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Original article

Association of genetic variants in enamel-formation genes with dental caries: A meta- and gene-cluster analysis

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ABSTRACT

Previous studies have reported the association between multiple genetic variants in the enamel-formation genes and the risk of dental caries with inconsistent results. We performed a systematic literature search of the PubMed, Cochrane Library, HuGE and Google Scholar databases for studies published before March 21, 2020 and conducted meta-, gene-based and gene-cluster analysis on the association between genetic variants in the enamel-formation genes and the risk of dental caries. We identified 21 relevant publications including a total of 24 studies for analysis. The genetic variant rs17878486 in *AMELX* was significantly associated with dental caries risk (OR = 1.40, 95% CI: 1.02–1.93, P = 0.037). We found no significant association between the risk of dental caries with rs12640848 in *ENAM* (OR = 1.15, 95% CI: 0.88–1.52, P = 0.310), rs1784418 in *MMP20* (OR = 1.07, 95% CI: 0.76–1.49, P = 0.702) and rs3796704 in *ENAM* (OR = 1.06, 95% CI: 0.96–1.17, P = 0.228). Gene-based analysis indicated that multiple genetic variants in *AMELX* showed joint association with the risk of dental caries (6 variants; $P < 10^{-5}$), so did genetic variants in *MMP13* (3 variants; P = 0.004), *MMP2* (3 variants; $P < 10^{-5}$), *MMP20* (2 variants; $P < 10^{-5}$) and *MMP3* (2 variants; $P < 10^{-5}$). The gene-cluster analysis indicated a significant association between the genetic variants in this enamel-formation gene cluster and the risk of dental caries ($P < 10^{-5}$). The present meta-analysis revealed that genetic variant rs17878486 in *AMELX* was associated with dental caries, and multiple genetic variants in the enamel-formation genes jointly contributed to the risk of dental caries, supporting the role of genetic variants in the enamel-formation genes in the etiology of dental caries.

1. Introduction

Dental caries is one of the most common oral diseases, with an age-standardized global prevalence of untreated dentine carious lesions being around 9% in the primary dentition and around 35% in the permanent dentition during the last three decades (Frencken et al., 2017). Dental caries is a major public health concern, leading to tooth pain or loss and many other concomitants such as trouble in learning, eating or sleeping (Gilchrist et al., 2015). Dental caries remains to be very common despite the adoption of various preventive measures.

Dental caries results from continued localized demineralization of the dental enamel and dentine. It is a chronic disease with a multi-factorial etiology, involving the complex interactions between genetic, environmental and behavioral factors such as flu-

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oride exposure, diet and oral hygiene (Shungin et al., 2019; Harris et al., 2004; Yildiz et al., 2016). Although previous studies have been successful in revealing the factors associated with the risk of dental caries (Costa et al., 2012; Piekoszewska-Zietek et al., 2017; Kumar et al., 2016), more studies are needed to validate these findings, identify additional factors, and elucidate their exact roles in the etiology of dental caries.

The quality and quantity of enamel plays a direct role in the susceptibility to dental caries. Enamel-formation genes, such as *AMBN*, *AMELX*, *TUFT1*, *KLK4* and *ENAM*, represent a cluster of genes that are involved in the pathway of odontogenesis of dentin-containing teeth (Shungin et al., 2019; Pang et al., 2017). Previous studies examined the association of genetic variants in this gene cluster with dental caries susceptibility, with inconsistent results (Gerreth et al., 2017; Olszowski et al., 2012; Ergoz et al., 2014). Therefore, we performed this meta-analysis to examine the association between multiple genetic variants in this gene cluster and the risk of dental caries. Considering that the effect of individual genetic variants may be small, we also performed a gene-based and gene-cluster analysis to explore the joint association of multiple genetic variants in this gene cluster with the risk of dental caries.

2. Materials and methods

Ethical approval and informed consent statements are not required due to the systematic review and meta-analytic nature of this study.

2.1. Eligibility criteria

We adopted the following inclusion criteria to determine study eligibility: 1) the studies were on human subjects; 2) the studies had a case and a control group, with the case group including subjects who had caries or high caries and the control group being care-free or having low/very low caries; and 3) the studies reported data on genetic variants in the enamel-formation genes for subjects in both the case group and the control group. We chose the studies with a larger sample size if multiple studies used overlapping data.

Two authors (XL and JY) performed an extensive literature search of the PubMed, Cochrane Library, HuGE and Google Scholar databases for studies published before March 21, 2020. The keywords used in the literature search are provided in the online supplementary file.

We retrieved all the potentially relevant publications to evaluate study eligibility. We manually searched the references in all the identified studies for research that might have been missed during the literature search. We also relied on Google Scholar's 'cited by' tool to search for potential publications that cited the studies identified in the literature search. The two authors performed the literature search independently. The search was limited to studies published in English. Any disagreement was resolved by group discussion (XL, SY and JY).

