPs) in *ENAM* (rs12640848), *MMP20* (rs1784418), *TAS2R38* (rs713598), and *LTF* (rs4547741) genes and early childhood caries (ECC) in Saudi preschool children. This case-control study enrolled 360 Saudi preschool children (262 with ECC and 98 caries-free). Data on environmental factors were collected through a questionnaire. However, caries experience and oral hygiene data were obtained during clinical examination. Buccal swab samples were collected for DNA extraction and SNPs were genotyped using PCR and DNA sequencing. Children with ECC were compared to caries free children (control), then they were categorized into two categories based on ECC severity as follows; non-severe ECC (NS-ECC), and severe-ECC (S-ECC). Association between the SNPs, ECC, NS-ECC, and S-ECC was reported as an odds ratio (OR) with a 95% confidence interval (CI). The majority of the children (72.8%) exhibited ECC (31.7% NS-ECC and 41.1% S-ECC) with mean dmft of 4.20 ± 4.05. Multivariate analyses of environmental factors showed that nocturnal feeding was a risk factor for ECC ($P = 0.008$). Poor oral hygiene was also a risk factor for both NS-ECC and S-ECC (ECC: $P < 0.0001$, NS-ECC: $P = 0.032$ and S-ECC: $P < 0.0001$). Univariate analysis showed that the AG genotype of rs1784418 of *MMP20* gene was protective against ECC (OR = 0.532; 95% CI = 0.316–0.897, $P = 0.018$) and against NS-ECC (OR = 0.436; 95% CI = 0.238–0.798, $P = 0.007$). When environmental risk factors for ECC were included as covariates during multivariate analysis, AG variant in rs1784418 of *MMP20* gene remained less frequent in NS-ECC cases compared to controls with borderline significance (OR = 0.542; 95% CI = 0.285–1.033, $P = 0.063$). Our findings concluded that *MMP20* rs1784418 SNP might be associated with protection against ECC in Saudi preschool children.

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1. Introduction

Dental caries is a complex, multifactorial, chronic, and an enormously prevailing illness both in developing and industrialized nations and it remains the most predominant disease of childhood (Gerreth et al., 2017; Petersen, 2003). Early childhood caries (ECC) as defined by the American Academy of Pediatric Dentistry (AAPD) is *"the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries) or filled tooth surfaces in any primary tooth in a child under the age of six"*. Furthermore, it is considered severe early childhood caries (S-ECC) when there is *"any sign of*

smooth surface caries in a child younger than 3 years of age and one or more decayed, missing or filled (dmf) smooth tooth surfaces in primary maxillary anterior teeth from 3 to 5 years or a dmf surface score more than or equal to four at age 3 years, five at age 4 years and greater than or equal to six at age 5 years" (Policy on Early Childhood Caries (ECC): Classifications, 2017).

Dental caries can be considered as a dysbiotic disease, occurring when there is an unfavorable shift in the demineralization and remineralization balance of the tooth structure (Divaris, 2016; Featherstone, 2008). Causes of this shift vary from individual to individual, hence, the best predictor for caries is past caries experience (Vieira et al., 2014). Distal risk factors such as socioeconomics, education, and access to dental care, are causes of population-based incidence, while proximal factors, like diet, oral hygiene (OH), past dental caries experience, fluoride exposure and genetics, are known to influence individual cases (Divaris, 2016).

Several studies have documented the impact of genetic variations on diseases associated with dental formation. For example, genes associated with enamel formation such as amelogenin (*AMELX*) (Gerreth et al., 2017; Piekoszewska-Zietek et al., 2017), enamelin (*ENAM*) (Abbasoglu et al., 2015; Gerreth et al., 2016), matrix metalloproteinase 20 (*MMP20*) (Antunes et al., 2016; Filho et al., 2017) and tuftelin (*TUFT1*) (Abbasoglu et al., 2015) have been linked to impact the risk of dental caries in children. Moreover, expression of genes that possibly modulate taste and dietary habits, salivary formation and immune responses in dental caries, such as aquaporin 5 (*AQP5*) (Piekoszewska-Zietek et al., 2017), lactoferrin (*LTF*) (Abbasoglu et al., 2015; Azevedo et al., 2010) and taste receptor 2 member 38 (*TAS2R38*) (Wendell et al., 2010), have also been shown to correlate with dental diseases. Additionally, it has been documented in genome-wide association studies (GWAS) that other genetic loci might influence risk of dental caries (Vieira et al., 2014).

