Aus dem Institut für Zahn-, Mund- und Kieferheilkunde,
Abteilung für Parodontologie und Synoptische Zahnmedizin
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Plasminogen is a Genetic Risk Factor of Periodontitis

zur Erlangung des akademischen Grades
Doctor medicinae dentariae (Dr. med. dent.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

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Zusammenfassung

Beider Parodontitis handelt es sich um eine Entzündung des Parodontium, welche durchbakterielle Infektionen hervorgerufen wird. Diese Infektionen können zurZerstörung des Zahnfleischgewebes, zur alveolaren Knochenresorption und schließlich zum Verlust der Zähne führen. Plasmin, das durch das Gen Plasminogen (*PLG*) codiert wird, hat eine wichtige Aufgabe in der Fibrinolyse und der Spaltung weiterer Plasmaproteine, sowie in der Aktivierung von Kollagenasen und Mediatoren des Komplementsystems. Es spieltdadurch eine entscheidende Rolle inder Wundheilung und hat eine entsprechend großeBedeutungfür den Erhalt eines gesunden Parodontium.

In einer vorangegangenen Arbeit wurde gezeigt, dass der Herzinfarkt-assoziierte Einzelnukleotid Polymorphismus (SNP) rs4252120 mit der Aggressiven Parodontitis (AgP) assoziiert ist. Es war unbekannt, ob diese oder andere Varianten auch eine Bedeutung für die Chronische Parodontitis (CP) haben. In der vorliegenden Arbeit sollte die Assoziation von rs4252120 mit CP in in 1.419 Fällen und 4.562 Kontrollen getestet werden. Zusätzlich wurde *PLG* mit imputierten Genotypen weiterer Fall-Kontrollstudien der AgP (896cases, 4.562 controls; HumanOmniBeadChips, GRCh37/hg19) und der CP (1.961cases, 1.864 controls; Genome-Wide Human SNP Array 6.0, NCBI36/hg18) feinkartiert, um weitere krankheitsassoziierte Varianten zu identifizieren. Potentielle Assoziationen wurden für das allelische genetische Model getestet und für die Covariaten Rauchen und Geschlecht adjustiert.

Die Assoziation von rs4252120 mit chronischer Parodontitis konnte nicht bestätigt werden($p = 0,05$). Allerdings wurde3‘ von *PLG*ein Haplotyp-Block als mitchronischer und aggressiver Parodontitis assoziiertgefunden (rs1247559; gepoolter p-Wert=$6.6x10^{-6}$), der jedoch nicht mit dem Herzinfarkt assoziiert ist.Daten zur Korrelation von SNP-Allelen und Expressionsdaten (eQTLs; GRASP)weisen darauf hin, dass der parodontitis-assoziierte Bereich 3‘ von *PLG* eine Osteoblasten-spezifische cis-regulatorische Aktivität auf die Expression von *PLG* hat (P=6.910-05, rs783176 [r2 zu rs1247559=0,87).

In dieser Arbeit konnte gezeigt werden, dass genetische Varianten 3‘ von *PLG*mit Chronischer und Aggressiver Parodontitis assoziiert sind und diese Varianten möglicherweise einen regulatorischen Effekt auf die Transkription von *PLG* in Osteoblasten haben. Diese Ergebnisse leisten einen wichtigen Beitrag für ein verbessertes Verständnis der Ätiologie der Parodontitis.

Abstract

Periodontitis involves inflammation of the periodontium, bacterial infection, degradation of gum tissue, and alveolar bone resorption, which eventually leads to teeth loss.Plasmin, which is encoded by the gene plasminogen (*PLG*) has a critical role in wound healing through fibrinolysis, the degradation of further plasma proteins, and the activation of collagenases and of mediators of the complement system.Therefore, *PLG* is essential for the maintenance of a healthy periodontium.The intronic*PLG*variant rs4252120was previously shown to be associated with aggressive periodontitis (AgP) and coronary artery disease (CAD) but it had not been tested for association with chronic periodontitis (CP). Furthermore, *PLG*had not comprehensively been analyzed for additional variants associated with AgP and CP. In this study, we tested rs4252120 for a potential association with CP in 1,419 casesand 4,562 controls. Additionally, to identify further disease associated variants,I mapped the chromosomal region at*PLG* with imputed genotypes of further case-control samples of AgP (896 cases, 4,562 controls; HumanOmniBeadChips, GRCh37/hg19) and of CP (1.961 cases, 1.864 controls; Genome-Wide Human SNP Array 6.0, NCBI36/hg18). Putative associations were adjusted for the covariates smoking and sex and tested using the allelic genetic model.

SNP rs4252120 was not associated with CP (p=0.05), showing a minor allele frequency of 27.9 % in the cases and 29.6 % in the controls.However, another haplotype block3' downstreamto*PLG*and tagged by rs1247559,was associated with both CP and AgP (rs1247559; pooled $p=6.6x10^{-6}$, odds ratio=0.81) but not with CAD.Expression quantitative trait locus data (GRASP) indicated that the associated haplotype block has a tissue specific regulatory role on the expression of *PLG* in osteoblasts (P=6.910-05, rs783176 [$r^2$with rs1247559=0.87).

These results suggest that genetic variations 3' downstream to*PLG*are associated with periodontitis and have a potential osteoblast specific cis-regulatory effect on the expression of *PLG*. The results of this study contribute to an improved understanding of the etiology of periodontitis.

Affidavit

I, Hong Chen, certify under penalty of perjury by my own signature that I have submitted the thesis on the topic **'Plasminogen is a Genetic Risk Factor of Periodontitis'**. I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The section on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) corresponds to the URM (s.o) and are answered by me. My contribution in the selected publication for this dissertation corresponds to those that are specified in the following joint declaration with the responsible person and supervisor.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

_____Hong Chen_____

Date Signature

Detailed Declaration of Contribution

Hong Chen had the following share in the following publication:

She planned the experiments. She performed the analysis and interpretation of the genotypes of the investigated single nucleotide polymorphisms. She additionally collected and interpreted the eQTL data. She wrote the manuscript.

