Original Article

Genetic Evidence for *PLASMINOGEN* as a Shared Genetic Risk Factor of Coronary Artery Disease and Periodontitis

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Background—Genetic studies demonstrated the presence of risk alleles in the genes *ANRIL* and *CAMTA1/VAMP3* that are shared between coronary artery disease (CAD) and periodontitis. We aimed to identify further shared genetic risk factors to better understand conjoint disease mechanisms.

Methods and Results—In-depth genotyping of 46 published CAD risk loci of genome-wide significance in the worldwide largest case–control sample of the severe early-onset phenotype aggressive periodontitis (AgP) with the Illumina Immunochip (600 German AgP cases, 1448 controls) and the Affymetrix 500K array set (283 German AgP cases and 972 controls) highlighted *ANRIL* as the major risk gene and revealed further associations with AgP for the gene *PLASMINOGEN* (*PLG*; rs4252120: $P=5.9\times10^{-5}$; odds ratio, 1.27; 95% confidence interval, 1.3–1.4 [adjusted for smoking and sex]; 818 cases; 5309 controls). Subsequent combined analyses of several genome-wide data sets of CAD and AgP suggested *TGFBRAP1* to be associated with AgP (rs2679895: $P=0.0016$; odds ratio, 1.27 [95% confidence interval, 1.1–1.5]; 703 cases; 2.143 controls) and CAD ($P=0.0003$; odds ratio, 0.84 [95% confidence interval, 0.8–0.9]; n=4117 cases; 5824 controls). The study further provides evidence that in addition to *PLG*, the currently known shared susceptibility loci of CAD and periodontitis, *ANRIL* and *CAMTA1/VAMP3*, are subjected to transforming growth factor-β regulation.

Conclusions—*PLG* is the third replicated shared genetic risk factor of atherosclerosis and periodontitis. All known shared risk genes of CAD and periodontitis are members of transforming growth factor-β signaling. (***Circ Cardiovasc Genet***. 2015;8:159-167. DOI: 10.1161/CIRCGENETICS.114.000554.)

Key Words: coronary artery disease ■ genetic association studies ■ periodontitis ■ plasminogen

Periodontitis is a chronic inflammatory disease of the oral cavity. The inflammation is elicited by the oral microbial biofilm that leads to gingival bleeding, pocket formation, alveolar bone loss, and eventually tooth loss as final outcome.[1] Periodontitis affects human populations worldwide at prevalence rates of 11% for the severe forms.[2] It is largely classified into the subforms chronic periodontitis (CP) and aggressive periodontitis (AgP). Whereas chronic periodontitis is mostly observed in adults and is characterized by a slow progress of the disease, AgP is the most early-onset and most extreme phenotype and found in young individuals aged <35 years. AgP is diagnosed based on rapid destruction of the alveolar bone and is comparatively rare in the general population with prevalence rates ≈0.1%. Shared with coronary artery disease (CAD), smoking, obesity, and mellitus diabetes contribute strongly to periodontitis[3–5] and both diseases are characterized by chronic inflammation.[6] Likewise, epidemiological studies demonstrated an association between the presence of CAD and periodontitis,[7] which is dependent on the severity of periodontal disease.[8] We previously showed that CAD and periodontitis are genetically related by ≥2 genetic susceptibility loci, the long noncoding antisense RNA (lncRNA) *ANRIL* (*CDKN2BAS*) at chromosome 9p21.3[9,10] and a

conserved noncoding element within *CAMTA1* upstream of *VAMP3*.[11] Molecular biological data placed ANRIL and VAMP3 into a regulatory network that integrates glucose and fatty acid metabolism with immune response,[11,12] providing evidence for a mechanistic link between CAD, periodontitis, obesity, and inflammation. The impairment of these pathways by genetic factors may be a common pathogenic denominator of CAD and periodontitis.

Clinical Perspective on p 167

In the present study, we aimed to identify further putatively shared genetic risk factors of CAD and periodontitis to better understand the common genetic architecture of these diseases. In the first stage, we genotyped 46 CAD risk loci, which gave published evidence for genome-wide association with CAD.[13] These chromosomal regions were genotyped on the Illumina Immunochip[14] and the Affymetrix 500K Genotyping Array Set[15] using cases of AgP. For AgP it is thought that smoking, obesity, diabetes mellitus, and age have a minor role in disease development and patient samples are considered enriched with genetic risk factors that are located in genes involved in the pathogenesis of periodontitis.

In a second stage of this study, we combined several genome-wide data sets of CAD and AgP from Germany and the UK and propose a significant association within *TGFBRAP1* for replication in future enlarged case–control samples of AgP.

Materials and Methods

Study Population

Details on the study populations are given in Table 1, in the Data Supplement, and in the Tables I to III in the Data Supplement. Written informed consent was obtained from all participants. The recruitment and experimental protocols were approved by the institutional ethics review board and data protection authorities.

Genotyping

Immunochips were genotyped and analyzed as described in Ref. 16 and Affymetrix 500K arrays as described in Refs. 15, 17, and 18. We included all single-nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) ≥5% and at a Hardy–Weinberg equilibrium for controls >5%, and with a call rate of >95%. SNP rs1981458 and rs17514846 (*FURIN*), rs4252120 (*PLASMINOGEN*) and rs2679895 (*TGFBRAP1*) were also genotyped on 384-well plates using TaqMan assays hCV11947689, hCV1244341, hCV11225947, and hCV27295110, respectively (Applied Biosystems) as described.[10]

Analysis of Candidate Genes

Twenty-seven chromosomal regions, which gave published evidence of genome-wide association with CAD, were genotyped using the Immunochip (Illumina) in 600 German AgP cases and 1443 population representative German controls (Table 1).[13] In addition, 18 further CAD risk loci of genome-wide significance were genotyped using the Affymetrix 500K Arrays set with 283 German AgP cases and 979 German controls.[15] See Tables IV and V in the Data Supplement for gene names, SNPs, genotypes, and association statistics of the associated genetic regions. Potential associations that were observed for genotype data of the immunochip were validated in silico in an independent case–control panel of 159 Dutch AgP cases and 679 population representative Dutch controls as described in [16]. Potential associations that were observed for genotype data of the 500K array genome-wide association study (GWAS) data were replicated using a panel of 424 German AgP cases and 3628 German controls and validated using 159 Dutch AgP cases and 352 periodontal healthy Dutch controls, as described in Table 1.