The tooth enamel development is an intricate interplay of several genetic and chemical processes. This includes enamel matrix deposition, mineralization and maturation. *ENAM* gene is essential for mineralization and microstructural development and organization of tooth enamel (Vieira et al., 2014). A previous study reported a statistically significant association between single nucleotide polymorphisms (SNPs) in the *ENAM* gene and dental caries in Polish children (Gerreth et al., 2016). Another study also reported rs3796703 variant of *ENAM* gene to be associated with dental caries risk in children aged < 4 years in a Chinese population (Wang et al., 2017). In addition, a possible association has been suggested between *ENAM* variants and the prevalence of *Streptococcus mutans* infection, which is the main pathogen in dental caries (Patir et al., 2008). Also, *MMP20* gene is required for the maturation process of the enamel (Vieira et al., 2014). Several studies have demonstrated contrasting evidence of *MMP20* association with dental caries (Abbasoglu et al., 2015; Antunes et al., 2016; Filho et al., 2017; Tannure et al., 2012). The results ranged from no association between MMP20 variant rs1784418 and dental caries (Abbasoglu et al., 2015) to a significant risk of dental caries with the same *MMP20* gene variant (Antunes et al., 2016; Tannure et al., 2012). However, a meta analysis has reported a protective role of *MMP20* variant rs1784418 C > T against dental caries (Filho et al., 2017).

TAS2R38 gene encodes a receptor that facilitates bitter taste sensing thus controling dietary habits via taste (Vieira et al., 2014; Wendell et al., 2010). A significant link between SNPs in *TAS2R38* gene and dental caries protection was demonstrated in primary dentition (Wendell et al., 2010). Also, lactoferrin is an iron-binding glycoprotein, encoded by *LTF* gene, that possesses broad-spectrum antimicrobial, immunoreactive and anti-inflammatory properties (Vieira et al., 2014; Wang et al., 2017). A

protective effect of rs4547741 variant of *LTF* gene was suggested previously (Abbasoglu et al., 2015), however, others reported a lack of significant association between rs1126478 and rs1126477 variants of *LTF* gene and the development of dental caries (Wang et al., 2017; Wang and Qin, 2018).

All above studies have been carried out outside of Saudi Arabia and the lack of enough studies in the country with rise in prevalence in dental carries among children (Al-Meedani and Al-Dlaigan, 2016) indicates more studies are needed to investigate the genetic markers in Saudi preschool children (Alyousef et al., 2017). In addition, most of the exisiting genetic studies have incomplete information concerning environmental factors that should be included as covariates in the genetics analysis. Therefore, the present study aimed to evaluate the genotypic frequencies of SNPs in *ENAM* (rs12640848), *MMP20* (rs1784418), *TAS2R38* (rs713598) and *LTF* (rs4547741) genes and to demonstrate their association with dental caries in preschool children in Saudi Arabia. Also, this study aimed to evaluate the interaction between such genetic variations and the surrounding environmental factors.

2. Material and methods

2.1. Subjects

In this case-control study, Saudi healthy, unrelated preschool children with complete primary dentition and no dental anomalies were recruited from preschools in Riyadh city. Demographic data related to age, andgender, socioeconomics, medical and dental history, infant feeding history, and current dietary habits were documented via questionnaire upon inclusion in the study. During the school visit, height and weight were taken to calculate the body mass index (BMI). Intraoral examination was performed while the child was seated in a supine position under a portable light, using disposable examination instruments. The caries status of the teeth was scored according to the modified criteria of the World Health Organization (WHO) using the index of "decayed-missing and-filled teeth/surfaces" (dmft/dmfs) for the primary teeth without using radiographs (Organization, 2013). Oral hygiene (OH) was recorded according to (James et al., 1960), and the plaque index (PI) according to (Loe, 1967). PI was divided into two groups (low: ≤ 1, high: >1). To determine the intra-examiner reliability, the examiner re-examined 10% of randomly selected children for dmft/s scoring after 2–3 weeks and Cronbach's Alpha was 0.961.

Children with ECC were compared to caries free children (control), then were categorized into two categories based on ECC severity as follows; non-severe ECC (NS-ECC), and severe-ECC (S-ECC), according to the AAPD definition (Policy on Early Childhood Caries (ECC): Classifications, 2017).

