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Common polymorphisms in the *IFI16* and *AIM2* genes are associated with periodontal disease

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Abstract

Background—We investigated the single nucleotide polymorphism (SNP) context of a previously identified periodontitis-associated locus and examined its association with microbial, biological and periodontal disease clinical parameters.

Methods—We annotated a 200Kb-spanning region of 1q12 previously highlighted in a genome-wide association scan among 4,910 European American individuals (SNP rs1633266). Two haplotype blocks were selected. We examined the association of these polymorphisms with data on microbial plaque composition, gingival crevicular fluid (GCF)-interleukin (IL)-1β levels and clinical parameters of periodontal disease. Descriptive analysis of *IFI16* and *AIM2* protein

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expression in gingival tissues from healthy (n=2) and chronic periodontitis individuals (n=2) was done via immunohistochemistry.

Results—The highlighted locus is a 100Kb region containing the *interferon gamma-inducible protein 16* (*IFI16*) and *absent in melanoma 2* (*AIM2*) genes. Two haplotype blocks rs6940 and rs1057028 were significantly associated with increased extent bleeding on probing and levels of microorganisms *Porphyromonas gingivalis*, *Tannerella forsythia* and *Campylobacter rectus* (p 0.05). Haplotype block rs1057028 was also significantly associated with pathogens *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans*, increased GCF-IL-1β levels and extent of probing depth 4mm (p 0.05). Prevalence of severe periodontitis (biofilm-gingival interface-P3 classification) was positively associated with haplotype block rs1057028. Similar trends were observed for haplotype block rs1057028. *IFI16* and *AIM2* protein expression was observed in multiple cell types of gingival tissues, including inflammatory cells.

Conclusion—This study found *IFI16* and *AIM2* SNPs associated with higher levels periodontal microorganisms and increased percentage of periodontal disease clinical parameters, suggesting the need for functional studies and additional fine-mapping of variants in the 1q12-locus.

Keywords

Pathogenesis of periodontal disease(s); genetics; host response

MeSH terms

Periodontitis; Innate Immune Response; Polymorphism; Genetic

INTRODUCTION

Periodontal disease is polygenic condition of the tooth supporting structures.[1–4] Early studies among monozygotic and dizygotic twins showed that 33–48% of the variance in periodontal disease expression was attributable to genetics.[1, 5] Notably, alterations in genes encoding proteins involved in the immune response are shown to influence the host microbiota and increase periodontal clinical parameters of disease. Individuals with variants in *interleukin* (*IL*)*-1α*, *IL-1β* and *IL-6* are shown to have a unique periodontal microbiome with high levels of classical "red" and "orange" complex species that are known to be significantly associated with periodontal inflammation.[6] *IL-6* polymorphisms have also been moderately associated with the diagnosis of periodontitis.[7] In addition, individuals with single gene mutations of β2 integrins leading to Leukocyte Adhesion Deficiency-1 show increased bacterial loads, decreased complexity of biofilms and severe periodontal bone loss.[8] Together, the evidence supports the concept that genetic alterations controlling the immune response of the host can lead to alterations of microbial communities and predispose individuals to periodontal disease.

Genome-wide association studies (GWAS) and candidate gene studies have been used in attempt to identify single nucleotide polymorphisms (SNP)s that either contribute to the pathogenesis and/or risk of developing periodontal disease. To date, 4 studies have conducted GWAS analysis to identify SNPs associated with the American Academy of

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Periodontology (AAP)[9] definition of chronic periodontal disease.[10–13] No single marker met the genome-wide significance criteria, although four loci [*ninein (NIN), abhydrolase domain containing 12B (ABD12B), WAS protein homolog associated with actin, golgi membranes and microtubules (WHAMM), adaptor-related protein complex-3 beta-2 subunit (AP3B2)*] met gene-centric statistical significance criteria.[12] Therefore, a new approach was utilized in order to identify SNPs that were relevant to the pathogenesis of periodontal disease. This approach utilized a combination of the levels of 8 classical periodontal pathogens and gingival crevicular fluid (GCF) IL-1β to derive periodontal complex traits (PCTs) via principal component analysis.[14] The objective of this approach is for identifying loci related to the biological background and pathogenesis of periodontal disease. Approximately 2.5 million markers were evaluated among 975 European American adults. Several traits were derived by this analysis, with each trait having different eigenvalues (loadings) of the 8 microorganisms and GCF-IL-1β. PCT1 (named the Socransky Trait) was defined by a microbial community structure with high positive loadings of all periodontal pathogens, and correlated with clinical measurements of periodontal disease.[15] Six loci were associated with PCT1, including *C-Type Lectin Domain Family 19 Member A (CLEC19A), T-Cell Receptor Alpha Locus (TRA), Glycoprotein, Alpha-Galactosyltransferase 2, Pseudogene (GGTA2P), Transmembrane 9 Superfamily Member 2 (TM9SF2), RNA Binding Motif Single Stranded Interacting Protein 3 (RBMS3)* and *interferon (IFN) gamma-inducible protein 16 (IFI16)/ absent in melanoma (AIM)2.*[14] The clinical, microbial and biological characterization of individuals with SNP variants in these 6 individual loci is currently unknown. This present study further investigates *IFI16/AIM2* loci using bioinformatics, clinical, microbial and biological data.

