



Integration of genome-wide association study and expression quantitative trait loci data identifies *AIM2* as a risk gene of periodontitis

Wenjing Li¹ | Qiwen Zheng² | Huanxin Meng¹ | Dafang Chen²

¹Department of Periodontology, National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing Key Laboratory of Digital Stomatology, Peking University School and Hospital of Stomatology, Beijing, China

²Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing, China

Correspondence

Huanxin Meng, Department of Periodontology, Peking University School and Hospital of Stomatology, 22 Zhongguancun Nandajie, Haidian District, 100081 Beijing, China.
Email: kqhxmeng@bjmu.edu.cn

Dafang Chen, Department of Epidemiology and Biostatistics, School of Public Health, Peking University, No.38 Xueyuan Road, Haidian District, 100191 Beijing, China.
Email: dafangchen@bjmu.edu.cn

Abstract

Aim: To identify risk variants associated with gene expression in peripheral blood and to identify genes whose expression change may contribute to the susceptibility to periodontitis.

Material and Methods: We systematically integrated the genetic associations from a recent large-scale periodontitis GWAS and blood expression quantitative trait loci (eQTL) data using *Sherlock*, a Bayesian statistical framework. We then validated the potential causal genes in independent gene expression data sets. Gene co-expression analysis was used to explore the functional relationship for the identified causal genes.

Results: *Sherlock* analysis identified 10 genes (rs7403881 for *MT1L*, rs12459542 for *SIGLEC5*, rs12459542 for *SIGLEC14*, rs6680386 for *S100A12*, rs10489524 for *TRIM33*, rs11962642 for *HIST1H3E*, rs2814770 for *AIM2*, rs7593959 for *FASTKD2*, rs10416904 for *PKN1*, and rs10508204 for *WDR37*) whose expression may influence periodontitis. Among these genes, *AIM2* was consistent significantly upregulated in periodontium of periodontitis patients across four data sets. The cis-eQTL (rs2814770, ~16 kb upstream of *AIM2*) showed significant association with *AIM2* ($p = 6.63 \times 10^{-6}$) and suggestive association with periodontitis ($p = 7.52 \times 10^{-4}$). We also validated the significant association between rs2814770 and *AIM2* expression in independent expression data set. Pathway analysis revealed that genes co-expressed with *AIM2* were significantly enriched in immune- and inflammation-related pathways.

Conclusion: Our findings implicate that *AIM2* is a susceptibility gene, expression of which in gingiva may influence periodontitis risk. Further functional investigation of *AIM2* may provide new insight for periodontitis pathogenesis.

KEYWORDS

AIM2, eQTL, GWAS, periodontitis, *Sherlock*

Wenjing Li and Qiwen Zheng are the co-first authors of this article.

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1 | INTRODUCTION

Periodontitis is a common oral disease with complex aetiology. The signs of periodontitis include periodontal pocket, attachment loss, and alveolar bone resorption on radiographs, often accompanied with gingival recession and associated tooth hypersensitivity, tooth mobility and drifting, and periodontal abscesses, which can eventually lead to tooth loss (Pihlstrom, Michalowicz, & Johnson, 2005). This disorder not only affects oral health, but also correlates with systemic disorders such as cardiovascular diseases, diabetes, pre-term birth, and aspiration pneumonia (Pihlstrom et al., 2005; Williams et al., 2008). Furthermore, a 16-year longitudinal study indicated that periodontitis is linked to the increased risk of premature death (Soder, Jin, Klinge, & Soder, 2007). The 2016 Global Burden of Diseases, Injuries and Risk Factors Study estimated that periodontitis was the 11th most prevalent causes of disease worldwide (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2017). Moreover, severe periodontitis was attributed with adding \$38.85 billion (21%) to the estimated global indirect cost in 2015 (Righolt, Jevdjevic, Marcenes, & Listl, 2018). Therefore, it is of a great importance to investigate the aetiology of periodontitis.

Accumulating evidence indicates that periodontitis is caused by a combination of genetic and environmental factors (Jin et al., 2011; Meng et al., 2011). Several environmental factors, including poor oral hygiene, tobacco smoking, socio-economic status, malnutrition, psychological conditions, drug use, and local conditions, were reported to contribute to the onset of periodontitis (Jin et al., 2011). In addition to environmental factors, genetic factors also have pivotal role in periodontitis. The heritability of periodontitis is reported as high as 50% (Michalowicz et al., 2000; Nibali et al., 2019), indicating the essential role of genetic factors in this disease.

To identify the genetic risk variants for periodontitis, several genome-wide association studies (GWAS) have been conducted worldwide (Hong, Shin, Ahn, Lee, & Kim, 2015; Masumoto et al., 2019; Munz et al., 2017; Offenbacher et al., 2016; Sanders et al., 2017; Teumer et al., 2013). For example, the GWAS by Munz et al identified nucleotide variants at *SIGLEC5* and *DEFA1A3* as risk loci for periodontitis (Munz et al., 2017). Another GWAS by Hong et al confirmed a previously reported association between *TENM2* gene and periodontitis and suggested a newly identified relationship to *LDLRAD4* (Hong et al., 2015). However, even though the data from GWAS support a number of loci involved in periodontitis, it is difficult to discriminate many of the causal genes from the background noise of the hundreds of thousands of SNPs in the assay. Considering the fact that most of the variants identified by GWAS are located in non-coding regions, it is reasonable to assume that these variants affect phenotypes through regulating gene expression. Previous studies (Luo et al., 2015; Wu et al., 2019; Yang et al., 2018; Zhong et al., 2019) have successfully prioritized plausible causal genes by using integrative approach of expression quantitative trait loci (eQTL) and disease association

Clinical Relevance

Scientific rationale for study: Recent large-scale GWAS have identified multiple risk variants associated with periodontitis. However, it is challenging to identify the causal genes at the risk loci. Since most of the variants are located in non-coding regions, it is reasonable to assume that these variants affect phenotypes by regulating gene expression.