2.2. Ethical declaration

This research study was approved by the Institutional Review Board (IRB) at King Saud University (KSU), Committee for Medical Research and was registered with the College of Dentistry Research Center (CDRC), KSU. In addition, the study was approved by the Ethical Review Committee (ERC) of King Faisal Specialist Hospital and Research Center (KFSH&RC), Riyadh, Saudi Arabia. The study followed the ethical guidelines proposed in the 1975 Declaration of Helsinki. Since the study involved children, their legal guardians were briefed and clarified about the objectives of the study and informed consents were obtained prior to enrollment. All the patient related data including biological samples were anonymized to ensure confidentiality.

2.3. Sample size calculation

Using a power of 0.95 at $\alpha = 0.05$, and effect size of 0.1, at least 246 children should be included and divided into 3 groups according to caries severity. Hence, each group had at least 82 preschool children. Based on the reported prevalence of dental caries in preschool children of Riyadh (approximately 70–75%) (Al-Meedani and Al-Dlaigan, 2016; Wyne et al., 2008), 300 preschool children need to be examined in order to get 25–30% who are caries free. Giving allowance of 15–25%, 345–375 children should be examined in total.

2.4. Genotyping of ENAM, MMP20, TAS2R38 and LTF gene SNPs

Buccal swab samples were collected and mixed with phosphate buffered saline (PBS). The solution was then instantly utilized to extract genomic DNA using the OmniSwab Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Thereafter, extracted DNA was quantified through a NanoDrop™ 8000 Spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). Primers used for the amplification of the regions surrounding the SNPs (Supplementary Table 1) were designed using Primer 3 Browser, version 4.1.0 (https://primer3.ut.ee/) and synthesized at the Oligo Synthesis Core Facility of KFSH&RC. Amplification was carried out using GoTaq® green master mix (Promega, Madison, WI, USA), 50 nM for each forward and reverse primers and 3 µl (20 ng/µl) of DNA extract with a total volume of 40 µl. The cycling parameters were 5 min at 95 °C for initial denaturation and 45 cycles at 95 °C/1 min, 60 °C/2 min, and 72 °C/2 min with a final extension at 72 °C/10 min.

The amplified product was run in 2% agarose gel (Sigma-Aldrich, St. Louis, MA, USA) and viewed under ultraviolet in a gel documentation system (Bio-Rad Laboratories, Hercules, CA, USA). Amplicons were gel extracted using QIAquick Gel Extraction Kit (Qiagen). Ten microliters (10 µl) PCR products tagged with M13 were sequenced using dideoxy Sanger Sequencing method. Sequence analyses were performed using DNA Star Lasergene Software (Lasergene, Madison, WI, USA).

2.5. Statistical analysis

Statistical package for social sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA) was used to perform data entry and analysis. Univariate analysis was conducted to determine associations of socio-demographic variables, medical variables, infant feeding practices, dietary habits, dental and OH variables to ECC, NS-ECC and S-ECC and was represented as odds ratio (OR) with 95% confidence interval (CI). The variables with P-values < 0.05 in the univariate analyses were included in regression models to determine the environmental ECC risk factors. Regression analysis of each genetic marker was performed and the strength of association between the studied SNPs and ECC was represented as odds ratio (OR) with 95% confidence interval (CI). The four SNPs were evaluated for the Hardy-Weinberg equilibrium (HWE) using HaploView software version 4.2 (Broad Institute, Cambridge, MA, USA). The environmental factors identified as possible risk factors for ECC were included as covariates during the multivariate analyses to detect gene-environment interactions. P- values of < 0.05 were considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics of studied children

The demographic and clinical data of the participants in the present study are shown in Table 1. In this study, genetic variants of

ENAM, MMP20, TAS2R38 and LTF genes were evaluated in a total of 360 preschool children with a mean age of 61 ± 8.1 months. About 56% of the children were female and 59.2% of children were from public schools. Only 27.2% of the studied children were caries-free and the majority of the children (72.8%) exhibited ECC in which varying degrees of severity were observed (31.7% with NS-ECC and 41.1% with S-ECC) with mean dmft of 4.20 ± 4.05. Only 27.2% of the children had low PI and only 20.6% of the children showed good OH.

