Received: 12 May 2020 | Revised: 18 January 2021 | Accepted: 9 March 2021

DOI: 10.1111/jcpe.13460

ORIGINAL ARTICLE CLINICAL PERIODONTOLOGY

Journal of Clinical Periodontology WILEY

Genome-wide association study of stage III/IV grade C periodontitis (former aggressive periodontitis) in a Spanish population

Alicia de Coo[1] | Raquel Cruz[1,2] | Inés Quintela[1,3] | David Herrera[4,5] | Mariano Sanz[4] | Pedro Diz[6] | Segundo Rodríguez Grandío[5] | Nuria Vallcorba[5] | Isabel Ramos[5] | Alfonso Oteo[5] | Cristina Serrano[5] | Alejandro Esmatges[5] | Francisco Enrile[5] | Leopoldo Mateos[5] | Roberto García[5] | Pablo Álvarez-Novoa[5] | Blas Noguerol[5] | Ion Zabalegui[5] | José Blanco-Moreno[5] | Ángel Alonso[5] | Ramón Lorenzo[5] | Angel Carracedo[1,2,3,7] | Juan Blanco[6]

[1]Grupo de Medicina Xenómica, Centro Singular de Investigación en Medicina Molecular y Enfermedades Crónicas (CIMUS), Universidade de Santiago de Compostela, Santiago de Compostela, Spain

[2]CIBERER-Instituto de Salud Carlos III, Centro Singular de Investigación en Medicina Molecular y Enfermedades Crónicas (CIMUS), Universidade de Santiago de Compostela, Santiago de Compostela, Spain

[3]Centro Nacional de Genotipado - Plataforma de Recursos Biomoleculares - Instituto de Salud Carlos III (CeGen-PRB3-ISCIII), Universidade de Santiago de Compostela, Santiago de Compostela, Spain

[4]ETEP (Etiology and Therapy of Periodontal and Peri-implant Diseases) Research Group, University Complutense of Madrid, Madrid, Spain

[5]SEPA Network of Research Clinics (Red de Clínicas de Investigación de la Sociedad Española de Periodoncia y Osteointegración, SEPA), Madrid, Spain

[6]Grupo de Investigación en Odontología Médico-Quirúrgica (OMEQUI), Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Universidade de Santiago de Compostela, Santiago de Compostela, Spain

[7]Fundación Pública Galega de Medicina Xenómica– SERGAS, Santiago de Compostela, Spain

Correspondence
Alicia de Coo, Avenida de Barcelona, s/n, Centro Singular de Investigación en Medicina Molecular y Enfermedades Crónicas (CIMUS), planta 2, 15782, Santiago de Compostela, España.
Email: alicia.diz@usc.es

Funding information
The genotyping service was carried out at CeGen-PRB3-ISCIII; it is supported by grant PT13/0001, ISCIII-SGEFI / FEDER. In addition, PerioGEN study was supported in part by Manohay dental and by the Spanish Ministry of Education, Culture and Sport by FPU grant (FPU15/05974). PerioGEN study was also supported by the Spanish Society of Periodontology (*Sociedad Española de Periodoncia*, SEPA) and by the Spanish Foundation of Periodontology and Dental Implants (*Fundación Española de Periodoncia e Implantes Dentales*) which

Abstract

Aim: To identify loci associated with stages III/IV, grade C periodontitis (PIII/IV-C) through a genome-wide association study (GWAS).

Materials and Methods: 441 Caucasian Spanish PIII/IV-C cases from the SEPA Network of Research Clinics and 1141 controls from the *Banco Nacional de ADN* were genotyped with "Axiom Spain Biobank Array," which contains 757836 markers, including rare and low-frequency Spanish variants. The analysis of the individual association and subsequently the gene-level analysis with Sequence Kernel Association Test (SKAT) were carried out adjusting for age, sex and PC1 covariates. Pathway Analysis was additionally performed with Ingenuity Pathway Analysis (IPA) software on the top associated genes.

Results: In the individual analyses, no genome-wide significant signals were detected. However, 8 SNPs of 8 loci reached suggestive evidence of association with PIII/IV-C, including *FAT3* rs35709256, *CSNK1G2* rs4807188, *MYH13* rs2074872, *CNTN2* rs116611488, *ANTXR1* rs4854545, 8p23.2 rs78672540, *ANGPT1* rs13439823 and

Carracedo and Blanco contributed equally to this study.

organized and coordinated the SEPA Network of Research Clinics, where patients were identified, and samples were taken

PLEC rs11993287 ($p < 5 \times 10^{-6}$). SKAT analysis identified other interesting signals at *CNTN2*, *FBXO44*, *AP1M2*, *RSPO4*, *KRI1*, *BPIFB1* and *INMT*, although their probability does not exceed the multiple-test correction. IPA indicated significant enrichment of pathways related to cAMP, IL-2, CD28, VDR/RXR and PI3K/Akt.