Principal findings: Our study identified *AIM2* as a potential causal gene whose expression level may play a role in periodontitis. Genes co-expressed with *AIM2* were enriched in immune- and inflammation-related pathways.

Practical implications: *AIM2* could be a potential diagnostic marker as well as therapeutics target after further experimental validations.

data. Nevertheless, to date, there is no relevant systematic study, incorporating data from different omics layers to identify periodontitis-associated genes. Therefore, we applied a Bayesian integrative approach (*Sherlock*) that combined a large GWAS summary statistics for periodontitis and blood eQTL data to investigate potential causal genes, as well as to explore the role of these genes involved in the pathogenesis.

2 | MATERIAL AND METHODS

2.1 | Ethics statement

Each contributing study obtained informed consent from all study participants. The studies complied with all relevant ethical regulations, including the Declaration of Helsinki, and ethical approval for data collection and analysis was obtained for each study from the local boards (Shungin et al., 2019).

2.2 | Periodontitis GWAS data

We used summary statistics from a large-scale periodontitis GWAS (Shungin et al., 2019). In this study, Shungin et al performed a meta-analysis of a recent periodontitis GWAS from the Gene-Lifestyle Interactions in Dental Endpoints (GLIDE) consortium. Briefly, genome-wide genotypes from nine independent samples of European ancestry (including ARIC, COHRA1, DRDR, MDC, NFBC1996, SHIP, SHIP trend, TWINGENE, and WGHS) with a final sample size up to 17,353 periodontitis cases and 28,210 controls were combined and subjected to a systematic meta-analysis. More detailed information about sample description, diagnosis, genotyping, and statistical analyses can be found in the original study (Shungin et al., 2019).

2.3 | Whole blood eQTL data

eQTLs are partially tissue-specific, and, hence, it is recommended to use pathologically relevant tissue for the assessment of gene expression. However, due to the unavailability of eQTL data of gingiva tissue, we used readily available tissues, typically whole blood, as a proxy for eQTL effect measurement. The released v.7 summary statistics eQTL results from the Genotype-Tissue Expression (GTEx) project database for the whole blood ($n = 369$) were adopted (GTEx Consortium, 2013, 2015). Gene expression was quantified using RNA-sequencing technology, and single-nucleotide polymorphism (SNP) genotyping was performed using the Illumina OMNI 5M SNP Array. In addition to whole blood eQTL data from GTEx, we also used another available well-characterized expression data to validate eQTL results. Consortium for the Architecture of Gene Expression (CAGE; Lloyd-Jones et al., 2017) used individual-level whole blood expression and genotype data on 2,765 individuals to explore the genetic architecture of gene expression in peripheral blood. Measurements of gene expression were performed using Illumina Whole-Genome Expression BeadChips, and genotype data were imputed using the 1,000 Genomes Phase 1 version 3 reference panel, resulting in 7,763,174 SNPs passing quality control. Whole-genome eQTL data (peripheral blood) of 2,765 samples from five cohorts were obtained and meta-analysed.

2.4 | *Sherlock* integrative analysis

On the basis of the underlying assumption that the expression change of a specific gene may contribute to periodontitis risk, we used *Sherlock* integrative analysis method, developed by He et al. (2013), to integrate SNP associations from CLIDE (Shungin et al., 2019) and whole blood eQTL from GTEx (GTEx Consortium, 2015). Briefly, *Sherlock* utilizes a Bayesian statistical framework to infer genes whose expression alterations would influence disease pathogenesis. The workflow of this algorithm is as follows: (a) identification of expression-associated SNPs (eSNPs) in whole blood tissue from GTEx v7 eQTL data based on *Sherlock* criteria ($p < 10^{-5}$ for trans- and $p < 10^{-4}$ for cis-associations); (b) evaluation of the association between eSNPs and periodontitis using the GWAS data from GLIDE consortium; (c) assignment of each eSNP with the score (Bayes factor, BF), according to the association significance between the eSNP and periodontitis; and (d) calculation of the logarithm of BF (LBFs) for each SNP and sums of LBFs for all SNPs to acquire the final LBF score for each gene. There are three different scenarios by assigning the score for each eSNP: (a) in case the eSNP of a specific gene is also associated with periodontitis in GWAS, it receives a positive score; (b) if the eSNP of the gene is not associated with periodontitis, it gets a negative score; and (c) if the SNP shows association only in GWAS, the score is not altered. The value of LBF score for each gene represents the strength evidence between the gene and disease. *Sherlock* utilizes

both strong and moderate SNPs in the eQTL and GWAS data instead of restricting focus on the few SNPs reaching genome-wide significant level.

2.5 | Expression analysis of candidate genes in gingiva of periodontitis patients

