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Identification of a Shared Genetic Susceptibility Locus for Coronary Heart Disease and Periodontitis

Arne S. Schaefer1*, Gesa M. Richter1, Birte Groessner-Schreiber2, Barbara Noack3, Michael Nothnagel4, Nour-Eddine El Mokhtari5, Bruno G. Loos6, Søren Jepsen7, Stefan Schreiber1

1 Institute for Clinical Molecular Biology, University Medical Center Schleswig-Holstein, Kiel, Germany, 2 Department of Operative Dentistry and Periodontology, University Medical Center Schleswig-Holstein, Kiel, Germany, 3 University Medical Center Carl Gustav Carus der Technischen Universität Dresden, Zentrum für Zahn-, Mund- und Kieferheilkunde, Poliklinik für Zahnerhaltung, Dresden, Germany, 4 Institute of Medical Informatics and Statistics, University Medical Center Schleswig-Holstein, Kiel, Germany, 5 Clinic of Cardiology, University Medical Center Schleswig-Holstein, Kiel, Germany, 6 Department of Periodontology, Academic Centre for Dentistry Amsterdam (ACTA), VU University Amsterdam, Amsterdam, The Netherlands, 7 Department of Periodontology, Operative and Preventive Dentistry, University of Bonn, Bonn, Germany

Abstract

Recent studies indicate a mutual epidemiological relationship between coronary heart disease (CHD) and periodontitis. Both diseases are associated with similar risk factors and are characterized by a chronic inflammatory process. In a candidate-gene association study, we identify an association of a genetic susceptibility locus shared by both diseases. We confirm the known association of two neighboring linkage disequilibrium regions on human chromosome 9p21.3 with CHD and show the additional strong association of these loci with the risk of aggressive periodontitis. For the lead SNP of the main associated linkage disequilibrium region, rs1333048, the odds ratio of the autosomal-recessive mode of inheritance is 1.99 (95% confidence interval 1.33–2.94; \( P = 6.9 \times 10^{-5} \)) for generalized aggressive periodontitis, and 1.72 (1.06–2.76; \( P = 2.6 \times 10^{-3} \)) for localized aggressive periodontitis. The two associated linkage disequilibrium regions map to the sequence of the large antisense noncoding RNA ANRIL, which partly overlaps regulatory and coding sequences of CDKN2A/CDKN2B. A closely located diabetes-associated variant was independent of the CHD and periodontitis risk haplotypes. Our study demonstrates that CHD and periodontitis are genetically related by at least one susceptibility locus, which is possibly involved in ANRIL activity and independent of diabetes associated risk variants within this region. Elucidation of the interplay of ANRIL transcript variants and their involvement in increased susceptibility to the interactive diseases CHD and periodontitis promises new insight into the underlying shared pathogenic mechanisms of these complex common diseases.

Introduction

Coronary Heart Disease (CHD) is the leading cause of death worldwide [1,2]. It is a systemic disease which is propagated by several environmental and behavioural risk factors [3–7] with inflammation as an important additional risk [8]. It has a strong genetic basis [9], but despite decades of intensive genetic research, until recently, the sequence variants that confer cardiovascular risk had remained largely unknown. Periodontitis is a complex chronic inflammatory disease, resulting in a loss of connective tissue and bone support of the teeth [10]. It is the major cause of tooth loss in adults above 40 years, and according to the WHO, affects human populations worldwide at prevalence rates of up to 10 to 20% for the most severe forms [11]. Moreover, it is associated with an increase in premature death in adults [12]. Formal genetic studies have demonstrated the genetic basis of periodontitis and indicated that about half of the population variance in chronic periodontitis can be attributed to genetic factors, with a concordance rate of 0.23–0.38 for monozygotic twins [13], and a heritability of 50% for dizygotic twins [14]. But hitherto, association studies with periodontitis have led to controversial results and its inherited components have remained unexplained [15].

Epidemiological studies demonstrated an association between the presence of CHD and periodontitis [16,17], which is dependent on the severity of periodontal disease [18,19]. CHD is also propagated by risk factors similar to periodontitis. Both diseases share smoking as a major environmental risk factor and relate to diabetes mellitus, obesity, and gender [6,7,20–22]. Recent studies also demonstrated similarities in the spectrum of bacteria in the oral cavity and in coronary plaques [23,24], and both diseases are characterized by an imbalanced immune reaction and a chronic inflammatory process [23,26]. Periodontitis is also associated with elevated C-reactive protein levels [27], a phenotype for which common genetic risk factors for periodontitis and atherosclerosis have been discussed [28]. These findings indicate a possible mutual genetic basis underlying both diseases.

