Saudi Journal of Biological Sciences 28 (2021) 4089–4095

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Mendelian randomization analysis identified genes potentially pleiotropically associated with periodontitis

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ARTICLE INFO

Article history:
Received 7 February 2021
Revised 26 February 2021
Accepted 8 April 2021
Available online 16 April 2021

Keywords:
Periodontitis
Pleotropic association
Expression quantitative trait loci
Summary Mendelian randomization

ABSTRACT

Objective: To prioritize genes that were pleiotropically or potentially causally associated with periodontitis.
Methods: We applied the summary data-based Mendelian randomization (SMR) method integrating genome-wide association study (GWAS) for periodontitis and expression quantitative trait loci (eQTL) data to identify genes that were pleiotropically associated with periodontitis. We performed separate SMR analysis using CAGE eQTL data and GTEx eQTL data. SMR analysis were done for participants of European and East Asian ancestries, separately.
Results: We identified multiple genes showing pleiotropic association with periodontitis in participants of European ancestry and participants of East Asian ancestry. *PDCD2* (corresponding probe: ILMN_1758915) was the top hit showing pleotropic association with periodontitis in the participants of European ancestry using CAGE eQTL data, and *BX093763* (corresponding probe: ILMN_1899903) and *AC104135.3* (corresponding probe: ENSG00000204792.2) were the top hits in the participants of East Asian ancestry using CAGE eQTL data and GTEx eQTL data, respectively.
Conclusion: We identified multiple genes that may be involved in the pathogenesis of periodontitis in participants of European ancestry and participants of East Asian ancestry. Our findings provided important leads to a better understanding of the mechanisms underlying periodontitis and revealed potential therapeutic targets for the effective treatment of periodontitis.

1. Introduction

Periodontitis is a common disease characterized by an inflammatory response to commensal and pathogenic oral bacteria (Berezow and Darveau, 2000). The primary clinical features of periodontitis include periodontal pocketing, alveolar bone loss (BL), clinical attachment loss (CAL), and gingival inflammation (Flemmig, 1999). Based on the 2009-2014 National Health and Nutrition Examination Surveys data, it was estimated that periodontitis affected about 42% of US adults aged 30 to 79 years (Eke et al., 2020). Periodontitis is considered as the main cause of tooth loss in adults. Moreover, it is also associated with various systemic conditions such as coronary heart disease (Humphrey et al., 2008), diabetes (Nascimento et al., 2018) and pre-term birth (Walia and Saini, 2015). Periodontitis not only affects a patient's

Abbreviations: eQTL, expression quantitative trait loci; GWAS, Genome-wide association studies; GO, Gene ontology; HEIDI, Heterogeneity in dependent instruments; IVs, Instrumental variables; KEGG, Kyoto Encyclopedia of Genes and Genomes; LD, Linkage disequilibrium; MR, Mendelian randomization; SMR, Summary data-based Mendelian randomization.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.04.028

life, it also brings tremendous economic burden to the society, with an estimated global productivity loss due to untreated severe periodontitis being around $38.85 billion in 2015 (Righolt et al., 2018).

Periodontitis is a complex, multi-factorial infectious disease with possible contributions from multiple factors, including immunological response (Cekici et al., 2000), oral bacterial infections (Slots, 2000), lifestyle factors such as smoking (Leite et al., 2018) and alcohol consumption (Wang et al., 2016), psychological factors such as stress (Hilgert et al., 2006) and depression (Nascimento et al., 2019), and systematic diseases such as diabetes (Preshaw and Bissett, 2019). Previous studies also suggested that genetics plays an important role in the pathogenesis of periodontitis. For example, genetically identical monozygotic twins have more than a twofold increased risk of early onset periodontitis, compared with dizygotic twins (Corey et al., 1993). Another population-based twin study estimated that the heritability of periodontitis was approximately 50% (Michalowicz et al., 2000). Moreover, many GWAS and candidate gene studies have identified a number of genetic loci associated with the susceptibility of periodontitis (Schaefer et al., 2010; Munz et al., 2017; Teumer et al., 2013; Divaris et al., 2013; Freitag-Wolf et al., 2014; Laine et al., 2010). However, the biological mechanisms of these findings remain largely unclear, and more studies are needed to explore genes that are potentially causally associated with periodontitis to better understand the pathogenesis of periodontitis.

Mendelian randomization (MR) uses genetic variants as the proxy to randomization and is a promising tool to search for pleotropic/potentially causal effect of an exposure (e.g., gene expression) on the outcome (e.g., periodontitis) without the need of conducting conventional randomized clinical trials (RCTs) (Davey Smith and Hemani, 2014). Confounding and reverse causation, which are commonly encountered in traditional association studies, can be greatly reduced by using MR. MR has been successful in identifying gene expression probes or DNA methylation loci that are pleiotropically/potentially causally associated with various phenotypes, such as neuropathologies of Alzheimer's disease and severity of COVID-19 (Liu et al., 2021, 2020).