2.2. Data extraction

Two authors (DL and JY) independently extracted the following data from the eligible studies according to a pre-specified protocol for data extraction: name of the first author, year of publication, characteristics of the study participants, including sample size, distribution of age and gender, race/country of origin of the participants, screening method for dental caries, and genotype data for participants in the case and the control group. Any discrepancies were resolved in a group meeting. The quality of the included studies was assessed by two authors (DL and JY) independently using the Newcastle–Ottawa Scale (NOS) (Wells et al., 2019). Extracted data were entered into a computerised spreadsheet for analysis.

2.3. Data analysis

We used odds ratios (ORs) as a measure of the association between genetic variants in the enamel-formation genes and the risk of dental caries. In all the meta-analyses, we used random-effects models to calculate the ORs and the corresponding 95% confidence intervals (CIs). The analyses were performed using different genetic models, including additive, allelic, dominant, recessive and co-dominant genetic models. Between-study heterogeneity was assessed using I^2, and publication bias was visually checked by a contour-enhanced funnel plot and evaluated by Egger's test. We performed meta-analysis for a single genetic variant when there were data from multiple studies for that genetic variant. However, we only reported meta-analysis results for genetic variants that had data from at least four studies. Meta-analysis results for genetic variants that had data from less than four studies were used only in the gene-based and gene-cluster analysis as described in detail below.

2.4. Gene-based and gene-cluster analysis

We conducted gene-based analyses to assess the overall association of multiple genetic variants in each enamel-formation gene with the risk of dental caries. We followed the methods as described in Dr. Li et al. 2020 (Li et al., 2020). Specifically, we used the P-values of all genetic variants within that gene obtained from our meta-analyses or from published literature when no meta-analysis could be done. Four different P-value combination methods were utilized—the Fisher's method (Fisher, 1932), the Simes method (Simes, 1986), the modified inverse normal method (Hartung, 1999) and the modified truncated product method (TPM) (Zaykin et al., 2002; Sheng and Yang, 2012). For the modified TPM, we calculated unweighted and weighted TPM, where the former did not consider the difference in sample sizes whereas the latter employed the sample sizes as its weight, thereby allowing studies with larger sample sizes to play a larger role in the calculation (Zaykin et al., 2002). A detailed description of the four methods has been given elsewhere (Sheng and Yang, 2012). To estimate the *P*-value for the modified TPM, we ran 100,000 simulations to account for the correlation between the *P*-values. Gene-cluster analysis followed a similar approach to the gene-based analyses.

2.5. Sensitivity/additional analysis

We examined the association by including only the studies in which the genetic data in the control group satisfied Hardy–Weinberg equilibrium (HWE). We also repeated the meta-analyses by excluding studies of low quality (NOS < six stars). And finally, we examined the association through meta-analysis by including only the studies that used data from children.

All statistical analyses were performed using R (https://www.r-project.org). A *P*-value < 0.05 was considered statistically significant. This study was reported according to the PRISMA guidelines (Moher et al., 2009).

3. Results

3.1. Study selection and characteristics

Fig. 1 shows the selection of eligible studies included in our meta-analyses. We identified 132 potential publications through