Matthias Munz[1,2*], Hong Chen[1,3*], Yvonne Jockel-Schneider[4], Knut Adam[5], Per Hoffman[6,7], Klaus Berger[8], Thomas Kocher[9], Jörg Meyle[10], Peter Eickholz[11], Christof Doerfer[12], Matthias Laudes[13], André Uitterlinden[14], Wolfgang Lieb[15], Andre Franke[16], Stefan Schreiber[16], Steven Offenbacher[17], Kimon Divaris[18,19], Corinna Bruckmann[20], Bruno G. Loos[21], Soeren Jepsen[22], Henrik Dommisch[1], Arne S. Schaefer[1]; **A haplotype block downstream of plasminogen is associated with chronic and aggressive periodontitis**; Jounal of Clinical Periodontology; 2017

Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

__Hong Chen_____

Statement about Hong Chen's contribution to the paper

Publication:

A haplotype block downstream of plasminogen is associated with chronic and aggressive periodontitis

Matthias Munz*, Hong Chen*, Yvonne Jockel-Schneider, Knut Adam, Per Hoffman, Klaus Berger, Thomas Kocher, Jörg Meyle, Peter Eickholz, Christof Doerfer, Matthias Laudes, André Uitterlinden, Wolfgang Lieb, Stefan Schreiber, Steven Offenbacher, Kimon Divaris, Corinna Bruckmann, Bruno G. Loos, Soeren Jepsen, Henrik Dommisch, Arne S. Schaefer; Jounal of Clinical Periodontology; 2017

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We, the candidate and the candidate's Principal Supervisor, certify that the contents of the paper specified above is my original investigation and achievement and has not been submitted for any academic degree elsewhere.

Hong Chen, planned the experiments, generated the genotypes of the investigated single nucleotide polymorphisms, collected and interpreted the eQTL data, conducted the data analysis and statistical evaluations, and wrote and amended the manuscript in the review process.

Signature, date and stamp of the supervising university lecturer / supervisor

University lecturer

_____

Signature of the doctoral candidate

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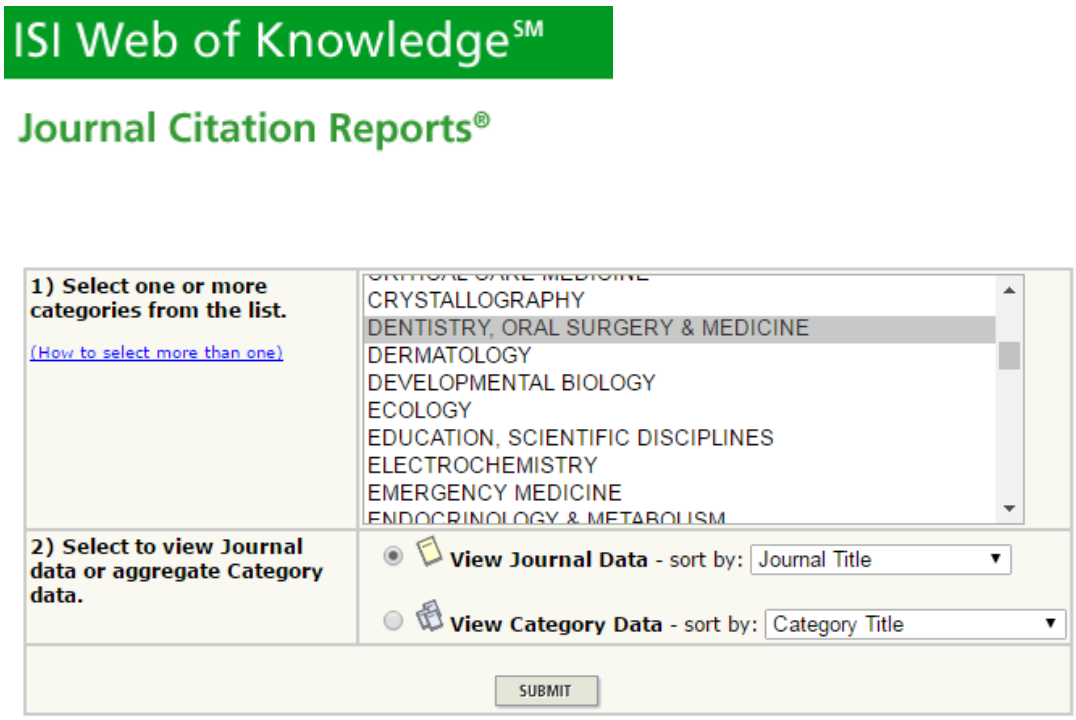
Journal Summary List (ISI Web of KnowledgeSM)

Technical journals of the category: Dentistry, Oral Surgery & Medicine

Jounal of Clinical Periodontology: Place 6 of 91

Impact factor:3.915

Eigenfactor:0.01397



Journal Summary List

Journal Title Changes

Journals from: subject categories DENTISTRY, ORAL SURGERY & MEDICINE VIEW CATEGORY SUMMARY LIST