Sherlock identifies disease-associated genes with the assumption that the expression level of a specific gene may have an influence on disease pathogenesis. To evaluate whether the risk genes identified by *Sherlock* are differentially expressed in periodontitis sites compared with unaffected site, we used publicly available expression data sets, including GSE16134 (Kebeschull et al., 2014), GSE10334 (Demmer et al., 2008), GSE27993 (Schminke et al., 2015), and GSE79705 (Taiete et al., 2018) from Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo/>) database. GSE16134 contains 310 gingival tissue samples (241 samples from affected site and 69 samples from unaffected site), obtained from 120 systemically healthy non-smokers with periodontitis. Gene expression data were generated with Affymetrix HG-U133Plus 2.0 microarrays. GSE10334 includes 90 systemically healthy non-smokers with moderate to advanced periodontitis (63 with chronic and 27 with aggressive periodontitis). A total of 247 individual tissue samples (183 from diseased and 64 from healthy sites) were submitted to the analysis. RNA was extracted, amplified, reverse transcribed, labelled, and hybridized with AffymetrixU133Plus 2.0 arrays. GSE27993 has periodontal ligament samples, obtained from five patients with periodontitis and five healthy participants with no pathologies in periodontal tissue. The transcriptome data were acquired using Affymetrix Human Gene 1.0 ST Array. GSE79705 contains 12 gingival tissue samples from patients with a history of generalized aggressive periodontitis (AgP, $n = 4$) or chronic periodontitis (CP, $n = 4$) and from individuals with no history of periodontitis ($n = 4$). Gene expression was measured using NimbleGen Homo sapiens Expression Array. Expression data were normalized and summarized using the Robust Multi-Array (RMA) analysis, and differences between cases and controls were tested using Wilcoxon rank-sum test. Further details about sample selection, RNA isolation, quantification, quality control, and statistical analysis can be found in original papers (Demmer et al., 2008; Kebeschull et al., 2014; Schminke et al., 2015; Taiete et al., 2018).

2.6 | Co-expression and enrichment analyses

Genes prioritized by *Sherlock* may exert their effect on disease susceptibility through expression alteration. Considering an epistasis process, the key genes should be considered as part of networks, not individually. Therefore, we constructed a co-expression networks for the identified genes (confirmed by both *Sherlock* and expression analysis) in order to explore the biological functions of these expression-associated genes and gain an understanding

of biological mechanisms, involved in disease pathogenesis. Genome-wide expression correlation analysis was also performed to identify co-expressed genes of disease-associated causal genes in 241 periodontitis-affected gingiva samples from GSE16134. After Bonferroni correction, Gene Ontology (GO; Ashburner et al., 2000) and Kyoto Encyclopedia of Genes and Genomes (KEGG; Kanehisa & Goto, 2000) enrichment analyses of the significantly co-expressed genes ($p < 2.3 \times 10^{-6}$, 0.05/21,755 genes tested) were conducted using “clusterProfiler” R package (Yu, Wang, Han, & He, 2012).

3 | RESULTS

3.1 | Sherlock integrative analysis prioritizes 10 periodontitis risk genes

The overview of study design is provided in Figure 1. To identify genes that influence the risk of periodontitis (through changes in their expression levels), we integrated genetic associations from the largest GWAS of periodontitis so far with whole blood eQTL from 369 peripheral blood samples through *Sherlock* approach. *Sherlock* integrative analysis identified 10 top genes, which may contribute to periodontitis susceptibility (Table 1). These genes include *MT1L*, *SIGLEC5*, *SIGLEC14*, *S100A12*, *TRIM33*, *HIST1H3E*, *AIM2*, *FASTKD2*, *PKN1*, and *WDR37*. For each gene, at least one SNP showed significant association with the

expression of the corresponding gene and periodontitis simultaneously, indicating that these SNPs may be the promising functional candidate SNPs and exert their influences on periodontitis risk through regulating gene expression. Of note, recent GWAS gave evidences that *SIGLEC5* (sialic acid binding Ig-like lectin 5) was a risk locus of aggressive and chronic periodontitis (Munz et al., 2017; Shungin et al., 2019). Rs12461706, a common intronic variant of *SIGLEC5*, was demonstrated to be the leading signal (EAF = 0.40 for T allele with OR = 1.05, $p = 3.9 \times 10^{-9}$) (Shungin et al., 2019). And rs4284742, also an intron variant of *SIGLEC5*, was found to be the leading signal as well (EAF = 0.76 for G allele with OR = 1.34, $p = 1.09 \times 10^{-8}$; Munz et al., 2017). However, although rs4284742 showed an eQTL effect of *SIGLEC5* in Blood eQTL browser ($p = 7.72 \times 10^{-14}$; Westra et al., 2013), it did not demonstrate the same eQTL effect in blood tissue of GTEx data. Additionally, we did not find evidence that rs12461706 had an eQTL effect of *SIGLEC5* in blood tissue in GTEx though it affected the expression of *SIGLEC5* in other various tissues, such as adipose, lung, and stomach. Therefore, *Sherlock* identified rs12459542, which was associated with both periodontitis and gene expression, as the supporting SNP of *SIGLEC5*. In addition, a previous study (Offenbacher et al., 2016) refined the phenotype via principal component analysis and identified that rs1633266 (in LD with rs2814770, $r^2 > .9$), an intron variant located at *IFI16*, was associated with one of the periodontal complex traits ($p = 3.1 \times 10^{-8}$). The variant rs1633266 was also significantly associated with *AIM2* expression in GTEx blood samples with P -value at 3.87×10^{-5} , which is slightly larger compared to the eQTL of rs2814770 ($p = 6.6 \times 10^{-6}$). The results put additional emphasis on association results that did not pass the genome-wide significance threshold in previous GWAS, thus enforcing previously suggestive associations like that of *AIM2*. This highlights the importance of integrating different layers of information to identify causal genes from the large number of associations with moderate significance that are difficult to distinguish from type 2 errors.