Both *IFI16* and *AIM2* are members of the IFN-inducible PYHIN protein family that contain C-terminal DNA-binding hematopoietic expression, interferon-inducible nature, and nuclear localization (HIN) domain(s) and an N-terminal Pyrin domain (PYD) that belongs to the death domain superfamily of signaling molecules.[16, 17] Both IFI16 and AIM2 are intracellular recognition sensors that trigger inflammatory responses against DNA from the host and microorganisms.[17] Increased expression of *AIM2* has been reported in a number of inflammatory diseases, including psoriasis, atopic dermatitis, venous ulcers, inflammatory disease and periodontitis suggesting involvement with inflammation.[18–22] Expression of *IFI16* in inflammatory diseases is less explored but increased expression is reported in inflammatory bowel disease.[22] To our knowledge, no study has explored the expression of *IFI16* in periodontal tissues. Because of the critical role of these proteins in innate immunity, the purpose of this study was to evaluate the relationship between SNPs in the *IFI16* and *AIM2* loci with periodontal microorganisms, levels of GCF-IL-1β and clinical parameters of periodontal disease. Meanwhile, descriptive analysis of *IFI16* and *AIM2* protein expression in gingival samples derived from healthy and individuals with periodontal disease showed expression in multiple cells, including inflammatory cells. Conclusively, our previous[14] and present study supports that variants in *IFI16/AIM2* are associated with increased loads of periodontal pathogens and increased parameters of clinical disease.

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MATERIALS AND METHODS

GWAS population

A total of 4,910 Northern European descendants were enrolled in the Dental Atherosclerosis Risk in Communities (DARIC) Cohort as described.[23, 24] Blood was collected as described for genotyping for ~2.5 million markers.[10] GCF was collected at 4 gingival sampling areas from the mesio-buccal region of each first molar from each individual and stored for further analysis of IL-1β levels.[23] Plaque samples were collected from the subgingival mesio-buccal site of the maxillary right first molar, and stored for further DNA whole chromosomal checkerboard for the 8 periodontal pathogens. Periodontal measurements (n=4,766) in all teeth at 6 sites per tooth were collected and included number of missing teeth, gingival index (GI), plaque index (PI), probing depths (PD), clinical attachment level (CAL) and bleeding on probing (BOP). All sites were examined by trained and calibrated examiners with >90% agreement.

Bioinformatic approaches

Initial analysis of genome-wide imputed SNP data was done with a software package.[25] The results of that analysis revealed one genome-wide significant SNP in the *IFI16* region (lead SNP rs1633266). We then identified and visualized markers in linkage disequilibrium with this polymorphism.14,26,[*]The criteria used to prioritize and select SNPs of interest for this analysis were: a) a statistical significance criterion ($p<5\times10^{-5}$ considered as 'suggestive' evidence of association), b) biological plausibility of genes in the region, c) functional significance and d) linkage disequilibrium, the non-random association in the occurrence of alleles at two loci, represented by the square of the correlation coefficient between two indicator variables (r^2). SNPs with suggestively association ($p<5\times10^{-5}$) with PCT1 were selected and were then carried forward for screening of missense SNPs with predicted functional protein damage.[27–29] Using these criteria, we identified 2 SNPs of interest, rs6940 and rs1057028 located in the IFI16 region. We then searched and evaluated SNPs in perfect linkage disequilibrium (Northern and Western European ancestry panel, $r^2=1$, $D'=1$ cutoff) with these 2 markers as previously described.30,[†] We further gathered protein information and performed alignment of the different IFI16 isoforms [clustal 0 (1.1.1) multiple sequence alignment].31,[‡] The SNPs within these loci were carried forward to tests of association with clinical and biological parameters, including plaque microorganisms, GCF-IL-1β levels, periodontal clinical measurements and biofilm-gingival interface (BGI)-periodontal disease classification.[32] The BGI classification was selected because it defines biological phenotypes based on 8 periodontal pathogens, serum immunoglobulin (Ig)G (17 bacteria), 16 GCF-mediators, PD and BOP, representing the individual's current disease activity and inflammatory condition. Previous GWAS studies from our group have already shown that traditional AAP/ADA periodontal disease classification[9] that utilize CAL, a measurement of the loss of tissue or history disease, does not allow identification of groups with similar biological characteristics.[10, 14]