Conclusions: GWAS found no SNPs significantly associated with PIII/IV-C.

KEYWORDS

epidemiology, genome-wide association study, periodontitis, rare variation, single-nucleotide polymorphisms

Clinical Relevance

Scientific rationale for study: Although the influence of genetic risk factors seems to be high in grade C periodontitis (rapid progression), the genetic basis of stages III/IV, grade C periodontitis (PIII/IV-C), remains unknown.

Principal findings: We did not find any genome-wide significantly associated SNPs. However, five pathways were identified, and, despite the low sample size, the previously reported *SIGLEC5* gene was re-discovered.

Practical implications: The failure of identifying SNPs with genome-wide significance association with PIII/IV-C indicates rather challenge of studying the genetic basis of periodontal disease, so collaboration between groups will be required to achieve very large sample sizes.

1 | INTRODUCTION

Periodontitis (PD) is a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus that eventually may result in tooth loss (Papapanou et al., 2018). In populations worldwide, severe periodontitis is reported to affect 11% (Kassebaum et al., 2014), while the estimated pooled prevalence for aggressive periodontitis (AgP) is 1.6%, compared to 0.1% of the European population (Bouziane et al., 2020). It is also influenced by genetic and environmental factors (Abusleme et al., 2013).

Currently, numerous candidate gene studies have been developed and several genes associated with risk or protection to the development of periodontitis were identified (Nibali et al., 2017). These studies were replaced for the Genome-Wide Association Studies (GWAS), which allowed testing thousands of polymorphisms across the genome. To date, more than a dozen GWAS have been published in relation to periodontitis, but few were carried out in relation to AgP (Schaefer et al., 2010; Divaris et al., 2012, 2013; Teumer et al., 2013; Feng et al., 2014; Freitag-Wolf et al., 2014; Rhodin et al., 2014; Shaffer et al., 2014; Hong et al., 2015; Schaefer et al., 2015; Shimizu et al., 2015; Offenbacher et al., 2016; Munz, Willenborg, et al., 2017; Sanders et al., 2017; Bevilacqua et al., 2018). Despite all association studies performed, few identified genes are currently considered true genetic factors (Schaefer, 2018). This is probably due to the small sample size of the studies, uncertain criteria for case selection used and lack of homogenous ethnic background of the studied populations. Moreover, association studies have been focussed on the analysis of common variants and did not take into account rare variants in the study design. It is known that common variants do not completely explain all the variability associated with genetic factors, hence the possible importance of the, poorly studied, rare variants in genetics of complex traits, such as periodontitis (Bomba et al., 2017). Rare variants, alone or in combination with common variants, have small to modest effect on phenotypic variation; thus, their analysis can enhance our understanding of genetic regulation and development of complex diseases (Auer & Lettre, 2015). Nowadays, only one GWAS has studied the rare variation in relation to periodontitis (Sanders et al., 2017). Sanders *et al.* identified one genome-wide significant association signal associated with interproximal clinical attachment level (iCAL) in a Hispanic/Latino population of 10,935 participants.

Therefore, in order to identify genetic risk factors that predispose to periodontitis, we conducted the first GWAS in patients with stages III/IV, grade C periodontitis (formerly known as aggressive periodontitis) (Papapanou et al., 2018; Tonetti et al., 2018), in a Spanish population, analysing common and rare variants.

2 | MATERIALS AND METHODS

2.1 | Study population

The PerioGEN study is a multicentre, population-based, case–control study of Spanish individuals, performed at the SEPA (Spanish Society of Periodontology) Research Network of Dental Clinics, a network of university and private dental clinics involved in clinical research projects under the supervision of highly trained specialists.

2.1.1 | Cases

Cases were patients with a severe phenotype, namely stages III/IV, grade C periodontitis (PIII/IV-C), that met the following inclusion criteria: age between 14 and 35 years at the time of the initial diagnosis, Spanish ancestry, CAL loss >4 mm in at least 2 non-adjacent teeth (see *Phenotype measurement and definition* section). Exclusion criteria were as follows: isolated CAL loss or having a systemic disorder that has a major impact on the loss of periodontal tissues.

2.1.2 | Controls

The control sample population were randomly selected from the Spanish National DNA BioBank. All of them were healthy unrelated individuals evenly distributed through the different geographic areas in Spain.