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Mark	Rank	Abbreviated Journal Title (linked to journal information)	ISSN	JCR Data						*Eigenfactor*® Metrics	
				Total Cites	Impact Factor	5-Year Impact Factor	Immediacy Index	Articles	Cited Half-life	*Eigenfactor*® Score	*Article Influence*® Score
☐	1	PERIODONTOL 2000	0906-6713	3376	4.949	5.108	1.311	45	9.7	0.00441	1.503
☐	2	J DENT RES	0022-0345	16401	4.602	5.024	0.837	233	9.9	0.02345	1.541
☐	3	ORAL ONCOL	1368-8375	7291	4.286	3.752	0.969	159	6.2	0.01490	1.131
☐	4	CLIN IMPLANT DENT R	1523-0899	3161	4.152	4.500	0.452	199	4.6	0.00771	1.301
☐	5	DENT MATER	0109-5641	10324	3.931	4.667	0.674	187	7.2	0.01364	1.039
☑	6	J CLIN PERIODONTOL	0303-6979	11767	3.915	4.435	0.658	155	9.5	0.01397	1.242
☐	7	CLIN ORAL IMPLAN RES	0905-7161	11968	3.464	4.522	0.619	236	7.2	0.01695	1.120
☐	8	J DENT	0300-5712	6199	3.109	3.254	0.543	184	6.7	0.01129	0.897
☐	9	MOL ORAL MICROBIOL	2041-1006	577	3.061	3.092	1.028	36	3.3	0.00261	0.992
☐	10	J ENDODONT	0099-2399	12519	2.904	3.357	0.394	335	6.7	0.01609	0.659
☐	11	J PERIODONTOL	0022-3492	14268	2.844	3.113	0.727	161	>10.0	0.01328	0.854
☐	12	INT ENDOD J	0143-2885	5253	2.842	2.794	0.928	139	7.7	0.00871	0.849
☐	13	J OROFAC PAIN	1064-6655	1280	2.824	2.678		0	9.9	0.00194	0.897
☐	14	OPER DENT	0361-7734	2593	2.819	2.207	0.537	95	7.9	0.00346	0.577
☐	15	INT J ORAL SCI	1674-2818	678	2.595	3.203	0.406	32	3.8	0.00252	0.941
☐	16	J PERIODONTAL RES	0022-3484	3662	2.474	2.394	0.622	98	9.1	0.00494	0.664
☐	17	J ORAL FACIAL PAIN H	2333-0384	102	2.444	2.444	0.359	39	1.4	0.00040	0.740
☐	18	EUR J ORAL IMPLANTOL	1756-2406	648	2.328	3.736	0.214	28	4.4	0.00242	1.143
☐	19	CARIES RES	0008-6568	3746	2.278	3.174	0.397	78	>10.0	0.00465	0.895
☐	20	COMMUNITY DENT ORAL	0301-5661	3686	2.233	2.357	0.311	61	>10.0	0.00407	0.768

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Article type : Epidemiology (Cohort study or case-control study)

A haplotype block downstream of plasminogen is associated with chronic and aggressive periodontitis

Running Title: Plasminogen and periodontitis

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COMPETING INTERESTS STATEMENT

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

ABSTRACT

Aim: The intronic variant rs4252120 in the plasminogen gene (*PLG*) is known to be associated with aggressive periodontitis (AgP) and atherosclerosis. Here, we examined the chromosomal region spanning *PLG* for associations with both chronic periodontitis (CP) and AgP.

Material and Methods: The association of *PLG* candidate rs4252120 was tested in a German case-control sample of 1,419 CP cases using the genotyping assay hCV11225947 and 4,562 controls, genotyped with HumanOmni BeadChips. The German and Dutch sample of AgP cases (N=851) and controls (N=6,836) were genotyped with HumanOmni BeadChips. The North-American CP sample (N=2,681 cases, 1,823 controls) was previously genotyped on the Genome-Wide Human SNP Array 6.0. Genotypes were imputed (software Impute v2) and association tests were performed using an additive genetic model adjusting for sex and smoking.

Results: Rs4252120 was not associated with CP. However, a haplotype block downstream of *PLG* and not in linkage disequilibrium with rs4252120 (r^2=0.08) was associated with both AgP (rs1247559; P=0.002, odds ratio [OR]=1.33) and CP (P=0.02, OR=1.15). That locus was also significantly associated with *PLG* expression in osteoblasts ($p=6.9x10^{-5}$).

Conclusions: Our findings support a role of genetic variants in *PLG* in the etiology of periodontitis.

CLINICAL RELEVANCE:

Scientific rationale for the study: Plasminogen is essential for the maintenance of a healthy periodontium because of its role in wound healing. The *PLG* variant rs4252120 is strongly associated with atherosclerosis and aggressive periodontitis. To date, *PLG* has not been tested for association with chronic periodontitis.

Principal findings: In two independent case-control samples, we showed that rs4252120 is not associated with chronic periodontitis. Instead, variants downstream of *PLG* showed

associations with chronic and with aggressive periodontitis, with reported effects on *PLG* expression.

Practical implications: Our findings indicate a general role of genetic variants downstream of *PLG*, which modulate the susceptibility of periodontitis.

Introduction

Plasmin is a blood circulating protease involved in protein degradation of fibrin with a central role in wound healing through fibrinolysis of blood clots and extracellular matrix (ECM) degradation. In addition, it facilitates macrophage migration (Ploplis et al., 1998), stimulates platelet degranulation and monocyte chemotaxis (Syrovets and Simmet, 2004), and modifies low density lipids (LDL) particles to increase their complement-activating capacity and uptake by macrophages (Torzewski et al., 2004). The interplay between hemostatic processes, such as coagulation and fibrinolysis, and the inflammatory response are essential components of host defense and bacterial invasion (Bhattacharya et al., 2012). Plasmin is formed at sites of inflammation by proteolysis of its inactive precursor, plasminogen (PLG), which circulates in blood plasma and interstitial fluids. Earlier, an association of the single nucleotide polymorphism (SNP) rs4252120 was identified for coronary artery disease (CAD) at a genome-wide significance level (4.88×10^{-10}) (Deloukas et al., 2013). In a large case-control study on the early-onset phenotype aggressive periodontitis (AgP) we found this variant associated with AgP ($P=5.9\times10^{-5}$) (Schaefer et al., 2015). This SNP is located in an intron of the coding region of the gene *PLG*.