[*]LocusZoom, http://locuszoom.org/
[†]SNPNexus, http://www.snp-nexus.org
[‡]UniProt, http://www.uniprot.org/

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Plaque microbial analysis

A subset of 909 participants of the DARIC cohort was evaluated for plaque microbial composition using DNA-DNA checkerboard as previously described.[32] One plaque sample was used from each individual and assayed by DNA checkerboard for the 8 periodontal pathogens. Microorganism levels were expressed as counts using known microbial standards for *Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Treponema denticola (Td), Tannerella forsythia (Tf), Campylobacter rectus (Cr), Fusobacterium nucleatum (Fn), Aggregatibacter actinomycetemcomitans (Aa)* and *Prevotella nigrescens (Pn)*. The total counts reflect a sum of these targeted pathogens for each individual.

Gingival crevicular fluid IL-1β levels

Four GCF strips were eluted and analyzed separately for each individual (n=4,407). IL-1β levels were evaluated by enzyme-linked immune-absorbent assay (ELISA) according to the manufacturer's instructions as previously described.[33] GCF analyte concentration data were pooled to provide a mean value for each individual in ng/mL.

Sample collection for immunohistochemistry

To describe the tissue distribution of IFI16 and AIM2 in human gingival tissues, gingival biopsies were taken from 2 individuals with healthy periodontium and 2 with chronic periodontal disease according to the AAP/ADA classification.[9] All enrolled participants into this study provided written informed consent, which was approved by the Institutional Review Board (IRB) of the University of North Carolina at Chapel Hill. Major exclusion criteria included symptom of any systemic disease, antibiotic use within 1 month prior to the examination, or medical treatment for any known disease that is associated with periodontal disease within the last 3 months. Gingival biopsies were harvested either during routine periodontal flap surgeries from participants clinically diagnosed with chronic periodontitis or crown lenthening surgeries and healthy volunteers. A tissue biopsy sample (~3mm×4mm) was removed from underneath the papillae, buccally or lingually, to include the col area of depth of the osseous crest. Upon removal, gingival tissues were fixed in 10% neutral buffered formalin overnight, dehydrated (70% alcohol), and embedded in paraffin for the immunohistochemical procedure.

Immunohistochemistry

Gingival tissue sections (5μm thick) were obtained in the sagittal direction including the epithelial and connective tissues. The slides were stained with rabbit polyclonal anti-IFI16[*] and rabbit polyclonal anti-AIM2[†]. Anti-rabbit horseradish peroxidase (HRP)-DAB staining‡ was used according to the manufacturer's instructions and the slides were counterstained with hematoxylin. Photo images were captured using an Olympus BX61 microscope[§].

Statistical analysis

General linear models (PROC GLM of SAS, version 9.4) were used to examine associations between the SNPs of interest and adjusted mean counts of microorganisms, GCF-IL-1β

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levels and clinical measurements, adjusting for microbial plaque levels (p 0.05). Chi-square tests were used to examine SNP associations with periodontal disease category (BGI-classification) (p 0.05).[32]