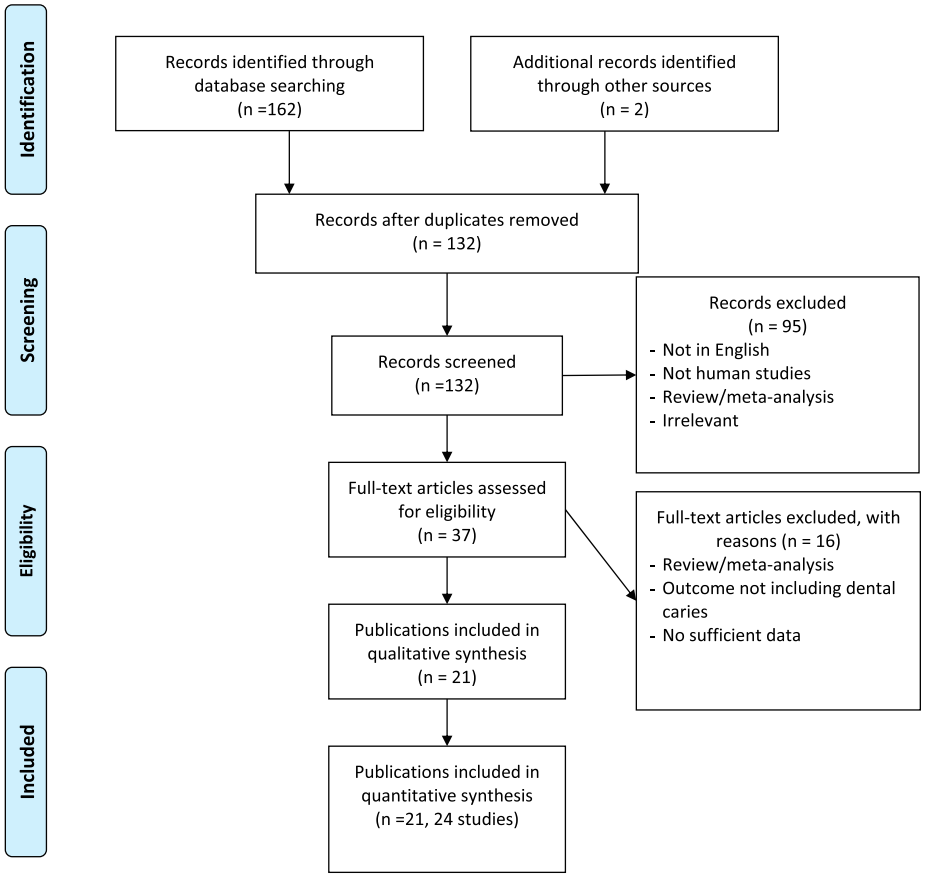

Fig. 1. Flow diagram of the process of selecting studies included in the meta-analyses. Note: Please see the Methods section for additional details.

our initial search. After screening the abstracts, 95 publications were excluded because they were not about human subjects, were not in English, were reviews/meta-analysis or were irrelevant. This left 37 publications that were retrieved for more detailed evaluation. We excluded an additional 16 publications because they were reviews or meta-analyses, the outcomes did not include dental caries, or because there were insufficient data. This resulted in 21 publications including a total of 24 studies that met the eligibility criteria and were included in our analyses (Yildiz et al., 2016; Gerreth et al., 2017; Olszowski et al., 2012; Ergoz et al., 2014; Ouryouji et al., 2008; Kang et al., 2011; Tannure et al., 2012; Tannure et al., 2012; Jeremias et al., 2013; Antunes et al., 2016; Gerreth et al., 2016; Karayasheva et al., 2016; Cavallari et al., 2017; Filho et al., 2017; Wang et al., 2017; Koohpeima et al., 2018; Borilova Linhartova et al., 2018; Devang Divakar et al., 2019; Hu et al., 2019; Vasconcelos et al., 2019; Duran-Merino et al., 2020; Nicoline et al., 2020). In summary, the meta-analyses of rs12640848 in *ENAM* included seven studies with a total of 1256 subjects in the case group and 710 subjects in the control group (Ergoz et al., 2014; Jeremias et al., 2013; Gerreth et al., 2016; Borilova Linhartova et al., 2018; Devang Divakar et al., 2019; Duran-Merino et al., 2020); the meta-analyses of rs1784418 in *MMP20* included five studies with a total of 699 subjects in the case group and 817 subjects in the control group (Gerreth et al., 2017; Tannure et al., 2012; Antunes et al., 2016; Filho et al., 2017; Vasconcelos et al., 2019); the meta-analyses of rs17878486 in *AMELX* included four studies with a total of 249 subjects in the case group and 193 subjects in the control group (Gerreth et al., 2017; Ergoz et al., 2014; Kang et al., 2011; Jeremias et al., 2013); and the meta-analyses of rs3796704 in *ENAM* included four studies with a total of 574 subjects in the case group and 533 subjects in the control group (Ergoz et al., 2014; Jeremias et al., 2013; Koohpeima et al., 2018; Devang Divakar et al., 2019). Data for other genetic variants came from fewer studies.

All included publications were published since 2008, with a sample size ranging from 71 to 1005. The basic characteristics of the included studies are presented in Table 1. The majority of the included studies were of good quality, except four studies which had NOS scores of 4 or 5 (Yildiz et al., 2016; Kang et al., 2011; Duran-Merino et al., 2020; Nicoline et al., 2020).

3.2. Assessment of publication bias

We did not find evidence of a significant publication bias for the meta-analysis of rs12640848 in *ENAM* (P = 0.053), rs1784418 in *MMP20* (P = 0.238), rs17878486 in *AMELX* (P = 0.521) and rs3796704 in *ENAM* (P = 0.194; Fig. 2). Assessment of publication bias for the meta-analysis of other genetic variants is not very meaningful due to the limited number of studies included in the corresponding meta-analysis.

3.3. Association with the risk of dental caries

For simplicity, we mainly reported results assuming an additive model for the meta-analyses. Complete results for meta-analyses and association analyses of individual genetic variants assuming different genetic models were presented in Supplementary Tables 1–5. The genetic variant rs17878486 in *AMELX* was significantly associated with dental caries risk (OR = 1.40, 95% CI: 1.02–1.93, P = 0.037; Table 2). We found that rs12640848 in *ENAM*, the genetic variant that had largest number of studies in our meta-

Table 1
Basic characteristics of all the studies included in the analyses.

