Association of genetic and environmental factors with dental caries among adolescents in south China: A cross-sectional study



K. Wang[1,2*], L. Pang[1,2*], Y. Tao[1,2], X. Li[3], J. Zhang[3], T. Cui[3], B. Zeng[1,2], H. Lin[1,2]

[1]*Department of Preventive Dentistry, Guanghua School of Stomatology, Hospital of Stomatology, Sun Yat-sen University, Guangzhou, PR China*
[2]*Guangdong Provincial Key Laboratory of Stomatology, Sun Yat-sen University, Guangzhou, PR China*
[3]*Foshan Stomatology Hospital, Foshan, School of Stomatology and Medicine, Foshan University, Foshan, PR China*

e-mail: linhc@mail.sysu.edu.cn

DOI 10.23804/ejpd.2020.21.02.07

Abstract

Aim The objective of this study was to investigative genetic and environmental factors that contribute to caries susceptibility among adolescents in south China.

Materials and methods A cross-sectional study was conducted among 1055 adolescents aged 13–14 years old in south China. The International Caries Detection and Assessment System (ICDAS) was used to identify caries. Environmental variables were analysed by regression models. Twenty-three single nucleotide polymorphisms (SNPs) in 14 genes were identified from saliva samples. Regression analysis was used for the evaluation of effects of SNP markers using the minor allele as the effect allele.

Results Our results suggest that gender, Cariostat score and Plaque Index were associated with dental caries. After the adjustment by age and gender, the G allele in AMBN (rs13115627) nominally was a protective factor for caries under additive model (P=0.028; OR=0.782; 95% CI, 0.627-0.974). However, the association did not meet the Bonferroni correction significance cut-off for multiple testing.

Conclusion Gender, Cariostat score and Plaque Index were associated with dental caries in this population. No selected SNPs showed a significant association with dental caries under either additive model or dominance model.

KEYWORDS Dental caries, SNP, Adolescent, ICDAS

Introduction

Dental caries is one of the most common chronic diseases among children and adults worldwide. The cost of direct treatment was estimated to account for an average of 4.6% of global health expenditures [Listl et al., 2015]. According to the results of the 4th National Oral Health Survey of China (conducted in 2015-2016), the prevalence of caries in 12-year-old children had increased from 28.9% to 38.5% in the last decade [Quan et al., 2018]. Dental caries is a great burden and challenge to China and the world.

Dental caries is a multifactorial disease. Environmental risk factors such as addiction to sugary snacks and drinks, poor oral hygiene, high levels of cariogenic bacteria, salivary dysfunction, and insufficient fluoride exposure are critical to its development [Giugliano et al., 2018; Fisher-Owens et al., 2007]. Evidences also found in studies estimated that 26-64% of caries susceptibility is genetically determined [Nibali et al., 2017]. Genes involved in enamel formation, the immune response, saliva proteins and food preferences have been considered to be involved in the aetiology of dental caries [Chapple et al., 2017]. Present studies have identified large numbers of single nucleotide polymorphisms (SNPs) associated with caries [Zeng et al., 2014; Shungin et al., 2019; Vieira et al., 2014]. However, only a few of these SNPs have been discussed in Chinese population [Hu et al., 2019]. There exist some differences in genes among different ethnic group. The objective of present study was preliminary to explore the genetic factors in combination with environmental factors related to dental caries of adolescents in south China.

Materials and Methods

Subjects

An ethical review was obtained before the study conducted. A written informed consent was obtained from each student's guardian before the study. The oral examination and saliva tests were performed from March to April 2018 in Foshan. Foshan is a medium-sized city in south China with a population of 7.6 million. The GDP was CNY 124,324 (USD 18,018) per capita in 2017 [Foshan Municipal Bureau of Statistics 2018]. The water fluoride concentration is 0.16 mg/L [Foshan Water Conservancy Bureau 2017].

According to Peduzzi, for logistic regression analysis, the number of events (death or illness) should be 5–10 times the number of independent variables [Peduzzi et al., 1996]. In total, 41 independent variables were investigated via logistic regression in this study, and the caries prevalence in 12-year-old adolescents was 38.5%; thus, the total sample size should be between 533 and 1065 [Peduzzi et al., 1996; Quan et al., 2018].

A multi-stage cluster sampling technique was employed. There were 39 middle schools in Foshan in 2018. From these middle schools, 6 were randomly sampled. Four classes of

Grade 7 were randomly sampled from each of the 6 schools. All students in these classes whose parents were all Han Chinese and were permanent residents in Foshan were recruited for this study, excluding those who reported having systematic illness or using antibiotic in the preceding 2 weeks.

Questionnaire

The questionnaire was mainly designed with reference to the Fisher-Owens conceptual model of influence on children's oral health [Fisher-Owens et al., 2007]. The structured questionnaire recorded demographic information, socioeconomic status, diet and self-reported oral health behaviours. The questionnaire was self-administered and completed in schools by students with the guidance of guardians. The reliability of the questionnaire was assessed by an internal consistency test.