RESULTS

SNP identification and analysis

A sample of 4,910 Northern European descendants was genotyped and evaluated as periodontal complex traits (PCT), being PCT1 associated with periodontal clinical parameters of disease.[14] To select variants correlation with clinical parameters, SNP prioritization was based upon statistical significance, linkage disequilibrium (r^2), biological relevance, gene proximity, coding sequences and functional prediction. *IFI16* rs1633266, an intron variant, was the lead SNP most significantly associated with the Socransky trait (p=3.1×10^{-8}, Supplemental Figure 1). Nine additional SNPs were located in the *IFI16* region and 1 in the *AIM2* region (rs2793845) that were in high disequilibrium (r^2 0.8) with the lead SNP in *IFI16,* rs1633266; 1 SNP in the *IFI16* region with an r^2 0.6 and 16 SNPs in the *IFI16* region with r^2>0.4 (Supplemental Figure 1). *IFI16* and *AIM2* are both localized in the 1q25.2 locus and are transcribed in opposite directions (Supplemental Figure 1). Among the SNPs that correlated with PCT1 (p 5×10^{-5}, suggestive evidence of association), 21 were localized in the *IFI16* locus and 1 closest to *AIM2* (within 2 kb downstream of the 3′ end of a transcript) (Supplemental table 1). Analysis with SNAP[30] indicated the existence of 2 tight haplotype blocks in perfect linkage disequilibrium (r^2=1 and D′=1), including *IFI16* rs6940 (missense) with neighboring gene AIM2 rs2793845 and several additional intronic SNPs, while the second block identified by missense rs1057028 included rs1057027 and additional introns, all located in the IFI16 region (Table 1). Bioinformatic analysis of functional damages potentially caused by missense SNPs demonstrated that rs6940 (T>S) affected all 4 isoforms with a prediction of possibly damaging (score 0.584–0.782), rs1057027 as benign and rs1057028 (Y>N) as probably damaging to the protein function of isoform 3 (score 0.995) (Supplemental Table 2). This analysis suggests that rs6940 (minor allele [T] frequency=0.22) and rs1057028 (minor allele [T] frequency =0.3) are potentially/probably damaging for the protein function and, therefore, were selected for correlations with periodontal clinical parameters. Sequence alignment of the isoforms shows that the SNPs do not localize in the known functional domains (PYRIN domain, HIN domains and p53 interaction domain) (Supplemental figure 2A–B). However, rs6940 is located between HIN200-1/p53 C-terminus binding and HIN200-2/p53 core domain binding and could be interfering with the 3-D structure and protein function. It is possible that not all 4 isoforms of IFI16 are affected to the same degree based on the different protein sizes (Supplemental figure 2C). Indeed rs1057028 is predicted to probably damage isoform 3 only (Supplemental table 2). Imputation quality was 0.9994 and 0.9999 for rs6940 and rs1057028, respectively. In sum, our analysis identified 2 tight haplotype blocks with several SNPs in the *IFI16/ AIM2* region that are potentially important in the pathogenesis of periodontal disease. SNPs rs6940 and rs1057028 are predicted to be potentially/probably damaging to the protein function.

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IFI16 SNPs are associated with parameters of periodontal disease

Correlation analysis of the clinical parameters showed that the both haplotype blocks rs6940 and rs1057028 had a significant increase in percent sites with extent bleeding on probing (EBOP), while rs1057028 also had significantly higher percentage of extent of probing depth greater or equal to 4mm (EPDGE4) (Table 2). Increased trends were observed for mean PD and percent of extent of gingival score greater or equal to 1 (EGIGE1) (Table 2). This suggests that SNPs in the region of *IFI16* and *AIM2* affects the biology of the tissues that leads to an increase in the extent of periodontal disease. Further analysis of the microbiological composition of plaque samples shows that several periodontal pathogen counts (plaque-adjusted) were also significantly higher in both haplotype blocks, including *Pg*, *Tf* and *Cr*. Loads of *Pg* were over 274 times higher for rs6940 and 90 times higher for rs1057028 when comparing 2.2 individuals (homozygous for the minor allele) to 1.1 individuals (homozygous for the major allele). Additional organisms were significantly increased in rs1057028, including of *Fn* and *Aa,* with similar trends observed for rs1057028 homozygous minor alleles (Table 3). This finding further supports that SNPs in the *IFI16* and *AIM2* region potentially affects the biological host response of the individual that leads to increased numbers of periodontal pathogens present in plaque samples. Cytokine analysis showed that individuals with haplotype block rs6940 had a 4-fold increased concentration in levels of GCF-IL-1β, with a trend of significance for rs1057028 of 2-fold increase (Table 4). Since IL-1β is a well-known pro-inflammatory cytokine implicated in periodontal disease progression [34], the finding of increased levels of this cytokine in the GCF in the presence of SNPs in the *IFI16* and *AIM2* regions suggest that potential defects in *IFI16/AIM2* can alter the inflammatory response of an individual and further influence disease status. Individuals with both haplotype blocks showed an increased percentage of severe periodontal disease (BGI-P3) when compared to healthy controls (Table 5), reaching statistical significance for haplotype block rs1057028 (p=0.02). The BGI classification accounts for PD and BOP (and not CAL), representing the individual's current disease activity and inflammation. Because the presence of SNPs in the *IFI16* and *AIM2* regions lead to an increase in number of individuals with periodontal disease/inflammation, it suggests that the functional defect increased the predisposition of the individual developing the multifactorial condition of periodontal disease. Taken together, the data suggest that the presence of these SNPs alter the normal host response that leads to an increased predisposition to develop periodontal disease, observed by higher numbers of periodontal microorganisms, increased measurements of periodontal disease and higher number of individuals with clinical disease.