Recently, four independent genome-wide association studies (GWAS) reported a strong association of a region of elevated linkage...
Coronary heart disease (CHD) and periodontitis are the most widespread diseases in the Western industrialized world and pose a substantial health threat to populations worldwide. CHD is a leading cause for premature death, and periodontitis is the major cause for tooth loss in adults over 40 years. Both diseases are associated with similar risk factors such as smoking, diabetes, and gender, and both diseases are further characterized by a chronic inflammatory process. In the last year, several genome studies have identified a region of the human genome near the CDKN2A and CDKN2B genes as having an influence on CHD. We show that this genetic region, being the most important susceptibility locus for CHD to date, is also associated with a substantial risk increase of aggressive periodontitis. The associated genetic region maps to a genomic region that codes for an “antisense RNA,” which partly overlaps regulatory and coding sequences of genes CDKN2A/CDKN2B. The interplay between these common inflammatory complex diseases could be partially due to the shared genetic risk variants of this antisense RNA.

**Results**

In the first stage of the experiment, we selected three SNPs of this LD region (rs2891168, rs1333042 and rs1333048), and verified the putative associations of these SNPs with CHD in a large population of 1,104 early-onset CHD patients (age at disease onset <55 years) and 736 healthy ethnically matched controls (Table 1). All SNPs gave evidence for association with CHD (Table 2, for genotypes refer to Table S1). With SNP rs2891168, which were in high LD in HapMap [33] CEU (r²=0.86), Two subsequent GWA studies [31,32] identified an association of rs1333049 (P=1.79×10⁻¹⁴ and P=5.4×10⁻¹⁶, respectively) which is in strong LD with rs10757278 in HapMap CEU and which is flanking the proximate border of the LD region (Figure 1). A subsequent meta-analysis in seven different populations confirmed the association of this region with CHD [34], making it the best replicated genetic risk locus of CHD to date. To investigate whether this locus may also relate to the risk for periodontitis, we investigated a possible association of this LD region with aggressive periodontitis (AgP), the most extreme form of periodontal diseases for which it is assumed that genetic factors play a greater role in the susceptibility than for chronic periodontitis [15].

**Association of the Chromosome 9p21.3 Locus with Localized AgP**

We replicated the associations in an independent population of 146 German periodontitis patients with the less severe AgP phenotype localized AgP (≥50% bone loss at ≥7 teeth below the age of 35), and further 368 ethnically matched healthy controls. All SNPs were found to be significantly associated at both the multiplicative and the genotypic level. SNP rs1333048 was again most significant, with a recessive OR of 1.70 (95% confidence interval 1.06–2.68), P=2.5×10⁻² (Table S2). After adjustment for the covariates smoking, type 2 diabetes and gender, all SNPs remained significant in the multiplicative genetic model, but not so under the recessive model. The smallest P-value was again observed for SNP rs1333048 (Pmultiplicative=5.9×10⁻⁵) (Table 3). However, genotypic ORs were similar, albeit slightly higher than those observed for generalized AgP (ORhet=1.56 [0.94–2.64], ORhom=2.30 [1.28–4.20]), and AIC values between the two models differed by less than 3.0 (Table S4), again suggesting a not completely autosomal-recessive model for the genetic effect of this SNP.

The minor allele of rs1333048 has a frequency of 54% in the combined AgP case samples and of 45% in the combined controls (Table S3). These frequencies correspond to those found in this LD region by the WTCCC study (53% in cases, 47% in controls) [31] and by the German myocard infarct study (54% in cases, 48% in controls) [32].

**Association Analysis of the Proximal CHD Associated LD Region**

The analyzed LD region is defined by a region of moderate LD (average D’ approximately 0.60) stretching at the 5’ site [34] (Figure 1). There, a second region of elevated LD, moderately in LD with the main CHD-associated LD region, harbours further CHD associated SNPs [32,34] (Figure 1). Three tagging SNPs (rs17044859, rs1292136 [recently renamed rs496892], and rs7655618) were identified to be sufficient to characterize the main haplotypes of this region [34]. To test these SNPs for possible associations with AgP, we performed a two step case-control analysis. If a shared genetic cause for both diseases is assumed,
association with the primary causal polymorphism will be consistent in clinical cohorts of related diseases, while SNP associations secondary to the main associated LD structure are much less likely to be consistently observed, due to different LD patterns in different cohorts. As with the GWA studies on CHD, in our study, the 3’ LD region gave stronger association signals than the second LD region. Among the three tagging SNPs, only SNP rs496892 was significant in both AgP populations after adjustment. Here, the OR was 1.36 (95% confidence interval 1.04–1.79), \( P = 2.4 \times 10^{-2} \) for generalized AgP, and 1.38 (95% confidence interval 1.02–1.86), \( P = 3.6 \times 10^{-2} \) for localized AgP (Table 4, Table S3) in the multiplicative model.