Study	Year of publication	Country/ origin	Ethnicity	Cases			Control			Diagnosis of dental caries	NOS
				n	Age	Male (%)	n	Age	Male (%)		
(Ouryouji et al., 2008)	2008	India	Asian	80	5.4 ± 0.92	63.8%	67	4.8 ± 1.2	43.3%	dmft	6
(Kang et al., 2011)	2011	Korea	Asian	87	23.2 ± 7.8	73.4%	33	21.3 ± 7.8	69.7%	DMFT	5
(Olszowski et al., 2012a)	2012	Poland	European	37	5	51.4%	34	5	47.1%	DMFT/dmft	7
(Olszowski et al., 2012b)	2012	Poland	European	58	13	31.0%	50	13	42.0%	DMFT/dmft	7
Tannure et al., 2012a	2012	Brazil	Mixed	293	8.83 ± 2.95	51.9%	212	9.40 ± 3.39	54.7%	DMFT/dmft	8
Tannure et al., 2012b	2012	Brazil	Mixed	227	8.73 ± 0.17	50.2%	161	9.45 ± 0.22	54.7%	DMFT/dmft	8
Ergoz et al., 2014	2013	Turkey	European	100	8.65 ± 1.94	50%	100	8.65 ± 1.94	50%	DMFT/DMFS, dfmt/ dmfs	8
(Jeremias et al., 2013)[#]	2013	Brazil	Mixed	–	–	–	–	–	–	DMFT/dmft	8
Yildiz et al., 2015	2015	Turkey	Mixed	77	20–60	–	77	20–60	–	DMFT	6
(Antunes et al., 2016)	2016	Brazil	Mixed	245	4.38 ± 1.18	53%	541	3.09 ± 1.49	53%	dmft	9
(Gerreth et al., 2016)	2016	Poland	European	48	20–42 months	47.9%	48	20–42 months	50.0%	*	6
(Karayasheva et al., 2016)	2016	Bulgaria	European	82	20–32	–	20	20–32	–	DMFT	6
(Cavallari et al., 2017)	2017	Brazil	Mixed	100	18.43	51.3%	100	18.52	48.7%	ICDAS	7
(Filho et al., 2017)	2017	Brazil	Mixed	103	4–7	49.5%	81	4–7	46.9%	dmft	6
(Gerreth et al., 2017)	2017	Poland	European	48	20–42 months	47.9%	48	20–42 months	50.0%	*	6
(Wang et al., 2017)	2017	China	Asian	505	41.7 ± 7.0	52.7%	500	43.7 ± 4.0	49.0%	DMFT	8
(Koohpeima et al., 2018)	2018	Iran	Asian	236	29.8 ± 7.9	37.3%	166	28.4 ± 9.5	54.2%	DMFT	6
Linhartova et al., 2018a	2018	Czech	European	109	2–6	58.7%	78	2–6	46.2%	DMFT/dmft	6
Linhartova et al., 2018b	2018	Czech	European	541	13–15	52.7%	177	13–15	53.1%	DMFT/dmft	6
Devang Divakar et al., 2019	2019	India	Asian	168	6.9 ± 1.9	52.4%	193	23.2 ± 2.5	52.3%	DMFT/DMFS, dfmt/ dffs	6
(Hu et al., 2019)	2019	China	Asian	161	12–15	52.2%	196	12–15	48.0%	DMFT	9
(Vasconcelos et al., 2019)	2019	Brazil	Mixed	131	10–12	–	85	10–12	–	DMFT/dmft	7
(Duran-Merino et al., 2020)	2020	Mexico	Mixed	39	11	–	32	11	–	–	4
(Nicoline et al., 2020)	2020	Indonesia	Asian	95	–	–	89	–	–	DMFT	5

Data for age were presented as mean, mean ± SD or range.
SD: standard deviation; NOS: the Newcastle–Ottawa scale; DMFT: decayed, missing and filled teeth index; DMFS, decayed, missing, and filled surfaces; ICDAS, International Decay Detection and Assessment System.
* Teeth were evaluated by one trained and calibrated dentist specialized in pediatric dentistry using an artificial light, a dental mirror and a probe, after calibration by an experienced specialist.
# Number of subjects varies for different genetic variants.

analysis, was not significantly associated with the risk of dental caries (OR = 1.15, 95% CI: 0.88–1.52, $P = 0.310$). Meta-analysis also revealed no significant association of rs1784418 (OR = 1.07, 95% CI: 0.76–1.49, $P = 0.702$) in *MMP20* and rs3796704 (OR = 1.06, 95% CI: 0.96–1.17, $P = 0.228$) in *ENAM* with the risk of dental caries. There was significant heterogeneity in all the meta-analyses except the meta-analysis of rs3796704 ($P = 0.091$; Fig. 3). We did not find significant association of the four genetic variants with the risk of dental caries assuming other genetic models (Supplementary Tables 2–5).

Meta-analyses of other genetic variants that included fewer studies revealed no significant association with the risk of dental caries. However, multiple genetic variants in *ENAM*, *TUFT1*, *MMP2*, *MMP3*, *MMP8*, *MMP13*, *MMP20* and *AMELX* showed significant association with the risk of dental caries in individual studies (Supplementary Table 1).

3.4. Gene-based and gene-cluster analysis

Gene-based analysis results are presented in Table 3. We found that genetic variants in *AMELX* showed joint association with the risk of dental caries (6 variants; all $Ps < 2 \times 10^{-4}$), so did genetic variants in *MMP13* (3 variants; all $Ps \leq 0.01$), *MMP2* (3 variants; all $Ps < 10^{-5}$), *MMP20* (2 variants; all $Ps < 10^{-5}$) and *MMP3* (2 variants; all $Ps < 10^{-5}$). The gene-cluster analysis indicated a significant association between the genetic variants in this enamel-formation cluster and the risk of dental caries (all $Ps < 10^{-5}$; Table 4).