Descriptive histological distribution of IFI16 and AIM2 in gingival tissues

The purpose of this approach was to describe the IFI16 and AIM2 protein expression in cells of the periodontium. IHC analysis was performed in gingival tissue samples of individuals with healthy tissues (n=2) and chronic periodontal disease (n=2). The demographics of this population are shown on Supplementary Table 3. Representative low-resolution (10X) images (Figure 1A, D, G, J, M, P) of healthy and periodontitis tissues show a similar pattern of expression among samples. Both proteins had a homogeneous distribution in the epithelial layer, with minimal-no expression in the keratin layer, among healthy and periodontitis samples (Figure 1B, E, K, N). IFI16 staining was dense in the basal layer (Figure 1A, B). Migrating neutrophils expressing IFI16 and AIM2 were observed in the

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epithelial layer in a sample derived from individuals with periodontal disease (Figure 1K, N). In the connective tissue (Figure 1C, F, L, O), endothelial cells and cells of the inflammatory infiltrate showed expression of both IFI16 and AIM2. Minor expression of these proteins was observed in fibroblasts in the connective tissue. All samples (n=4) demonstrated a similar pattern of staining for both proteins. This descriptive analysis demonstrated that cells of the periodontal apparatus express IFI16 and AIM2 and, therefore, further supports a potential role of these proteins in the pathogenesis of periodontitis.

DISCUSSION

Our study characterized clinical and biological periodontal data among a sizeable group of participants and examined their association with SNPs in the *IFI16/AIM2* locus. We found 2 haplotype blocks, one including a missense SNP rs6940 and a variant close to neighboring gene *AIM2,* and a second block including missense SNP rs1057028 that were significantly associated with periodontal disease parameters, including increased extent PD and BOP, increased GCF-IL-1β levels, higher loads of periodontal pathogens and higher prevalence of severe periodontal disease. Prediction analysis indicated that the function of IFI16 is altered by the presence of rs6940 and rs1057028. Several additional SNPs were in perfect linkage disequilibrium with the index SNPs. These polymorphisms are quite common in the general population with minor allele frequencies of 0.22 for rs6940 (minor allele: T) and 0.3 for rs1057028 (minor allele: T). We identified that IFI16 and AIM2 are expressed in epithelial cells, fibroblasts, endothelial cells and leukocytes of gingival tissues. The presence of these proteins in inflammatory cells of gingival tissues, including the finding of neutrophils in the epithelial layer (Figure 1K, N) suggests a role of IFI16 and AIM2 in the response to periodontal pathogens. Taken together, these findings propose that these proteins and specific polymorphisms may have an important role in periodontal disease pathogenesis.

Previous GWAS analysis by our group have highlighted loci potentially associated with clinically derived-disease definitions, like chronic periodontal disease,[10, 12] and high levels of specific periodontal pathogens.[35] However, no single marker met the strict genome-wide significance. Only 4 loci met gene-centric statistical significance. [12] This suggested the existence of several distinct conditions with different genetic and biological backgrounds with similar overlapping clinical presentations of periodontal tissue loss. Therefore, a new approach was utilized by defining the disease phenotype as complex traits as previously described.[14, 36] With this approach, six PCT were identified by levels of 8 periodontal pathogens, local inflammatory response (GCF-IL-1β) and clinical data.[14] PCT1 was defined by a uniformly high periodontal pathogen load and significantly correlated with clinical periodontal disease parameters. Six loci were associated with PCT1, which included *IFI16/AIM2*. While there was a degree of anticipation that the *IFI16/AIM2* haplotype blocks would be correlated with some periodontal microorganisms since the lead SNP was identified from an analysis that used a microbial community structure, the individual microorganisms correlated with the haplotype blocks were not known. In addition, the clinical measurements and disease significance in this population, and tissue distribution of IFI16 and AIM2 were further characterized. Therefore, the present study further refines the findings derived from the PCT1 analysis and defines the clinical and biological characteristics of individuals with SNPs in the *IFI16/AIM2* region.