PerioGEN investigators enrolled 1,644 Spanish individuals older than 18 years. The case population was composed of 465 subjects (160 males and 305 females) and the control group was composed of 1179 subjects (582 males and 597 females) (Table 1). All participants in the study were Caucasians.

2.1.3 | Statistical power calculation

The statistical power was calculated with PS Power and Sample Size Program (Dupont & Plummer, 1998). Considering our actual sample size (465 cases and 1,179 controls), a significance threshold of $p < 5 \times 10^{-8}$ (conventional genome-wide threshold) and an allele frequency of 0.3 we would have >82% power to detect as significant an odds ratio (OR) of 0.55 (protector allele) or 1.68 (risk allele). For SNPs showing a lower minor allele frequency (MAF) in controls (0.1), the detectable OR would be 0.31 (protector) or 2.05 (risk).

As far as the gene-set analysis is concerned—which will be performed in about 20,000 genes—we estimate that our sample size will allow us to achieve an 81% of power to detect as significant ($p < 2.6 \times 10^{-6}$) a small effect size ($d = 0.3$) in the comparison of genetic score between cases and controls.

2.2 | Phenotype measurement and definition

Dental PerioGEN clinical examiners recorded information of visual dichotomous plaque index, probing depth, gingival recession, and bleeding on probing (BOP), evaluated at 6 sites per tooth, and number of missing teeth, for all patients with PIII/IV-C. The examiners defined AgP phenotype, which nowadays corresponds to stage III/IV, grade C periodontitis based on criteria of the current classification of periodontal diseases (Papapanou et al., 2018; Tonetti et al., 2018). Furthermore, cases were classified according to CAL loss and extent and distribution in two categories: localized stage III/IV, grade C periodontitis (<30% of teeth involved) or molar/incisor pattern and generalized stage III/IV, grade C periodontitis (>30% of teeth involved).

TABLE 1 Characteristics of the PerioGEN population

	Cases (PIII/IV-C)	Controls
Individuals	465	1179
Gender		
Female (*n*)	305	597
Female (%)	65.59	50.64
Male (*n*)	160	582
Male (%)	34.41	49.36
Unknown gender, *n* (%)	0 (0)	0 (0)
Age		
Mean age at sample intake	40.75[a]	46.32
Unknown age, *n* (%)	14 (3.01)	15 (1.27)
Smoking habit		
Current smoker, *n* (%)	160 (34.41)	n.a.
Non-smoker, *n* (%)	294 (63.22)	n.a.
Former smoker, *n* (%)	6 (1.29)	n.a.
Unknown smoking habit, *n* (%)	5 (1.08)	1179 (100)
Phenotype		
Localized PIII/IV-C, *n* (%)	40 (8.60)	
Generalized PIII/IV-C, *n* (%)	340 (73.12)	
Unknown PIII/IV-C phenotype, *n* (%)	85 (17.28)	

Note: PIII/IV-C diagnosis was established by clinicians following criteria of the "World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions 2018" (Papapanou et al., 2018; Tonetti et al., 2018), and smoking habit was estimated by self-reporting.
PIII/IV-C: stages III/IV, grade C periodontitis; n.a.: not available.
[a]Although the age of patients was >40 on average, patients had originally been diagnosed earlier (patients were between 14 and 35 years at the time of initial diagnosis).

2.3 | DNA isolation and genotyping

The collection of case samples (saliva) was done with the ORAcollect®DNA kit (DNA Genotek Inc.), and DNA was extracted from spit samples according to standard protocols (DNA Genotek, 2018). Control samples (blood) were provided by the Banco Nacional de ADN-Instituto de Salud Carlos III (BNADN-ISCIII; Universidad de Salamanca, Spain, www.bancoadn.org). Samples from cases and controls were randomly placed on the same plates and genotyped using the Axiom Spain Biobank Array (Thermo Fisher Scientific), a panel specifically designed for Spanish population that contains 757836 markers and allows to explore the rare functional variation, because they include rare variants selected in the Spanish population (50,536 markers). Genotyping was performed at the Centro Nacional de Genotipado-Universidade de Santiago de Compostela (CeGen-USC), following the protocol established by Thermo Fisher

(Axiom™ 2.0 Assay Manual Workflow). Genotyping was done blinded to case–control status, and one sample of a European male with known genotype was included in each plate as positive control. GWAS genotype data were automatically called by AxiomTM Genotyping Algorithm version 1 (Axiom GT1) available through Axiom™ Analysis Suite v 3.0.1.4.

Several markers with evidence of association at individual analysis and markers with importance at gene-based analysis were validated in a subset of 416 individuals of PerioGEN study using SEQUENOM MassARRAY matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry platform.