3.5. Sensitivity analysis

The association of rs17878486 in *AMELX* remained to be significant when we excluded studies in which genetic data in the control group violated HWE (OR = 1.59, 95% CI: 1.15–2.20, $P = 0.006$; Supplementary Table 6), so was the association of rs1784418 in *MMP20* (OR = 1.24, 95% CI: 1.05–1.46, $P = 0.012$). The association disappeared after excluding studies of low quality and after excluding studies that used adult data (Supplementary Tables 7–8). It should be noted that the total sample sizes for these sensitivity analyses were very limited. We did not find a significant association of the other two genetic variants (rs12640848 and rs3796704) with the risk of dental caries in all the sensitivity analyses (Supplementary Tables 6–8).

4. Discussions

In this manuscript, we performed a systematic literature search and conducted meta-, gene-based and gene-cluster analysis to examine the association of multiple genetic variants in the enamel-formation genes with the risk of dental caries. We found that rs17878486 in *AMELX* was significantly associated with the

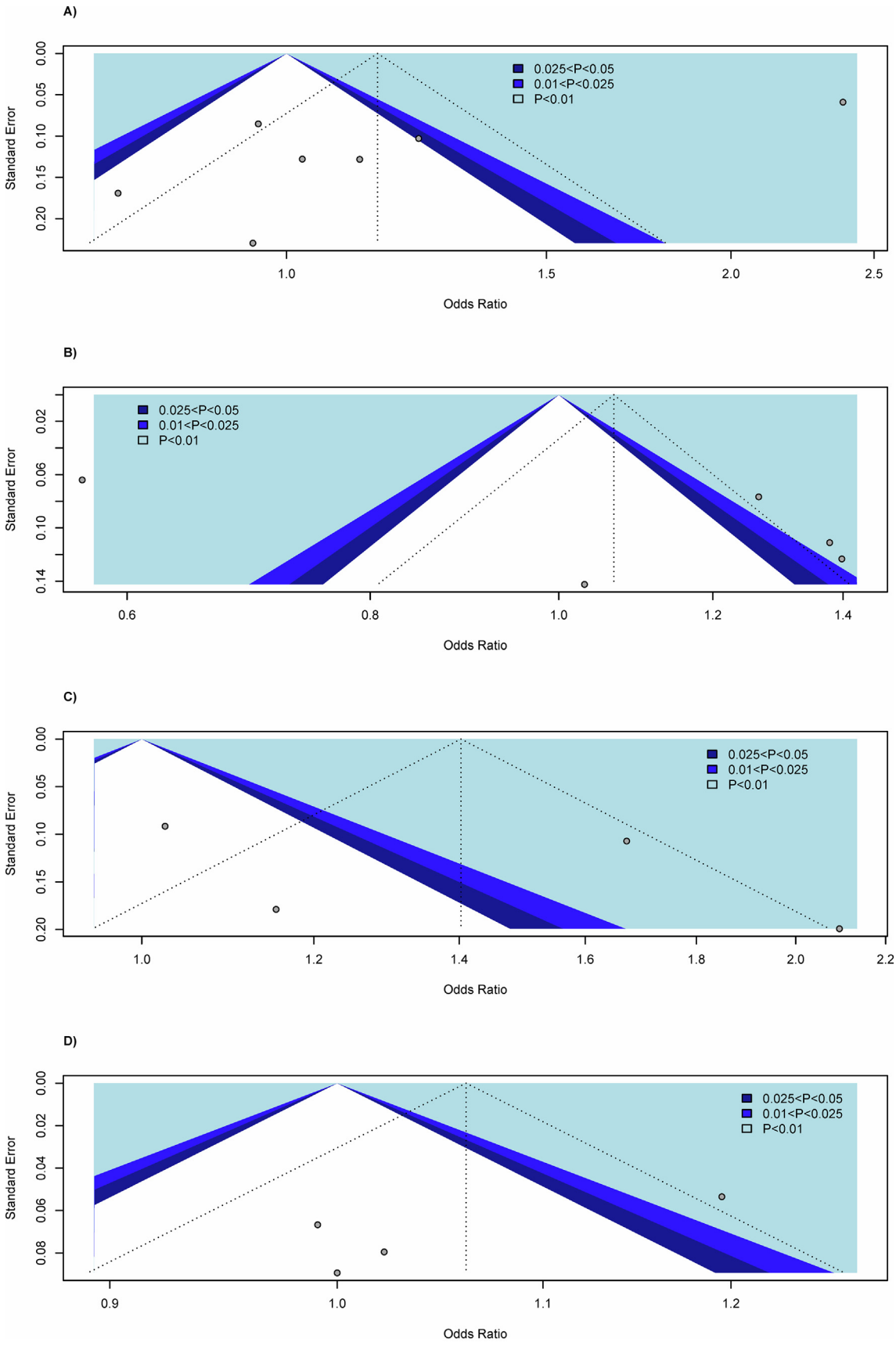
A)
Standard Error
0.00
0.05
0.10
0.15
0.20
0.025<P<0.05
0.01<P<0.025
P<0.01
1.0
1.5
2.0
2.5
Odds Ratio
B)
Standard Error
0.02
0.06
0.10
0.14
0.025<P<0.05
0.01<P<0.025
P<0.01
0.6
0.8
1.0
1.2
1.4
Odds Ratio
C)
Standard Error
0.00
0.05
0.10
0.15
0.20
0.025<P<0.05
0.01<P<0.025
P<0.01
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Odds Ratio
D)
Standard Error
0.00
0.02
0.04
0.06
0.08
0.025<P<0.05
0.01<P<0.025
P<0.01
0.9
1.0
1.1
1.2
Odds Ratio