In this study, we applied the summary data-based MR (SMR) method integrating summarized GWAS data for periodontitis and cis- eQTL (expression quantitative trait loci) data to prioritize genes that are pleiotropically/potentially causally associated with periodontitis.

2. Methods

2.1. Data sources

2.1.1. eQTL data

In the SMR analysis, *cis*-eQTL genetic variants were used as the instrumental variables (IVs) for gene expression. We performed SMR analysis using gene expression data in blood due to the unavailability of eQTL data of the gum. Specifically, we used the CAGE eQTL summarized data (Lloyd-Jones et al., 2017), which included 2,765 participants, and the V7 release of the GTEx eQTL summarized data (GTEx Consortium, 2017), which included 338 participants. The eQTL data can be downloaded at https://cnsgenomics.com/data/SMR/#eQTLsummarydata.

2.1.2. GWAS data for periodontitis

The GWAS summarized data were provided by a recent genome-wide association *meta*-analysis of periodontitis (Shungin et al., 2019). The results were based on *meta*-analyses of 1000 genomes phase 1 version 2/3 imputed GWASs on periodontitis, with a total of nine cohorts from the Gene-Lifestyle Interactions in Dental Endpoints (GLIDE) consortium (Shungin et al., 2015). Specifically, the *meta*-analysis for participants of European ancestry included seven cohorts with a total sample size of 45,563 (17,353 cases and 28,210 controls), and the *meta*-analysis for participants of East Asian ancestry included two cohorts with a total sample size of 17,350 (1,680 cases and 15,670 controls). All participating studies assumed an additive genetic model, adjusting for age, age-squared and other study-specific covariates. The GWAS summarized data can be downloaded at https://data.bris.ac.uk/data/dataset/2j2rqgzedxlq02oqbb4vmycnc2.

2.1.3. SMR analysis

We conducted the SMR analysis with *cis*-eQTL as the IV, gene expression as the exposure, and periodontitis as the outcome. The analysis was done using the method as implemented in the software SMR. Detailed information regarding the SMR method was reported in a previous publication (Zhu et al., 2016). In brief, SMR applies the principles of MR to jointly analyze GWAS and eQTL summary statistics in order to test for pleotropic association between gene expression and a trait due to a shared and potentially causal variant at a locus. We also conducted the heterogeneity in dependent instruments (HEIDI) test to evaluate the existence of linkage in the observed association. A P_{HEIDI} of less than 0.05 indicates that the observed association could be due to two distinct genetic variants in high linkage disequilibrium with each other. We adopted the default settings in SMR (e.g., minor allele frequency [MAF] > 0.01, removing SNPs in very strong linkage disequilibrium [LD, r^2 > 0.9] with the top associated eQTL, and removing SNPs in low LD or not in LD [r^2 less than 0.05] with the top associated eQTL) except relaxing the threshold of eQTL P-value (P_{eQTL} <10^{-4}) due to the exploratory nature of this study, and used false discovery rate (FDR) to adjust for multiple testing. We performed SMR analysis for participants of European and East Asian ancestries, separately, using CAGE and GTEx eQTL data, respectively, comprising a total of four SMR analyses.

We used Affymetrix exon array S1.0 platforms to annotate the transcripts. We conducted functional enrichment analysis using the functional annotation tool "Metascape" for the top tagged genes to functionally annotate putative transcripts (Zhou et al., 2019). Gene symbols corresponding to the ten top hit genes were used as the input of the gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis.

Data cleaning and statistical/bioinformatical analysis was performed using R version 4.0.3 (https://www.r-project.org/), PLINK 1.9 (https://www.cog-genomics.org/plink/1.9/) and SMR (https://cnsgenomics.com/software/smr/).

3. Results

3.1. Basic information of the summarized data

The number of participants of the CAGE eQTL data is much larger than that of the GTEx eQTL data, so is the number of eligible probes. The sample size of the GWAS data for the European ancestry is much larger than that for the East Asian ancestry, so is the number of eligible genetic variants. The detailed information was shown in Table 1.

3.2. SMR analysis in participants of European ancestry

In participants of European ancestry, we identified two genes showing pleiotropic association with periodontitis after correction for multiple testing using FDR (Table 2). Specifically, using the CAGE eQTL data, our SMR analysis identified two genes that were pleiotropically/potentially causally associated with periodontitis, including *PDCD2* (ILMN_1758915; $P_{SMR} = 3.77 \times 10^{-5}$; Fig. 1) and

Table 1
Basic information of the GWAS and eQTL data.