2.4 | Quality control and filtering

Genetic markers with calling rates <98% and markers with genotypes not following the Hardy–Weinberg equilibrium (FDR, False Discovery Rate <0.2) were discarded. No MAF threshold was applied because rare variant will be included in gene-based analysis. Samples with unsuccessful genotyping <98% were also excluded. Additionally, familiar relationship was evaluated by calculating pairwise identity-by-descent (IBD) and existence of stratification was explored by principal component analysis (PCA), after linkage disequilibrium pruning of markers. Individuals with familiar relationship (proportion of IBD ≥0.125) and those identified as outliers in the PCA (those showing a PC value deviated more than 5 standard deviations from the mean) were identified and excluded. Quality control (QC) analyses were conducted with PLINK v1.90 (Purcell et al., 2007) and GenABEL R package (Aulchenko et al., 2007).

2.5 | Statistical analysis

We assessed the significance of associations between genotypes and PIII/IV-C using logistic regression analysis, under different genetic models in the statistical software environment R v2.8. (RcoreTeam, 2008). All analyses were adjusted for the covariates gender and age to avoid possible cofounding in the logistic regression model. The first principal component (PC1) of the PCA previously performed (above) was also retained and included as covariate because cases and controls were significantly different for this PC. The genetic models considered were (a) log-additive model, with genotypes coded as 0, 1 and 2 in function of the number of minor alleles, (b) dominant model, in which both heterozygous and rare allele homozygous were combined and compared with the genotype homozygous for the most frequent allele, and (c) recessive model, in which rare allele homozygous is compared with the rest of genotypes. The dependent variable was the case/control status, coded as 1 or 0, respectively. The logistic regression analysis was individually performed for each SNP; rare variants were not individually analysed because of lack of power. A conventional $p < 5 \times 10^{-8}$ genome-wide could be used as significance threshold (Manolio, 2010). However, as we tested several genetic models and for taking into account the correlation between them, we used the correction of significance level proposed in González et al. (2008) and we considered as effective number of tests the number of markers * 2.2. p-values $<5 \times 10^{-6}$ were considered suggestive evidence of association.

Since the assignment of an erroneous genotypes has the potential to introduce artefacts of false positive associations (Clayton et al., 2005), we carried out a visual inspection of the clusterplots (graphic representation of the genotypes intensities for each SNP) for all markers showing a suggestive association signal ($p < 5 \times 10^{-6}$) and those belonging to the top associated genes.

2.6 | Sequence kernel association test

To examine the combined effect of rare and common variants, we used sequence kernel association test (SKAT) (Ionita-Laza et al., 2013). We carried out SKAT with the default "beta" weights (upweighting the rarer variants) combining common and rare variants and only for rare variants (SKAT and SKAT rares), and SKAT with the same weight for all variants (SKAT w1) and a simpler collapsing method, burden test (BURDEN). All SKAT analyses alternatives were also adjusted for covariates gender, age and PC1. Correction for multiple testing was performed with a conservative Bonferroni threshold that guaranteed an experiment-wise significance level of $\alpha = 0.05$. Additionally, we ranked genes according to uncorrected p-value and selected a set of "focus genes" with p-values <0.01, for subsequent pathway analysis.

2.7 | Ingenuity pathway analysis (IPA)

The pathways analyses were generated through the use of IPA (QIAGEN Inc., IPA) identifying gene sets that are enriched for focus genes. Canonical signalling pathways are based on IPA curated databases (Krämer et al., 2014). We performed a Core Analysis to obtain a list of enriched pathways ranked by p-value and percentage of overlapping genes mapped against the total number of those in each pathway. Fisher's exact test was utilized to calculate a p-value across the process of IPA analysis.

2.8 | Association with PD susceptibility variants

2.8.1 | Genotype imputation

To check the existence of genetic association for those variants previously found associated at genome-wide significant level with periodontitis (Schaefer et al., 2010; Munz, Willenborg, et al., 2017; Bevilacqua et al., 2018), which were not included in the Spain Biobank array, we carried out genotype imputation. Also we performed the imputation of variants associated at significant level $p < 10^{-6}$ with PD in the recent meta-analysis of Munz et al., (2018). Imputation was conducted for each sample using SHAPEIT (Delaneau et al., 2012) for the phasing step and IMPUTE2 (Howie et al., 2009) for the imputation using 1000 Genomes Phase 3 b37

reference set. We performed a post-imputation QC filtering and only variants which passed the imputation quality (INFO) cut-off of >0.8 were maintained. SNPs with alleles A/T or C/G were excluded to avoid strand ambiguity.