Strong evidence of associations between the presence of CAD and chronic periodontitis (CP) is derived from multiple longitudinal epidemiological studies (Dietrich et al., 2008). However, at the time being, a causative relationship between CAD and CP has not been supported by clear experimental evidence (Lockhart et al., 2012). Given the high incidences of CAD and CP, the identification of shared genetic risk factors has clinical importance, because it will help to improve the understanding of the molecular mechanisms that underlie both diseases. Currently, although several genome-wide association studies (GWAS) on CP have been published in recent years, no studies reported the association of genetic variants at *PLG* with CP. Single GWAS or meta-analyses of several GWAS have limitations to detect risk variants with low allele frequencies or moderate effect sizes. This is because by exploring many SNPs there is a high risk of finding false significant associations according to conventional criteria of $P<0.05$. In fact, when 500,000 SNPs were analyzed, one would

expect to find over 25,000 disease associated with a P-value 0<0.05 simply by chance. To avoid this error, a correction for multiple comparisons is employed, so that in GWAS, associations are considered significant with a $P<5x10^{-8}$. This approach reduces false positives, but also markedly decreases the power to detect SNPs associated with disease (Riancho, 2012). Very likely periodontitis is a polygenic disease, with hundreds of risk variants each of small effects and most of them presumably cannot directly be identified by GWAS that comprise few thousands of cases (Manolio et al., 2009). Association studies of well-prioritized candidate genes can provide an alternative, as confined genetic regions require a smaller number of tests and thus are more efficient. However, the power of candidate-gene studies largely relies on the selection of the right candidate gene as well as the appropriate selection of the right SNP out of thousands of known variants within a specific chromosomal region. Different sub-phenotypes of a complex polygenetic disease such as periodontitis can be regarded as parts of a range of similar conditions that are attributed to the effects of different genetic risk loci, which form the individual genetic constitution (Gibson, 2011). In this view, different manifestation such as AgP and CP can share genetic risk loci. To test *PLG* for associations with CP, at first, we selected SNP rs4252120 and performed an association study with in 1,419 German CP cases and 4,562 German controls. In a second step, we analyzed the *PLG* locus for additional CP- and AgP associated variants, using available genotype data from a sample of white North-American CP cases and controls, and of AgP cases and controls of North-West European descent. To reduce the number of multiple parallel tests, we applied a two-step design. In the hypothesis-generating step, we searched the chromosomal region of the entire *PLG* locus, including the neighboring genes *LPA* and *MAP3K4* to identify candidate SNPs. The SNP with the strongest association in the region was selected and its association was validated in our AgP sample.

Here, we provide evidence that a confined haplotype block upstream *PLG*, which has a reported cis-effect on gene-expression, is associated with periodontitis.

Material and Methods

Northwest-European AgP sample

The AgP cases (324 males, 527 females) were recruited across Germany and The Netherlands as described before (Schaefer et al., 2015). In brief, inclusion criteria for the AgP cases were ≥2 teeth with 30% alveolar bone loss documented by x-ray radiographs before the age of 35.

The German controls consisted of three independent population representative samples from North-Germany (FOCUS [Food Chain Plus]) (Muller et al., 2015) and from West-Germany (Dortmund Health Study [DHS] (Berger, 2012) and Heinz Nixdorf Recall Studie [HNR1-3]) (Schmermund et al., 2002). In total, the controls comprised 4,562 individuals (2,226 males, 2,304 females, 32 unknown sex). The Dutch AgP controls (N= 2,274, 50% males) consisted of the The B-PROOF (B-Vitamins for the PRevention Of Osteoporotic Fractures) study and were described before (van Wijngaarden et al., 2011).

Northwest-European CP sample

The German CP cases were earlier described in detail (Schaefer et al., 2011). In brief, 1,419 German CP patients (611 males, 782 females, 26 unknown sex) were recruited across Germany at the University hospitals in Kiel, Bonn, Dresden, and Munich. All patients were older than 40 years at the time of diagnosis. Probing pocket depth, attachment level and furcation involvement were measured and bleeding upon probing was registered. The dimension of bone loss was assessed by means of periapical and/or panoramic radiographs. The percentage of bone loss was measured at each tooth based on the length of the roots. Patients presenting probing depths ≥5 mm with more than 30% bone loss at least at three teeth were included in the study.

The German AgP controls were used to match the German CP cases in the association test of candidate SNP rs4252120.

North-American CP sample

This case-control sample comprised non-diabetic white American individuals with moderate CP (N=1,920) and severe CP (N=761), as diagnosed in terms of Centers of Disease Control (CDC) and American Academy of Periodontology (AAP) consensus three-level classifications (Page and Eke, 2007). For controls, 1,823 healthy individuals were selected among non-diabetic participants of the Atherosclerosis Risk in Communities (ARIC) study as previously described in detail (Divaris et al., 2013).

Written informed consent was obtained from all participants of this study. The institutional ethics review boards and data protection authorities of the respective centers and institutions, approved the recruitment and experimental protocols.

Genotyping

The German CP cases were genotyped for candidate SNP rs4252120 using the TaqMan SNP Genotyping assay hCV11225947 (Thermo Fisher Scientific, Waltham, MA, USA) with a

TaqMan Genotyping System (Applied Biosystems, Foster City, CA, USA). Genotypes were generated by automatic calling using the Genemapper 4.0 software as recently described (Schaefer et al., 2011).

The North-West European controls and AgP cases were genotyped on an IScan system with HumanOmni BeadChips (Illumina, San Diego, CA, USA). SNPs, which were not directly genotyped by the subtype HumanOmniExpressBead Chips, were imputed using 1000G Phase 3 SNPs of Northern Europeans from the HapMap CEPH reference populations (Utah residents with ancestry from northern and western Europe) and the software Impute v2 (Howie et al., 2009). After imputation, the control studies were merged using the genetic analysis software Gtools (http://www.stats.ox.ac.uk/~marchini/software/gwas/gwas.html).