Statistical Analysis

SNP imputation was performed in silico with the BEAGLE v.3.1.141 software package[19] and HapMap3 reference haplotypes from the Central Europeans of Utah (CEU) cohorts[20] to predict missing autosomal genotypes in silico. We subsequently analyzed only those SNPs that could be imputed with moderate confidence ($r^2 > 0.3$) and had a minor allele frequency >1% in cases or in controls as previously described.[21] Genotypes were analyzed using the software PLINK v2.[22] Significance of association was assessed using χ^2 tests. Power calculations were performed using PS Power and Sample Size Calculations software.[23] Linkage disequilibrium measures were calculated with Haploview 4.1.[24] Logistic regression analysis was performed to adjust for possible confounding of the covariates smoking and sex in the R statistical environment.[25] For the regression analyses, smoking was coded as a binary variable according to whether a patient or control person had ever smoked. The genetic models considered were (1) an allelic model (genotypes aa, aA, and AA coded as 0, 1, and 2, respectively), (2) a dominant model (genotypes coded as 0, 1, and 1), and (3) a recessive model (genotypes coded as 0, 0, and 1). Thus, each logistic model included 3 independent variables: 2 binary covariates (sex and smoking), plus the genetic variable that was either binary (dominant or recessive genetic model) or considered continuous (allelic model). The dependent variable was always the case/control status, coded as 1 or 0, respectively.

Details on the cutoff criteria for the selection of SNPs to be taken into replication and the statistical analyses and imputation methods are described in the Data Supplement.

Quantitative Real-Time Polymerase Chain Reaction

Transcript levels were analyzed by quantitative real-time reverse transcription polymerase chain reaction. Primers and conditions are described in the Data Supplement.

Cell Culture Conditions and Transforming Growth Factor-β Stimulation

Human gingival fibroblastic primary cells were derived from 3 different individuals during dental surgery. The cells of each donor were cultured in DMEM containing 1% penicillin/streptomycin and 10% heat inactivated fetal bovine serum. For each of the 3 different donors, 6 cell cultures were grown independently as biological replicates. After reaching 50% to 60% confluence, the medium of 3 cultures of each donor was replaced by fresh medium supplemented with 20 ng/mL transforming growth factor (TGF)-β for 48 hours. Three cultures of each donor continued to grow for 48 hours in replaced fresh medium that lacked the TGF-β supplement. For each biological replicate, the quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) was performed in triplicates. For each donor, the mean for 3 replicates was calculated and standard 1- and 2-tailed *t* tests were performed. For *ANRIL*, *C11ORF10*, and VAMP3, where the effect directions were unknown, 2-tailed tests were performed. For *ADIPOR1* and *CDKN2B*, where the effect directions after TGF-β stimulation were known from literature, a 1-tailed test was performed.

Results

Candidate Gene Association Study of Known CAD Risk Loci in AgP

Immunochip

In an explorative step of the first stage of this study, 27 established CAD risk loci of genome-wide significance were genotyped with the Immunochip. Two loci, *ANRIL* and *FURIN*, suggested association with periodontitis and fulfilled the

Table 1. Assignment of the Analysis Populations to the Different Stages of the Study

	Candidate Gene Study						Joint GWAS Analysis			
	Stage 1			Stage 2			Stage 1	Stage 2	Stage 3	Stage 4
	Immunochip		TaqMan (*FURIN*; rs1981458, rs17514846)	Affymetrix 500K	TaqMan (*PLG*; rs4252120)		Affymetrix 500K			TaqMan (*TGFBRAP1*; rs2679895)
Samples	Exploration (D)	Validation (NL)	D	Exploration (D)	Replication (D)	Validation (NL)	Exploration AgP/CAD (D)	Replication CAD (D)	Validation CAD (UK)	Replication AgP (D)
Cases										
AgP (German)	600	…	600/628	283	…	…	283	…	…	…
AgP (Dutch)	…	164	159	…	424	159	…	…	…	427
MI (GerMIF-I)	…	…	…	…	…	…	…	970	…	…
MI (GerMIF-II)	…	…	…	…	…	…	1222	…	…	…
MI (WTCCC)	…	…	…	…	…	…	…	…	1925	…
Sum cases	600	164	600/159	283	424	159	283/1222	970	1925	427
Controls										
Popgen (SPCs)	471	…	471	500	…	…	500	…	…	…
Popgen (PopCONs)	977	…	…	…	…	…	…	…	…	…
Popgen (BSPs)	…	…	449	479	…	…	479*	…	…	…
KORA	…	…	3028†/3259‡	…	3259	…	820	1644	…	…
MICK	…	…	…	…	…	…	…	…	…	1292
Periofree (Munich)	…	…	…	…	423	…	…	…	…	222
UMCG (NL)	…	679	…	…	…	…	…	…	…	…
ACTA (NL)	…	…	352	…	…	352	…	…	…	…
WTCCC (UK)	…	…	…	…	…	…	…	…	2936	…
Sum controls	1443	679	3948/4179/352	979	3682	352	979/1299	1644	2936	1514

Detailed characteristics of the individual samples are given in the Data Supplement. ACTA indicates Academic Centre for Dentistry Amsterdam; AgP, aggressive periodontitis; BSP, blood donors special phenotyped; CAD, coronary artery disease; GerMIF, German Myocard Infarct Family Study; KORA, kooperative Gesundheitsforschung in der Region Augsburg; MI, myocardial infarction; MICK, Metabolic Intervention Cohort Kiel; PopCON, biobank popgen controls; SPC, special phenotpyed controls; UMCG, University Medical Center Groningen; and WTCCC, Wellcome Trust Case-Control Consortium.

*Part of the GWAS of AgP and the GERMIF-II; †metabochip; and ‡OmniExpress array. For details on the study samples please refer to the supplemental materials.

preassigned significant threshold of $P<6\times10^{-3}$ (Table VI in the Data Supplement; Table 2). The associations of *ANRIL* with AgP were in accordance with our previous studies.[9,10] To show the independence of this association from established covariates, we adjusted rs1981458 for smoking and sex by logistic regression analysis. Because data on smoking were not available for 977 of the popgen PopCon control sample, we excluded these controls from the analysis. To keep sufficient statistical power (SP) for analysis of this low frequency variant, we added another 449 German blood donors (BSP; sample of Blood donors Special Phenotyped) from the biobank popgen and additional 3028 population representative German controls from the biobank KORA (Kooperative Gesundheitsforschung in der Region Augsburg) , for which data on smoking and sex were available. Before and after adjustment, this association remained significant (Table 2).