3.2. Multiple logistic regression analysis of environmental factors in ECC children as compared to caries free children

Several factors, including the area of the preschool, history of a previuos dental visit, history of infant feeding [nocturnal feeding (bottle/breast milk), having sugary drinks in the bottle at night], PI and OH, were considered as confounding factors for the development of ECC and were included in this analysis. The results demonstrated that children with no reported dental visit (OR = 0.367; CI = 0.204–0.662, and $P = 0.001$), no history of nocturnal feeding (OR = 0.445; CI = 0.244–0.813, and $P = 0.008$) and good OH (OR = 0.345; CI = 0.230–0.519 and $P < 0.0001$) were significantly less associated with ECC (Table 2).

3.3. Genotypic analysis of SNPs of ENAM, MMP20, TAS2R38 and LTF in ECC children versus caries free children

A total of four genes, with one SNP for each gene, were analyzed for association with dental caries in the study population. These were rs12640848, rs1784418, rs713598 and rs4547741 in ENAM, MMP20, TAS2R38 and LTF genes, respectively.

When children where dichotomized into ECC cases and controls (caries free), the heterozygous AG genotype of rs1784418 of MMP20 was protective against ECC (OR = 0.532; 95% CI = 0.316–0.897, and $P = 0.018$) (Table 3). A multivariate analysis of these SNPs after adjusting for OH, history of dental visit, and history of nocturnal feeding indicated that none of the SNPs showed any significance in frequency and distribution in these two groups (Table 4).

3.4. Multinomial logistic regression analysis of environmental factors in NS-ECC and S-ECC children as compared to caries free children

ECC children were classified into NS-ECC and S-ECC groups. Several variables were considered and included in the analysis to assess their contribution to ECC susceptibility and severity (Table 5). Findings showed that children with no history of a pre-

Table 1
Demographics and clinical data of studied children

	Categories	No. (%)	Mean ± SD
Age groups	36–47 months	26 (7.2%)	
	48–59 months	90 (25%)	61 months ± 8.1
	60–71 months	244 (67.8%)	
Gender	Male	159 (44.2%)	–
	Female	201(55.8%)	
Pre-school	Public	213 (59.2%)	–
	Private	147 (40.8%)	
Caries	Caries free	98 (27.2%)	
	NS-ECC	114 (31.7%)	dmft 4.20 ± 4.05
	S-ECC	148 (41.1%)	
Plaque Index (PI)	Low: ≤1	98 (27.2%)	–
	High: >1	262 (72.8%)	
Oral Hygiene (OH)	Poor	104 (28.9%)	–
	Fair	182 (50.6%)	
	Good	74 (20.6%)	

*NS-ECC: Non-Severe Early Childhood Caries, S-ECC: Severe Early Childhood Caries, SD: Standard Deviation, dmft: decayed missing and filled teeth (primary).

Table 2
Multiple logistic regression analysis of environmental factors among ECC children when compared to caries free children.

Factor	Level	Reference	OR	95% C.I.	P value
Pre-school area	West	Central	0.915	(0.759–1.104)	0.353
History of dental visit	No	Yes	0.367	(0.204–0.662)	**0.001**
Nocturnal feeding (bottle/breast milk) at infancy	No	Yes	0.445	(0.244–0.813)	**0.008**
Sugary drinks in bottle at night at infancy	No	Yes	0.226	(0.395–1.246)	0.226
Plaque Index (PI)	High	Low	1.282	(0.705–2.333)	0.416
Oral Hygiene (OH)	Good	Poor	0.345	(0.230–0.519)	**<0.0001**

Note: Pre-School Area (Central, East, North, South, West), PI (low: ≤1, high: >1) OH (poor, fair, good).

Table 3
Univariate analyses of genotypes among ECC children when compared to caries free children.

Gene	Genetic marker	Genotype	Univariate analysis		
			OR	95% C.I.	P value
MMP20	rs1784418	AA	Ref		
		AG	0.532	(0.316–0.897)	**0.018**
		GG	0.863	(0.417–1.785)	0.691
ENAM	rs12640848	AA	Ref		
		AG	0.98	(0.565–1.700)	0.943
		GG	1.568	(0.790–3.112)	0.198
TAS2R38	rs713598	GG	Ref		
		CG	0.686	(0.409–1.152)	0.154
		CC	1.12	(0.524–2.394)	0.77
LTF	rs4547741	CC	Ref		
		CT	0.96	(0.493–1.868)	0.904
		TT	0.242	(0.04–1.475)	0.124

Table 4
Multivariate analyses of genotypes among ECC children when compared to caries free children.