2.8.2 | Polygenic risk score (PRS)

To test the contribution of a polygenic effect to PD risk, we performed predictive modelling using polygenic score based upon SNPs of the recent meta-analysis of Munz et al., (2018) as predictor variable. The individual polygenic risk scores (PRS) were generated by PLINK v1.90 as sum of the risk alleles weighted by effect sizes as in the meta-analysis. The models were fitted using the above mentioned individual PRS and predicting PIII/IV-C /control status in our study. Significance of the PRS was evaluated by logistic regression, using case–control status as dependent variable and sex, age and PC1 as covariates.

3 | RESULTS

3.1 | GWAS for PIII/IV-C

We conducted a GWAS in 465 Spanish cases affected with PIII/IV-C and 1,179 Spanish controls. During the previous QC axiom analysis, a total of 19 samples were removed (8 cases and 11 controls) and 43 samples were removed too on subsequent QC analysis (15 cases and 27 controls). After QC and visual inspection of 6045 Affymetrix assigned genotypes (those showing a suggestive association signal or belonging to the top associated genes), 645,639 genetic markers were maintained. In addition, a subset of 416 individuals of PerioGEN study (237 cases and 179 controls) were re-genotyped with SEQUENOM MassARRAY for 9 loci (*BPIFB1* rs1884885 and rs1999663, *CHTF18* rs201005922, *CNTN2* rs116611488, *CTSO* rs10020154, *FBXO44* rs2294641, *MYH13* rs2074872 and rs4791401 and *TBCK* rs35363222) to validate obtained results. These loci are those that presented doubtful clusterplots and evidence of association at individual analysis or importance at SKAT analysis.

Individual association analysis was performed in 579,301 markers (those showing MAF >1%) for 441 cases and 1,141 controls. Table S1 shows a summary of demographic characteristics for the studied population. No SNP reached genome-wide significance in GWAS analysis ($p > 3.9 \times 10^{-08}$; Bonferroni threshold for 573301*2.2 number effective of tests), after visual inspection of the clusterplots and re-genotyping validation (Figure 1). Nonetheless, eight markers kept suggestive evidence of association (Table 2). The eight SNPs were rs35709256 (*FAT3*), rs4807188 (*CSNK1G2*), rs2074872 (*MYH13*), rs116611488 (*CNTN2*), rs4854545 (*ANTXR1*), rs78672540 (8p23.2), rs13439823 (*ANGPT1*) and rs11993287 (*PLEC*). None of SNPs were significant after this multi-test adjustment (Bonferroni threshold = 3.9×10^{-08}).

SKAT analyses were done using all markers that passed previous QC. Only results from genes with at least two genotyped markers were considered, resulting in a total of 19,293 genes. Seven associations were found for the four SKAT alternatives performed: *CNTN2* ($p = 1.25 \times 10^{-05}$, SKAT rares), *FBXO44* ($p = 1.55 \times 10^{-05}$, BURDEN), *AP1M2* ($p = 2.78 \times 10^{-05}$, SKAT w1), *RSPO4* ($p = 5.53 \times 10^{-05}$, BURDEN), *KRI1* ($p = 5.56 \times 10^{-05}$, SKAT w1), *BPIFB1* ($p = 7.08 \times 10^{-05}$, SKAT w1) and *INMT* ($p = 8.90 \times 10^{-05}$, SKAT rares) (Table 3). However, none of these focus genes would remain significant at the Bonferroni threshold