Statistical evidence of the CHD association signals suggested that the main associated LD region alone did not fully explain the association with CHD [34]. To test this, we performed a haplotype analysis [35] and pooled the two AgP populations to increase statistical power. The analysis indicated that rs1333048 accounted for most of the association with periodontitis (data not shown), similar to the genetic effect observed for the same haplotypic backgrounds in the CHD populations [34].

**Association Analysis with T2D Associated SNP rs10811661**

The analyzed LD region is further defined by a recombination hot spot 3’ to rs1333049. (Figure 1). To our knowledge, there is no evidence in the literature for an association with CHD distal to rs1333049 [29–32,34,36]. Three studies demonstrated an association of rs10811661 with type 2 diabetes [37–39] (T2D; 8.6 kb downstream of rs1333049; Figure 1). However, no association of this variant was observed with coronary heart disease [36].

Because T2D is an established risk factor for periodontitis we tested the T2D associated SNP rs10811661 for an additional

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**Figure 1. LD Structure \( (r^2) \) of the Chromosome 9p21.3 Locus in HapMap CEU.** The tagging SNPs of the CDKN2A/2B genes (1–13) are given to indicate the extent of these genes on the LD map. They are the only known genes within this genetic region (NCBI build36). Further SNPs are placed on the LD map: the three tagging SNPs of the adjacent AgP/CHD associated LD region (14–16), the four GWAS lead-SNPs of the main AgP/CHD associated LD region (18, 21, 22, 24), the three candidate SNPs selected for this study (19, 20, 23), and the T2D associated SNP (26). SNPs which were genotyped in this study are underlined, the four CHD-associated GWAS lead SNPs and the three CHD associated SNPs of the second LD region are marked by asterisks. SNP 17 and 25 are included into the figure to describe the distinct borders of the LD region. They are located immediately 5’ and 3’ to rs4977574 and rs1333049, respectively. All RefSeq genes and transcripts of this region are placed on the physical gene map, which has been aligned to the LD map and SNP positions below.

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association with AgP. No association with neither AgP status, prior or after adjustment for smoking, diabetes and gender was detected (Table 4).

Discussion

In this candidate gene association study we provide evidence for a shared association of the CHD high-risk locus on chromosome 9p21.3 with AgP. We selected SNPs which were located on the same LD region as the lead SNPs of previous GWA studies on CHD, by which they were flanked. We verified the selected SNPs for their association with CHD prior to the replication in the AgP populations. For the initial verification panel we used CHD cases for an age older than the general age of onset of mild forms of periodontitis (~60 years). We considered this matching as appropriate to minimize a possible stratification by individuals with undiagnosed periodontal diseases because less severe forms of periodontitis are not easily diagnosed at a young age and the information of the periodontal status of the controls was self-reported. Because the locus on chromosome 9p21.3 is associated with CHD, it could have an effect on survival. Hence, older controls may represent “survivors” and do not comprise the complete gene pool of the population. This could cause stratification. To assess this problem, we matched the controls in the replication panel for the age of the AgP cases to better mirror the gene pool of the average population. The independence of the controls may represent “survivors” and do not comprise the complete gene pool of the population. This could cause stratification. To assess this problem, we matched the controls in the replication panel for the age of the AgP cases to better mirror the gene pool of the average population. This could cause.

The robustness of these significant findings was supported by the fact that the inclusion of covariates such as smoking and gender did not notably alter P-values or effect sizes. In the explorative analysis we used a population with the most severe phenotype of periodontitis, generalized AgP. Similar to the early-onset CHD phenotype, this most severe form is suspected to be determined by genetic risk factors in particular. In this case panel, we matched the controls for an age older than the general age of onset of mild forms of periodontitis (~60 years).