3.2 | Significant upregulation of *AIM2* in periodontitis

Sherlock infers disease-associated genes under the assumption that the expression levels of candidate genes are altered in patients. Therefore, if the genes identified by *Sherlock* are true risk genes, the expression of these genes should be dysregulated in periodontitis. To validate this, we tested whether genes listed in Table 1 are dysregulated in the affected site among periodontitis patients using GSE16134 and GSE10334 data. We found that *TRIM33*, *FASTKD2*, and *WDR37* were significantly downregulated in the affected sites compared with unaffected sites of gingival tissue in both data sets (Figure 2a,b), while *AIM2* and *PKN1* showed consistent upregulation effect on affected sites among periodontitis patients (Figure 2a,b). We further examined the expression of the above-mentioned genes in periodontitis cases and healthy controls. We found that *AIM2* is significantly upregulated in the periodontal ligament ($p = .008$) of periodontitis cases compared with controls in GSE27993 data set (Figure 3c). Additionally, although the results were insignificant due to the small sample size, we observed

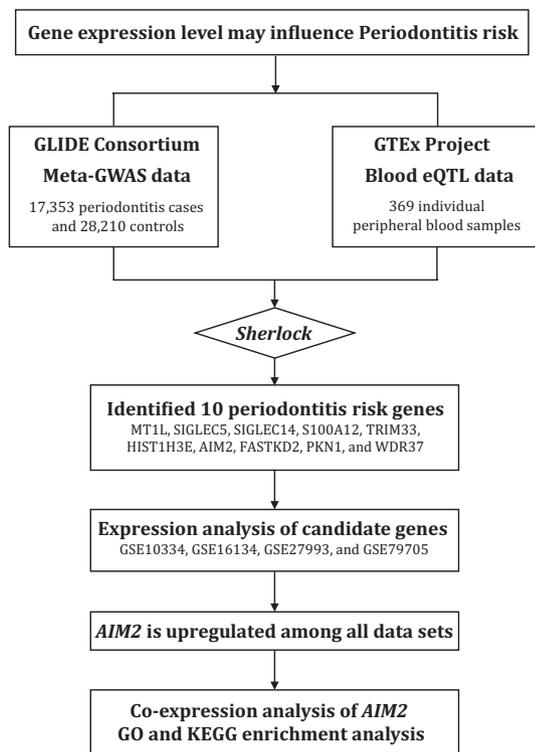


FIGURE 1 Overview of study workflow for integrative analysis of genome-wide association studies and expression quantitative trait loci data

TABLE 1 Top periodontitis-associated genes predicted by Sherlock integrative analysis

Susceptible gene	Gene position (hg19)	Position	LBF ^a	p-Value ^b	Supporting SNP ^c	Nearest gene	GWAS p-value ^d	eQTL p-value ^e	FDR ^f
MTIL	16q13, chr16: 56651373– 56652730	16q13	5.62	6.55×10^{-5}	rs7403881	MTIL	1.41×10^{-5}	4.74×10^{-7}	4.52×10^{-3}
SIGLEC5	19q13.41, chr19: 52114756– 52133727	19q13.41	4.99	1.48×10^{-4}	rs12459542	RPL7P51	8.76×10^{-5}	2.20×10^{-8}	5.11×10^{-3}
SIGLEC14	19q13.41, chr19: 52145806– 52150132	19q13.41	4.73	1.93×10^{-4}	rs12459542	RPL7P51	8.76×10^{-5}	3.22×10^{-17}	4.44×10^{-3}
S100A12	1q21.3, chr1: 153346184– 153348075	1q21.3	3.86	4.52×10^{-4}	rs6680386	S100A8	3.38×10^{-4}	1.81×10^{-12}	6.23×10^{-3}
TRIM33	1p13.2, chr1: 114935399– 115053781	1p13.2	3.70	5.62×10^{-4}	rs10489524	DENND2C	6.08×10^{-4}	3.96×10^{-6}	6.46×10^{-3}
HIST1H3E	6p22.2, chr6: 26224427– 26227706	6p22.2	3.56	6.00×10^{-4}	rs11962642	LOC105374986	1.69×10^{-4}	1.58×10^{-7}	5.91×10^{-3}
AIM2	1q23.2, chr1: 159028790– 159046685	1q23.2	3.15	8.96×10^{-4}	rs2814770	IFI16	7.52×10^{-4}	6.63×10^{-6}	6.87×10^{-3}
FASTKD2	2q33.3, chr2: 207630112– 207660913	2q33.3	3.15	8.96×10^{-4}	rs7593959	CPO	1.01×10^{-3}	3.65×10^{-6}	6.18×10^{-3}
PKN1	19p13.12, chr19: 14544166– 14582679	19p13.12	3.10	9.34×10^{-4}	rs10416904	PKN1	2.46×10^{-3}	1.78×10^{-9}	5.86×10^{-3}
WDR37	10p15.3, chr10: 1095423–1178237	10p15.3	3.10	9.45×10^{-4}	rs10508204	IDI2-AS1	2.31×10^{-3}	9.28×10^{-7}	5.43×10^{-3}

Abbreviations: eQTL, expression quantitative trait loci; FDR, false discovery rate; GWAS, genome-wide association studies; LBF, log Bayes factor for each gene.

^aLBF reflects the association strength between this gene and periodontitis. For instance, a LBF of 5.0 means that the gene is more likely to be associated with periodontitis (exp (5.0) = ~148 times) than no association.

^bp-Value from Sherlock integrative analysis.

^cSNP with the highest LBF score.

^dp-Value for eQTL SNP from the GWAS of periodontitis.

^ep-Value for eQTL SNP from the eQTL whole blood v7 data.

^fFDR was calculated at $p = 10^{-3}$ threshold.