Gene	Genetic marker	Genotype	Multivariate analysis		
			OR	95% C.I.	P value
MMP20	rs1784418	AA	Ref		
		AG	0.606	(0.342–1.074)	0.086
		GG	1.039	(0.465–2.324)	0.925
ENAM	rs12640848	AA	Ref		
		AG	1.019	(0.554–1.873)	0.952
		GG	1.54	(0.726–3.266)	0.26
TAS2R38	rs713598	GG	Ref		
		CG	0.777	(0.440–1.371)	0.384
		CC	1.264	(0.545–2.928)	0.585
LTF	rs4547741	CC	Ref		
		CT	0.669	(0.312–1.434)	0.301
		TT	0.283	(0.039–2.048)	0.211

*The analyses were adjusted for history of dental visit, nocturnal feeding (bottle/breast milk) at infancy and Oral Hygiene (OH).

viuos dental visit and good OH were significantly less likely to have NS-ECC (OR = 0.494; CI = 0.251–0.971, and P = 0.041) and (OR = 0.595; CI = 0.371–0.956, and P = 0.032) respectively, and were significantly less likely to have S-ECC (OR = 0.406; CI = 0.205–0.805, and P = 0.01) and (OR = 0.247; CI = 0.149–0.407, and P < 0.0001) respectively. In addition children with history of higher daily feeding frequency as an infant (≥7 times /day) were significantly more likley to have S-ECC (OR = 2.15; CI = 1.102–4.191, and P = 0.025).

3.5. Genotypic analysis of SNPs of ENAM, MMP20, TAS2R38 and LTF in NS-ECC and S-ECC children versus caries free children

When the children with dental caries were clustered based on ECC severity, upon comparison with caries free children, results showed significant differences for one of the four evaluated SNPs. The heterozygous AG genotype of the rs1784418 marker of *MMP20* gene was less frequent in the NS-ECC cohort compared to caries free controls (OR = 0.436; 95% CI = 0.238–0.798, and P = 0.007) (Table 6). Similarly, the CG heterozygous genotype of

the rs713598 for *TAS2R38* gene, was also less frequent in the S-ECC cohort compared with the controls with border line significance (OR = 0.582; 95% CI = 0.329–1. 028, and P = 0.062). When all four SNPs were compared in the NS-ECC and S-ECC children for differences in genotype distribution after adjusting for history of dental visit, history of infant daily feeding frequency and OH, no statistically significant differences were observed (Table 7). However, AG variant in rs1784418 of *MMP20* gene remained less frequent in NS-ECC cases compared to controls with borderline significance (OR = 0.542; 95% CI = 0.285–1.033, and P = 0.063).

4. Discussion

Factors such as tooth morphology, buffering ability, salivary flow, dietary habits, oral microbiome, OH and past history of dental caries clearly play an important part in development of carious lesions (Alyousef et al., 2017; Kang et al., 2011; Vieira et al., 2014). Nonetheless, there is indeed a convincing evidence that host genetic background may drive the predisposition of an individual

Table 5

Multiple logistic regression analysis of environmental factors among NS-ECC and S-ECC children when compared to caries free children.

Factor	Level	Reference	NS-ECC			S-ECC		
			OR	95% C.I.	P value	OR	95% C.I.	P value
Age Groups	60–71 months	36–47 months	1.391	(0.873–2.218)	0.165	1.505	(0.924–2.449)	1
Pre- school Area	West	Central	0.909	(0.730–1.132)	0.395	0.929	(0.743–1.161)	0.519
Preterm Birth	No	Yes	0.392	(0.136–1.134)	0.084	0.491	(0.166–1.453)	0.199
Frequency of Brushing	One or more	No/irregular	0.857	(0.455–1.614)	0.633	0.59	(0.311–1.122)	0.108
History of Dental Visit	No	Yes	0.494	(0.251–0.971)	**0.041**	0.406	(0.205–0.805)	**0.01**
Daily feeding frequency (bottle/breast milk) at infancy	7 or more	<7/day	1.128	(0.576–2.205)	0.726	2.15	(1.102–4.191)	**0.025**
Nocturnal feeding (bottle/breast milk) at infancy	No	Yes	0.596	(0.285–1.244)	0.168	0.534	(0.244–1.166)	0.155
Sugary drinks in bottle at night at infancy	No	Yes	0.928	(0.473–1.822)	0.829	0.6	(0.304–1.183)	0.14
Current consumption of Sweets & Soft Drinks between Meals	>1/day	No	1.248	(0.827–1.884)	0.291	1.197	(0.781–1.836)	0.409
Plaque Index (PI)	High	Low	1.269	(0.634–2.541)	0.502	1.469	(0.703–3.071)	0.306
Oral Hygiene (OH)	Good	Poor	0.595	(0.371–0.956)	**0.032**	0.247	(0.149–0.407)	**<0.0001**