The robustness of these findings which indicate a shared susceptibility locus for CHD and AgP was also reflected in the similar frequency difference of the rare alleles in both the WTCCC study on CHD and the presented study on AgP, which both differed by approximately 10% between cases and controls. The observed association of a shared susceptibility

Table 1. Baseline Characteristics of the Study Populations.

| Subject characteristic | Verification | Replication | Verification | Replication |
|------------------------|--------------|-------------|--------------|-------------|
|                        | CHD cases    | Controls    | CHD cases    | Controls    | Generalized AgP | Controls | Localized AgP | Controls |
| Individuals, n         | 1,097        | 736         | 1,104        | 736         | 151           | 736      | 137           | 368      |
| Men (%)                | 918 (83.7)   | 222 (30.2)  | 829 (75.1)   | 366 (49.7)  | 53 (35.1)     | 385 (52.3)| 54 (39.4)     | 184 (50.0)|
| Women (%)              | 179 (16.3)   | 510 (69.3)  | 275 (24.9)   | 370 (50.3)  | 96 (63.6)     | 351 (47.7)| 83 (61)       | 184 (50.0)|
| Mean age (SD) at diagnosis | 48 (5.7)    | -           | 57 (6.4)     | -           | 30 (5.6)      | -        | 29 (5.1)      | -        |
| Mean (SD) age at examination | 57 (5.9)    | 42 (12.7)   | 65 (6.1)     | 46 (13.1)   | 38 (6.6)      | 63 (7.4)  | 37 (7.0)      | 37 (9.6) |

Values are given as mean (standard deviation [SD]) when appropriate). Affection status was based on reported history (except for the number of affected teeth which was analyzed on the basis of radiographs by the clinicians, smoking was estimated by self-report). CHD – Coronary Heart Disease, AgP – Aggressive Periodontitis.
Table 2. Candidate SNP Associations of the Main CHD Associated LD Region in Coronary Heart Disease.

| CHD panels | SNP         | Genotypic | Recessive | Multiplicative | Dominant |
|------------|-------------|-----------|-----------|----------------|----------|
| Verification | rs2891168   | 5.8 × 10^{-6} | 1.44 | 1.13–1.84 | 2.02 | 1.52–2.68 |
|            | rs1333042   | 5.8 × 10^{-6} | 1.49 | 1.16–1.91 | 2.02 | 1.52–2.68 |
|            | rs1333048   | 3.9 × 10^{-5} | 1.36 | 1.06–1.75 | 1.92 | 1.44–2.55 |
| Replication | rs2891168   | 3.4 × 10^{-4} | 1.36 | 1.09–1.69 | 1.69 | 1.30–2.21 |
|            | rs1333042   | 7.3 × 10^{-5} | 1.46 | 1.17–1.83 | 1.74 | 1.34–2.27 |
|            | rs1333048   | 2.1 × 10^{-3} | 1.31 | 1.05–1.64 | 1.59 | 1.22–2.07 |

Association Statistics are shown for the two case and control panels of Coronary Heart Disease (CHD). Given are the odds ratios (OR), their 95% confidence intervals (CI 95%), and the P values which were obtained either from a likelihood-ratio test (genotypic model) or from a Wald test (autosomal-dominant, multiplicative, and recessive models). Values are given after adjustment for the covariates smoking, diabetes, and gender in a logistic regression model. All other markers showed no significance at the 5% test level under either of the models.

Table 3. Candidate SNP Associations of the Main CHD Associated LD Region in Aggressive Periodontitis.

| AgP panels | SNP         | Genotypic | Recessive | Multiplicative | Dominant |
|------------|-------------|-----------|-----------|----------------|----------|
| Gen.       | rs2891168   | 4.9 × 10^{-3} | 1.06 | 0.68–1.69 | 1.05 | 1.26–3.34 |
|            | rs1333042   | 7.7 × 10^{-3} | 1.10 | 0.70–1.77 | 2.00 | 1.24–2.98 |
|            | rs1333048   | 3.6 × 10^{-3} | 1.12 | 0.70–1.80 | 2.13 | 1.31–3.50 |
| Local.     | rs2891168   | 4.6 × 10^{-2} | 1.40 | 0.86–2.34 | 2.09 | 1.17–3.77 |
|            | rs1333042   | 7.1 × 10^{-2} | 1.44 | 0.87–2.42 | 1.97 | 1.10–3.54 |
|            | rs1333048   | 2.1 × 10^{-2} | 1.56 | 0.94–2.64 | 2.30 | 1.28–4.20 |