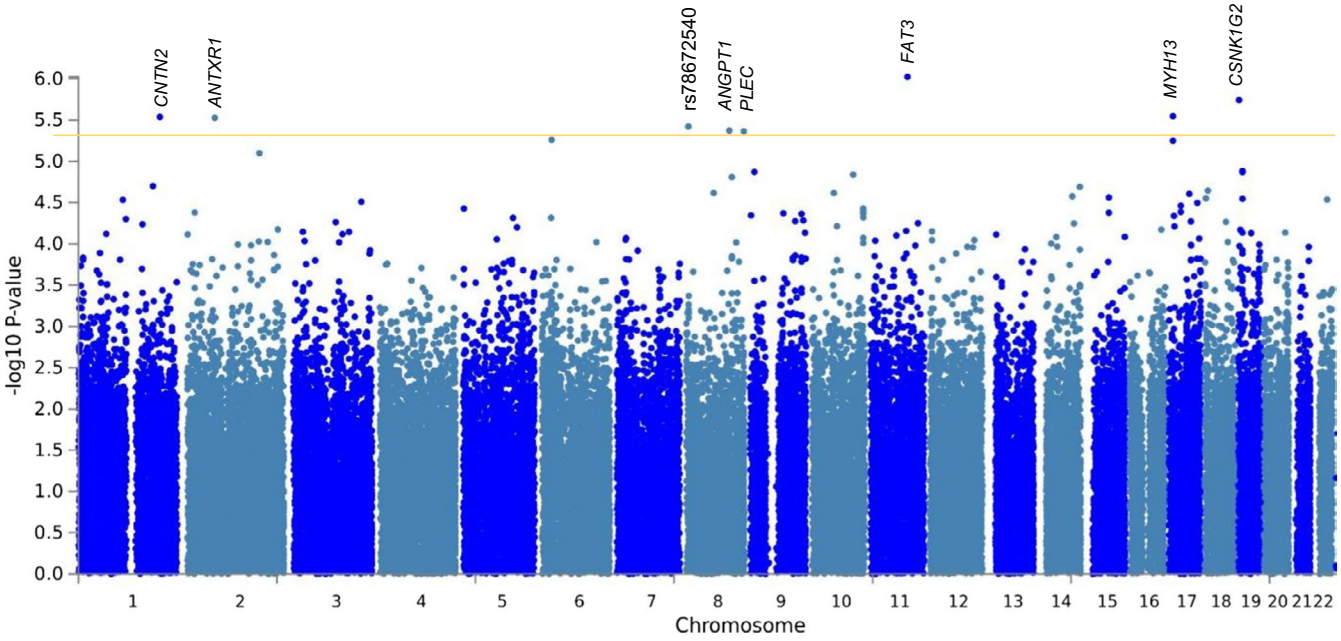

FIGURE 1 Manhattan plot for markers association results, after reviewing of assigned genotypes and re-genotyping confirmation. Horizontal axis: genomic position of the analysed variants; Vertical axis: $-\log_{10}$ (*P*-value) for each variant. The line indicates the suggestive association threshold at *p*-value = 5×10^{-6}

TABLE 2 Association results, adjusted for covariates, of top SNPs in loci with suggestive association ($p < 5 \times 10^{-06}$) with PIII/IV-C in the PerioGEN study (all markers represented passed visual clusterplots inspection)

SNP	Location	Position (bp)[a]	Gene (role)	*N* total	Effect allele	MAF (Cases/controls)	OR (95% CI)	*p*-value
rs35709256	11q14.3	92612282	*FAT3* (intron)	1547	A	0.12/0.07	2.07 (1.55–2.77)	9.48×10^{-07}
rs4807188	19p13.3	1975217	*CSNK1G2* (intron)	1551	A	0.05/0.02	2.80 (1.83–4.27)	1.81×10^{-06}
rs2074872	17p13.1	10222061	*MYH13* (intron)	1553	A	0.41/0.33	2.12 (1.55–2.91)	2.84×10^{-06}
rs116611488	1q32.1	205005227	*CNTN2* (upstream)	1559	T	0.03/0.009	4.22 (2.31–7.72)	2.90×10^{-06}
rs4854545	2p13.3	69313773	*ANTXR1* (intron)	1553	G	0.03/0.009	4.34 (2.34–8.02)	2.97×10^{-06}
rs78672540	8p23.2	5247045	none	1558	C	0.08/0.04	2.32 (1.62–3.31)	3.78×10^{-06}
rs13439823	8q23.1	108440377	*ANGPT1* (intron)	1552	A	0.43/0.37	2.01 (1.49–2.70)	4.22×10^{-06}
rs11993287	8q24.3	144966243	*PLEC* (downstream)	1559	A	0.31/0.38	0.58 (0.46–0.73)	4.31×10^{-06}

Note: PIII/IV-C: stages III/IV, grade C periodontitis.

Abbreviations: bp, base pairs; CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.

[a]Chromosomal positions are based on hg19/GRCh37.

TABLE 3 Association results, adjusted for covariates, of top genes for association with PIII/IV-C in the PerioGEN study (all markers that contribute to the association of these genes passed manual clusterplots inspection)

Chr	Location	Gene	*N* SNPs	cMAF	*p*-value	SKAT model
1	1q32.1	CNTN2	18	0.25	1.25×10^{-05}	SKAT rares
1	1p36.22	*FBXO44*	7	0.94	1.55×10^{-05}	BURDEN
19	19p13.2	*AP1M2*	7	0.98	2.78×10^{-05}	SKAT w1
20	20p13	*RSPO4*	60	9.50	5.53×10^{-05}	BURDEN
19	19p13.2	*KRI1*	16	3.29	5.56×10^{-05}	SKAT w1
20	20q11.21	*BPIFB1*	19	2.43	7.08×10^{-05}	SKAT w1
7	7p14.3	*INMT*	17	0.36	8.90×10^{-05}	SKAT rares

Note: PIII/IV-C: stages III/IV, grade C periodontitis.