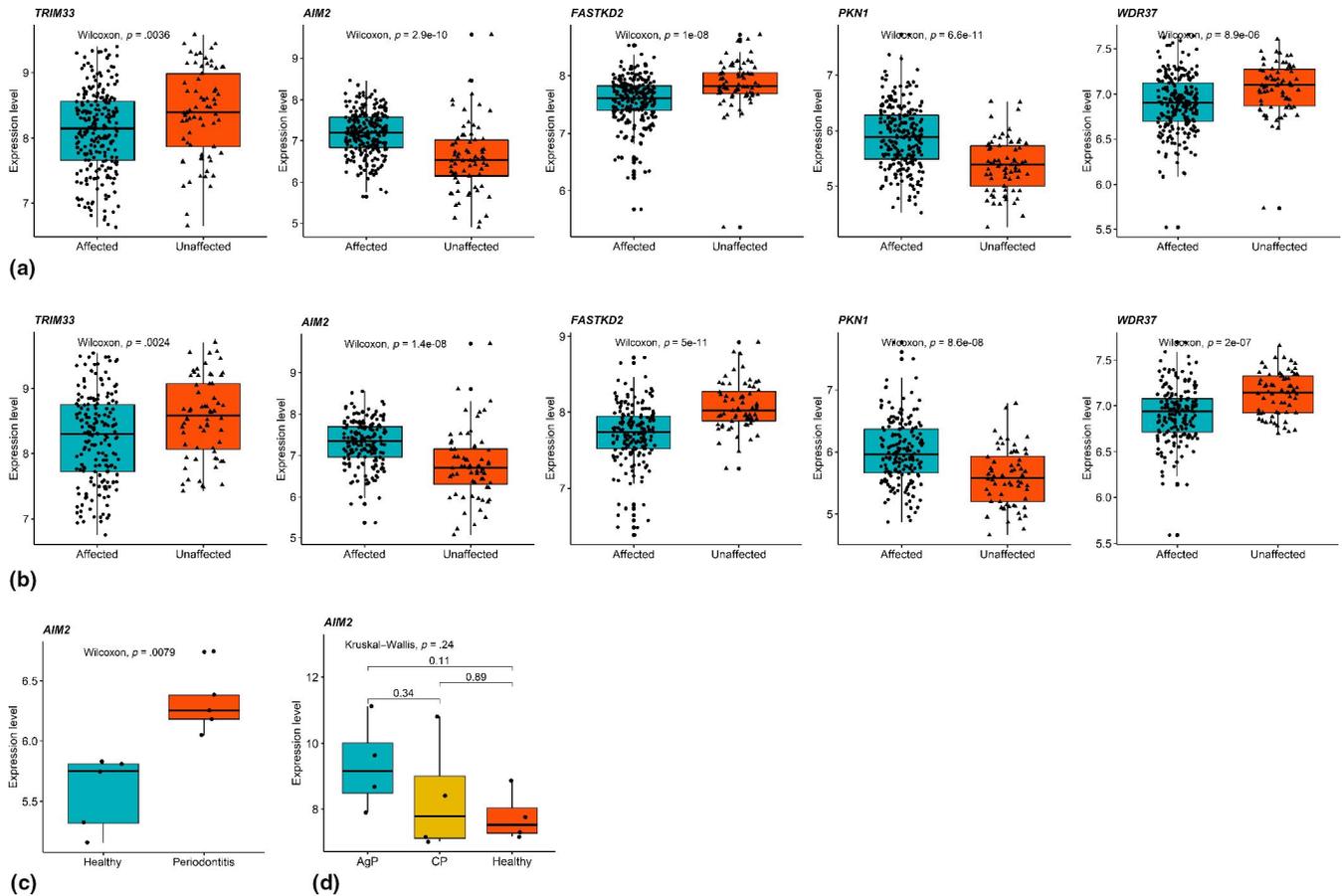


FIGURE 2 Dysregulation of top causal genes in periodontitis. Top causal genes identified by *Sherlock* were dysregulated in affected sites and unaffected sites of gingival tissue among periodontitis patients, data from GSE16134 (a) and GSE10334 (b). *AIM2* was significantly upregulated in periodontal ligament of periodontitis cases compared with controls in GSE27993 (c). The expression level of *AIM2* was associated with disease severity in GSE79705 (d)

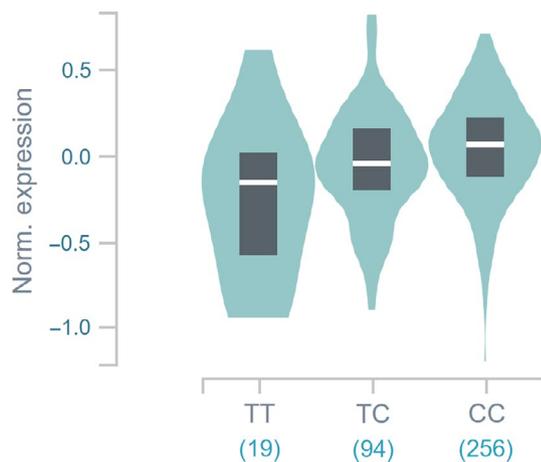


FIGURE 3 SNP rs2814770 is significantly associated with *AIM2* expression in whole blood ($p = 6.63 \times 10^{-6}$). Expression data were extracted from GTEx v7, which contained 369 normal subjects. Of note, the individuals with CC genotype have higher *AIM2* expression level

that *AIM2* expression was positively correlated with disease severity. Patients with aggressive periodontitis had the highest *AIM2* expression level, while healthy controls showed the lowest *AIM2* expression level

in GSE79705 data set (Figure 3d). Taken together, our *Sherlock* integrative analysis suggested that periodontitis-associated genetic variants might confer risk of periodontitis through affecting *AIM2* expression. Consistent with this hypothesis, we found that *AIM2* showed a significant upregulation in periodontitis cases, suggesting *AIM2* upregulation might play a pivotal role in periodontitis pathogenesis.