Oral examination

The oral examination was conducted in the schools. The examinee was lying in a portable dental chair. The teeth were examined visually with the help of a plain mouth mirror, a head-mounted light, a CPI probe and compressed air. The International Caries Detection and Assessment System (ICDAS) criteria were used to record dental caries [Pitts et al., 2013]. All tooth surfaces were first examined with a wet surface and then re-examined after the teeth were dried with compressed air. Filled and missing teeth due to caries were also recorded. The DMFT was calculated both at the early clinical stage of decay, with enamel and dentine caries (from the D1 level, as in the ICDAS method), and at the later stages of decay, with dentine-only caries (from the D3 level, as in the ICDAS method), and these values were recorded as D1MFT and D3MFT, respectively. Individuals with lesions scoring 3–6 were classified into the caries group, and the others were classified into the caries-free group in this study [Banting et al., 2005]. The Plaque Index (PI) of Silness and Löe scale was used to record dental plaque [Löe, 1967].

Three examiners had taken 12-hour e-learning courses on ICDAS before the study. About 10% of the subjects were randomly re-examined to monitor the consistency of the examination and evaluate the interexaminer reliability.

Microbiological assessment

The microbiological assessment was performed using the Cariostat kit (GangDa Medical Technology Co. LTD., Beijing, China), which is a colorimetric test for measuring the presence of acidogenic microorganisms [Ramesh et al., 2013]. According to the instructions of the products, the examiners used the sterile cotton swab to scrub the buccal surfaces of the maxillary molars and mandibular incisors 3–5 times and immersed the swab into the culture medium ampule. The samples were incubated at 37 °C for 48 hours. The colour of the culture medium was compared with the reference colour on the colour chart supplied. The reference colour was scored from 0 to 3 with every 0.5 grade as an interval. The colour turns from blue to green and, ultimately, to yellow, indicating an increased acid production ability of the plaque in the sample.

Saliva tests

All saliva was collected after the students had rinsed their mouths with tap water. The unstimulated saliva was collected for 15 minutes. Students were asked to spit the saliva through a funnel into a scaled tube every 3 minutes. Then, the unstimulated saliva flow rate (ml/min) was calculated. The saliva buffering capability was measured by the Ericsson method [Ericsson, 1959]. One millilitre of saliva was added to 3 ml of 3.3 mmol HCl within 5 minutes after collection and then allowed to stand for 20 minutes to remove CO2. The final pH of the saliva was evaluated by an electrical pH meter. The buffering capability of unstimulated saliva was recorded as 'low', 'normal' and 'high' when the pH value fell into the ranges of <4.25, 4.25–4.75 and >4.75, respectively.

Candidate genes selection

The candidate gene categories identified in this exploring study included enamel formation genes, immune response genes, genes related to saliva, and genes related to taste and dietary habits [Vieira et al., 2014]. The criterion for gene selection was previously reported associations with caries within these four categories. According to IGSR and the 1000 Genomes Project database of Chinese population (https://www.internationalgenome.org), functional SNPs of selected genes was included. The Haploview 4.2 software package (http://www.broad.mit.edu/mpg/haploview/) was used to exclude SNPs with a minor allele frequency of less than 0.05 or r2 less than 0.8 based on HapMap data from the southern Chinese Han population. The candidate genes and their SNPs selected for this study are listed in Table 1.

DNA analysis

Genomic DNA was extracted from 1-ml saliva samples according to the manufacturer's instructions (Saliva DNA Sample Collection Kit, ZEESAN, Xiamen, China). The DNA concentration and purity of each sample were determined by spectrophotometry. Twenty-three SNPs in 14 genes were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Statistical analysis

The data were analysed by SPSS version 22 for Windows (IBM Inc., Chicago, IL, USA). The differences in the environmental factors between the caries and caries-free groups were compared by binary regression analysis or an independent samples t-test. All environmental risk factors with a P value of less than 0.2 in the univariate analysis were applied to stepwise multivariate regression analysis. The Hardy-Weinberg equilibrium was estimated by the Chi-square test. Genotypes of the candidate genes were analysed under the hypothesis of an additive model and using the major allele as the reference. Each SNP was treated as a continuous exposure under additive model, coded 0,1, or 2(minor alleles). A binary regression model was applied to estimate the direct effects of SNPs. The highly conservative Bonferroni correction considering the number of tested SNPs was applied to both direct effects. The corrected significance level of multiple testing was set at $P<0.002$ (0.05/23) for direct effects.

RESULTS

In total, 1055 13–14 years old students participated in the survey. Of them, 615 were boys and 440 girls. The Cronbach's alpha of the questionnaire was 0.75, indicating a good reliability. For the inter-examiner reliability of the caries examination, the Cohen's kappa values were 0.85 (Examiner A v.s. B), 0.83 (Examiner B v.s. C) and 0.91 (Examiner C v.s. A).