SNP rs1981458 is located 55 bp downstream of the common GWAS lead SNP rs17514846 (MAF=47%; HapMap CEU) and is not associated with CAD in the CARDIoGRAMplusC4D Consortium, encompassing >63 000 CAD cases and >130 000 controls.[13] rs17514846 was not included on the Immunochip. To test whether rs17514846 was also associated with AgP, we genotyped the 600 German AgP cases, which were also used for the

immunochip and 4179 German healthy controls from the biobanks popgen and KORA (Table 1; Table I in the Data Supplement). The association of rs17514846 was not significant here.

Affymetrix 500K
Genetic risk loci of CAD[13] that were represented on the Immunochip by ≤2 SNPs were analyzed using Affymetrix 500K arrays (Table V in the Data Supplement). Of those 28 loci, only associations within the gene *PLG* fulfilled the preassigned selection criteria. The best association signals were shown by *PLG* SNP rs4252135 (MAF=28.3%, HapMap CEU) and the *PLG* GWAS lead SNP of CAD, rs4252120 (Table VII in the Data Supplement). After covariate adjustment for sex and smoking, the association of rs4252120 with AgP was significant with $P_{allelic}$=0.00019 (odds ratio [OR], 1.46; 95% confidence interval [CI], 1.20–1.78; Table 2). In a further sample of 424 less severe German AgP cases and 3682 controls, this association slightly missed significance (Table 1). After adjustment for the covariates smoking and sex, the association was nominally significant under the dominant genetic model but not under the allelic model (Table 2). To increase the SP, both German samples were pooled (n=706 cases, 4957 controls). After adjustment for sex and smoking, the association

Table 2.   AgP Association Statistics for the Best Associated SNPs at *FURIN* and *PLG*

Gene, SNP	Population	SNP	P Values	OR (95% CI)	Cases					Controls				
					11% (n)	12% (n)	22% (n)	Sum	MAF (%)	11% (n)	12% (n)	22% (n)	Sum	MAF (%)
FURIN, rs1981458	German	Unadjusted	0.00581	1.34 (1.1–1.7)	76.2 (457)	22.5 (135)	1.3 (8)	600	12.6	81.6 (1168)	17.5 (251)	0.9 (13)	1432	9.7
		Adjusted (smoking, sex)	0.0115*	1.28 (1.1–1.6)	76.2 (457)	22.5 (135)	1.3 (8)	600	12.6	81.0 (3197)	17.7 (700)	1.3 (51)	3948†	10.2
	Dutch	Unadjusted	0.96088	1.01 (0.7–1.5)	81.1 (133)	17.7 (29)	1.2 (2)	164	10.1	81.2 (550)	17.6 (119)	1.2 (8)	677	10.0
PLG, rs4252120	GWAS (German)	Unadjusted	0.00018‡	1.44 (1.2–1.8)	40.4 (114)	47.5 (134)	12.1 (34)	282	35.8	51.7 (659)	40.9 (521)	7.5 (95)	1275	27.9
		Adjusted (smoking, sex)	0.00019	1.46 (1.2–1.8)	...	...	...	...	...	...	...	...	...	...
	Replication (German)	Unadjusted	0.05524	1.2 (1.0–1.4)	44.3 (188)	45.1 (191)	10.6 (45)	424	33.1	49.1 (1809)	41.9 (1541)	9.0 (332)	3682	29.9
		Adjusted (smoking, sex)	0.0571||	1.18 (1.0–1.4)	...	...	...	...	...	...	...	...	...	...
	Pooled (German)	Unadjusted	0.00024	1.25 (1.1–1.4)	42.8 (302)	46.0 (325)	11.2 (79)	706	34.2	49.8 (2468)	41.6 (2062)	8.6 (427)	4957	29.4
		Adjusted (smoking, sex)	0.00071	1.25 (1.1–1.4)	...	...	...	...	...	...	...	...	...	...
	Replication (Dutch)	Unadjusted	0.01158	1.44 (1.1–1.9)	42.8 (68)	44.0 (70)	13.2 (21)	159	35.2	53.7 (189)	37.8 (133)	8.5 (30)	352	27.4
		Adjusted (smoking, sex)	0.00964	1.51 (1.1–2.1)	...	...	...	...	...	...	...	...	...	...
	Pooled all (German, Dutch)	Adjusted (smoking, sex)	5.9×10^{-5}	1.27 (1.1–1.4)	42.8 (370)	45.7 (395)	11.6 (100)	865	34.4	50.1 (2657)	41.4 (2195)	8.6 (457)	5309	29.3

CI indicates confidence interval; MAF, minor allele frequency; OR, odds ratio; and SNP, single-nucleotide polymorphism.

*The *P* value for the dominant model is *P*=0.00625; OR, 1.35 (95% CI, 1.1–1.7).

†The control sample that was used for the covariate adjustment consisted of 922 North-German controls (popgen) and an additional sample of 3026 South-German controls (KORA).

‡A total of 296 additional popgen blood donor controls were used in the TaqMan genotyping (n=1275) compared with Affymetrix 500K genotyping (n=979) to increase the statistical power. This explains the discrepancy with the *P* value in the text of the article (*P*=0.00059; see also Table VII in the Data Supplement).

§The *P* value for the dominant model is *P*=0.0336; OR, 1.27 (95% CI, 1.0–1.6).

||*P* value is given for the dominant genetic model.

of rs4252120 was significant with $P=0.00071$ and a genetic effect of OR of 1.25 and 95% CI of 1.1 to 1.4.

We validated the association in the smaller Dutch AgP panel of high severity and found the same genetic effect direction (Table 2). Next, to increase the SP, the German and Dutch samples were pooled (865 cases, 5309 controls) and adjusted for sex and smoking. The association became more significant with $P_{allelic}=5.9\times10^{-5}$ (Table 2).