3.3 | SNP rs2814770 is associated with *AIM2* expression in independent data sets

Sherlock and subsequent expression analysis suggested that *AIM2* might be a causal periodontitis risk gene. We noticed that individuals with CC genotype at rs2814770 have higher *AIM2* expression compared with TT carriers (Figure 3). Intriguingly, C allele of SNP rs2814770 is the risk allele in our GWAS sample (OR = 1.08, 95% CI: 1.03–1.13, $p = 7.52 \times 10^{-4}$). Moreover, higher expression of *AIM2* was also observed in periodontitis patients, aggressive periodontitis patients, and affected sites in the above-described expression analysis. For the further validation of the association between rs2814770 and *AIM2*, we examined an independent expression data set (Lloyd-Jones et al., 2017). We found that rs2814770 is significantly associated

with *AIM2* expression ($p = 1.9 \times 10^{-8}$) in CAGE data set ($n = 2,765$ individuals). SNP rs2814770 is located in ~16 kb upstream of *AIM2*, and, according to the criteria described in previous paper (Bryois et al., 2014), rs2814770 is a cis-eQTL of *AIM2*. These consistent results suggested that rs2814770 is an authentic eQTL for *AIM2* gene and a promising functional candidate SNP involved in pathogenesis with a regulatory effect.

3.4 | Co-expression analysis of *AIM2* and enrichment analysis

Identification of genes whose expression levels are associated with *AIM2* could enhance our knowledge of periodontitis pathogenesis. Using expression data in GSE16134, we identified 2,228 genes that were significantly co-expressed with *AIM2* after Bonferroni correction. In the GO enrichment analysis, the expression of *AIM2* was closely correlated with the neutrophil activation, leucocyte migration, T-cell activation, regulation of lymphocyte activation and lymphocyte differentiation, and others (Figure 4a). In the KEGG pathway, the expression of *AIM2* was closely associated with protein processing in endoplasmic reticulum, chemokine signalling pathway, and osteoclast differentiation (Figure 4b).

4 | DISCUSSION

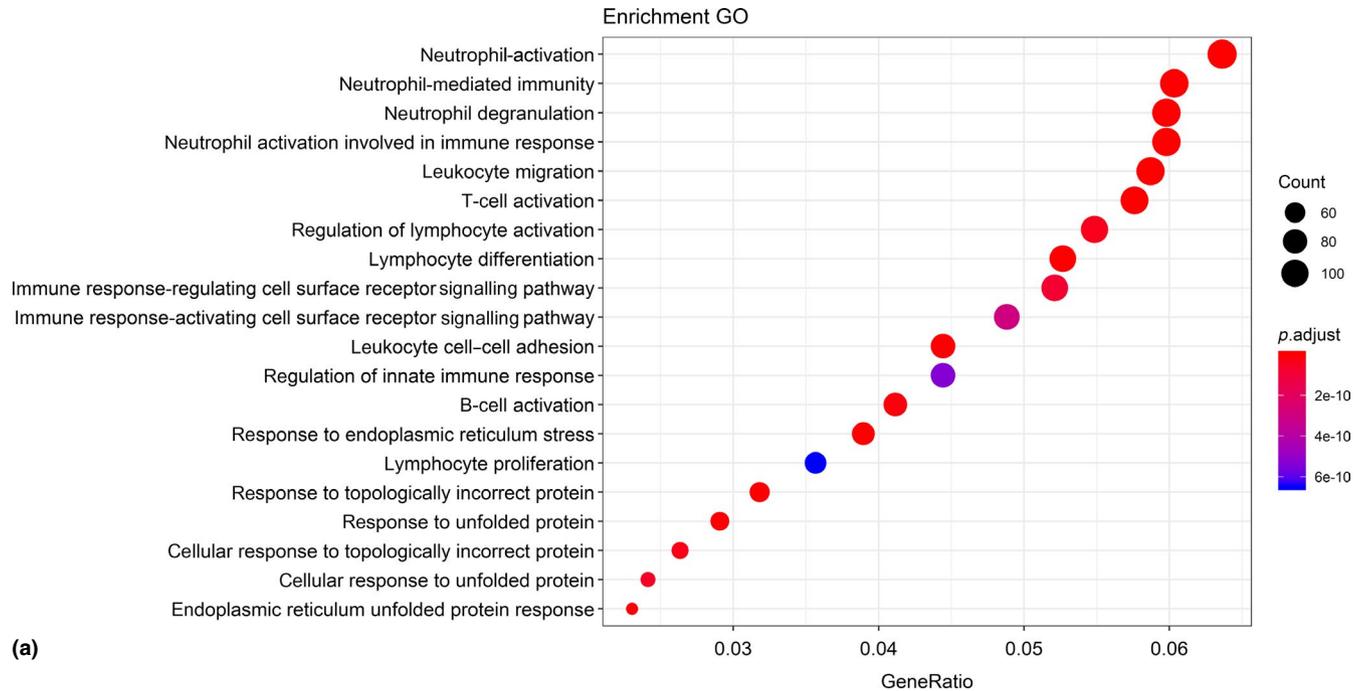
In this study, we used *Sherlock* to systematically integrate genetic association signals from the largest to date GWAS of periodontitis and independent blood eQTL data. We identified 10 genes, in which perturbations may have a role in periodontitis. Among those genes, *AIM2*, *SIGLEC5*, and *SIGLEC14* have been reported in previous GWAS, which strengthen evidence for their role as risk factors for periodontitis. In addition, we found consistent results that *AIM2* was significantly upregulated in gingiva tissue among periodontitis cases, compared with healthy controls. Furthermore, genes co-expressed with *AIM2* were significantly enriched in immune- and inflammation-related pathways. These convergent lines of evidence supported the potential involvement of *AIM2* in periodontitis.