Dental caries status

The mean D1-6MFT was 4.42±0.11, and the mean D3-6MFT

Gene	Locus	Marker public ID	Base pair exchange (MAF)	Functional implication	HWE test	
					χ^2	P
Enamel formation genes						
ENAM	4q13.3	rs12640848	A/G (0.33)	intron variant	0.206	0.650
		rs3796703	C/T (0.01)	missense variant	88.863	<0.001
AMBN	4q21	rs13115627	A/G (0.30)	intron variant	0.680	0.410
AMELX	Xp22.2	rs946252	C/T (0.31)	intron variant	425.798	<0.001
TFIP11	22q12.1	rs134143	T/C (0.35)	noncoding transcript exon variant	1.199	0.273
		rs2097470	C/T (0.29)	intron variant	3.478	0.062
MMP20	11q22.3-q23	rs1612069	G/T (0.48)	intron variant	2.959	0.085
		rs1784418	C/T (0.42)	intron variant	0.846	0.358
TUFT1	1q21	rs17640579	A/G (0.22)	intron variant	0.039	0.844
		rs3790506	G/A (0.25)	intron variant	2.862	0.091
Immune response genes						
DEFB1	8p23.1	rs11362	C/T (0.40)	5′ UTR variant	0.331	0.565
		rs1800972	G/C (0.14)	5′ UTR variant	0.367	0.545
LTF	3p21.31	rs4547741	C/T (0.07)	intron variant	0.905	0.314
		rs1126478	C/T (0.37)	missense variant	4.117	0.042
MBL2	10q21.1	rs1800450	C/T (0.12)	missense variant	2.421	0.120
		rs11003125	G/C (0.31)	regulatory region variant	4.255	0.039
MASP2	1p36.22	rs10779570	G/T (0.36)	intron variant	3.280	0.070
Saliva genes						
AQP5	12q13.12	rs1996315	G/A (0.43)	intron variant	0.480	0.488
		rs923911	C/A (0.22)	intron variant	0.182	0.670
CA6	1p36.23	rs2274327	C/T (0.27)	missense variant	0.226	0.634
Taste receptor genes						
TAS1R2	1p36.13	rs35874116	T/C (0.27)	missense variant	2.879	0.090
		rs9701796	G/C (0.20)	missense variant	0.016	0.901
TAS2R38	7q34	rs713598	C/G (0.50)	missense variant	0.705	0.401

TABLE 1 Candidate genetic markers evaluated in this study.

was 0.72±0.40. A total of 382 (36.2%) students had caries scored from 3–6 (categorised into the caries group in this study), and 673 (63.8%) students had caries scored from 0-2 (categorised into the caries-free group in this study), among which 129 (12.2%) scored 0.

Environmental factors

Results of the univariate analysis showed that environmental factors of gender, household monthly income, one-child family, Plaque Index and Cariostat score were associated with caries ($p<0.05$) (Table 2). The frequency of snacking, pit and fissure sealant, as well as saliva buffering capability showed a p-value between 0.05 and less than 0.2. All above factors were applied to a multivariate regression analysis, which indicated that those were girls, with higher Plaque Index score, with higher Cariostat score had a higher probability with caries in this population.

Linkage disequilibrium

A total of 23 SNPs distributed across the 14 genes were investigated (Table 1), among which rs3796703 (ENAM), rs946252 (AMLEX), rs11003125 (MBL2) and rs1126478 (LTF) were inconsistent with the Hardy-Weinberg equilibrium distribution and were thus excluded from further analysis.

Genetic factors

The associations between genotypes and dental caries under additive model and dominance model were analysed by logistic regression analysis, both univariate analyses and following multiple analyses adjusted by age and gender (Table 3, 4). The