Statistical Tests

SNPs were tested for deviation from Hardy-Weinberg Equilibrium (HWE) in controls and cases as previously described (Wigginton et al., 2005). The significance of the association of rs4252120 was tested for an additive genetic model. Association tests for other SNPs were performed for the AgP case-control sample with SNPTEST v2.5.2 (Marchini et al., 2007) assuming an additive genetic model with sex and a binary variable smoking status (never smoked = 1, ever smoked = 0) as covariates.

The statistical power that was provided by the given sample size of this study was calculated using the Power and Sample Size Program (Dupont and Plummer, 1998) with the following settings: Minor allele frequency (MAF) in controls = 25%, the odds ratio for disease in allele carriers relative to non-carriers = 1.3, and a Type I error probability associated with this test of this null hypothesis = 0.05. Association tests for the North-American sample were described before (Divaris et al., 2013).

eQTL Analysis

To identify putative effects of the disease-associated SNPs we searched the data bases haploreg (v4.1) (Ward and Kellis, 2012) and GTEx (v.6) (Consortium, 2015) for expression quantitative trait loci (eQTL).

Linkage Disequilibrium (LD) Analysis

LD between SNPs was analysed using the 1000GENOMES:phase_3 sub-population CEU (Utah Residents [centre d'etude du polymorphisme humain; CEPH] with Northern and Western Ancestry) as provided by The Ensembl Project (www.ensembl.org) and the

LocusZoom software (Pruim et al., 2010). SNPs that are located on the associated haplotype block were identified by haploreg and are listed in the **Supplementary Table 1**.

RESULTS

Rs4252120 is not associated with CP

PLG SNP rs4252120 was genotyped in 1,419 CP cases and 4,562 controls. We considered an additive model for the rare marker allele. In these tests, SNP rs4252120 showed nominal significant association with CP with P=0.047 and for the T-allele an odds ratio (OR) = 1.13 (95% confidence interval [95% CI] = 1.002-1.27), showing a minor allele frequency of 27.9 % in the cases and 29.6 % in the controls (**Table 1, Table 2**). The statistical power of this finding was 97.5%. Next, we validated our results using association data of white non-diabetic North-American CP cases (N = 1,920 cases) and healthy controls (N = 1,823), which were imputed to the genome build NCBI36/hg18 (Divaris et al., 2013). In this sample, rs4252120 was not associated with CP, with p = 0.47. After adding an additional sample of non-diabetic North-American cases of severe CP (N = 762), the association remained not significant before and after covariate adjustment (P = 0.80; Table 2).

A haplotype block downstream PLG is associated with CP and AgP

To further investigate if other genetic variants at *PLG* were associated with CP, we used the association data of 1,920 non-diabetic CP cases and 1,823 healthy controls from the recent GWAS of CP (Divaris et al., 2013), to analyze the chromosomal region of the entire *PLG* locus, including the neighboring genes *LPA* and *MAP3K4* (**Figure 1**). We identified several SNPs, which were associated with $P < 0.05$ downstream of the coding region of *PLG* and not in LD with rs4252120 ($r^2 = 0.08$). Rs1247559 showed the most significant association with P = 0.025 and a genetic effect of the C-allele with OR = 1.17 (95% CI = 1.02-1.34). After adding the additional 762 non-diabetic severe CP cases, the association slightly became more significant with P = 0.022 prior to adjustment. The association did not change after covariate adjustment (P = 0.02, OR = 1.15 (95% CI = 1.02 – 1.30; **Table 3**).

Next, we analyzed the same chromosomal region with GWAS data of our case-control sample of AgP (N = 851 cases, N = 6,836 controls), which was imputed to the genome build GRCh37/hg19. In this data set, the AgP and CAD associated candidate rs4252120, intronic of *PLG*, showed an association with AgP after adjustment for the covariates smoking and sex with P=0.0038 and a genetic risk effect for the C allele of OR=1.18 (95% CI = 1.05-1.32). However, the CP associated region downstream of *PLG* showed stronger association, with

haplotype tagging SNP rs1247559 showing an association of p = 0.0003 and a genetic effect of the C-allele with OR = 1.35 (95% CI = 1.15-1.59) prior to adjustment for sex and smoking, and of P = 0.002, OR = 1.33 (95% CI = 1.14-1.59) after covariate adjustment (**Table 3**). We next inquired if rs1247559 was significantly associated with CAD in the CARDIoGRAMplusC4D Consortium meta-analysis sample (Deloukas et al., 2013). This SNP showed no association with P = 0.09 (**Figure 1 c,** personal communication).

DISCUSSION

In the current study, we did not find an association of the AgP risk variant rs4252120 with CP. However, we identified a haplotype block downstream of *PLG*, which showed shared association with CP and AgP. The statistical power was >0.9 for our samples, making false negative findings unlikely. Of note, this haplotype block is not in LD with rs4252120 (r^2 = 0.08) and showed a protective effect for AgP and CP associated with the rare alleles. Furthermore, rs1247559 was not significantly associated with CAD. This was in contrast to rs4252120, for which the rare C-allele showed a risk-increasing effect for AgP, indicating independence of the effects of both SNPs. Data on the effects of specific alleles that contribute to variation in gene expression levels of mRNAs (eQTLs) as available from Haploreg and GTEx indicated effects of rs4252120 on the expression of the gene *DDR1* (Discoidin Domain Receptor Tyrosine Kinase 1; located at chr6:2,143,681-2,160,922; human genome build GRCh37/hg19) in peripheral blood monocytes with p=3.35 x10^{-6} (Zeller et al., 2010). Phenotypes that have been associated with DDR1 are negative regulation of vascular associated smooth muscle cell migration, collagen binding and regulation of matrix metalloproteinase-2 (MMP-2) and MMP-9 activities, but also wound healing (Hou et al., 2002). These phenotypes may indicate mechanistic pathways linking the association of rs4252120 with AgP and CAD to DDR1. However, genome-wide significant disease associations of variants within the DDR1 gene were not reported for CAD and periodontitis, but for psoriasis (Feng et al., 2009), age-related macular degeneration (Fritsche et al., 2013), breast neoplasm (Hunter et al., 2007), systemic lupus erythematosus (Hom et al., 2008), type 1 diabetes (Tomer et al., 2015), and HIV control (Fellay et al., 2009). Computational analysis of the location and score of transcription factor binding sites at rs4252120 (TFBS; computed with the Transfac Matrix Database v7.0, created by the public database Biobase) indicated a TFBS of the transcription factor *CUX1*, ending two nucleotides upstream of rs4252120. This