Combined GWAS Analysis of CAD and AgP

In the second stage of this study, SNPs that suggested association with CAD in imputed genotypes of GWAS data of the German Myocard Infarct Family Study (GerMIFS)-II were tested if they showed a similar level of significance in imputed genotypes of the GWAS on AgP. A total of 30 383 SNPs showed a *P* value of <0.05 in the GerMIF-II GWAS and 99 154 SNPs showed a *P* value of <0.05 in the AgP GWAS. Of these SNPs, 1182 SNPs were shared between both GWAS (Figure 1). Next, these SNPs were tested for association in a second imputed GWAS of CAD, the GerMIFS-I. At the same significance threshold, 128 SNPs showed nominal association. To further reduce the number of suggestive SNP associations, we strengthened the selection criteria. SNPs were selected, which showed a genetic effect in the same direction between the 2 CAD GWAS samples at a level of significance of $P<0.01$, and additionally SNPs, which showed a level of significance at a $P<0.01$ between the replication panel GerMIFS-I and the AgP GWAS sample, regardless of the effect direction (Figure 1). Twenty-one SNPs fulfilled these selection criteria (Table VIII in the Data Supplement). These associations were subsequently replicated in the Wellcome Trust Case-Control Consortium CAD GWAS sample (Table VIII in the Data Supplement). Five SNPs were located within *ANRIL* and 2 within *TGFBRAP1* (Table 3). Whereas the shared association of *ANRIL* with AgP and CAD had repeatedly been observed by us and others,[9,10,16,26] an association of *TGFBRAP1* with CAD or periodontitis has not been reported previously. The ORs of the 2 *TGFBRAP1*-associated SNPs rs920217 and rs2679895 were identical in the 3 CAD samples (OR<0.9), which was opposite to the genetic effect in the AgP sample (Table 3). The MAFs of both SNPs were similar in all CAD and AgP control samples. After adjustment of rs2679895 for
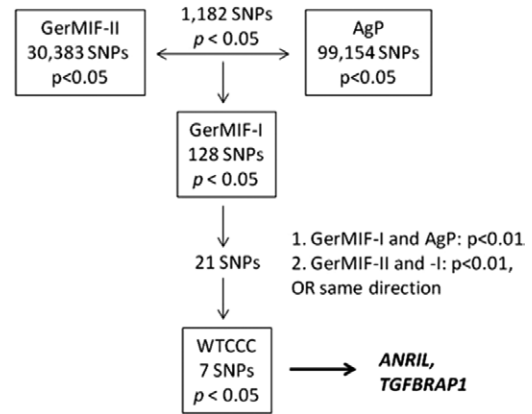

Figure 1. Study design of the combined GWAS analyses. To generate hypotheses on candidate genes that are shared in the pathogenesis of coronary artery disease and periodontitis, the GWAS were combined as illustrated. The individual GWAS samples are published in Refs. 15, 17, and 18. AgP indicates aggressive periodontitis; GerMIF, German Myocard Infarct Family Study; SNP, single-nucleotide polymorphism; and WTCCC, Wellcome Trust Case-Control Consortium.

the covariates smoking and sex, the effect of the rare allele of rs2679895 remained significantly associated with AgP ($P_{allelic}=0.0169$; OR, 1.33; 95% CI, 1.05–1.68; Table 4).

To localize the association with *TGFBRAP1* more precisely, all SNPs that were represented on the Affymetrix 500K arrays were tested for association with the CAD-Wellcome Trust Case-Control Consortium sample and the AgP GWAS sample (28 SNPs; Figure 2). Notably, a GWAS on progression of AIDS (Acquired Immune Deficiency Syndrome) reported an association with rs1020064, which was not genotyped in our samples. This SNP is located ≈1000 bp upstream of rs2576742 (Figure 2),[27] which was genotyped in our samples. SNP rs2576742 showed a similar level of association as rs2679895 (CAD-Wellcome Trust Case-Control Consortium: $P=0.0056$; OR, 0.87 [95% CI, 0.79–0.96] and AgP: $P=0.0041$; OR, 1.36 [95% CI, 1.10–1.68]). Analysis of the linkage disequilibrium structure of rs2679895, rs2576742, and the AIDS-associated SNP rs1020064 in a sample of German population representative controls (n=211), for which genotypes of these 3 SNPs were available, showed complete linkage ($r^2>0.98$;

Table 3. Summary Statistics of Candidate SNP Associations of the Joint GWAS Analysis of Coronary Artery Disease and AgP (Affymetrix 500K)

Gene	SNP	GerMIF-II* (German)		GerMIF-I* (German)		WTCCC† (British)		AgP† (German)	
		P Value	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)
ANRIL	rs10965212	0.0028	1.19 (1.06–1.33)	4.39E–04	1.22 (1.09–1.37)	6.94E–10	1.29 (1.2–1.4)	8.26E–04	1.38 (1.14–1.66)
	rs10965215	0.0061	1.17 (1.05–1.31)	3.40E–04	1.23 (1.10–1.37)	4.32E–10	1.30 (1.2–1.4)	0.0011	1.37 (1.13–1.65)
	rs564398	1.22E–04	0.80 (0.71–0.90)	7.12E–04	0.82 (0.73–0.92)	1.65E–08	0.79 (0.7–0.9)	5.26E–04	0.71 (0.59–0.86)
	rs10965219	0.0016	1.20 (1.07–1.34)	1.11E–03	1.21 (1.08–1.35)	3.94E–11	1.32 (1.2–1.4)	2.82E–04	1.42 (1.17–1.71)
	rs6475606	5.25E–07	1.33 (1.19–1.48)	1.11E–05	1.29 (1.15–1.44)	2.44E–14	1.37 (1.3–1.5)	2.56E–04	1.42 (1.18–1.71)
TGFBRAP1	rs920217	0.0435	0.87 (0.76–0.996)	0.0095	0.84 (0.73–0.96)	0.0262	0.89 (0.81–0.99)	0.02427	1.30 (1.03–1.63)
	rs2679895	0.0435	0.87 (0.76–0.996)	0.0095	0.84 (0.73–0.96)	0.0083	0.87 (0.79–0.97)	0.0081	1.36 (1.08–1.70)

AgP indicates aggressive periodontitis; CI, confidence interval; GerMIF, German Myocard Infarct Family Study; OR, odds ratio; SNP, single-nucleotide polymorphism; and WTCCC, Wellcome Trust Case-Control Consortium.

*Imputed data; †genotyped data.