	Caries-free (n=673) N (%)	Caries (n=382) N (%)	Univariate		Multivariate	
			P*	OR (95% CI)	P*	OR (95% CI)
Gender			0.011a		0.010a	
Female	261 (38.8)	179 (46.9)		1.392 (1.080,1.794)		1.432 (1.089,1.883)
Male	412 (61.2)	203 (53.1)		1		1
Residence			0.743			
Suburban	333 (49.5)	185 (48.4)		1.043 (0.811,1.341)		--
Urban	340 (50.5)	197 (51.6)		1		--
Caregiver			0.688		--	
Other people	187 (27.8)	99 (25.9)		0.893 (0.668,1.195)		--
Father	76 (11.3)	40 (10.5)		0.888 (0.587,1.344)		--
Mother	410 (60.9)	243 (63.6)		1		--
Education of caregiver			0.379		--	
≥9 years	300 (44.6)	181 (47.4)		1.120 (0.870,1.440)		--
<9 years	373 (55.4)	201 (52.6)		1		--
Household monthly income (RMB)			0.034a		0.051	
<3000	157 (23.3)	89 (23.3)		1.484 (0.970,2.269)		1.531 (0.978,2.396)
3000-7000	393 (58.4)	246 (64.4)		1.638 (1.129,2.376)		1.633 (1.100,2.424)
≥7000	123 (18.3)	47 (12.3)		1		1
One-child family			0.030a		0.227	
No	507 (75.3)	310 (81.2)		1.410 (1.033,1.923)		1.225 (0.881,1.703)
Yes	166 (24.7)	72 (18.8)		1		1
Frequency of toothbrushing			0.638		--	
<2 times per day	276 (41.0)	151 (39.5)		0.940 (0.728,1.215)		--
≥2 times per day	397 (59.0)	231 (60.5)		1		--
Dental flossing			0.202		--	
No	612 (90.9)	356 (93.2)		1.365 (0.847,2.200)		--
Yes	61 (9.1)	26(6.8)		1		--
Toothpaste			0.377		--	
Non fluoride	170 (25.3)	106 (27.7)		1.136 (0.856,1.509)		--
Fluoride	503 (74.7)	276 (72.3)		1		--
Frequency of snacking			0.122		0.370	
≥2 per day	68 (10.1)	51 (13.4)		1.625 (1.020,2.589)		1.420 (0.867,2.327)
1 per day	462 (68.6)	265 (69.4)		1.243 (0.895,1.726)		1.109 (0.784,1.569)
<1 per day	143 (21.2)	66 (17.3)		1		1
Frequency of drinking sweet drinks			0.768		--	
≥2 per day	59 (8.8)	36 (9.4)		1.157 (0.715,1.874)		--
1 per day	430 (63.9)	249 (65.2)		1.098 (0.821,1.470)		--
<1 per day	184 (27.3)	974 (25.4)		1		--
Pit and fissure sealant			0.177		0.347	
No	640 (95.1)	370 (96.9)		1.590 (0.811,3.116)		1.401 (0.694,2.827)
Yes	33 (4.9)	12 (3.1)		1		1
Orthodontics appliance			0.470		--	
Yes	17 (2.5)	7 (1.8)		0.720 (0.296,1.753)		--
No	656 (97.5)	375 (98.2)		1		--
Saliva secretion(ml/min)			0.465		--	
<0.25	178 (26.4)	109 (28.5)		1.110 (0.839,1.470)		--
≥0.25	495 (73.6)	273 (71.5)		1		--
Saliva buffering capability (pH)			0.191		0.463	
<3.5	213 (31.6)	126 (33.0)		1.201 (0.888,1.623)		1.103 (0.803,1.516)
3.5-4.25	186 (27.6)	121 (31.7)		1.320 (0.970,1.797)		1.265 (0.917,1.744)
≥4.25	274 (40.7)	135 (35.3)		1		1
Plaque Index			0.001a		0.003a	
2-3	49 (7.3)	51 (13.4)		2.212 (1.437,3.405)		2.183 (1.389,3.431)
1-2	252 (37.4)	156 (40.8)		1.316 (1.006,1.721)		1.238 (0.932,1.645)
0-1	372 (55.3)	175 (45.8)		1		1
Cariostat score			<0.001a		<0.001a	
2-3	79 (11.7)	124 (32.5)		5.012 (3.326,7.552)		5.034 (3.301,7.676)
1-2	412 (61.2)	201 (52.6)		1.558 (1.107,2.193)		1.530 (1.079,2.169)
0-1	182 (27.0)	57 (14.9)		1		1
	Mean (SD)	Mean (SD)	P**	OR (95% CI)	P*	OR (95% CI)
Age (years)	13.16 (0.40)	13.18 (0.40)	0.345	0.026 (-0.075,0.026)	--	--

**logistic regression analysis - **Independent samples t-test - aP<0.05*

TABLE 2 Caries distribution among characteristics.

Gene	SNP	Caries-free (n=673) N (%)	Caries (n=382) N (%)	Univariate			Adjusted#	
				P	OR (95% CI)		P	OR (95% CI)
Enamel formation genes								
ENAM	rs12640848			0.766	1.034 (0.831,1.286)		0.720	1.041 (0.836,1.296)
	AA	429 (63.7)	243 (63.6)					
	AG	217 (32.2)	120 (31.4)					
	GG	27 (4.1)	19 (5.0)					
AMBN	rs13115627			0.033	0.788 (0.633,0.981)		0.028	0.782 (0.627,0.974)
	AA	382 (56.8)	239 (62.6)					
	AG	253 (37.6)	130 (34.0)					
	GG	38 (5.6)	13 (3.4)					
TFIP11	rs134143			0.541	0.944 (0.783,1.137)		0.470	0.933 (0.774,1.125)
	TT	304 (45.2)	175 (45.8)					
	CT	285 (42.3)	167 (43.7)					
	CC	84 (12.5)	40 (10.5)					
	rs2097470			0.309	0.897 (0.728,1.106)		0.255	0.885 (0.717,1.092)
	CC	353 (52.5)	209 (54.7)					
	CT	277 (41.2)	155 (40.6)					
	TT	43 (6.4)	18 (4.7)					
MMP20	rs1612069			0.572	1.054 (0.878,1.266)		0.575	1.054 (0.877,1.267)
	GG	191 (28.4)	102 (26.7)					
	GT	350 (52.0)	202 (52.9)					
	TT	132 (19.6)	78 (20.4)					
	rs1784418			0.741	0.970 (0.810,1.162)		0.791	0.976 (0.813,1.170)
	CC	189 (28.1)	111 (29.1)					
	CT	345 (51.3)	194 (50.8)					
	TT	139 (20.6)	77 (20.1)					
TUFT1	rs17640579			0.701	0.961 (0.786,1.175)		0.676	0.958 (0.783,1.172)
	AA	360 (53.5)	200 (52.4)					
	AG	258 (38.3)	161 (42.1)					
	GG	55 (8.2)	21 (5.5)					
	rs3790506			0.354	1.097 (0.902,1.335)		0.413	1.086 (0.892,1.322)
	GG	375 (55.7)	213 (55.8)					
	AG	255(37.9)	130 (34.0)					
	AA	43 (6.4)	39 (10.2)					
Immune response genes								
DEFB1	rs11362			0.552	1.056 (0.883,1.263)		0.544	1.057 (0.884,1.265)
	CC	240 (35.7)	133 (34.8)					
	CT	322 (47.8)	179 (46.9)					
	TT	111 (16.5)	70 (18.3)					
	rs1800972			0.516	0.906 (0.672,1.221)		0.565	0.916 (0.678,1.236)
	GG	543 (80.7)	316 (82.7)					
	GC	123 (18.3)	61 (16.0)					
	CC	7 (1.0)	5 (1.3)					
LTF	rs4547741			0.986	0.997 (0.682,1.457)		0.997	1.001 (0.683,1.466)
	CC	600 (89.2)	341 (89.3)					
	CT	70 (10.4)	39 (10.2)					
	TT	3 (0.4)	2 (0.5)					
MBL2	rs1800450			0.880	1.020 (0.791,1.314)		0.881	1.020 (0.791,1.315)
	CC	508 (75.5)	288 (75.4)					
	CT	150 (22.3)	84 (22.0)					
	TT	15 (2.2)	10 (2.6)					
MASP2	rs10779570			0.704	1.040 (0.851,1.270)		0.830	1.022 (0.836,1.250)
	TT	391 (58.1)	219 (57.3)					
	TG	236 (35.1)	134 (35.1)					
	GG	46 (6.8)	29 (7.6)					
Saliva genes								
AQP5	rs1996315			0.982	0.998 (0.831,1.199)		0.932	1.008 (0.839,1.212)
	GG	240 (35.7)	135 (35.3)					