SNPs: single-nucleotide polymorphisms; Chr: chromosome; cMAF: cumulative minor allele frequency.

SKAT rares: SKAT with "beta" weights only for rare variants; SKAT w1: SKAT with same weights for common and rare variants; BURDEN: Burden test.

of 2.59×10^{-6} for 19,293 genes. Table 3 shows the top results of SKAT after clusterplots revision and re-genotyping confirmation.

3.2 | Ingenuity pathway analysis (IPA)

Using IPA, we identified 38 pathways enriched for focus genes (SKAT *p*-value <0.01) significantly related to PIII/IV-C (Fisher's exact test $p < 0.05$) (Table S2). The 5 most significantly enriched pathways ($p < 4 \times 10^{-03}$) are shown in Table 4. Figures S1–S5 show the top five canonical pathways: cAMP-mediated signalling (Figure S1), regulation of interleukin (IL)-2 expression in activated T lymphocytes (Figure S2), CD28 signalling in T helper cells (Figure S3), VDR/RXR activation (Figure S4) and PI3K/Akt signalling (Figure S5).

3.3 | Association with PD susceptibility variants

Of the total of 9 susceptibility variants with genome-wide significant association with PD, three were already included in the Axiom Spain Biobank Array (rs1537415 at *GLT6D1*, rs16870060 at *MTND1P5* and rs242016 at *EFCAB4B*). The other six variants were imputed; all of them passed QC filtering (INFO >0.8 and there was not strand ambiguity).

Among the four susceptibility variants with genome-wide significant association with AgP, which were previously reported (Schaefer et al., 2010; Munz, Willenborg, et al., 2017), only rs42284742 at *SIGLEC5* reached association at nominal significance ($p < 0.05$) in PerioGEN and with same effect direction as Munz et al. study (Table S3).

About the 6 periodontitis risk loci ($p < 10^{-06}$) identified by Munz et al. (2018), only 2 SNPs passed the nominal significance threshold for association in our data. rs11084095 at *SIGLEC5*-AC018755.18 met nominal statistical significance criteria with the same direction effect in our study. On the other hand, rs2064712 showed nominal significance but with opposite effect direction (Table S3).

Moreover, our GWAS had sufficient statistical power to detect as significant the *EFCAB4B* genetic variations as it has been previously reported (Bevilacqua et al., 2018). However, none of the four identified SNPs showed significant association ($p > 0.4$).

TABLE 4 Top five of most significant pathways ($p < 4 \times 10^{-03}$) enriched with respect to focus genes identified by Ingenuity Pathway Analysis (IPA)

Pathway name	*p*-value*	Contributing genes[a]
cAMP mediated signalling	3.6×10^{-05}	*AKAP1*, *CALM*, Gi-coupled Receptor, Gs-Coupled Receptor, *MAP2K1*, *PDE4A*, *PKIA*, *PPP3* and *RGS*
Regulation of IL–2 expression in activated T lymphocytes	6.4×10^{-04}	*CALM*, *IKBKE*, *MAPK12*, *MAP2K1*, *NFAT5*, *PPP3* and *RAC1*
CD28 signalling in T helper cells	1.3×10^{-03}	*CALM*, *CD86*, *IKBKE*, *MAPK12*, *MAP2 K1*, *NFAT5*, *PPP3*, *RAC1* and *TCRA*
VDR/RXR activation	2.6×10^{-03}	*CDKN1A*, *CST6*, *GTF2B*, *MED1*, *PPARD*, *SULT2A1* and *WT1*
PI3K/Akt signalling	3.1×10^{-03}	*CDKN1A*, *EIF4EBP1*, *IKBKE*, *ITGA3*, JAK3, *MAP2K1*, *MTOR*, *THEM4* and *TSC2*

Abbreviation: IL: interleukin.

[a]Contributing genes: focus genes that contribute to identified pathways by Ingenuity Pathway Analysis (IPA); **p*-value determinates the probability that the association between the focus genes and the canonical pathway is explained by chance alone.

Noteworthy, the two risk loci that reached nominal association in the current study (rs4284742 and rs11084095) and with same effect direction as in previous studies (Munz, Willenborg, et al., 2017; Munz et al., 2018) were located in the same gene, *SIGLEC5*.

The regression analysis reveals lack of significance for PRS based on SNPs of Munz et al. meta-analysis (Table S4). So, this PRS is not able to differentiate PIII/IV-C cases from controls in our Spanish cohort.