Table 4. Summary Statistics of *TGFBRAP1* SNP rs2679895 for the Different Aggressive Periodontitis Samples After Covariate Adjustments

Genetic Model	German 1*		German 2†		German Pooled†		Dutch*		Turkish*	
	P Value (AIC)	OR (95% CI)	P Value (AIC)	OR 5% CI)	P Value (AIC)	OR (95% CI)	P Value (AIC)	OR (95% CI)	P Value (AIC)	OR (95% CI)
Allelic	0.0169 (1074)	1.33 (1.05–1.68)	0.0443 (1562)	1.22 (1.004–485)	0.0016 (2692)	1.27 (1.09–1.47)	0.0305 (588)	0.68 (0.48–0.96)	0.0332 (113)	0.44 (0.20–0.92)
Recessive	n.s.	1.49 (0.82–2.66)	0.0123 (1560)	1.80 (1.13–2.84)	0.0077 (2695)	1.62 (1.13–2.31)	0.0269 (584)	0.63 (0.41–0.94)	0.0126 (111)	0.29 (0.11–0.76)

AIC indicates Akaike's Information Criterion; CI, confidence interval; and OR, odds ratio.

*Adjusted for smoking and sex.

†Adjusted for smoking.

Figure I in the Data Supplement). This is supported by the data of the HapMap CEU reference population.

Associations of *TGFBRAP1* SNP rs2679895 With AgP in Samples of Different Geographical and Ethnical Background

We replicated the association of rs2679895 with AgP in 427 German AgP cases of a less severe phenotype (Table 1; Table III in the Data Supplement). Because the KORA control sample was part of the GerMIF-I and II studies and the popgen controls were part of the 500K Array GWAS on AgP, we used an independent sample of population representative German controls from the Metabolic Intervention Cohort Kiel (n=1292) and additional 222 periodontitis-free controls. In the replication, rs2679895 showed similar association

Figure 2. $-\log_{10} P$ values of the genotyped single-nucleotide polymorphisms (SNPs) of *TGFBRAP1* aligned to the chromosomal map. The top diagram shows the nominal $-\log^{10} P$ of the 26 SNPs, which were genotyped at this chromosomal region in the German aggressive periodontitis (AgP) GWAS sample and the Wellcome Trust Case-Control Consortium (WTCCC) coronary artery disease (CAD) GWAS sample (Affymetrix 500K). The P values are aligned to the genomic SNP position (National Center for Biotechnology Information build 36). The panel below shows the chromosomal position of *TGFBRAP1*. SNPs that were genotyped in the AgP sample are shown in blue, SNPs from the WTCCC CAD sample are shown in red. SNP rs2679895 is depicted by the green circle. Dashed horizontal red line, Nominal significance threshold of P=0.05. Three SNPs passed this threshold in both samples: rs2576742 (in complete linkage disequilibrium with the AIDS (Acquired Immune Deficiency Syndrome)-associated SNP rs1020064, see Figure I in the Data Supplement), rs2679895, and rs2033727 (depicted below in **bold** letters). SNP-IDs from **left** to **right**: rs4851743, rs17636399, rs17687727, rs4851752, rs2460255, rs17030722, rs3816133, rs2576742, rs17688608, rs3792048, rs2679895, rs17689220, rs1985599, rs873738, rs893249, rs17695158, rs17638586, rs2033727, rs2576776, rs880427, rs6543286, rs10193943, rs6543288, rs10190186, rs6543289, and rs6753046.

with AgP as was observed in the first German AgP sample (Table 5 and see Table 6 for allele frequencies and genotypes). Because the Metabolic Intervention Cohort Kiel control sample was selected for diabetes mellitus–free men, adjustment for potential sex effects would have introduced a bias. Therefore, we adjusted for smoking only. The association remained significant (Table 4). To increase the SP, we pooled all German AgP cases and controls and adjusted for smoking. After adjustment of this largest sample, the allelic model showed an association with P=0.0016; OR, 1.27 (95% CI; 1.09–1.47; Table 4).

We tested the association of rs2679895 with the smaller Dutch AgP sample (Table III in the Data Supplement). In this sample, SNP rs2679895 was significant (Table 5), but with a reversed OR compared with the discovery sample, also after adjustment for smoking and sex (Table 4).

Finally, we tested the association of SNP rs2679895 in a Turkish AgP sample (91 cases, 75 healthy controls; Table 4). Here, the association was significant, also with a reversed OR compared with the German case–control samples (Table 4).

Shared Risk Loci of CAD and Periodontitis Are Regulated by TGF-β

TGFBRAP1 associates with the TGFBR complex[28] and was reported to act as an inhibitor of TGF-β signaling.[29] *PLG* was reported to activate TGF-β signalling[30,31] and *FURIN* was also shown to be regulated by TGF-β.[32] Recently, we showed that *ANRIL* regulates the distant genes *ADIPOR1*, *VAMP3*, and *C11ORF10* and an earlier study demonstrated that the expression of *ADIPOR1* was negatively regulated by TGF-β.[33] This indicated that genes that are related to the disease pathogenesis of both CAD and AgP may act together in the same biological pathway. We tested if, in addition to *PLG*, *TGFBRAP1*, *FURIN*, and *ADIPOR1* and also the expression of *ANRIL, VAMP3*, and *C11ORF10* were regulated by TGF-β signaling. After TGF-β stimulation of human gingival fibroblasts, the transcriptional isoform of *ANRIL* that was previously shown to be positively correlated with *ADIPOR1*,
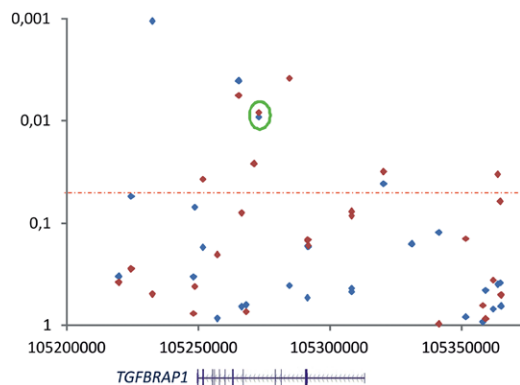
Table 5. Summary Statistics of *TGFBRAP1* Single-Nucleotide Polymorphism rs2679895 in 3 Aggressive Periodontitis Case–Control Samples

Germany/Austria		Dutch/Irish		Turkish	
P Value	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)
0.0099	1.26 (1.06–1.49)	0.0260	0.71 (0.52–0.96)	0.0450	0.59 (0.35–0.99)

CI indicates confidence interval; and OR, odds ratio.