AIM2, also known as absent in melanoma 2, is a protein encoded by the *AIM2* gene in humans (DeYoung et al., 1997). *AIM2* is a member of the *Ifi202/IFI16* family. It is an intracellular recognition sensor that triggers the inflammasome to activate the proteolytic maturation and release of inflammatory cytokines to initiate immune and repair responses (Di Micco et al., 2016). The role of *AIM2* in periodontitis has been suggested in a previous genetic association study (Marchesan et al., 2017), and its importance in assembling the inflammasome has been reported (Lamkanfi & Dixit, 2009). Increased expression of *AIM2* has been shown in a number of inflammatory diseases, including psoriasis, atopic dermatitis, venous ulcers, and inflammatory bowel disease, suggesting its involvement in inflammation regulation (Dombrowski et al., 2011;

de Koning et al., 2012; Sahingur, Xia, Voth, Yeudall, & Gunsolley, 2013; Vanhove et al., 2015). The present study revealed that *AIM2* is significantly associated with periodontitis. Furthermore, we validated the association between *AIM2* at both the gene expression level and at the level of the association with periodontitis. The present findings were consistent with previous reports on the expression of *AIM2* in gingival tissues (Bostanci, Meier, Guggenheim, & Belibasakis, 2011; Marchesan et al., 2017; Sahingur et al., 2013; Xue, Shu, & Xie, 2015). For example, Xue et al. (2015) observed a significantly increased *AIM2* expression in chronic periodontitis lesions. The increased expression of *AIM2* was also demonstrated in gingival fibroblasts in response to oral biofilm ex vivo (Bostanci et al., 2011). Additional support for the role of *AIM2* and periodontitis is the upregulation of *AIM2* and subsequent inflammasome activation during the *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*-mediated infection, which are the major pathogenic bacteria of periodontitis infection (Kim, Park, Song, Na, & Chung, 2016; Okano et al., 2018). Taken together, our findings are consistent with the previous in vitro and in vivo studies, demonstrating that *AIM2* is the potential risk gene of periodontitis (Bostanci et al., 2011; Xue et al., 2015). However, further studies on the exact role of *AIM2* in the pathogenesis of periodontitis are required.

Besides the identification of a potential risk gene for periodontitis, we also determined the *AIM2* expression-associated alleles at rs2814770 in whole blood ($p = 6.63 \times 10^{-6}$). The supporting eSNP of *AIM2*, rs2814770, has moderate p -value ($p = 7.52 \times 10^{-4}$) in GLIDE GWAS data and, therefore, is generally ignored in the traditional GWAS analyses. In contrast, *Sherlock* utilizes both strong and moderate SNPs in the eQTL and GWAS data to make comprehensive assessment. SNP rs2814770 is an intron variant of *IFI16* (gamma interferon inducible protein 16), located ~16 kb upstream of *AIM2*. Interestingly, the SNP rs1633266 (in LD with the supporting eSNP rs2814770 of *AIM2*, $r^2 > .9$) was reported to be strongly associated with both Scoransky trait, part of the periodontal complex traits, which is characterized by a uniformly high pathogen load (Offenbacher et al., 2016), and blood expression of *AIM2*. The replication of this finding along with eSNP from our analysis provides independent evidence of *AIM2*. Previous study reported that *IFI16* is a risk gene of periodontitis (Marchesan et al., 2017), and some experiments have suggested that the inhibition of *IFI16* could increase the expression of *AIM2*. Hence, we speculate that *IFI16* rs2814770 might regulate the expression of *AIM2*, explaining thereby its association with periodontitis. Therefore, the crosstalk mechanisms between the *AIM2* inflammasome and *IFI16* signalling need further investigations. In addition, genes co-expressed with *AIM2* correlate with a number of pathways, including neutrophil activation, leucocyte migration, T-cell activation, regulation of lymphocyte activation, and lymphocyte differentiation, according to GO analysis, and protein processing in endoplasmic reticulum, chemokine signalling pathway, and osteoclast differentiation in KEGG analysis. Considering the close association between immune inflammation and periodontitis (Bunte & Beikler, 2019), and