Data Source	Total number of participants	Number of eligible genetic variants or probes
European ancestry		
eQTL data		
CAGE	2,765	8,230
GTEx	338	2,162
GWAS data	45,563	779,1334
East Asian ancestry		
eQTL data		
CAGE	2,765	7,304
GTEx	338	2,010
GWAS data	17,350	418,8352

GWAS: genome-wide association studies; QTL, quantitative trait loci

D4S234E (i.e., *NSG1*, ILMN_1772627; $P_{SMR} = 9.08 \times 10^{-4}$; Fig. 2). GO enrichment analysis of biological process and molecular function showed that the ten top hit genes were involved in two GO terms, including positive regulation of cysteine-type endopeptidase activity (GO:2001056) and positive regulation of defense response (GO:0031349; Fig. S1A). Concept network analysis of the ten top hit genes also revealed multiple domains related with endopeptidase activity (Fig. S1B). More information could be found in **Table S1**.

Using the GTEx eQTL data, we did not identify any genes that were pleiotropically/potentially causally associated with periodontitis, after correction for multiple testing using FDR (Table 2). However, we found that two genes, *NSG1* (CAGE eQTL: ILMN_1772627, $P_{SMR} = 9.08 \times 10^{-4}$; GETx eQTL: ENSG00000168824.10, $P_{SMR} = 1.35 \times 10^{-3}$) and *S100A12* (CAGE eQTL: ILMN_1748915, $P_{SMR} = 1.58 \times 10^{-3}$; GETx eQTL: ENSG00000163221.7, $P_{SMR} = 1.90 \times 10^{-3}$) were among the top hits in both SMR analyses. GO enrichment analysis of biological process and molecular function showed that the ten top hit genes were involved in two MAP kinase-related GO terms (GO:0043405 and GO:0043406; Fig. S1C). Concept network analysis of the genes revealed multiple domains related with MAP kinase activity and inflammation (Fig. S1D). More information could be found in **Table S2**.

3.3. SMR analysis in participants of East Asian ancestry

In participants of East Asian ancestry, we identified two genes showing significant pleiotropic association with periodontitis after correction for multiple testing using FDR (Table 3). Specifically, using the CAGE eQTL data, our SMR analysis identified one gene, *BX093763* (ILMN_1899903, $P_{SMR} = 2.33 \times 10^{-4}$). GO enrichment analysis of biological process and molecular function showed that the ten top hit genes were involved in one GO terms, axon development (GO:0061564; Fig. S2**A**). Concept network analysis of the genes revealed multiple domains related with inflammation (Fig. S2B). More information could be found in **Table S3**. Using the GTEx eQTL data, our SMR analysis identified one gene, *AC104135.3*, that was pleiotropically/potentially causally associated with periodontitis, after correction for multiple testing using FDR (ENSG00000204792.2, $P_{SMR} = 7.46 \times 10^{-4}$; Fig. 3). GO enrichment analysis of biological process and molecular function did not find any significant GO terms. Concept network analysis of the genes revealed multiple domains related with endogenous peptide antigen (Fig. S2**C**). More information could be found in **Table S4**.

We found that two genes were among the top hits in both SMR analyses, including *PSD4* (CAGE eQTL: ILMN_2154115, $P_{SMR} = 1.79 \times 10^{-3}$; GTEx: ENSG00000125637.11, $P_{SMR} = 1.84 \times 10^{-3}$) and *GFRA2* (CAGE eQTL: ILMN_1656300, $P_{SMR} = 3.55 \times 10^{-3}$; GTEx: ENSG00000168546.6, $P_{SMR} = 7.07 \times 10^{-3}$). More information could be found in **Table S3-4**.

4. Discussion

In the present study, we explored putative genes that showed pleiotropic/potentially causal association with periodontitis by integrating GWAS and eQTL data in the SMR analysis. We identi-

Table 2
The top ten probes identified in the SMR analysis in the participants of European ancestry.