TABLE 3 Logistic regression analysis of SNPs under additive model.

	GA	328 (48.7)	189 (49.5)					
	AA	105 (15.6)	58 (15.2)					
	rs923911			0.592	1.062 (0.852,1.325)		0.537	1.072 (0.859,1.339)
	CC	429 (63.7)	254 (66.5)					
	CA	228 (33.9)	101 (26.4)					
	AA	16 (2.4)	27 (7.1)					
CA6	rs2274327			0.372	0.914 (0.749,1.114)		0.312	0.902 (0.739,1.101)
	CC	336 (49.9)	194 (50.8)					
	CT	276 (41.0)	164 (42.9)					
	TT	61 (9.1)	24 (6.3)					
Taste receptor genes								
TAS1R2	rs35874116			0.269	0.848 (0.632,1.136)		0.248	0.840 (0.626,1.129)
	TT	521 (77.4)	303 (79.3)					
	TC	144 (21.4)	79 (20.7)					
	CC	8 (1.2)	0 (0.0)					
	rs9701796			0.431	1.091 (0.878,1.357)		0.505	1.077 (0.865,1.341)
	CC	419 (62.3)	242 (63.4)					
	CG	235 (34.9)	114 (29.8)					
	GG	19 (2.8)	26 (6.8)					
TAS2R38	rs713598			0.989	0.999 (0.827,1.206)		0.943	0.993 (0.822,1.200)
	GG	317 (47.1)	183 (47.9)					
	GC	287 (42.6)	157 (41.1)					
	CC	69 (10.3)	42 (11.0)					
#The results are shown adjusted for the covariates of gender and age								

TABLE 3 Logistic regression analysis of SNPs under additive model.

SNP rs13115627 in AMBN had a suggestive P value of 0.028 in additive model after adjusted by gender and age (Table 3). However, none of the P value met the significance cut-off P value (0.002) after the Bonferroni correction for multiple testing in either additive models or dominance models.

Discussion

The present study gives a profile of dental caries of adolescents in Foshan, China, using the scales of International Caries Detection and Assessment System (ICDAS). In order to minimise the miscellaneous of population, permanent residents from Foshan in Guangdong Province were chosen in the investigation. Environmental factors including demographic factors, oral health behaviours, saliva and microbiological results were analysed in this study. Four categories of genes enrolled in caries aetiology were taken into consideration, and totally 23 SNPs were identified in this population.

Individuals with a high Plaque Index score were considered to be associated with caries experience [Obregón-Rodríguez et al., 2019]. Our results showed that students with a score of Plaque Index between 2 to 3 had an odd of 2.183 times compared with those whose Plaque Index were between 0 to 1. Since only 52.2% of students had a Plaque Index score under 1, the efficient self plaque control method should be conveyed to them. Microbiology factors, including Streptococcus mutans and lactobacilli were also recognised as critical factors in caries aetiology. Cariostat is a simple colorimetric test developed to assess the acid produced by bacteria in plaque [Ramesh et al., 2013]. Previous longitudinal studies have found Cariostat score as a risk indicator in primary dentition among Chinese children [Xuan et al., 2017]. Our present results showed a significant association between caries of permanent dentition and Cariostat score. However, longitudinal studies are needed to verify whether it is a risk factor in this population.

As observed in other populations [Martinez-Mier and Zandona, 2013], a higher proportion of girls had caries experience. The different salivary composition and flow rate, dietary habits, genetic variations, early eruption of teeth, and particular social roles in families were most common factors in explaining the consistent trend of caries rates being higher in females than males [Ferraro and Vieira, 2010]. Our results showed no significant associations between caries and saliva factors or diets, while gender was an independent risk indicator for caries. Thus the main reason for gender bias in caries risk for girls investigated in this study could be the particular social roles in their families, and the earlier eruptions of teeth than boys. Another possible reason for this bias could be genetic variations. Previous studies have found caries susceptible and caries protective loci, some of which are X-linked [Lukacs, 2011]. Variations in these genes would mainly change the caries susceptibility. The enamel formation gene AMELX on X chromosome was most investigated, and was selected for this study. Unfortunately, rs946252 (AMLEX) was inconsistent with the Hardy-Weinberg equilibrium distribution in this population. Further investigations of more loci on genes of X or Y chromosome in different populations should be conducted to verify the genetic contributions to gender bias in caries risk.