Note: Age groups (36–47 months, 48–59 months, 60–71 months), Pre-school Area (Central, East, North, South, West), Current consumption of Sweets & soft drinks (No, Not daily, 1/day, >1/day), PI (low: ≤1, high: >1) OH (poor, fair, good).
*NS-ECC: Non-severe Early Childhood Caries, S-ECC: Severe Early Childhood Caries.

Table 6

Univariate analyses of genotypes among NS-ECC and S-ECC children when compared to caries free children.

Gene	Genetic marker	Genotype	Univariate analysis					
			NS-ECC			S-ECC		
			OR	95% C.I.	p value	OR	95% C.I.	p value
MMP20	**rs1784418**	**AA**	Ref			Ref		
		AG	0.436	(0.238–0.798)	**0.007**	0.621	(0.350–1.103)	0.104
		GG	0.757	(0.331–1.729)	0.509	0.962	(0.437–2.118)	0.923
ENAM	**rs12640848**	**AA**	Ref			Ref		
		AG	0.9	(0.472–1.714)	0.749	1.047	(0.568–1.929)	0.882
		GG	1.55	(0.711–3.380)	0.271	1.583	(0.747–3.354)	0.23
TAS2R38	**rs713598**	**GG**	Ref			Ref		
		CG	0.846	(0.464–1.544)	0.585	0.582	(0.329–1.028)	0.062
		CC	1.053	(0.433–2.559)	0.91	1.164	(0.518–2.617)	0.714
LTF	**rs4547741**	**CC**	Ref			Ref		
		CT	0.877	(0.400–1.923)	0.742	1.026	(0.497–2.122)	0.944
		TT*	0	0	0	0.435	(0.071–2.664)	0.368

*NS-ECC: Non-severe Early Childhood Caries, S-ECC: Severe Early Childhood Caries.

Table 7

Multivariate analyses of genotypes among NS-ECC and S-ECC children when compared to caries free children.

Gene	Genetic marker	Genotype	Multivariate analysis					
			NS-ECC			S-ECC		
			OR	95% C.I.	p value	OR	95% C.I.	P value
MMP20	**rs1784418**	**AA**	Ref			Ref		
		AG	0.542	(0.285–1.033)	0.063	0.99	(0.508–1.932)	0.977
		GG	0.911	(0.377–2.204)	0.837	1.475	(0.589–3.695)	0.406
ENAM	**rs12640848**	**AA**	Ref			Ref		
		AG	1.016	(0.511–2.021)	0.964	1.686	(0.819–3.473)	0.156
		GG	1.709	(0.742–3.938)	0.208	2.207	(0.912–5.340)	0.079
TAS2R38	**rs713598**	**GG**	Ref			Ref		
		CG	0.87	(0.455–1.662)	0.673	0.634	(0.328–1.227)	0.176
		CC	1.249	(0.484–3.224)	0.646	1.387	(0.537–3.583)	0.5
LTF	**rs4547741**	**CC**	Ref			Ref		
		CT	0.748	(0.314–1.780)	0.511	0.955	(0.402–2.272)	0.917
		TT*	0	0	0	0.919	(0.114–7.400)	0.937

*The analyses were adjusted for history of dental visit, daily feeding frequency at infancy and Oral Hygiene (OH).
*NS-ECC: Non-severe Early Childhood Caries, S-ECC: Severe Early Childhood Caries.

to development of dental caries (Abbasoglu et al., 2015; Antunes et al., 2016; Filho et al., 2017; Gerreth et al., 2016; Gerreth et al., 2017; Piekoszewska-Zietek et al., 2017). This study was conducted in Saudi Arabia and, to the best of our knowledge, this is one of the few studies considering genetic variations in multiple genes with respect to ECC among Saudi children. Here, we examined the association between SNPs in ENAM, MMP20, TAS2R38 and LTF genes, important for enamel formation, taste preference and immune response, and their role in protection and/or susceptibility to develop ECC, and evaluated their interaction with environmental risk factors.