eQTL data	Probe ID	Gene	CHR	Top SNP	P_{eQTL}	P_{GWAS}	Beta	SE	P_{SMR}	P_{HEIDI}	N_{SNP}
CAGE	ILMN_1758915	*PDCD2*	6	rs17875294	7.50×10^{-74}	2.34×10^{-5}	0.15	0.04	$\mathbf{3.77 \times 10^{-5}}$	4.52×10^{-1}	20
	ILMN_1772627	*NSG1*	4	rs6843595	7.10×10^{-291}	8.45×10^{-4}	0.05	0.02	$\mathbf{9.08 \times 10^{-4}}$	1.33×10^{-1}	20
	ILMN_1823130	*F01764*	4	rs2369111	1.74×10^{-17}	3.62×10^{-4}	0.24	0.07	1.00×10^{-3}	3.31×10^{-2}	20
	ILMN_1808251	*C9orf38*	9	rs4556138	6.67×10^{-10}	1.94×10^{-4}	0.35	0.11	1.40×10^{-3}	1.51×10^{-2}	20
	ILMN_1748915	*S100A12*	1	rs3014878	2.17×10^{-177}	1.45×10^{-3}	−0.06	0.02	1.58×10^{-3}	3.77×10^{-1}	20
	ILMN_1748221	*PADI6*	1	rs1535876	2.45×10^{-50}	1.69×10^{-3}	−0.12	0.04	2.13×10^{-3}	3.50×10^{-1}	20
	ILMN_1659511	*LOC645652*	1	rs10927894	3.79×10^{-7}	1.38×10^{-4}	0.60	0.20	2.30×10^{-3}	2.88×10^{-2}	20
	ILMN_1710937	*IFI16*	1	rs12122315	5.38×10^{-19}	1.27×10^{-3}	−0.22	0.07	2.46×10^{-3}	1.37×10^{-1}	20
	ILMN_1729801	*S100A8*	1	rs58644524	1.93×10^{-19}	1.36×10^{-3}	−0.20	0.06	2.52×10^{-3}	3.72×10^{-1}	20
	ILMN_2096405	*WDR37*	10	rs12768746	3.40×10^{-7}	2.40×10^{-4}	0.41	0.14	2.91×10^{-3}	4.89×10^{-2}	20
GTEx	ENSG00000168824.10	*NSG1*	4	rs6414635	1.94×10^{-41}	9.57×10^{-4}	0.08	0.02	1.35×10^{-3}	2.34×10^{-1}	20
	ENSG00000256049.2	*PADI6*	1	rs10888031	2.95×10^{-42}	1.32×10^{-3}	−0.06	0.02	1.74×10^{-3}	3.22×10^{-1}	20
	ENSG00000163221.7	*S100A12*	1	rs57572338	1.56×10^{-15}	7.58×10^{-4}	−0.37	0.12	1.90×10^{-3}	5.90×10^{-1}	9
	ENSG00000184985.12	*SORCS2*	4	rs62289059	8.07×10^{-15}	8.95×10^{-4}	0.15	0.05	2.23×10^{-3}	4.30×10^{-1}	20
	ENSG00000127952.12	*STYXL1*	7	rs115332207	3.41×10^{-39}	2.22×10^{-3}	0.12	0.04	2.94×10^{-3}	9.10×10^{-1}	20
	ENSG00000233609.3	*RP11-62H7.2*	8	rs13259143	5.63×10^{-19}	2.60×10^{-3}	0.16	0.06	4.40×10^{-3}	6.75×10^{-2}	20
	ENSG00000106804.6	*C5*	9	rs7036980	2.84×10^{-10}	1.63×10^{-3}	0.19	0.07	4.76×10^{-3}	6.56×10^{-1}	20
	ENSG00000213523.5	*SRA1*	5	rs76128141	7.51×10^{-13}	2.88×10^{-3}	−0.27	0.10	5.91×10^{-3}	4.22×10^{-2}	14
	ENSG00000163421.4	*PROK2*	3	rs6777956	3.52×10^{-17}	4.01×10^{-3}	−0.21	0.08	6.40×10^{-3}	5.76×10^{-1}	17
	ENSG00000138835.18	*RGS3*	9	rs41306506	6.49×10^{-20}	5.77×10^{-3}	0.20	0.08	8.22×10^{-3}	9.88×10^{-1}	20

*The GWAS summarized data were provided by the study of Shungin et al. and can be downloaded at https://data.bris.ac.uk/data/dataset/2j2rqgzedxlq02oqbb4vmycnc2. The CAGE and GTEx eQTL data can be downloaded at https://cnsgenomics.com/data/SMR/#eQTLsummarydata.

P_{eQTL} is the P-value of the top associated *cis*-eQTL in the eQTL analysis, and P_{GWAS} is the P-value for the top associated *cis*-eQTL in the GWAS analysis, Beta is the estimated effect size in SMR analysis, SE is the corresponding standard error, P_{SMR} is the P-value for SMR analysis, P_{HEIDI} is the P-value for the HEIDI test and Nsnp is the number of SNPs involved in the HEIDI test.

FDR was calculated at $P = 10^{-3}$ threshold.

Bold font means statistical significance after correction for multiple testing using FDR.

CHR, chromosome; HEIDI, heterogeneity in dependent instruments; SNP, single-nucleotide polymorphism; SMR, summary data-based Mendelian randomization; QTL, quantitative trait loci; FDR, false discovery rate; GWAS, genome-wide association studies