Table 6. Allele Frequencies and Genotypes for *TGFBRAP1* Single-Nucleotide Polymorphism rs2679895 in the AgP and CAD Case–Control Samples

Disease	Population	Cases					Controls				
		11% (n)	12% (n)	22% (n)	MAF%	Sum	11% (n)	12% (n)	22% (n)	MAF%	Sum
AgP	Germany	50.0 (138)	42.4 (117)	7.6 (21)	28.8	276	59.5 (374)	35.1 (221)	5.4 (34)	23.0	629
	Germany, Austria (replication)	53.9 (230)	37.7 (161)	8.4 (36)	27.3	427	59.5 (901)	34.9 (529)	5.5 (84)	23.0	1.514
	NL, Ireland (validation1)	66.5 (135)	30.0 (61)	3.4 (7)	18.5	203	57.7 (205)	36.1 (128)	6.2 (22)	24.2	355
	Turkey (validation2)	68.1 (62)	27.5 (25)	4.4 (4)	18.1	91	53.3 (40)	38.7 (29)	8.0 (6)	27.3	75
CAD	GerMIF-I† (Affy 500K)	62.3 (604)	32.9 (319)	4.9 (47)	21.3	970	56.7 (909)	37.8 (606)	5.6 (89)	23.4	1604
	GerMIF-II† (Affy 500K)	62.8 (767)	32.4 (396)	4.8 (59)	21.0	1.222	59.6 (765)	34.0 (437)	6.4 (82)	24.4	1284
	WTCCC (Affy 500K)	64.4 (1.240)	31.5 (607)	4.1 (78)	19.8	1.925	60.5 (1.776)	34.9 (1.025)	4.6 (135)	22.1	2.936

AgP indicates aggressive periodontitis; CAD, coronary artery disease; GerMIF, German Myocard Infarct Family Study; MAF, minor allele frequency; and WTCCC, Wellcome Trust Case-Control Consortium.

VAMP3, and *C11OF10* expression[11] was significantly reduced (*P*=0.0209). *VAMP3* expression was also significantly reduced (*P*=0.0023), whereas the expression of *C11ORF10* did not significantly change before and after 48 hours of TGF-β stimulation (Figure 3).

Discussion

In the past, various risk alleles of *ANRIL* gave repeated evidence for association with CAD as well as with periodontitis.[9,10,18,34–37] In both parts of the current study, the candidate gene and joint GWAS analyses, we identified several variants within the coding region of *ANRIL* as risk factors shared between both diseases, showing the power of both approaches.

We identified the CAD GWAS lead SNP rs4252120, located within the coding region of *PLG*, to be associated with AgP, replicated this association, and demonstrated independence of the genetic effect from the confounding factors smoking and sex. In the context of this study, it is of interest that the PLG-plasmin system has an important function for degradation of tissue barriers and cell migration.[38] Various pathogenic bacteria were found to bind human PLG on bacterial PLG receptors,[39,40] which turns them into proteolytic organisms,[41] for
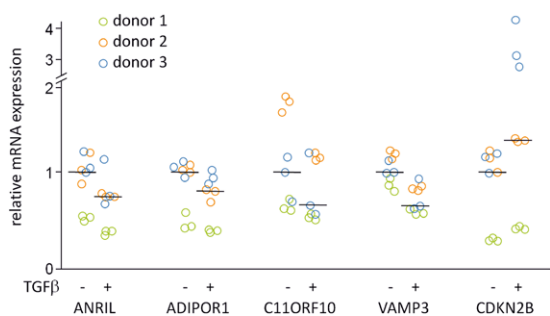

Figure 3. Relative expression of *ANRIL*, *ADIPOR1*, *C11ORF10*, *VAMP3*, and *CDKN2B* in response to transforming growth factor (TGF)-β stimulation. Human gingival fibroblasts from 3 different donors were stimulated with TGF-β for 48 hours (20 ng/mL). The figure shows the relative changes of transcript levels for each replicate of each donor (median, horizontal lines). After TGF-β stimulation, transcript levels were significantly reduced for *ANRIL* (*P*=0.02089), *ADIPOR1* (*P*=0.03649), and *VAMP3* (*P*<0.002344). The transcript levels of C11ORF10 and *CDKN2B* did not significantly change after TGF-β stimulation (C11ORF10: *P*=0.1623; CDKN2B: *P*=0.1068).

example, the common periodontal pathogen *Porphyromonas gingivalis* is able to activate human PLG expression and to inactivate human plasmin inhibitors, causing uncontrolled plasmin activity.[41] Along with other periodontal pathogens, *P. gingivalis* was also detected in thrombi at the sites of acute myocardial infarction.[42] We could not replicate the observed association of SNPs at *FURIN*. However, the associated rare allele of rs1981458 is a less frequent variant with MAF=10% and the Dutch replication panel lacked SP to reject or accept the null-hypothesis of no association. Yet, the function of FURIN makes it an interesting candidate for future research into putatively shared disease mechanisms, because FURIN, similar to PLG, is required for many pathogens to become fully pathogenic, as previously reviewed.[43]

In the second stage of this study, we found an association of SNP rs2679895 within *TGFBRAP1* in 3 independent CAD case–control samples and 4 independent AgP samples and demonstrated independence of the genetic effect from the confounding factors smoking and sex. In all CAD samples, the genetic effect of the association was in the same direction but in opposite direction to the 2 large German AgP samples. Similarly, the genetic effect of rs2679895 was in opposite direction in the Dutch–Irish AgP and Turkish AgP compared with the German AgP samples. Between the different control groups, the MAFs were similar, indicating that the observed effect was transmitted by the cases. We did not identify a putative causative disease associated variant at this genetic locus. It is possible that the opposite genetic effects observed in the German compared with the Dutch and Turkish AgP samples could be because of different linkage disequilibrium structures between these samples, which have different geographical and historical as well as ethnical background, respectively. Variants with both the same and opposite effects in the same pairs of different diseases have been reported before.[44] Replication studies in larger samples and finemapping of this genetic locus are required to fully elucidate the nature of the observed associations at *TGFBRAP1*. Our data suggest TGF-β signaling to have a role in the shared pathogenesis of CAD and periodontitis. This is in accordance with various studies that indicate that TGF-β signaling may represent a common site of intersection between the various downstream pathologies of obesity-induced complications[37] that comprise CAD and periodontitis. Interestingly, the identification of the adipokine

plasminogen activator inhibitor-1, an inhibitor of the PLG-plasmin system, to be strongly upregulated in visceral adipose depots in obesity also suggests a link of the PLG-plasmin system and obesity.