Our results showed that the heterozygous genotype AG of rs1784418 in MMP20 may be involved in the protection against ECC. It was noticed in our results that the genetic-environmental interaction was more pronounced when subjects were categorized based on severity of the ECC. Thus, it is plausible that the host genetic background may protect the teeth during caries initiation and the environmental factors could then have a stronger influence on the caries progression. MMPs are a family of genes involved in extracellular matrix degradation prior to mineralization. In the process of odontogenesis, MMPs, particularly enamelysin (MMP20), take part in the transformation of the early protease (amelogenin) secreted during enamel matrix development, implicating MMP20 in the formation of enamel and dentin during histo-differentiation of ameloblasts (Filho et al., 2017; Tannure et al., 2012; Vieira et al., 2014). MMP20 variant rs1784418 resides in the intronic region of the DNA. Although introns are non-coding sequences of DNA, they can influence gene expression and/or RNA splicing (Chorev and Carmel, 2012). In addition, although a complete understanding is still undeway inrtronic non coding RNA weather short non-coding RNAs, such as microRNAs (miRNAs), or long non-coding RNAs were found to facilitate many physiological processes and any defect may contribute to the emergence of pathologies and infectouise diseases (Catalanotto et al., 2016; Fernandes et al., 2019). Earlier reports have shown that rs1784418 variant of the MMP20 gene is associated with tooth agenesis (Küchler et al., 2011) and dental caries development (Tannure et al., 2012). We therefore anticipated that MMP20 variant rs1784418 may influence the risk of dental caries development possibly through the transcriptional regulation of MMP20 gene and consequently the MMP20 protein expression. Our results showed the protective effect of this gene variant was no longer present when covariate factors are taken into consideration. This result was at variance with a finding in 5–14 years old children (Tannure et al., 2012), upon comparison of results from different ethnicities, it was concluded that rs1784418 in MMP20 gene may contribute to increased risk of developing dental caries only in children of Caucasian decent and this was confirmed when adjusting for poor dietary habits (Tannure et al., 2012). Another study also implicated rs1784418 SNP to have a role in development of non-cavitated and cavitated carious lesions in children aged 2–6 years even when other cofactors were considered in multivariate analysis (Antunes et al., 2016). Our results are similar to a study by Filho et al. reporting a protective role of rs1784418 variants in children (Filho et al., 2017). In contrast, Abbasoğlu et al. (2015) did not find any association between MMP20 variant rs1784418 and ECC in Turkish children. In addition, Gerreth et al. (2017) reported that MMP20 SNP (rs1784418) was not associated with dental caries in Polish children aged 20–42 months. Recently, Borilova Linhartova et al, (2020) reported a lack of association between MMP20 SNP (rs1784418) and the susceptibility to or severity of dental caries in primary dentition in Czech children. As such, rs1784418 SNP may have a different effect on different populations depending on their ethnicity and environmental background.

ENAM encodes the enamelin protein, which is one of the major proteins involved in enamel matrix formation. It contributes to many different stages of enamel formation such as the initiation of tooth mineralization and regulation of crystal growth (Robinson et al., 1998). Due to the important role played by deficiency in enamel formation and caries development, we evaluated SNP rs12640848 in ENAM gene for association with dental caries; however, we did not find any significant result. Likewise, Wang et al. (2012) did not report any correlation between polymorphisms in this variant and dental caries in caucasian American children (Wang et al., 2012). Borilova Linhartova et al. (2018) also reported no association between polymorphism at this marker and dental caries in both the primary and permanent dentition in Czech children (Borilova Linhartova et al., 2018). Conversely, a highly significant association between the rs12640848 GG genotype and protection against dental caries in primary teeth was reported in Polish children (Gerreth et al., 2016). Also, another study reported a significant protective association in the same genetic locus in Turkish children against ECC, even after the results were adjusted for environmental factors (Abbasoglu et al., 2015). Variations in results in different studies could be explained by differences in populations with different genetic backgrounds and different environmental factors.