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Both IFI16 and AIM2 are PYHIN inflammasome proteins and mediators of innate immune responses.[17] Inflammasomes are multiprotein oligomers that promote activation of inflammatory cytokines IL-1β and IL-18.[37] The PYHIN inflammasome proteins bind microbial DNA and form caspase-1-activating inflammasomes (AIM2) or drive type I *IFN* gene transcription (IFI16).[17] IFI16 is also a mediator of the AIM2 inflammasome-dependent pathway by directly binding to AIM2.[38] Therefore, IFI16 has shown anti-inflammatory effects and AIM2 proinflammatory effects. This suggests that defects in the expression or protein function of IFI16 could dampen the anti-inflammatory response and, thereby increase the proinflammatory response. Studies show that AIM2 is increased in wound healing and in several inflammatory conditions, including psoriasis, atopic dermatitis and venous ulcers.[18, 19] Additional support for the role of AIM2 and IFI16 in inflammation is a recent demonstration of a strong increase of both proteins in the mucosa of individuals with active inflammatory bowel disease.[22] In accordance to our findings, 3 studies have previously demonstrated the presence of AIM2 in gingival tissues.[20, 21, 39] AIM2 expression was increased in gingival tissues from individuals with chronic periodontitis compared to healthy controls and generalized aggressive periodontitis. In addition, *Pg* infection can activate the AIM2 inflammasome in vitro.[39] These findings further support an importance of AIM2 in the pathogenesis of periodontal disease. To our knowledge, no other study has evaluated the expression of *IFI16* in periodontal tissues. Further analysis and quantification of this protein in periodontal cells and tissues in various disease states is warranted.

The concept that the host genotype can influence the microbiota and lead to disease has been reported previously. Counts of periodontal pathogens from the red and orange complex are reported to be significantly higher at PD>6mm in *IL-1* genotype positive individuals compared to genotype negative individuals.[6] *IL-6* polymorphisms and haplotypes have also been associated with periodontitis, possibly due to the transcription of *IL-6* that then alters tissue levels.[7, 40] An alteration in the immune response observed in leukocyte adhesion deficiency also leads to significant changes in the subgingival flora and severe periodontitis.[8] These results are in support of our data and the concept that changes in the innate immune functions can alter the host response and facilitate the development of diseases. A recent study found that *IFI16* rs6940 (also identified in this study) and *AIM2* rs855873 (also upstream of *AIM2*) were associated with increased susceptibility to Behcet disease, a systemic immune-mediated disease characterized by vasculitits and recurrent mucosal ulcerations.[41] The study shows rs6940 decreases the expression of anti-inflammatory IFI16 and increases susceptibility to an immune-mediated disease.[41] The fact that Behcet is associated with epithelial ulceration and our results show associations of these SNPs with BOP suggests that these variants may impair innate immune responses that maintain epithelial integrity at mucosal surfaces. In fact, individuals with Behcet disease have increased periodontal disease severity compared to healthy controls, suggesting both diseases share pathogenic aspects.[42, 43] These findings support the hypothesis of a role of anti-inflammatory IFI16 in periodontal disease pathogenesis and that variants of this gene predispose individuals for an altered immune response to infection that ultimately leads to disease.

Functional analysis was done to help identify SNPs that have a high potential of altering the protein function. Our current analysis indicates that both missense SNPs rs6940 and

rs1057028 are not localized in the known PYRIN, HIN-200 or p53-binding domains. However, the predictions suggest that the protein function is potentially/probably altered. Since rs1057028 is localized between both HIN domains it is possible that the variant induces a 3D conformation change to the protein structure. SNP rs6940 is predicted to alter only isoform 3, which is probably related to the different sizes of the isoforms. A previous study suggests that instead of the protein function, SNP IFI16 rs6940 decreases the expression of *IFI16*.[41] It is possible that additional SNPs in high linkage disequilibrium with rs6940 are leading to this effect. However, no functional assays were performed in the study.[41] Since the SNP associated with AIM2 is not present in a missense region, predictions for potential alterations could not be performed. Future studies defining the impact of the SNPs present in the 2 haplotype blocks in protein expression and host response should provide additional evidence for the pathogenesis of periodontitis.

Although we identified a biologically-relevant region associated with elevated periodontitis parameters, our results have limitations. The validity of the reported SNP associations will need to be further examined and replicated in another new study. However, it is important to note that the identification of these genes was not based on traditional AAP/ADA classification for chronic and aggressive periodontitis, which is utilized in other GWAS studies.[10, 44–46] Instead, these genes were first identified in the context of a PCT that utilized a combination of microorganisms and GCF-IL-1β levels.[14] In this model, periodontal disease is a group of distinct biological conditions with overlapping clinical presentations. Therefore, future GWAS analysis may consider periodontal complex traits for identifying biologically relevant genes.