In conclusion, we give evidence that *PLG* is a shared genetic susceptibility factor of CAD and AgP. We further propose *FURIN* and *TGFBRAP1* as good candidates for future replication studies in larger samples. The current study emphasizes the role of TGF-β signaling in the shared pathogenesis of CAD and periodontitis.

Disclosures

None.

Appendix

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References

1. Buchwald S, Kocher T, Biffar R, Harb A, Holtfreter B, Meisel P. Tooth loss and periodontitis by socio-economic status and inflammation in a longitudinal population-based study. *J Clin Periodontol*. 2013;40:203–211. doi:10.1111/jcpe.12056.
2. Marcenes W, Kassebaum NJ, Bernabé E, Flaxman A, Naghavi M, Lopez A, et al. Global burden of oral conditions in 1990-2010: a systematic analysis. *J Dent Res*. 2013;92:592–597. doi:10.1177/0022034513490168.
3. Mealey BL, Oates TW; American Academy of Periodontology. Diabetes mellitus and periodontal diseases. *J Periodontol*. 2006;77:1289–1303. doi:10.1902/jop.2006.050459.
4. Sangwan A, Tewari S, Singh H, Sharma RK, Narula SC. Periodontal status and hyperlipidemia: statin users versus non-users. *J Periodontol*. 2013;84:3–12. doi:10.1902/jop.2012.110756.
5. Schenkein HA, Loos BG. Inflammatory mechanisms linking periodontal diseases to cardiovascular diseases. *J Periodontol*. 2013;84(4 Suppl):S51–S69. doi:10.1902/jop.2013.134006.
6. Pant S, Deshmukh A, Gurumurthy GS, Pothineni NV, Watts TE, Romeo F, et al. Inflammation and atherosclerosis–revisited. *J Cardiovasc Pharmacol Ther*. 2014;19:170–178. doi:10.1177/1074248413504994.
7. Dietrich T, Jimenez M, Krall Kaye EA, Vokonas PS, Garcia RI. Age-dependent associations between chronic periodontitis/edentulism and risk of coronary heart disease. *Circulation*. 2008;117:1668–1674. doi:10.1161/CIRCULATIONAHA.107.711507.
8. Seinost G, Wimmer G, Skerget M, Thaller E, Brodmann M, Gasser R, et al. Periodontal treatment improves endothelial dysfunction in patients with severe periodontitis. *Am Heart J*. 2005;149:1050–1054. doi:10.1016/j.ahj.2004.09.059.
9. Schaefer AS, Richter GM, Dommisch H, Reinartz M, Nothnagel M, Noack B, et al. CDKN2BAS is associated with periodontitis in different European populations and is activated by bacterial infection. *J Med Genet*. 2011;48:38–47. doi:10.1136/jmg.2010.078998.
10. Schaefer AS, Richter GM, Groessner-Schreiber B, Noack B, Nothnagel M, El Mokhtari NE, et al. Identification of a shared genetic susceptibility locus for coronary heart disease and periodontitis. *PLoS Genet*. 2009;5:e1000378. doi:10.1371/journal.pgen.1000378.
11. Bochenek G, Häsler R, El Mokhtari NE, König IR, Loos BG, Jepsen S, et al. The large non-coding RNA ANRIL, which is associated with atherosclerosis, periodontitis and several forms of cancer, regulates ADIPOR1, VAMP3 and C11ORF10. *Hum Mol Genet*. 2013;22:4516–4527. doi:10.1093/hmg/ddt299.
12. Schwenk RW, Angin Y, Steinbusch LK, Dirkx E, Hoebers N, Coumans WA, et al. Overexpression of vesicle-associated membrane protein (VAMP) 3, but not VAMP2, protects glucose transporter (GLUT) 4 protein translocation in an *in vitro* model of cardiac insulin resistance. *J Biol Chem*. 2012;287:37530–37539. doi:10.1074/jbc.M112.363630.
13. Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet*. 2013;45:25–33.
14. Cortes A, Brown MA. Promise and pitfalls of the Immunochip. *Arthritis Res Ther*. 2011;13:101. doi:10.1186/ar3204.
15. Schaefer AS, Richter GM, Nothnagel M, Manke T, Dommisch H, Jacobs G, et al. A genome-wide association study identifies GLT6D1 as a susceptibility locus for periodontitis. *Hum Mol Genet*. 2010;19:553–562. doi:10.1093/hmg/ddp508.
16. Schaefer AS, Bochenek G, Manke T, Nothnagel M, Graetz C, Thien A, et al. Validation of reported genetic risk factors for periodontitis in a large-scale replication study. *J Clin Periodontol*. 2013;40:563–572. doi:10.1111/jcpe.12092.

17. Erdmann J, Grosshennig A, Braund PS, König IR, Hengstenberg C, Hall AS, et al.; Italian Atherosclerosis, Thrombosis, and Vascular Biology Working Group; Myocardial Infarction Genetics Consortium; Wellcome Trust Case Control Consortium; Cardiogenics Consortium. New susceptibility locus for coronary artery disease on chromosome 3q22.3. *Nat Genet*. 2009;41:280–282. doi:10.1038/ng.307.

18. WTCCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*. 2007;447:661–678.

19. Browning BL, Browning SR. A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. *Am J Hum Genet*. 2009;84:210–223. doi:10.1016/j.ajhg.2009.01.005.

20. Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, et al. Integrating common and rare genetic variation in diverse human populations. *Nature*. 2010;467:52–58.

21. Ellinghaus D, Ellinghaus E, Nair RP, Stuart PE, Esko T, Metspalu A, et al. Combined analysis of genome-wide association studies for Crohn disease and psoriasis identifies seven shared susceptibility loci. *Am J Hum Genet*. 2012;90:636–647. doi:10.1016/j.ajhg.2012.02.020.

22. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559–575. doi:10.1086/519795.

23. Dupont WD, Plummer WD Jr. Power and sample size calculations for studies involving linear regression. *Control Clin Trials*. 1998;19:589–601.

24. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005;21:263–265. doi:10.1093/bioinformatics/bth457.

25. The-R-Development-Core-Team. http://www.r-project.org. 2008. Accessed 2012.

26. Ernst FD, Uhr K, Teumer A, Fanghänel J, Schulz S, Noack B, et al. Replication of the association of chromosomal region 9p21.3 with generalized aggressive periodontitis (gAgP) using an independent case-control cohort. *BMC Med Genet*. 2010;11:119. doi:10.1186/1471-2350-11-119.

27. Le Clerc S, Limou S, Coulonges C, Carpentier W, Dina C, Taing L, et al; ANRS Genomic Group. Genomewide association study of a rapid progression cohort identifies new susceptibility alleles for AIDS (ANRS Genomewide Association Study 03). *J Infect Dis*. 2009;200:1194–1201. doi:10.1086/605892.

28. Wurthner JU, Frank DB, Felici A, Green HM, Cao Z, Schneider MD, et al. Transforming growth factor-beta receptor-associated protein 1 is a Smad4 chaperone. *J Biol Chem*. 2001;276:19495–19502. doi:10.1074/jbc.M006473200.

29. Charng MJ, Zhang D, Kinnunen P, Schneider MD. A novel protein distinguishes between quiescent and activated forms of the type I transforming growth factor beta receptor. *J Biol Chem*. 1998;273:9365–9368.

30. Wileman SM, Booth NA, Moore N, Redmill B, Forrester JV, Knott RM. Regulation of plasminogen activation by TGF-beta in cultured human retinal endothelial cells. *Br J Ophthalmol*. 2000;84:417–422.

31. Yee JA, Yan L, Dominguez JC, Allan EH, Martin TJ. Plasminogen-dependent activation of latent transforming growth factor beta (TGF beta) by growing cultures of osteoblast-like cells. *J Cell Physiol*. 1993;157:528–534. doi:10.1002/jcp.1041570312.

32. Blanchette F, Day R, Dong W, Laprise MH, Dubois CM. TGFbeta1 regulates gene expression of its own converting enzyme furin. *J Clin Invest*. 1997;99:1974–1983. doi:10.1172/JCI119365.

33. Otani K, Kitayama J, Kamei T, Soma D, Miyato H, Yamauchi T, et al. Adiponectin receptors are downregulated in human gastric cancer. *J Gastroenterol*. 2010;45:918–927. doi:10.1007/s00535-010-0228-2.

34. Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, Jonasdottir A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science*. 2007;316:1491–1493. doi:10.1126/science.1142842.

35. McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, et al. A common allele on chromosome 9 associated with coronary heart disease. *Science*. 2007;316:1488–1491. doi:10.1126/science.1142447.

36. Schunkert H, Götz A, Braund P, McGinnis R, Tregouet DA, Mangino M, et al; Cardiogenics Consortium. Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease. *Circulation*. 2008;117:1675–1684. doi:10.1161/CIRCULATIONAHA.107.730614.

37. Schunkert H, König IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, et al; CARDIoGRAM Consortium. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat Genet*. 2011;43:333–338. doi:10.1038/ng.784.

38. Lähteenmäki K, Edelman S, Korhonen TK. Bacterial metastasis: the host plasminogen system in bacterial invasion. *Trends Microbiol*. 2005;13:79–85. doi:10.1016/j.tim.2004.12.003.

39. Boyle MD, Lottenberg R. Plasminogen activation by invasive human pathogens. *Thromb Haemost*. 1997;77:1–10.

40. Lähteenmäki K, Kuusela P, Korhonen TK. Bacterial plasminogen activators and receptors. *FEMS Microbiol Rev*. 2001;25:531–552.

41. Grenier D. Degradation of host protease inhibitors and activation of plasminogen by proteolytic enzymes from Porphyromonas gingivalis and Treponema denticola. *Microbiology*. 1996;142 (Pt 4):955–961.

42. Ohki T, Itabashi Y, Kohno T, Yoshizawa A, Nishikubo S, Watanabe S, et al. Detection of periodontal bacteria in thrombi of patients with acute myocardial infarction by polymerase chain reaction. *Am Heart J*. 2012;163:164–167. doi:10.1016/j.ahj.2011.10.012.

43. Thomas G. Furin at the cutting edge: from protein traffic to embryogenesis and disease. *Nat Rev Mol Cell Biol*. 2002;3:753–766. doi:10.1038/nrm934.

44. Wang K, Baldassano R, Zhang H, Qu HQ, Imielinski M, Kugathasan S, et al. Comparative genetic analysis of inflammatory bowel disease and type 1 diabetes implicates multiple loci with opposite effects. *Hum Mol Genet*. 2010;19:2059–2067. doi:10.1093/hmg/ddq078.

CLINICAL PERSPECTIVE

Periodontitis is an inflammatory disease of the oral cavity caused by bacteria forming a biofilm on the gingiva. For the severe forms, periodontitis affects human populations worldwide at prevalence rates of 11%. Strong evidence of associations between the presence of periodontitis and coronary artery disease (CAD) was derived from multiple randomized clinical trials. Shared risk alleles of periodontitis and CAD within the 2 genes *ANRIL* and *CAMTA1/VAMP3* had been described. To better understand the conjoint disease mechanisms, this study aimed to systematically identify further shared genetic risk variants. In-depth genotyping of all major CAD risk loci of genome-wide significance and combined analyses of several genome-wide data sets of CAD and of the severe phenotype aggressive periodontitis highlighted *ANRIL* as the major shared risk gene of both diseases and established the gene *PLASMINOGEN* (*PLG*) as a newly discovered shared susceptibility gene (rs4252120, P=5.9×10^{-5}; odds ratio, 1.27; 95% confidence interval, 1.3–1.4). The PLG-plasmin system has an important function for degradation of tissue barriers and in previous studies various oral pathogenic bacteria were found to bind human PLG, which turns them into proteolytic organisms. These pathogens had been detected in thrombi at the sites of acute myocardial infarction and are discussed to directly or indirectly induce inflammatory responses with possible impacts on the pathogenesis of CAD. The study further provides evidence that all currently shared genetic risk loci of CAD and periodontitis are subjected to transforming growth factor-β regulation. These findings underpin future hypothesis-driven research into the inflammatory disease mechanisms of CAD.