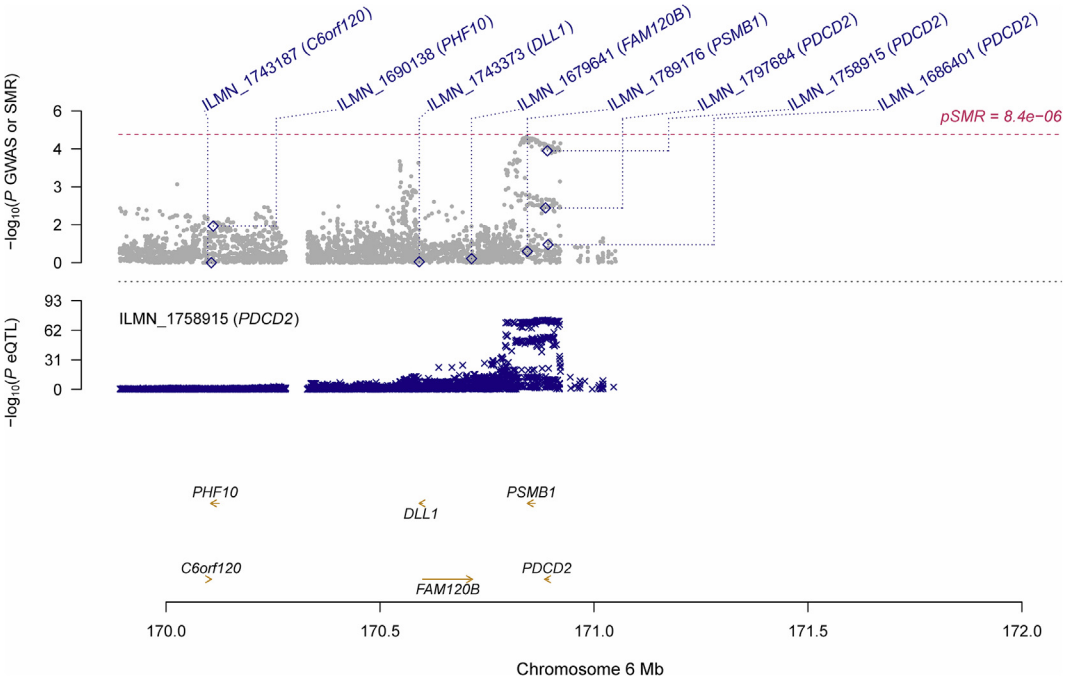

Fig. 1. Prioritizing gene around *PDCD2* in pleiotropic association with periodontitis in the participants of European ancestry.Results were obtained using CAGE eQTL data. Top plot, grey dots represent the -$\log_{10}$(*P* values) for SNPs from the GWAS of periodontitis, and rhombuses represent the -$\log_{10}$(*P* values) for probes from the SMR test with hollow rhombuses indicating that the probes do not pass the HEIDI test. Middle plot, eQTL results for ILMN_1758915 probe, tagging *PDCD2*. Bottom plot, location of genes tagged by the probes. GWAS, genome-wide association studies; SMR, summary data-based Mendelian randomization; HEIDI, heterogeneity in dependent instruments; eQTL, expression quantitative trait loci.