CONCLUSION

Together, our results support a role of for IFI16/AIM2 in the pathogenesis of periodontal disease. This association was observed by the correlation of SNP variants with increased measurements of microorganisms in the subgingival plaque, increased levels of GCF-IL-1β, increased periodontal clinical parameters of disease and increased prevalence of severe disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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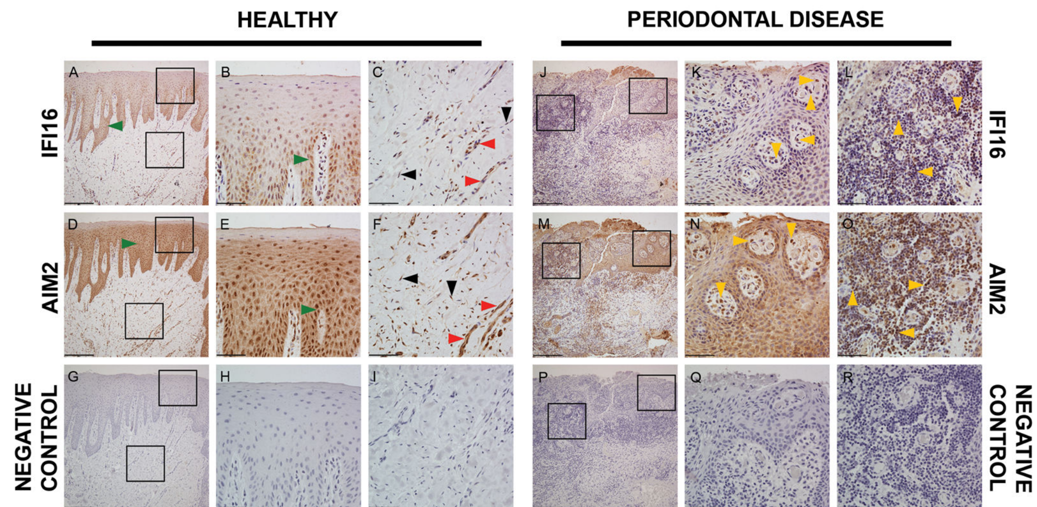

Figure 1.

Immunohistochemical detection of IFI16 and AIM2 in human gingival tissues. Representative images of tissue sections from a healthy individual (A–I) and an individual with periodontal disease (J–R) according to the ADA/AAP classification. stained with the indicated antibodies (left and right rows). A, D, G, J, M, P represent 10× magnification (scale bar = 200.00μm); B, C, E, F, H, I, K, L, N, O, Q, R represent 40× magnification (scale bar = 50.00μm) of the square inserts located in the figures with 10× magnification in the epithelial layer and connective tissue layer. Bottom row are negative controls. Green arrows=epithelial cells; black arrows=fibroblasts; yellow arrows=leukocytes; red arrows=endothelial cells.

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Table 1

SNPs in haplotype blocks associated with the missense SNPs rs6940 and rs1057028 from individuals with Northern and Western European ancestry.

SNP	Proxy	Distance to lead SNP (bp)	Predicted function
rs6940	rs7532207	244	intronic
	rs74359395	2335	3′ downstream IFI16
	rs3737522	3223	intronic
	rs3018316	6710	intronic
	rs2793845	7587	3′downstream *AIM2*
	rs2852695	8735	intronic
	rs2814770	11896	intronic
	rs2814771	12430	intronic
	rs12098223	12974	intronic
	rs3754460	17822	intronic
	rs74122232	18245	intronic
	rs1633266	18691	intronic
	rs1772407	18744	intronic
	rs3768519	19444	intronic
	rs1616024	20370	intronic
	rs3768523	20670	intronic
rs1057028	rs1057027	12	coding
	rs861318	167	intronic
	rs1633256	168	intronic
	rs1772415	389	intronic
	rs856057	654	intronic
	rs856056	680	intronic
	rs856055	796	intronic
	rs856054	868	intronic
	rs856053	885	intronic
	rs1417804	1594	intronic
	rs1614182	2137	intronic
	rs1633262	2499	intronic
	rs1772408	3260	intronic
	rs1633265	3338	intronic
	rs2570916	10257	intronic
	rs855865	25989	5′ upstream

Linkage disequilibrium with lead SNP: CEU D′=1, r^2=1

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Table 2

Clinical measurements among individuals with SNPs in haplotype blocks, mean (standard deviation).