TF is reported, amongst other genes, to regulate Matrix metalloproteinase 10 (*MMP10*), Interleukin1-alpha (*IL1A*), and Cyclooxygenase 2 (*COX2*) (Wilson et al., 2009).

No expression QTL data were available for the CP- and AgP- associated haploblock tagging SNP rs1247559, but for rs783176, which is in strong linkage disequilibrium to rs1247559 (r^2=0.9) and located within large intron 17 of *PLG*. For this SNP, effects on gene expression of *PLG* were reported for osteoblasts ($p = 6.9 \times 10^{-5}$), which were treated with Prostaglandin E_2 (PGE2) (Grundberg et al., 2011). Crosstalk between plasminogen activation and PGE_2 has been experimentally demonstrated (Bauman et al., 2010). The different eQTL effects further point to independent effects of the genomic regions tagged by rs1247559 and rs4252120, which could indicate the involvement of different pathophysiological mechanisms that could result in different manifestations of the phenotype.

AgP is a severe and rapidly progressing form of periodontal disease, commonly diagnosed in otherwise systemically healthy adolescents and young adults. Clinically, patients with AgP show more progressive attachment loss and bone destruction compared to CP patients, and more severe bleeding. *DDR1* knockdown was shown to slow epithelial repair and to be associated with a reduction in levels of the matrix metalloproteinase MMP-7 (Roberts et al., 2011), which is among other functions, involved in wound healing (Letra et al., 2013). In the context of the association of rs4252120 with CAD, it is interesting to note that DDR1 also promotes smooth muscle cell migration, and thereby contributes to arterial wound healing (Hou et al., 2002). The eQTL data that were available for SNP rs783176, which tags the AgP and CP associated haplotype block, indicated a cis-effect on *PLG* expression. Results from studies with *PLG* deficient mice showed that plasminogen is essential for the maintenance of a healthy periodontium and plays an important role in combating the spontaneous development of chronic periodontitis (Sulniute et al., 2011).

In addition to a role in wound healing, PLG has been shown to bind to bacterial surface-expressed elongation factor Tuf, where it can be proteolytically activated. It was proposed (Kunert et al., 2007) that Tuf acts as a virulence factor by acquiring host proteins to the pathogen surface, controlling complement, and possibly facilitating tissue invasion (OMIM [Online Mendelian Inheritance in Man] entry 173350).

The periodontitis-associated haplotype block downstream of *PLG* is not associated with CAD. However, as shown in Figure 1, other SNPs downstream PLG, which span the periodontitis-associated region, are associated with CAD at a genome-wide significance level (Deloukas et al., 2013). This could indicate different disease mechanisms for periodontitis and CAD, which each mediate specific upstream signals to PLG transcriptional regulation.

Most of the AgP and CP cases and controls of the current study have previously been reported in other case-control studies. Those studies independently identified several chromosomal regions, which were associated with both phenotypes. These loci were the large noncoding RNA ANRIL (Bochenek et al., 2013, Schaefer et al., 2011, Schaefer et al., 2009) [ANRIL is also nominally significant associated with CP in the US-American CP sample, (Divaris et al., 2013) supplementary information], the chromosomal region at *NPY* (Divaris et al., 2013, Freitag-Wolf et al., 2014), and *CAMTA1/VAMP3* (Bochenek et al., 2013, Divaris et al., 2012), and very recently, an association downstream of the genes *PF4/PPBP/CXCL5* (Shusterman et al., 2017).

We also noticed that the level of association of rs4252120 with AgP was less in the current study compared to our previous study. Although we used the same AgP cases, the control sample of the current study was much larger and the individuals were recruited from different geographical regions. Estimates of the genetic effect based on new association findings commonly tend to be upwardly biased due to a phenomenon known as the ‘‘winner’s curse’’ (Lohmueller et al., 2003). It is possible that the reduced genetic effect of the current study is closer to the real genetic effect.

In conclusion, we demonstrated that the AgP associated *PLG* risk SNP rs4252120 is not associated with late-onset and slowly progressing forms of periodontitis (i.e. CP). Interestingly, rs4252120 showed a proposed effect on the expression of the distant gene *DDR1* that, like *PLG*, plays a role in wound healing. We identified a protective haplotype block downstream of the coding region of *PLG*, which showed association with both AgP and CP, but not with CAD. This association was independent of rs4252120 and it is reported to have cis-effects on the expression of *PLG*. Our findings add to the general role of *PLG* in the etiology of CP and AgP.