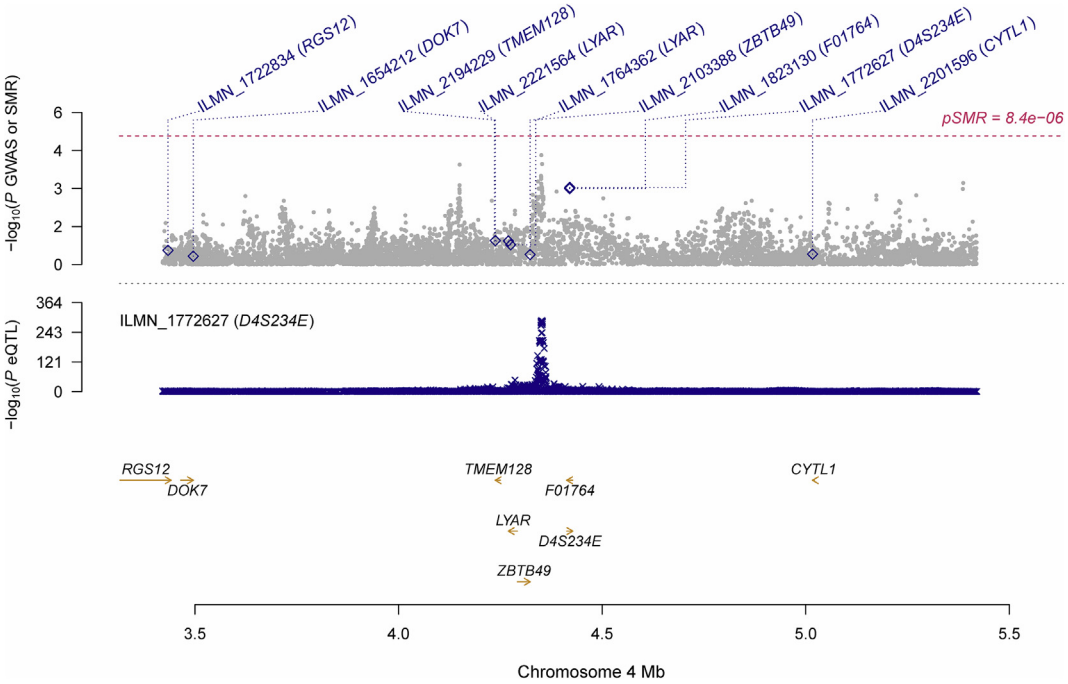

Fig. 2. Prioritizing gene around *D4S234E* (i.e., *NSG1*) in pleiotropic association with periodontitis in the participants of European ancestry. Results were obtained using CAGE eQTL data. Top plot, grey dots represent the -$\log_{10}$(*P* values) for SNPs from the GWAS of periodontitis, and rhombuses represent the -$\log_{10}$(*P* values) for probes from the SMR test with hollow rhombuses indicating that the probes do not pass the HEIDI test. Middle plot, eQTL results for ILMN_1772627 probe, tagging *D4S234E* (i.e., *NSG1*). Bottom plot, location of genes tagged by the probes. GWAS, genome-wide association studies; SMR, summary data-based Mendelian randomization; HEIDI, heterogeneity in dependent instruments; eQTL, expression quantitative trait loci.

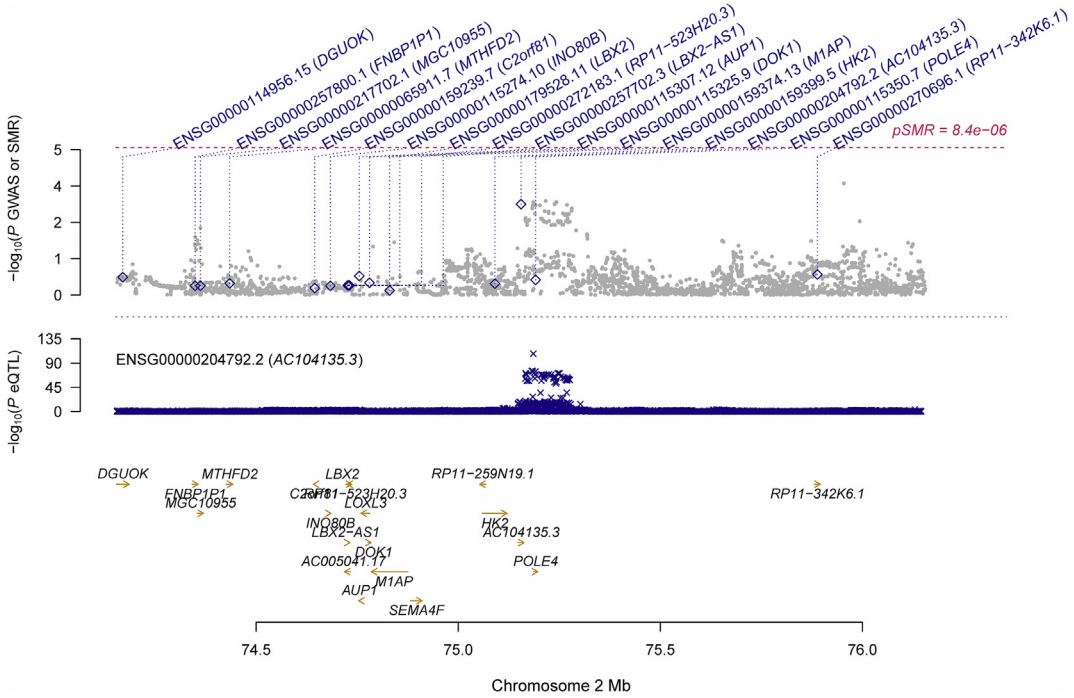

Fig. 3. Prioritizing gene around *AC104135.3* in pleiotropic association with periodontitis in the participants of East Asian ancestry. Results were obtained using GTEx eQTL data. Top plot, grey dots represent the -$\log_{10}$(*P* values) for SNPs from the GWAS of periodontitis, and rhombuses represent the -$\log_{10}$(*P* values) for probes from the SMR test with hollow rhombuses indicating that the probes do not pass the HEIDI test. Middle plot, eQTL results for ENSG00000204792.2 probe, tagging *AC104135.3*. Bottom plot, location of genes tagged by the probes. GWAS, genome-wide association studies; SMR, summary data-based Mendelian randomization; HEIDI, heterogeneity in dependent instruments; eQTL, expression quantitative trait loci.

Table 3
The top ten probes identified in the SMR analysis in the participants of East Asian ancestry.