TAS2R38 gene mainly mediates perception of bitter taste. The proposed mechanism of this gene in dental health and development involves its influence on dietary preference and taste, modulating insensitivity or sensitivity to cariogenic food products (Opal et al., 2015). Studies describing the possible role of TAS2R38 gene polymorphisms and dental caries in children are scarce. We found a border line significance suggesting a protective effect of TAS2R38 rs713598 against S-ECC and this association was no longer apparent after controlling for environmental covariates. One study compared the association of taste genes with dental caries in primary, mixed and permanent dentitions and concluded that TAS2R38 gene variants were associated with lower caries development in children with primary dentition (Wendell et al., 2010). No association was found in the permanent dentition in an older age group, suggesting that the association becomes less relevant as age increases and possibly other environmental factors could have a stronger influence on caries severity (Wendell et al., 2010). However, it is recommended to compare genetic results within the same age group who have similar dentition (e.g: primary teeth only) as difference in the effect of the genetic elements in dental caries between primary and permanent dentition is well established (Wang et al., 2010). Similar to our findings, Novák et al. (2017) did not find any relationship between rs713598 of TAS2R38 and ECC (Novák et al., 2017).

LTF gene encodes for LTF glycoprotein, a prominent part of the oral immune system that acts as a secretory molecule in salivary fluid (Gonzalez-Chavez et al., 2009). It possesses antimicrobial property, especially in human infants. Our results indicated no considerable independent significance between LTF gene variant rs4547741 and risk of ECC susceptibility or severity among Saudi preschool children. Abbasoğlu et al. (2015) investigated the same marker (rs4547741) in a similar age group to that of our study, and reported a protective effect of this variant against ECC for younger children (Abbasoglu et al., 2015). Candida albicans is one of the most frequent pathogens of dental biofilm and many studies have found it to be associated with dental caries in children. There are multiple theories on the role played by C. albicans in the caries formation, the most common of which is the ability of the yeast to produce and have great tolerance to acids, thus, having a potential to exacerbate carious lesions. Interestingly, LTF provides defense against C. albicans (Abbasoglu et al., 2015; Klinke et al., 2011; Yang et al., 2012). Therefore, SNPs in LTF gene may have a potential role in dissuading carious lesions. However, we were unable to find any significant independent association between the studied LTF SNP and ECC in this study.

In early childhood, caries exhibit a multifactorial etiology. There are socioeconomic risk factors, microbiological risk factors, and dietary considerations regarding feeding practice and sugar intake (Colak et al., 2013). Thus, some of these variables were thoroughly considered in our study and genetic factors were adjusted through regression analysis to control for confounding effect (Avila et al., 2015; Tiano et al., 2009). Children reporting a dental visit and poor OH, were statistically associated with ECC. These factors are interrelated, the first dental visit is recommended at the age of the first tooth eruption before any symptoms arise (Policy on Early Childhood Caries (ECC): Classifications, 2017). During this dental visit the focus will be on prevention, OH instructions, dietary counselling, risk assessment and determening the frequency of future dental visits and preventive measures needed such as fluoride application (Baker et al., 2019). Despite the importance of the first dental visit, in our study children who have visited the dentists were significantly more likely to have caries and more severe caries than those who had not visited the dentist before. This may be due to the observation that many of the children who have gone to a dental clinic were not going for checkup, rather, they were seeking treatment for an existing dental problem. Infant feeding practices including increased daily feeding frequenv and nocturnal bottle feeding are also important factors. In a 5-year prospective cohort study bottle feeding at night was found to be significantly associated with ECC (Gussy et al., 2020), therefore, AAPD has recommended against this practice (Policy on Early Childhood Caries (ECC): Classifications, 2017).

A limitation of this study is that only four SNPs were investigated. Also, candidate gene association studies are dependent on the current understanding of the biology of dental caries, a process that could change as more information becomes available (Werneck et al., 2010). Accordingly, it is hoped that this study can be used to build a foundation for future investigations on a wider variety of genes and genetic markers and encourage the conduction of regional genome wide association studies investigating ECC and dental caries in the Arab population.

In conclusion, the present study demonstrated that *MMP20* rs1784418 SNP might be associated with protection against ECC in Saudi preschool children, however, genetic polymorphisms in *ENAM* (rs12640848), MMP20 (rs1784418), *TAS2R38* (rs713598) and *LTF* (rs4547741) may not be independently predictive of risk or protection from ECC in Riyadh, Saudi Arabia. In fact, environmental factors had a stronger effect in the multivariate analysis and factors such as; history of dental visits, nocturnal feeding during infancy, daily infant feeding frequency and poor OH were associated with increased risk of ECC. In the light of our report, further work is needed to validate our findings and include other genetic variants to understand the role of genetic traits in ECC susceptibility and/or protection.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.01.036.

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