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Tables

Table 1. Allele frequencies and genotypes of rs4252120 and rs1247559 in the CP and AgP samples

SNP common/rare allele	**phenotype**	**sample**	**MAF (%)**	**F 11% (N)**	**F 12 % (N)**	**F22 % (N)**	**total**
rs4252120 (T / C)	**CP (German)**	**cases**	27.9	53.1 (753)	38.1 (541)	8.8 (125)	1,419
		ctrls.	29.6	50.0 (2,283)	40.8 (1,862)	9.1 (417)	4,562
	CP (US-American)	**cases (mod.)**	28.88	51.1 (981)	40.1 (769)	8.9 (170)	1,920
		cases (sev.)	28.84	51.1 (389)	40.1 (305)	8.8 (67)	761
		ctrls.	29.4	50.0 (910)	41.4 (754)	8.7 (159)	1,823
	AgP (German/ Dutch)	**cases**	33.3	44.5 (379)	44.2 (376)	11.3 (96)	851
		ctrls.	30.1	49.3 (3,368)	41.3 (2,826)	9.4 (642)	6836
rs1247559 (C / T)	**CP (US-American)**	**cases (mod.)**	16.4	70.0 (1,344)	27.2 (522)	2.8 (54)	1,920
		cases (sev.)	17.2	69.4 (528)	26.8 (204)	3.8 (29)	761
		ctrls.	18.4	66.6 (1.214)	30.1 (548)	3.4 (61)	1.823
	AgP (German/ Dutch)	**Cases**	13.8	74.6 (635)	23 (196)	2.4 (20)	851

		controls	17.1	68.6 (4,687)	28.7 (1,961)	2.8 (188)	6,836

1 = common allele, 2 = rare allele, MAF = minor (rare) allele frequency

Table 2. Allele frequencies and genotypes of rs4252120 (effect allele T/rare allele C) in the German and US-American CP samples

	crude (allelic)		**adjusted**	
sample	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**
CP (GER)	0.047	1.13 (1.002-1.27)		
CP mod. (US)	0.47	1.05 (0.92-1.19)		
CP sev. (US)	0.58	1.05 (0.89-1.24)		
CP pooled (US)	0.17	1.08 (0.97-1.22)	0.80	0.99 (0.89-1.10)
AgP (GER+NL)	0.0092	1.21 (105-1.40)	0.0038	1.18 (1.05-1.32).

Table 3. Association statistics of the CP and AgP associated haplotype tagging SNP rs1247559 (effect allele C/rare allele T) downstream *PLG*

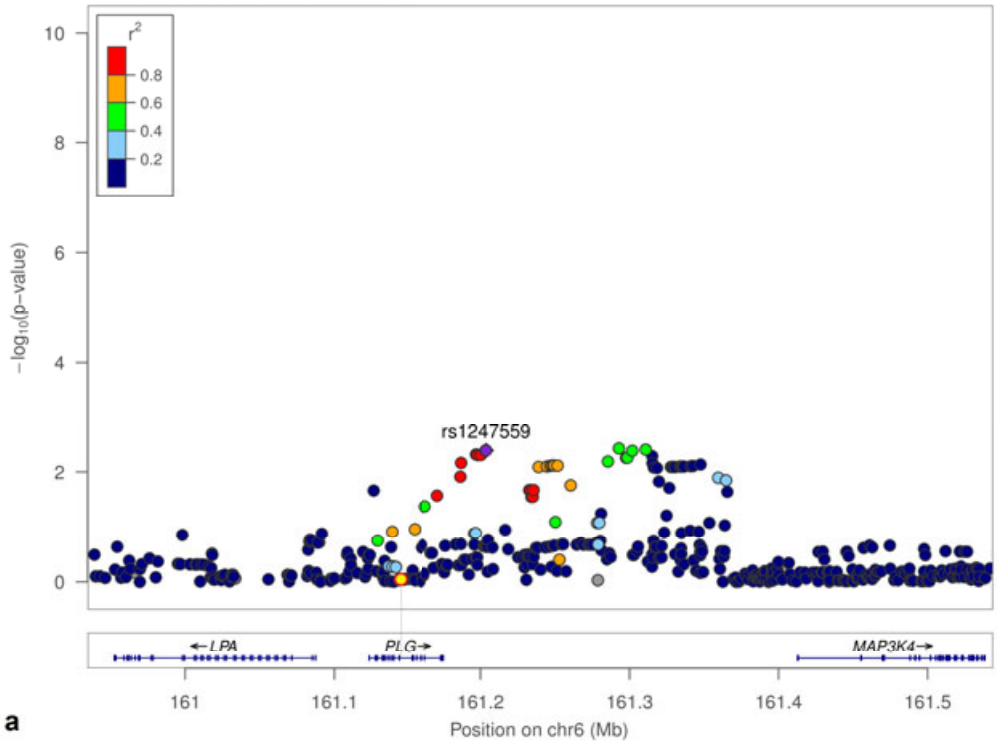
	crude (allelic)		**adjusted**	
sample	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**
CP mod. (US)	0.025	1.17 (1.02-1.34)		
CP sev. (US)	0.17	1.14 (0.95-1.36)		
CP pooled (US)	0.022	1.16 (1.021-1.32)	0.02 †	1.15 (1.02-1.30)
AgP (GER+NL)	0.0003	1.35 (1.15-1.59)	0.002	1.33 (1.14-1.59)