Current evidence from independently replicated results in multiple populations suggests that those genes with the largest impact on caries susceptibility are involved in enamel formation, immune regulation, salivary function, and dietary preferences [Chapple et al., 2017]. The enamel formation genes chosen for this study including AMELX, AMBN, ENAM, TUFT and TFIP, encode the secreted proteins necessary for early enamel formation, while MMP20 cleaved the extracellular matrix. All these matrix proteins are crucial for healthy enamel formation and their orchestrated removal is critical to allow for expansion of the mineral phase and completion of enamel mineralisation

Gene	SNP	Caries-free (n=673) N (%)	Caries (n=382) N (%)	Univariate			Adjusted#	
				P	OR (95% CI)		P	OR (95% CI)
Enamel formation genes								
ENAM	rs12640848			0.966	1.006 (0.775,1.306)		0.947	1.009 (0.776,1.311)
	AA	429 (63.7)	243 (63.6)					
	AG+GG	244 (36.3)	139 (36.4)					
AMBN	rs13115627			0.066	0.785 (0.607,1.016)		0.054	0.776 (0.599,1.005)
	AA	382 (56.8)	239 (62.6)					
	AG+GG	291 (43.2)	143 (37.4)					
TFIP11	rs134143			0.841	0.974 (0.757,1.254)		0.773	0.963 (0.748,1.241)
	TT	304 (45.2)	175 (45.8)					
	CT+CC	369 (54.8)	207(54.2)					
	rs2097470			0.479	0.913 (0.710,1.175)		0.437	0.905 (0.702,1.165)
	CC	353 (52.5)	209 (54.7)					
	CT+TT	320 (47.5)	173 (45.3)					
MMP20	rs1612069			0.559	1.088 (0.821,1.442)		0.576	1.084 (0.817,1.440)
	GG	191 (28.4)	102 (26.7)					
	GT+TT	482 (71.6)	280 (73.3)					
	rs1784418			0.736	0.953 (0.722,1.258)		0.764	0.958 (0.725,1.266)
	CC	189 (28.1)	111 (29.1)					
	CT	484 (71.9)	271 (79.0)					
TUFT1	rs17640579			0.722	1.047 (0.814,1.346)		0.715	1.048 (0.814,1.350)
	AA	360 (53.5)	200 (52.4)					
	AG+GG	313 (46.5)	182 (47.6)					
	rs3790506			0.990	0.998 (0.775,1.286)		0.919	0.987 (0.766,1.272)
	GG	375 (55.7)	213 (55.8)					
	AG+AA	298 (37.9)	169 (34.0)					
Immune response genes								
DEFB1	rs11362			0.783	1.038 (0.798,1.350)		0.733	1.047 (0.804,1.363)
	CC	240 (35.7)	133 (34.8)					
	CT+TT	433 (64.3)	249 (65.2)					
	rs1800972			0.413	0.872 (0.629,1.210)		0.454	0.882 (0.635,1.225)
	GG	543 (80.7)	316 (82.7)					
	GC+CC	130 (19.3)	66 (17.3)					
LTF	rs4547741			0.954	0.988 (0.659,1.482)		0.952	0.988 (0.657,1.484)
	CC	600 (89.2)	341 (89.3)					
	CT+TT	73 (10.8)	41 (10.7)					
MBL2	rs1800450			0.974	1.005 (0.751,1.345)		0.940	1.011 (0.755,1.355)
	CC	508 (75.5)	288 (75.4)					
	CT+TT	165 (24.5)	94 (24.6)					
MASP2	rs10779570			0.808	1.032 (0.800,1.331)		0.943	1.009 (0.782,1.303)
	TT	391 (58.1)	219 (57.3)					
	TG+GG	282 (41.9)	163 (42.7)					
Saliva genes								
AQP5	rs1996315			0.917	1.014 (0.780,1.318)		0.821	1.031 (0.972,1.342)
	GG	240 (35.7)	135 (35.3)					
	GA+AA	433 (64.3)	247 (64.7)					
	rs923911			0.369	0.886 (0.680,1.154)		0.392	0.891 (0.683,1.161)
	CC	429 (63.7)	254 (66.5)					
	CA+AA	244 (36.3)	128 (33.5)					
CA6	rs2274327			0.788	0.966 (0.752,1.242)		0.721	0.955 (0.742,1.229)
	CC	336 (49.9)	194 (50.8)					
	CT+TT	337 (50.1)	188 (49.2)					
Taste receptor genes								
TAS1R2	rs35874116			0.472	0.894 (0.658,1.214)		0.428	0.883 (0.649,1.201)
	TT	521 (77.4)	303 (79.3)					
	TC+CC	152 (22.6)	79 (20.7)					
	rs9701796			0.724	0.954 (0.736,1.238)		0.634	0.939 (0.723,1.219)
	CC	419 (62.3)	242 (63.4)					
	CG+GG	254 (34.9)	140 (29.8)					
TAS2R38	rs713598			0.802	0.968 (0.753,1.245)		0.749	0.960 (0.745,1.235)
	GG	317 (47.1)	183 (47.9)					
	GC+CC	287 (42.6)	157 (41.1)					

#The results are shown adjusted for the covariates of gender and age

TABLE 4 Logistic regression analysis of SNPs under dominance model.