Table 2
Meta-analysis of the association of genetic variants in enamel-formation genes with the risk of dental caries.*

Genetic variant*	Gene	Number of studies	Number of study subjects		Test of heterogeneity	OR (95% CI)	*P*
			Dental caries	Control			
rs12640848	*ENAM*	7	1256	710	7.69×10^{-25}	1.15 (0.88–1.52)	0.310
rs1784418	*MMP20*	5	699	817	1.38×10^{-20}	1.07 (0.76–1.49)	0.702
rs17878486	*AMELX*	4	249	193	3.58×10^{-4}	1.40 (1.02–1.93)	0.037
rs3796704	*ENAM*	4	574	533	0.091	1.06 (0.96–1.17)	0.228

We only reported results for genetic variants that had data from at least four studies. Results for other genetic variants assuming different genetic models were reported in Supplementary Tables 1–5.
OR: odds ratio; CI: confidence interval.
* Assuming an additive genetic model.

risk of dental caries, but no significant association of other genetic variants in the meta-analyses including at least four studies. However, gene-based analysis and gene-cluster analysis indicated that genetic variants in the enamel-formation genes were jointly associated with the risk of dental caries. To the best of our knowledge, this is the first meta-analysis on some of the genetic variants in the enamel-formation genes, and the first gene-based and gene-cluster analysis on the joint association of genetic variants in this gene cluster with the risk of dental caries.

AMELX is a gene located on the X chromosome (Lau et al., 1989) and encodes a set of isoforms of amelogenin, a major structural protein of the enamel organic matrix protein. Previous research using genetically engineered mice indicated that *AMELX* was crucial for proper enamel formation (Gibson et al., 2001). Our meta-analysis of four studies including a total of 249 subjects with caries and 193 subjects without caries indicated that the genetic variant rs17878486 in *AMELX* was significantly associated with the risk of dental caries assuming an additive model; however, it showed no significant association under other genetic models (Supplementary Tables 2–5). Moreover, no other genetic variants showed significant association except rs5933871 (Supplementary Table 1). Given the important role of *AMELX* in amelogenesis, future research is greatly needed to validate the relationship of the reported genetic variants and explore other genetic variants in this gene that may be associated with the risk of dental caries.

The gene *ENAM* is located on chromosome 4q 13.3 and has 10 exons. It encodes enamelin protein which is critical for the formation and elongation of enamel crystallites (Hu et al., 2000). Meta-analysis of the genetic variant rs12640848 in *ENAM* has the largest number of included studies. Previous research suggested that this polymorphism was associated with the risk of dental caries (Shaffer et al., 2015; Abbasoglu et al., 2015). In our meta-analysis assuming an additive model, of the six publications including a total of seven studies, only one study including 541 caries-affected children and 177 caries-free children aged 13–15 years old in Czech indicated a significant positive association of the G allele with an increased risk of dental caries (OR = 2.38, 95% CI: 2.12–2.67, $P < 0.0001$) (Borilova Linhartova et al., 2018). However, we did notice that several included studies indicated a significant association of this variant under other genetic models (Gerreth et al., 2016; Devang Divakar et al., 2019), although the overall meta-analysis results were still not significant (data not shown). Most of the other studied genetic variants in this gene were not associated with the risk of dental caries except one SNP rs3806804 which showed marginal association (OR = 0.80, 95% CI: 0.65–1.00; $P = 0.049$; Supplementary Table 1). More studies are needed to elucidate the role of rs12640848 and other genetic variants in *ENAM* in the formation of dental caries.

Matrix metalloproteinases (MMPs) refer to a large family of zinc-dependent endoproteinases which are fundamental in tooth formation and mineralization of dental tissue (Fanchon et al., 2004). More than 25 vertebrate MMPs have been identified, and 24 of them are present in humans (Fanjul-Fernandez et al., 2010). Genetic variants in many of the MMP genes, such as *MMP2*, *MMP3*, *MMP13* and *MMP20*, have been reported to be associated with the risk of dental caries (Tannure et al., 2012; Tannure et al., 2012; Karayasheva et al., 2016). Meta-analysis of rs1784418 in *MMP20* including a total of five studies showed no significant association of this genetic variant with the risk of dental caries (Table 1). However, many other genetic variants in this gene cluster were significantly associated with the risk of dental caries (Supplementary Table 1). These results should be interpreted with caution because they were based on data from single studies with limited sample sizes. Gene-based analysis indicated that genetic variants within *MMP2*, *MMP3*, *MMP8*, *MMP13* and *MMP20* were jointly associated with the risk of dental caries, further supporting the involvement of genetic variants in these genes in influencing the risk of dental caries. Future studies are warranted to reveal the function of these genetic variants in the etiology of dental caries.