eQTL data	Probe ID	Gene	CHR	Top SNP	P_{eQTL}	P_{GWAS}	Beta	SE	P_{SMR}	P_{HEIDI}	N_{SNP}
CAGE	ILMN_1899903	*BX093763*	5	rs984976	4.96×10^{-39}	1.27×10^{-4}	0.42	0.11	$\mathbf{2.33 \times 10^{-4}}$	2.67×10^{-1}	20
	ILMN_1734231	*DDOST*	1	rs6893	8.13×10^{-40}	1.17×10^{-3}	0.27	0.09	1.62×10^{-3}	9.21×10^{-2}	20
	ILMN_2154115	*PSD4*	2	rs2241976	1.10×10^{-77}	1.53×10^{-3}	0.26	0.08	1.79×10^{-3}	2.64×10^{-6}	20
	ILMN_2388155	*CASP3*	4	rs11721363	1.33×10^{-39}	2.07×10^{-3}	−0.88	0.29	2.72×10^{-3}	1.80×10^{-6}	20
	ILMN_1764522	*LMBR1*	7	rs73167977	3.88×10^{-22}	2.01×10^{-3}	−0.74	0.25	3.26×10^{-3}	9.03×10^{-4}	20
	ILMN_1656300	*GFRA2*	8	rs1479056	2.14×10^{-62}	3.07×10^{-3}	0.30	0.10	3.55×10^{-3}	5.51×10^{-1}	20
	ILMN_1791211	*DOK2*	8	rs1479056	1.14×10^{-47}	3.07×10^{-3}	0.34	0.12	3.72×10^{-3}	1.65×10^{-1}	20
	ILMN_1808661	*TOMM5*	9	rs7018807	9.27×10^{-61}	3.58×10^{-3}	−0.34	0.12	4.09×10^{-3}	4.20×10^{-3}	20
	ILMN_1910292	*BX094911*	4	rs3111820	3.02×10^{-5}	7.91×10^{-5}	−1.46	0.51	4.13×10^{-3}	2.69×10^{-1}	4
	ILMN_1805590	*NAA38*	7	rs7799229	1.28×10^{-67}	3.93×10^{-3}	0.24	0.08	4.44×10^{-3}	9.13×10^{-5}	20
GTEx	ENSG00000204792.2	*AC104135.3*	2	rs12366	5.06×10^{-108}	6.39×10^{-4}	0.12	0.03	$\mathbf{7.46 \times 10^{-4}}$	3.11×10^{-1}	20
	ENSG00000204469.8	*PRRC2A*	6	rs2075800	3.28×10^{-8}	6.54×10^{-5}	1.72	0.53	1.22×10^{-3}	8.73×10^{-2}	20
	ENSG00000243753.1	*HLA-L*	6	rs3094204	1.45×10^{-17}	6.48×10^{-4}	−0.27	0.09	1.54×10^{-3}	1.97×10^{-3}	20
	ENSG00000224769.1	*AC069213.1*	3	rs6804822	3.13×10^{-14}	5.05×10^{-4}	0.42	0.13	1.56×10^{-3}	1.34×10^{-3}	20
	ENSG00000125637.11	*PSD4*	2	rs2241976	2.71×10^{-66}	1.53×10^{-3}	0.34	0.11	1.84×10^{-3}	1.96×10^{-5}	20
	ENSG00000144791.5	*LIMD1*	3	rs34448158	4.35×10^{-11}	5.24×10^{-4}	0.71	0.23	2.16×10^{-3}	5.00×10^{-1}	20
	ENSG00000261490.1	*RP11-448G15.3*	4	rs3756218	6.00×10^{-12}	1.23×10^{-3}	−0.92	0.31	3.42×10^{-3}	3.45×10^{-3}	20
	ENSG00000164307.8	*ERAP1*	5	rs26490	3.17×10^{-51}	3.21×10^{-3}	−0.17	0.06	3.85×10^{-3}	8.25×10^{-1}	20
	ENSG00000164039.10	*BDH2*	4	rs3775972	7.75×10^{-11}	1.71×10^{-3}	0.55	0.20	4.74×10^{-3}	1.11×10^{-1}	20
	ENSG00000168546.6	*GFRA2*	8	rs1479057	8.81×10^{-11}	3.07×10^{-3}	0.50	0.19	7.07×10^{-3}	5.34×10^{-1}	19

*The GWAS summarized data were provided by the study of Shungin et al. and can be downloaded at https://data.bris.ac.uk/data/dataset/2j2rqgzedxlq02oqbb4vmycnc2. The CAGE and GTEx eQTL data can be downloaded at https://cnsgenomics.com/data/SMR/#eQTLsummarydata.
P_{eQTL} is the P-value of the top associated *cis*-eQTL in the eQTL analysis, and P_{GWAS} is the P-value for the top associated *cis*-eQTL in the GWAS analysis, Beta is the estimated effect size in SMR analysis, SE is the corresponding standard error, P_{SMR} is the P-value for SMR analysis, P_{HEIDI} is the P-value for the HEIDI test and Nsnp is the number of SNPs involved in the HEIDI test.
FDR was calculated at $P = 10^{-3}$ threshold.
Bold font means statistical significance after correction for multiple testing using FDR.
CHR, chromosome; HEIDI, heterogeneity in dependent instruments; SNP, single-nucleotide polymorphism; SMR, summary data-based Mendelian randomization; QTL, quantitative trait loci; FDR, false discovery rate; GWAS, genome-wide association studies

fied multiple genes, some of which represented novel genes, that might be involved in the pathogenesis of periodontitis in participants of European ancestry and participants of East Asian ancestry. Our findings provided helpful leads to a better understanding of the mechanisms underlying periodontitis and suggested potential therapeutic targets for the treatment of periodontitis.