[Lacruz et al., 2017]. DEFB1, LTF, MASP2 and MBL2 are major immune response genes encoding bactericidal factors. AQP5 and CA6 are genes that mainly influence saliva buffering capability. Genes determining dietary preferences include TAS2R38 and TAS1R2 [Vieira et al., 2014]. Because these genes are reported involved in caries aetiology, they were selected in this exploring study and a candidate gene association study was used in this research. The SNP rs13115627 in AMBN showed a nominal significant P value after adjusted by gender and age under the additive model. Individuals with allele G were less likely to experience cavious caries. However, the P value did not meet the significance level after multiple testing correction. Our previous study has found that the GG genotype of AMBN (rs13115627) was significantly associated with an increased calcium-phosphorus ratio in enamel, which could lead to lower caries risk [Pang et al., 2017; Das et al., 2016]. As AMBN is functionally relevant to calcification and caries, further studies should be carried out to verify whether rs13115627 is correlated with caries experience. Although the sample size of 1055 has enough statistical power to detect small odds ratios of genetic factors, this study found no significant association between dental caries and selected SNPs after the multivariate analysis. We failed to verify direct effect of genetic factors may be caused by two reasons. Firstly, the genetic background of studied population was different from those in previous studies. The effects of the SNPs tested in this study may be compensated by some other SNPs. Secondly, it has been recognised that enamel formation genes, immune response genes, genes related to saliva, and genes related to taste and dietary habits are related to caries, but the functional sites could be numerous and diffusely distributed, and large effect variants are rare, so the variants found in previous candidate gene association studies were hard to be replicated [Duncan et al., 2019]. This study aims to initially explore the association between genetic factors and caries, while genome-wide association studies should be applied to further verify the genetic contributions to dental caries.

Conclusion

Gender, Cariostat score and Plaque Index were associated with dental caries of adolescents in South China. No selected SNPs showed a significant association with dental caries under either additive model or dominance model.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

The present study was supported by the National Natural Science Foundation of China (no. 81570967) and The Forth National Oral Health Survey of China (no. 201502002).