Association Statistics are shown for the two case and control panels of Generalized Aggressive Periodontitis (Gen.) and Localized Aggressive Periodontitis (Local.). Given are the odds ratios (OR), their 95% confidence intervals (CI 95%), and the P values which were obtained either from a likelihood-ratio test (genotypic model) or from a Wald test (autosomal-dominant, multiplicative, and recessive models). Values are given after adjustment for the covariates smoking, diabetes, and gender in a logistic regression model. All other markers showed no significance at the 5% test level under either of the models.
Table 4. Candidate SNP Associations of the Second CHD Associated LD Region and the T2D Associated SNP.

| SNP          | Genotype | Dominant | Multiplicative | Recombinant |
|--------------|----------|----------|----------------|-------------|
|              | P        | CI 95%   | P              | CI 95%      |
| rs496892     | 0.77     | 0.49–1.23| 0.48           | 0.28–0.83   |
|              | OR       | CI 95%   | 0.38–0.89      |             |
|              | 0.15     | 10.9–17  | 0.76           | 0.47–1.20   |
|              | 0.66     | 0.41–1.16| 0.68           | 0.47–1.22   |
|              | 0.65     | 0.40–1.09| 0.66           | 0.40–1.15   |
|              | 0.65     | 0.40–1.09| 0.65           | 0.40–1.15   |
|              | 0.65     | 0.40–1.09| 0.65           | 0.40–1.15   |
| rs7865618    | 0.59     | 0.22–1.79| 0.68           | 0.45–0.96   |
|              | OR       | CI 95%   | 0.45–0.96      |             |
|              | 1.02     | 0.60–1.80| 0.68           | 0.45–0.96   |
|              | 0.91     | 0.55–1.55| 0.68           | 0.45–0.96   |
|              | 0.91     | 0.55–1.55| 0.68           | 0.45–0.96   |
|              | 0.91     | 0.55–1.55| 0.68           | 0.45–0.96   |
|              | 0.91     | 0.55–1.55| 0.68           | 0.45–0.96   |
|              | 0.91     | 0.55–1.55| 0.68           | 0.45–0.96   |
| rs10811661   | 0.64     | 0.40–1.02| 0.64           | 0.40–1.02   |
|              | OR       | CI 95%   | 0.39–0.95      |             |
|              | 0.64     | 0.40–1.02| 0.64           | 0.40–1.02   |
|              | 0.64     | 0.40–1.02| 0.64           | 0.40–1.02   |
|              | 0.64     | 0.40–1.02| 0.64           | 0.40–1.02   |
|              | 0.64     | 0.40–1.02| 0.64           | 0.40–1.02   |
|              | 0.64     | 0.40–1.02| 0.64           | 0.40–1.02   |

Local. rs7044859

| SNP          | Genotype | Dominant | Multiplicative | Recombinant |
|--------------|----------|----------|----------------|-------------|
|              | P        | CI 95%   | P              | CI 95%      |
| rs496892     | 0.59     | 0.22–1.79| 0.68           | 0.45–0.96   |
|              | OR       | CI 95%   | 0.45–0.96      |             |
| rs7865618    | 0.64     | 0.40–1.02| 0.64           | 0.40–1.02   |
|              | OR       | CI 95%   | 0.39–0.95      |             |
| rs10811661   | 0.64     | 0.40–1.02| 0.64           | 0.40–1.02   |
|              | OR       | CI 95%   | 0.39–0.95      |             |

Aggressive Periodontitis (Gen.) and Localized Aggressive Periodontitis (Local.). Given are the odds ratios (OR), their 95% confidence intervals (CI 95%), and the P values which were obtained either from a likelihood-ratio test (genotypic model) or from a Wald test (autosomal-dominant, multiplicative, and recessive models). Values are given after adjustment for the covariates smoking, diabetes, and gender in a logistic regression model. All other markers correspond with the same associated SNPs, but do not share any coding nucleotide sequences. Interestingly, mRNA AF109294, which is thought to encode a hypothetical MTAP fusion protein mRNA, also partially overlaps ANRIL, sharing two exons. Expression data suggested a coordinated transcriptional regulation of ANRIL, CDKN2A and CDKN2B [47], and expression in tissues involved in atherosclerosis, like vascular endothelial cells, macrophages, and coronary smooth muscle cells, had been shown [40]. The function and mechanism of action for this mRNA is yet unknown. Full length deletion of ANRIL was associated with cutaneous melanoma [47] and the CHD and AgP high-risk haplotype, overlapping exons 13–19 of ANRIL, is associated with diseases that also share a phenotype of abnormal cell proliferation. A shared function of the neighbouring genes CDKN2A/2B and MTAP is the regulation of cell proliferation/tumor suppression. It is tentative to speculate a mechanistic link between the overlapping disease phenotypes of impaired cell...
proliferation, and a potential transcriptional regulatory role of the ncRNA ANRIL. The interplay of these genes in the tissue specific regulation of cell proliferation could be mediated by this antisense RNA. It is intriguing that the CHD and AgP phenotypes share high-risk LD regions, whereas T2D has apparently independent risk variants within this region. Elucidation of the interplay of ANRIL transcript variants and their involvement in increased susceptibility to interactive diseases like CHD and periodontitis promises new insight into the underlying partially shared pathogenic mechanisms, and will open up new avenues in the understanding of the development of these complex common diseases.