We observed significant heterogeneities in all of the meta-analyses except the meta-analysis of rs3796704 in *ENAM* (P = 0.091; Table 2). The heterogeneity disappeared in some sensitivity analyses by removing the studies that violated HWE in the control group, the studies of low quality and the studies that used adult data, indicating that HWE, study quality which included several items such as selection of the study subjects, and age of study participants might be potential sources of heterogeneity. However, because of the limited availability of data from each individual study, the exact source of the heterogeneity could not be tracked, and meta-regression is also not feasible and/or meaningful, again due to the limited number of studies.

For the gene-based and gene-cluster analyses, we adopted the same approach as in our previous work which revealed that, despite weak evidence of individual genetic variants in lactotransferrin (*LTF*), multiple genetic variants in *LTF* showed joint contribu-

Fig. 2. Contour-enhanced funnel plots for meta-analyses of the association with dental caries assuming an additive model. The x-axis is the odds ratio, and the y-axis is the standard error of the estimated effect on the risk of dental caries. The vertical line in the figure represents the overall estimated odds ratio. The two diagonal lines represent the pseudo 95% confidence limits of the effect estimate. Levels of statistical significance of the individual studies are indicated by the shaded regions: the white region corresponds to p-values greater than 0.05, the dark blue-shaded region corresponds to *P*-values between 0.025 and 0.05, the blue-shaded region corresponds to *P*-values between 0.01 and 0.025, and the light blue-shaded region corresponds to *P*-values below 0.01. A) Funnel plot for meta-analysis of rs12640848; B) Funnel plot for meta-analysis of rs1784418; C) Funnel plot for meta-analysis of rs17878486; and D) Funnel plot for meta-analysis of rs3796704.