References

› Banting D, Eggertsson H, Ekstrand KR, Ferreira-Zandoná A, Ismail AI, Longbottom C, Pitts N.B, Reich E, Ricketts D, and Selwitz R. Rationale and evidence for the international caries detection and assessment system (ICDAS II). Ann Arbor 2005; 1001: 48109–1078.
› Chapple ILC, Bouchard P, Cagetti MG, Campus G, Carra M-C, Cocco F, Nibali L, Hujoel P, Laine M L, Lingström P, Manton DJ, Montero E, Pitts N, Rangé H, Schlueter N, Teughels W, Twetman S, Van Loveren C, Van der Weijden F, Vieira AR, Schulte AG. Interaction of lifestyle, behaviour or systemic diseases with dental caries and periodontal diseases: consensus report of group 2 of the joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. J Clin Periodontol 2017; 44: S39–S51.
› Das B, Muthu MS, Farzan JM. Comparison of the chemical composition of normal enamel from exfoliated primary teeth and teeth affected with early childhood caries: an in vitro study. Int J Paediatr Dent 2016; 26 (1): 20–25.
› Duncan LE, Ostacher M, Ballon J. How genome-wide association studies (GWAS) made traditional candidate gene studies obsolete. Neuropsychopharmacology 2019; 44 (9): 1518–1523.
› Ericsson Y. Clinical investigations of the salivary buffering action. Acta Odontologica Scandinavica 1959; 17 (2): 131–165.
› Ferraro M, Vieira A. R. Explaining Gender Differences in Caries: A Multifactorial Approach to a Multifactorial Disease. Int J Dent [online] 2010. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2840374/ [Accessed 12 Sep 2019].
› Fisher-Owens SA, Gansky SA, Platt LJ, Weintraub JA, Soobader M-J, Bramlett MD, Newacheck P W. Influences on children's oral health: a conceptual model. Pediatrics 2007; 120 (3): e510–e520.
› Foshan Municipal Bureau of Statistics, 2018. Statistical Communique of 2017 National Economic and Social Development of Foshan City - China Statistics Information Network [online]. Statistical Communique of 2017 National Economic and Social Development of Foshan City - China Statistics Information Network. Available from: http://www.tjcn.org/tjgb/19gd/35445.html [Accessed 11 Jan 2019].
› Foshan Water Conservancy Bureau, 2017. Foshan Municipal Water Affairs Bureau - Foshan City Water Supply Monthly Announcement (June 2017) [online]. Foshan Municipal Water Affairs Bureau - Foshan City Water Supply Monthly Announcement (June 2017). Available from: http://www.fswater.gov.cn/zhengwu/ywxx/gongshui/gs_szgb/201707/t20170714_6258352.html [Accessed 11 Jan 2019].
› Giugliano D, d'Apuzzo F, Majorana A, Campus G, Nucci F, Flores-Mir C, Perillo L. Influence of occlusal characteristics, food intake and oral hygiene habits on dental caries in adolescents: a cross-sectional study. Eur J Paediatr Dent 2018; 19 (2): 95–100.
› Hu X-P, Song T-Z, Zhu Y-Y, Wu L-L, Zhang X, Zhou J-Y, Li Z-Q. Association of ENAM, TUFT1, MMP13, IL1B, IL10 and IL1RN gene polymorphism and dental caries susceptibility in Chinese children. J Int Med Res 2019; 47 (4): 1696–1704.
› Lacruz RS, Habelitz S, Wright JT, Paine ML. Dental enamel formation and implications for oral health and disease. Physiological Reviews 2017; 97 (3): 939–993.
› Listl S, Galloway J, Mossey PA, Marcenes W. Global economic impact of dental diseases. JDR 2015; 94 (10): 1355–1361.
› Löe H. The gingival index, the plaque index and the retention index systems. J Periodontol 1967; 38 (6P2): 610–616.
› Lukacs JR. Sex differences in dental caries experience: clinical evidence, complex etiology. Clin Oral Invest 2011; 15 (5): 649–656.
› Martinez-Mier EA, Zandona AF. The impact of gender on caries prevalence and risk assessment. Dent Clin North Am 2013; 57 (2): 301–315.
› Nibali L, Di Iorio A, Tu,Y-K, Vieira AR. Host genetics role in the pathogenesis of periodontal disease and caries. J Clin Periodontol 2017; 44 Suppl 18: S52–S78.
› Obregón-Rodríguez N, Fernández-Riveiro P, Piñeiro-Lamas M, Smyth-Chamosa E, Montes-Martínez A, Suárez-Cunqueiro MM. Prevalence and caries-related risk factors in schoolchildren of 12- and 15-year-old: a cross-sectional study. BMC Oral Health 2019; 19 (1): 120.
› Pang L, Zhi Q, Zhuang P, Yu L, Tao Y, Lin H. Variation in Enamel Formation Genes Influences Enamel Demineralization In Vitro in a Streptococcus mutans Biofilm Model. Frontiers in Physiology [online] 2017; 8. Available from: http://journal.frontiersin.org/article/10.3389/fphys.2017.00851/full [Accessed 11 Jan 2019].
› Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol 1996; 49 (12): 1373–1379.
› Pitts NB, Ekstrand KR, ICDAS Foundation. International Caries Detection and Assessment System (ICDAS) and its International Caries Classification and Management System (ICCMS) - methods for staging of the caries process and enabling dentists to manage caries. Community Dent Oral Epidemiol 2013; 41 (1): e41-52.
› Quan JK, Wang XZ, Sun XY, Yuan C, Liu XN, Wang X, Feng XP, Tai BJ, Hu DY, Lin HC, Wang B, Si Y, Wang CX, Wang WJ, Rong WS, Zheng SG. Permanent Teeth Caries Status of 12- to 15-year-olds in China: Findings from the 4th National Oral Health Survey. The Chinese journal of dental research: the official journal of the Scientific Section of the Chinese Stomatological Association (CSA) 2018; 21 (3): 181–193.
› Ramesh K, Kunjappan S, Ramesh M, Shankar S, Reddy S. Comparative evaluation of predictive value of three caries activity tests-snyder, lactobacillus count and cariostat in mixed dentition children with and without caries. Journal of Pharmacy Bioallied Sciences 2013; 5 (Suppl 1): S63-68.
› Shungin D, Haworth S, Divaris K, Agler CS, Kamatani Y, Keun Lee M, Grinde K, Hindy G, Alaraudanjoki V, Pesonen P, Teumer A, Holtfreter B, Sakaue S, Hirata J, Yu,Y-H, Ridker P. M, Giulianini F, Chasman DI, Magnusson PKE, Sudo T, Okada Y, Völker U, Kocher T, Anttonen V, Laitala M-L, Orho-Melander M, Sofer T, Shaffer JR, Vieira A, Marazita ML, Kubo M, Furuichi Y, North KE, Offenbacher S, Ingelsson E, Franks PW, Timpson NJ, Johansson I. Genome-wide analysis of dental caries and periodontitis combining clinical and self-reported data. Nature Communications 2019; 10 (1): 2773.
› Vieira AR, Modesto A, Marazita ML. Caries: review of human genetics research. Caries Res 2014; 48 (5): 491–506.
› Xuan SY, Yuan JW, Wang J, Guan XL, Ge LH, Shimono YM. A 2-year cohort study on the caries risk assessment of 3-year-old caries-free children using Cariostat caries activity test. Zhonghua Kou Qiang Yi Xue Za Zhi = Zhonghua Kouqiang Yixue Zazhi = Chinese Journal of Stomatology2017; 52 (11): 667–671.
› Zeng Z, Feingold E, Wang X, Weeks,DE, Lee M, Cuenco KT, Broffitt B, Weyant RJ, Crout R, McNeil DW, Levy SM, Marazita ML, Shaffer John R. Genome-wide association study of primary dentition pit-and-fissure and smooth surface caries. Caries Res 2014; 48 (4): 330–338.