A recent study investigated molecular biomarker candidates and biological pathways of chronic periodontitis using pooled datasets in the Gene Expression Omnibus (GEO) database, and identified 123 common differently expressed genes (DEGs), including 81 upregulated genes and 42 downregulated genes (Suzuki et al., 2019). Several of the identified genes were also among the top hits in our SMR analysis. For example, the gene *NSG1* (Neuronal Vesicle Trafficking Associated 1) was found to be downregulated in persons with chronic periodontitis. It also showed significant pleiotropic association with periodontitis in our study of participants of European ancestry (Table 2). *NSG1*, also known as *D4S234E* or *NEEP21*, is located on 4p16.3 in human and is a member of the neuron-specific gene (NSG) family. It is the most important in regulating receptor recycling and synaptic transmission among the NSG family (Rengaraj et al., 2011). p53, an important tumor suppressor gene, binds to the promoter region of *NSG1* and regulates its expression in response to DNA damage. Inhibition of *NSG1* expression suppressed apoptosis (Kudoh et al., 2010). The exact role of *NSG1* in the pathogenesis of periodontitis is unclear and warrants further research.

Another research integrating GWAS and eQTL data identified 10 genes whose expression might influence periodontitis (Li et al., 2020). Of them, the gene *S100A12* (S calcium-binding protein A12) also appeared among the top hits in participants of European ancestry in the SMR analysis using CAGE and GTEx eQTL data (Table 2). *S100A12*, located on 1q21.3, is a member of the S100 family of EF-hand calcium-binding proteins (Guignard et al., 1995). Previous studies indicated that it played a prominent role in the regulation of inflammatory processes and immune response (Pietzsch and Hoppmann, 2009). It was reported that the levels of *S100A12* were higher in participants with high periodontal inflammatory burden and were associated with the percentage of bleeding on probing (Holmstrom et al., 2019). In gingival crevicular fluid and serum, the levels of *S100A12* increased with the inflammation of periodontium (Pradeep et al., 2014). These findings, together with ours, demonstrated the important role of *S100A12* in influencing periodontitis and highlighted the potential of this gene as a promising target for the prevention and treatment of periodontitis.

Our study was different from the previous study integrating GWAS and eQTL data (Li et al., 2020). We used the GWAS summarized data for both European and East Asian ancestries, while the previous research only analyzed GWAS data of European ancestry. Similarly, we used both CAGE and GTEx eQTL data, while the previous research used only GTEx data. Moreover, we undertook a SMR analytic framework which focused on exploring genes showing pleiotropic association/potentially causal association with periodontitis while the previous research adopted a Sherlock approach which is a Bayesian statistical framework aiming to identify genes whose expression was associated with periodontitis susceptibility (He et al., 2013).

Our study was also very different from another MR research on periodontitis (Shungin et al., 2015). Although both studies aimed to explore potentially causal factors for periodontitis, the previous research focused on examining the causal role of total adiposity in the pathogenesis of periodontitis, while our study attempted to identify genes that were pleiotropically/potentially causally associated with periodontitis. The analytic approaches were also different: in the previous research, the IVs were based on genetic risk scores calculated from three genes (*FTO*, *MC4R* and *TMEM18*) by summing the number of BMI increasing alleles; while in our study, we used all the genetic variants from GWAS summarized data as the potential instrumental variables.

Our study has some limitations. The number of probes used in our SMR analysis was limited, especially in the SMR analysis of participants of East Asian ancestry. As a result, we may have missed some genes which played important roles in the pathogenesis of periodontitis. The HEIDI test was significant for some of the identified genes (Table 2-3). Therefore, we could not rule out the possibility of horizontal pleiotropy, i.e., the identified association might be due to two distinct genetic variants in high linkage disequilibrium with each other. In addition, we only performed SMR analysis for participants of European and East Asian ancestries, and our findings might not be generalized to other populations. More studies are needed to validate our findings in independent populations. Due to the exploratory nature of study, we adopted correction for multiple testing to reduce false positive rate; however, we may have missed important SNPs or genes. We only used eQTL data in the blood due to the unavailability of eQTL data from the gum. Our findings need to be validated in the future when eQTL data from the gum is available. Finally, we could not quantify the changes in gene expression in subjects with periodontitis in comparison with the control due to the unavailability of individual eQTL data.

5. Conclusions

In conclusion, our SMR analysis revealed multiple genes that were potentially pleiotropically associated with periodontitis. More studies are needed to explore the underlying physiological mechanisms in the etiology of periodontitis.

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..

Acknowledgements

The study was supported by NIH/NIA grants P30AG10161, R01AG15819, R01AG17917, R01AG36042, U01AG61356 and 1RF1AG064312-01. Di Liu was supported by China Scholarship Council (CSC 201908110339).

The authors confirmed that all authors have reviewed the contents of the article being submitted, approved its contents, and validated the accuracy of the data.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.04.028.

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