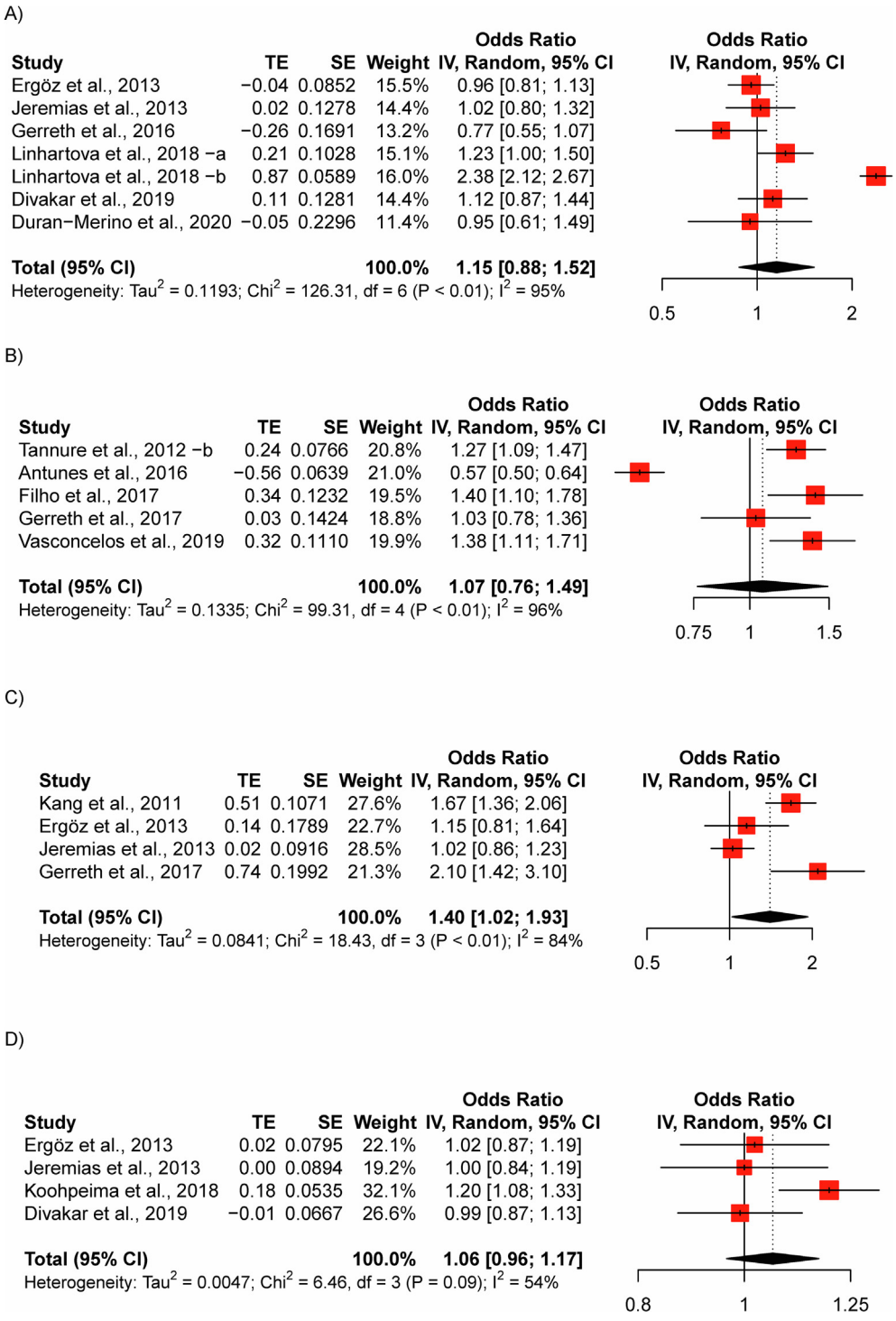

Fig. 3. Forest plots for meta-analysis of the association with dental caries assuming an additive model. Each study is represented by a square whose area is proportional to the weight of the study. The overall effect from meta-analysis is represented by a diamond whose width represents the 95% CI for the estimated OR. A) Forest plot for meta-analysis of rs12640848; B) Forest plot for meta-analysis of rs1784418; C) Forest plot for meta-analysis of rs17878486; and D) Forest plot for meta-analysis of rs3796704. OR, odds ratio; CI, confidence interval.

tion to the risk of dental caries (Li et al., 2020). Both of our studies indicated that although the effect of a single genetic variant may be small or insignificant, with proper methods we could still capture the joint contribution of multiple genetic variants. However, our previous study was limited to the exploration of genetic variants within a single gene, whereas in the present study, we examined multiple genetic variants in multiple genes. As a result, we were able to examine a larger number of genetic variants from a larger number of publications, and findings from our gene-based and gene-cluster analysis represented the joint effects from a larger number of genetic variants.

Our study has some limitations. Despite efforts in the systematic literature search, the sample sizes for many meta-analyses were limited. As a result, our findings need to be validated by future studies with larger sample sizes. The association of the risk of dental caries with many genetic variants was based on individ-

Table 3
Gene-based analysis of the association of genetic variants in enamel-formation genes with the risk of dental caries.

Gene	Number of variants	Fisher	Simes	Inverse	TPM (unweighted)	TPM (weighted)
ENAM	6	0.395	0.296	0.485	0.305	0.324
AMELX	6	1.26×10^{-4}	2.98×10^{-5}	1.40×10^{-5}	$<10^{-5}$	$<10^{-5}$
KLK4	5	0.494	0.276	0.599	0.275	0.381
TUFT1	4	0.103	0.077	0.064	0.090	0.088
MMP13	3	0.006	0.002	0.010	0.002	0.004
AMBN	3	0.452	0.262	0.998	0.261	0.199
MMP2	3	$<10^{-5}$	4.60×10^{-18}	2.00×10^{-51}	$<10^{-5}$	$<10^{-5}$
TFIP11	2	0.514	0.605	0.459	0.149	0.150
MMP20	2	$<10^{-5}$	3.48×10^{-19}	8.73×10^{-18}	$<10^{-5}$	$<10^{-5}$
MMP8	2	1.71×10^{-4}	0.005	0.004	0.004	0.003
MMP3	2	1.51×10^{-13}	1.59×10^{-8}	7.55×10^{-9}	$<10^{-5}$	$<10^{-5}$
MMP9	1	0.705	0.705	0.705	0.705	0.705

TPM, the modified truncated product method.

Table 4
Gene-cluster analysis of the association of genetic variants in the enamel-formation genes with the risk of dental caries.

Gene cluster	Fisher	Simes	Inverse	TPM
Enamel-formation genes	$<10^{-5}$	4.17×10^{-18}	2.77×10^{-45}	$<10^{-5}$

TPM, the modified truncated product method.

ual studies, which may be subject to biases due to a number of factors such as small sample size and the genetic background of the study participants. The exact relationship of these genetic variants with the risk of dental caries warrants further research. We did not perform analysis by type of dentition because of limited information and limited number of studies. Finally, due to a lack of data for individual subjects, out meta-analyses did not control for important factors that may affect the risk of dental caries. The estimated effect of the reported genetic variants on the risk of dental caries could be greatly confounded by such factors, a limitation of any meta-analysis that uses unadjusted results. Future research on the relationship between enamel-formation genes and the risk of dental caries should take into account the important confounding factors.

5. Conclusions

The present meta-analysis revealed that genetic variant rs17878486 in *AMELX* was associated with dental caries, and multiple genetic variants in the enamel-formation genes jointly contribute to the risk of dental caries. Future studies with large sample sizes that control for important confounding factors, such as diet, microbial and host characteristics, are needed to validate our findings and to explore additional genetic loci in this gene cluster that might also affect the risk of dental caries.

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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2020.11.071.

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