Methods

Study Populations

The CHD patients comprised a population-representative collection of unrelated Germans (Table 1). They were recruited from Schleswig-Holstein, the northernmost region in Germany, through the population-based PopGen biobank [49]. In the recruitment area, all coronary angiograms of any of the five cardiac catheterization laboratories were screened. Study subjects were required to have coronary catheterization demonstrating significant CHD (at least a 70% stenosis in one major epicardial coronary vessel). 1,104 cases had a diagnosed disease onset <55 years, of whom a subset of 396 individuals had suffered a myocardial infarction. The majority (90.3%) had a history of severe CHD and had undergone a coronary revascularization procedure (percutaneous coronary intervention or coronary artery bypass grafting). The controls were obtained from the Blood Service of the University Hospital Schleswig-Holstein. Information about the age and gender was available. Written informed consent was obtained from all participants and the recruitment and the experimental protocols were approved by the institutional ethics review board and data protection authorities.

The AgP patients were recruited from throughout Germany. Only patients of German ethnicity were included, determined by the location of both parental birthplaces. Prior to the study, the genetic sub-structure of the German population had been assessed [50], indicating only negligible sub-structures and therefore allowing a joint analysis of all individuals. A further inclusion criterion was age at diagnosis ≤35 years. A set of full-mouth dental radiographs was available for confirmative periodontal bone scoring. ≥2 teeth with ≥50% periodontal bone loss was defined as inclusion criterion. The sub-phenotype of localized AgP was characterized by ≥50% bone loss at 2–6 teeth; the sub-phenotype generalized AgP was characterized by ≥50% bone loss at ≥7 teeth. The ethnically matched controls used for generalized AgP association study were randomly identified on the basis of the local population registry and the controls used for localized AgP were obtained from the Blood Service of the University Hospital Schleswig-Holstein. All controls underwent an additional physical examination at the PopGen facilities to obtain information on general health status. Information on the oral health status and physical risk factors (e.g. smoking, diabetes) was obtained from questionnaires completed during medical consultation. Additionally, a clinical checkup was subsequently performed. All controls self-reported to be free of periodontitis.

Genotyping

Genomic DNA was extracted from blood samples (Invisorb Blood Universal Kit, Invitek, Berlin, Germany) and amplified by whole genome amplification (GenomiPhi, Amersham, Uppsala, Sweden). Genotyping was performed using the SNPlex and TaqMan Genotyping System (Applied Biosystems, Foster City, CA, USA) on an automated platform, employing TECAN Freedom EVO and 96-well and 384-well TETM liquid handling robots (TECAN, Männedorf, Switzerland). Genotypes were generated by automatic calling using the Genemapper 4.0 software (Applied Biosystems) with the following settings: sigma separation >6, angle separation for 2 cluster SNPs <1.2 radians, median cluster intensity >2.2, 2 × 3 contingency tables where applicable. Logistic regression analysis was performed in R v2.7.2 [35]. Significance was assessed by a Wald test and by a likelihood-ratio test.

Supporting Information

Table S1 Genotype Frequencies for the CHD Cases and Controls.

Table S2 SNP Associations in Aggressive Periodontitis Prior to Adjustment for Covariates.

Table S3 Genotype Frequencies for the AgP Cases and Controls.

Table S4 AIC Values for the Genetic Models Tested in the Study.

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Author Contributions

Conceived and designed the experiments: ASS. Performed the experiments: ASS GMR. Analyzed the data: ASS GMR MN. Contributed reagents/materials/analysis tools: BGS BN NEEM BGL SJ SS. Wrote the paper: ASS.
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