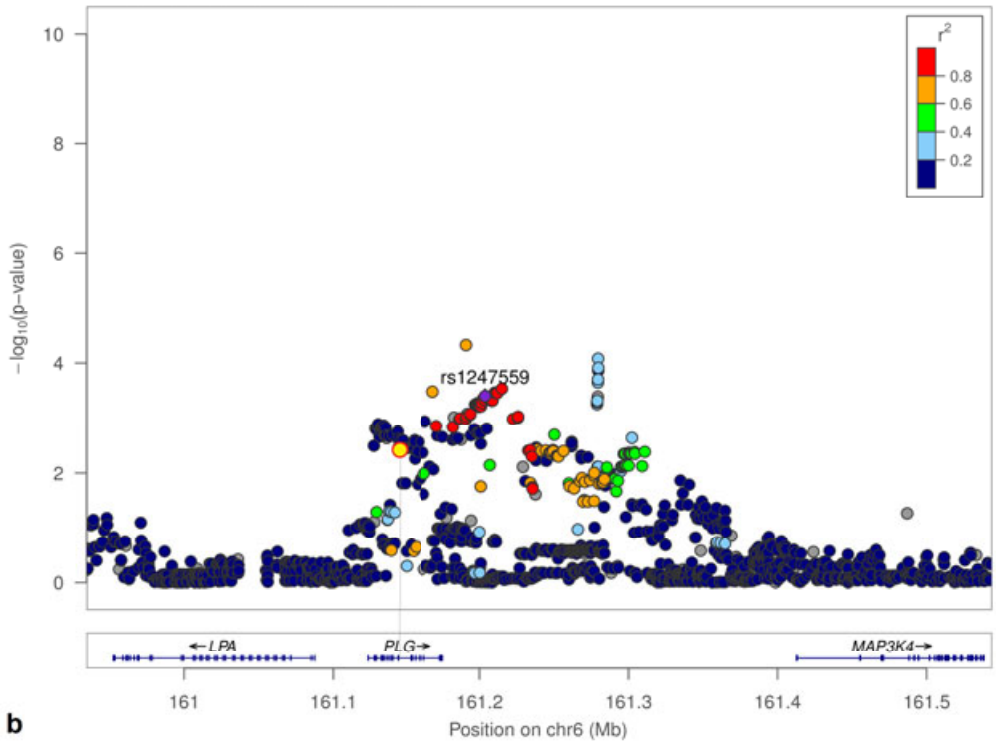
† Results for Severe Perio, Moderate Perio and 'Any Perio' vs. Health analyses, among non-diabetic participants of ARIC.

Figure Legends

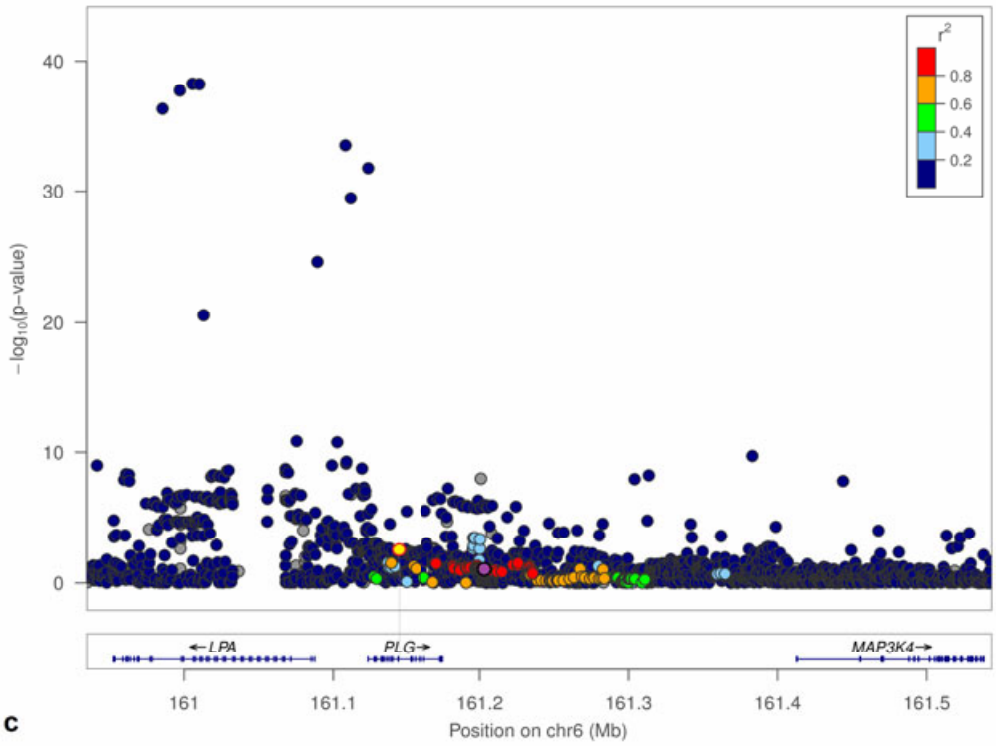
Figure 1. Association plots of imputed genotypes at the *PLG* locus for AgP, CP and CAD

Regional association plots of imputed genotypes for **(a)** moderate CP (1,920 cases, 1,823 controls), **(b)** AgP (851 cases, 6,836 controls), and **(c)** CAD (60,801 cases, 123,504 controls); data from the CARDIoGRAMplusC4D 1000 Genomes-based GWAS, a meta-analysis of GWAS studies of individuals from European, South Asian, and East Asian descent; published in (Nikpay et al., 2015). The -log10 p-values of the analyzed SNPs are plotted as a function of the genomic SNP position (NCBI build 37, hg19/1000 Genomes 2012, CEU). SNP annotation provided by Locuszoom databases (Purple diamond (Index SNP) = rs1247559, red filling indicates strong linkage to the index SNP, yellow circle= rs4252120).





b



c

Curriculum Vitae

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

Curriculum Vitae

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Complete list of publications

1 **A haplotype block downstream of plasminogen is associated with chronic and aggressive periodontitis.** Matthias Munz*, Hong Chen*, Yvonne Jockel-Schneider, Knut Adam, Per Hoffman, Klaus Berger, Thomas Kocher, Jörg Meyle, Peter Eickholz, Christof Doerfer, Matthias Laudes, André Uitterlinden, Wolfgang Lieb, Andre Franke, Stefan Schreiber, Steven Offenbacher, Kimon Divaris, Corinna Bruckmann, Bruno G. Loos, Soeren Jepsen, Henrik Dommisch, Arne S. Schaefer; Journal of Clinical Periodontology; May 26, 2017. doi: 10.1111/jcpe.12749. [Epub ahead of print]

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