SNP	n	EPDGE4 (%)	Mean PD (mm)	EBOP (%)	EGIGE1 (%)	Mean CAL (mm)
rs6940*						
1.1	3590	6.84 (0.16)	1.86 (0.01)	23.1 (0.33)	22.0 (0.46)	1.64 (0.01)
1.2	1057	6.84 (0.30)	1.84 (0.02)	23.7 (0.61)	22.4 (0.84)	1.65 (0.02)
2.2	80	6.28 (1.13)	1.82 (0.06)	28.3 (2.28)†	24.9 (3.21)	1.51 (0.1)
rs1057028*						
1.1	3199	6.84 (0.17)	1.86 (0.01)	23.1 (0.35)	22.1 (0.49)	1.64 (0.01)
1.2	1367	6.64 (0.27)	1.84 (0.01)	23.1 (0.54)	22.1 (0.74)	1.63 (0.02)
2.2	161	8.34 (0.79)†	1.92 (0.04)	28.8 (1.60)‡	25.1 (2.24)	1.75 (0.07)

* haplotype blocks for both SNPs include additional SNPs shown on table 1

chi-square p-values using 1.1 as the referent category:

† *p* 0.05,

‡ *p* 0.01.

Data was adjusted for plaque index.

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Table 3

Mean levels of periodontal microorganism (standard errors) and relative (fold) changes for rs6940 and rs1057028 haplotype blocks (adjusted for plaque levels).

SNP	N	Pg	Pi	Pn	Tf	Td	Cr	Fn	Aa
rs6940*									
1.1	781	2.22 (0.06)	2.55 (0.07)	2.68 (0.07)	2.43 (0.06)	2.61 (0.07)	2.66 (0.07)	2.86 (0.08)	2.57 (0.06)
1.2	255	2.27 (0.10)	2.71 (0.13)	2.51 (0.13)	2.49 (0.11)	2.53 (0.12)	2.72 (0.11)	2.96 (0.13)	2.74 (0.10)
2.2	17	3.54 (0.43) ‡	3.01 (0.54)	3.47 (0.52)	3.50 (0.45)†	3.11 (0.48)	3.69 (0.48)†	3.94 (0.55)	2.99 (0.44)
Fold change	274.34	58.41	120.34	191.54	64.87	180.11	194.47	52.20	
rs1057028*									
1.1	711	2.24 (0.06)	2.62 (0.08)	2.71 (0.08)	2.42 (0.07)	2.63 (0.07)	2.67 (0.07)	2.86 (0.08)	2.59 (0.06)
1.2	300	2.21 (0.10)	2.48 (0.12)	2.42 (0.12)	2.46 (0.10)	2.47 (0.11)	2.65 (0.11)	2.90 (0.12)	2.58 (0.10)
2.2	42	2.89 (0.26)†	3.07 (0.32)	3.31 (0.31)	3.13 (0.27)†	2.92 (0.29)	3.34 (0.29)†	3.55 (0.33)†	3.25 (0.26)†
Fold change	91.55	56.83	82.21	103.40	33.64	95.42	99.37	93.48	

*haplotype blocks for both SNPs include additional SNPs shown on Table 1.

Genotypes, 1.1: homozygous for the major allele, 1.2: heterozygous, 2.2: homozygous for the minor allele.

Chi-square p-values using 1.1 as the referent category:

†*p* 0.05,

‡*p* 0.01; fold change comparing 2.2 vs. 1.1

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Table 4

Mean log gingival crevicular fluid IL-1β levels (standard errors) and relative (fold) changes contrasting 2.2 vs. 1.1, according to rs6940 and rs1057028 genotypes (adjusted for plaque levels).

SNP	n	Mean log IL-1β (SE)
rs6940 *		
1.1	3359	2.05 (0.01)
1.2	976	2.04 (0.01)
2.2	73	2.08 (0.04)
Fold change		3.05
rs1057028 *		
1.1	3006	2.05 (0.01)
1.2	1255	2.04 (0.01)
2.2	147	2.10 (0.03) †
Fold change		5.13

* haplotype blocks for both SNPs include additional SNPs shown on table 1

† chi-square p 0.05 using 1.1 as the referent category; fold change comparing 2.2 vs. 1.1

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Table 5

Distribution of rs6940 and rs1057028 haplotype blocks according to the biofilm-gingival interface (BGI) periodontal disease classification (healthy—n=571 vs. severe periodontitis—n=531).

	Individuals (%)	
SNP	**Healthy**	**Severe Periodontitis (P3)**
rs6940[*]		
1.1	76.01	73.63
1.2	23.12	24.11
2.2	0.88	2.26
rs1057028[*]		
1.1	67.78	65.35
1.2	29.95	29.19
2.2	2.28	5.46[†]

[*] haplotype blocks for both SNPs include additional SNPs shown on table 1

[†] chi-square p 0.05