GO term enrichment analysis



KEGG pathways analysis

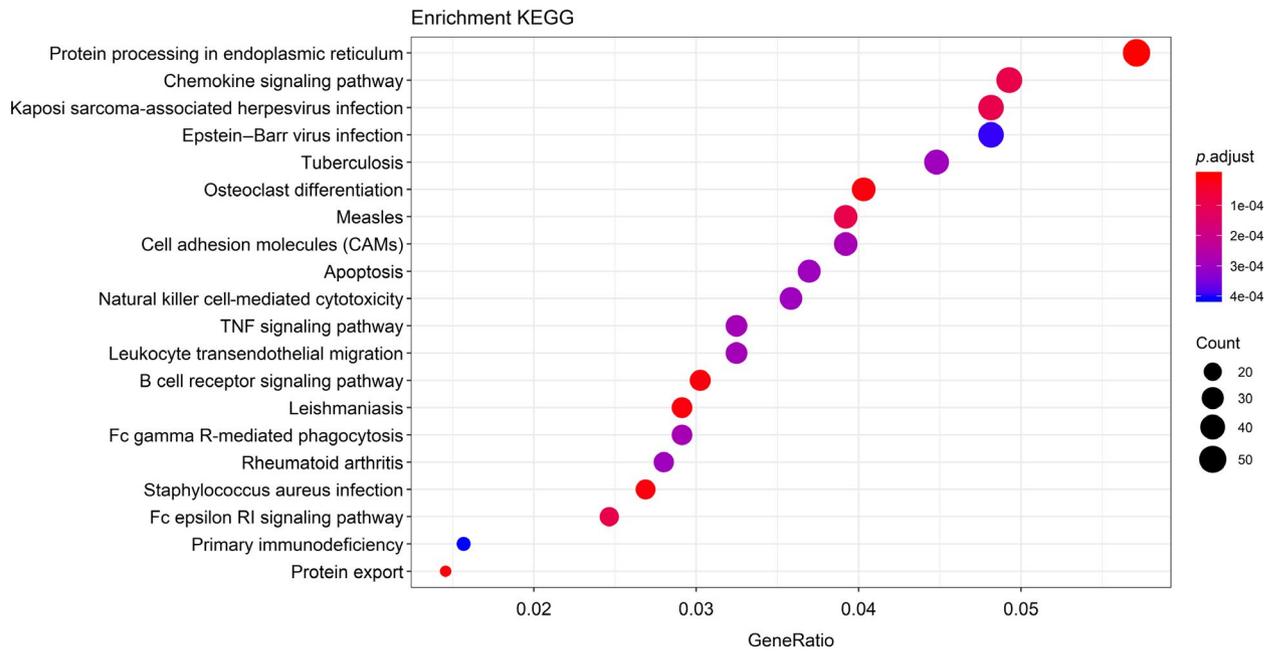


FIGURE 4 Top enriched GO terms and KEGG pathways for co-expression genes of *AIM2*. Node size represents gene ratio; node colour represents adjusted *p*-value. Abbreviations: GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes

the correlation between protein processing in the endoplasmic reticulum and periodontitis (P. Xue et al., 2016), the results of GO enrichment and KEGG analysis further support a potential role of *AIM2* in the pathogenesis of periodontitis.

From the systems biology perspective, *Sherlock* integrative analysis identified 10 risk genes (Table 1) whose expression level influences susceptibility to periodontitis. Further differential expression analysis revealed that only *AIM2* demonstrated a consistent upregulated trend in the gingival tissue among periodontitis patients. It is of

particular importance that other *Sherlock*-prioritized genes might be also essential for periodontitis, although some of the identified genes are not expressed in gingival tissues, but are almost exclusively expressed in leucocytes, for example *SIGLEC5*, *SIGLEC14*, and *S100A12*. Previous studies identified *SIGLEC5* (Munz et al., 2017; Shungin et al., 2019) as a genetic risk factor for both chronic periodontitis and aggressive periodontitis (Masumoto et al., 2019). Generally, the expression of *SIGLEC5* is restricted to blood cells, spleen, and tonsils. For instance, the median TPM value for *SIGLEC5* in whole blood in

GTE_x data sets is 101.8, whereas the value of AIM2 is 7.7. However, the containing of invaded leucocytes in gingival tissues, such as B cells and dendritic cells, results in the finding of *SIGLEC5* expression in GSE16134 and GSE10334 data sets. This spurious expression might lead to a fail to obtain the solid analysis for the differential expression of *SIGLEC5* between affected and unaffected gingiva tissues. Therefore, more information about differential expression of *SIGLEC5* in other tissue between periodontitis cases and controls is needed before we can draw reliable conclusions. In general, as a member of the human CD33-related Siglecs, *SIGLEC5* is widely expressed in myeloid cells of the innate immune system and B cells (Crocker & Varki, 2001). It was reported that *SIGLEC5* seemed to modulate the activation of myeloid cells to prevent inappropriate reactivity against self-tissues (Crocker, Paulson, & Varki, 2007). Since periodontitis is an inflammatory disease, characterized by alveolar bone resorption, we hypothesized that it may affect periodontitis by activating the inflammatory response. Furthermore, a recent study reported that the levels of S calcium-binding protein A12 (*S100A12*) were higher in participants with high periodontal inflammatory burden and were associated with percentage of bleeding on probing and presence of any type of tumour (Holmstrom et al., 2019). The levels of *S100A12* in gingival crevicular fluid and serum increased with the inflammation of periodontium (Pradeep et al., 2014). Therefore, these lines of evidence additionally underline the validity of *Sherlock* in identifying disease susceptibility genes. In the current study, we found that GWAS leading SNP rs4284742 had an eQTL effect of *SIGLEC5* identified by Blood eQTL browser but missed by GTE_x database. What's more, our further differential expression validation analysis focused on gingiva tissue and might miss other important risk genes, such as *SIGLEC5*. Therefore, more comprehensive studies are needed to identify genes that were missed by the current study. For instance, future studies need to explore other eQTL databases in addition to GTE_x and validate the differential expression genes not only in gingival tissues but also in other disease-related tissues, such as blood samples.

This study has some limitations. First, the plausible causal genes identified by *Sherlock* were dependent on gene expression profiles of a single eQTL data set of blood. However, the GTE_x project, which planned to generate RNA-seq expression profiles from human tissues in a large genotyped human cohort, did not include gingival tissue. We thus cannot get eQTL data from gingival-related cells/tissue from GTE_x for our analyses. Second, *Sherlock* integrative approach is a hypothesis-generating approach and the *Sherlock*-identified genes require subsequent validation by functional studies. Although we used a series of in silico gene expression data sets to support our findings, the biological mechanisms are remained to be elucidated. Third, differential gene expression analysis was only conducted in gingival tissue among periodontitis patients and healthy controls due to the limited data availability. However, we were unable to validate the *Sherlock*-identified genes that were dysregulated in other tissues, except for gingival. Future studies with large-scale transcriptome profiling in blood from periodontitis patients may provide additional knowledge for periodontitis susceptible genes.

In conclusion, the present study links periodontitis risk variants to specific genes, as well as provides convergent lines of evidence that AIM2 may be involved in pathophysiology of periodontitis. Further experimental studies are needed to validate our results and elucidate the potential biological mechanisms.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

ORCID

Wenjing Li  <https://orcid.org/0000-0002-9905-3836>

Qiwen Zheng  <https://orcid.org/0000-0001-6921-6254>

Huanxin Meng  <https://orcid.org/0000-0002-2954-818X>

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