4 | DISCUSSION

The PerioGEN study explores for the first time the genetic basis of PIII/IV-C in a Spanish population, through a GWAS of common and population-specific rare variants, analysing the GWAS data at SNP, gene and pathway level. We did not find any genome-wide significance association; however, eight SNPs (rs35709256 *FAT3*, rs4807188 *CSNK1G2*, rs2074872 *MYH13*, rs116611488 *CNTN2*, rs4854545 *ANTXR1*, rs78672540 8p23.2, rs13439823 *ANGPT1* and rs11993287 *PLEC*) showed suggestive evidence of association, only one, rs11993287, with protector effect. None of the eight suggestive loci have been previously investigated in the context of periodontitis.

In the current study, we also found three markers, rs4284742, rs11084095 and rs2064712, previously associated with PD that reached nominal statistical significance in PerioGEN study. The markers rs4284742 at *SIGLEC5* and rs11084095 at *SIGLEC5-AC018755.18* had been previously reported by Munz et al. in 2017 and 2018, respectively (Munz, Willenborg, et al., 2017; Munz et al., 2018). An association was detected with nominal statistical significance and same effect direction than that described by Munz et al. (Munz, Willenborg, et al., 2017; Munz et al., 2018) between both markers and PIII/IV-C. *SIGLEC5* encodes a group of immunoglobulins that mediate protein–carbohydrate interactions. SIGLEC5 protein seems to be involved in the activation of myeloid cells avoiding inappropriate reactivity against self-tissues, which is correlated with the existence in AgP of severe inflammation without the presence of a particular pathogenic burden (Munz, Willenborg, et al., 2017). Similarly, in addition to the association at *SIGLEC5*, rs2064712 at chr.6 showed a nominal significant association, but with opposite direction than that in the study by Munz et al. (2018). This variant locates downstream of the gene plasminogen, and associations with both AgP and chronic periodontitis (CP) were reported before (Munz, Chen, et al., 2017; Munz et al., 2018). Plasminogen is important in the maintenance of a healthy periodontium, as it is the inactive precursor of plasmin, which is involved in wound healing (Schaefer, 2018). The moderate p-value of both associations is probably related to the limitations of the sample size.

In addition, and considering that periodontitis is a common complex disease, numerous SNPs (mostly of small size effect) genetic interactions between them, and effects of rare variants contribute to its susceptibility, making it difficult to discover individual variants with genome-wide statistical significance. The gene-based test is a good complement because they have higher power to detect small effects by combining the effects of variants in the same gene. We performed a gene analysis, including SNPs and rare variants. Seven genes (*CNTN2*, *FBXO44*, *AP1M2*, *RSPO4*, *KRI1*, *BPIFB1* and *INMT*) were identified by gene-based analysis. Noteworthy that *CNTN2* showed association at both SNP and gene-level analysis in the present study. Protein encoded by *CNTN2* interacts with proteins involved in Alzheimer's Disease (AD) pathogenesis (APP and BACE1) (Bamford et al., 2020), a disease recently linked to *Porphyromonas gingivalis* (Dominy et al., 2019). Furthermore, the second most upregulated gene in the current study was *RSPO4*. This gene has previously been related to rapid progression of alveolar bone loss as a result of oral infection with *P. gingivalis* in a mouse model (Nashef et al., 2020). Despite the possible relationship of both genes with periodontitis, their probability in our study does not exceed the multiple-test correction.

In order to study the susceptibility associated with the cumulative effects of common risk alleles, we also performed predictive modelling using polygenic risk score based upon SNPs of the recent meta-analysis of Munz et al. (Munz et al., 2018). Through regression analysis, we found that PRS based on the SNPs with suggestive evidence of association identified by Munz et al. is not able to differentiate PIII/IV-C cases from controls depending on this PRS. Hence, these variants together do not explain the susceptibility to PIII/IV-C in our study population.

Finally, we identified 38 pathways enriched for focus genes significantly related to PIII/IV-C. The top five identified pathways were involved in the immune response, calcium homeostasis, bone development and the last of which is exploited by pathogens such as *P. gingivalis*, causing periodontal inflammation (Liu et al., 2018).

This study has some limitations. The small population size limits the statistical power and may result in false negative genome-wide significant findings and the inability to re-discovery more GWAS hit signals for periodontitis. In a post hoc power analysis, if we consider a true odds ratio of 1.28 (the OR we found for *DEFA1A3* in (Munz, Willenborg, et al., 2017), a MAF of 0.43 (that of the rs2738058) and the same control:cases ratio we had, we would had needed to study at least 3,620 cases and 9,367 controls to be able to identify this effect as statistically significant with a power of 80%. Otherwise, considering our actual sample size, number of cases and controls, and a significance threshold of $p < 5 \times 10^{-08}$, we had 80% power to detect an OR of >1.655 for SNPs with a MAF of 0.44. Furthermore, our study is limited by the fact that it is based on a single cohort, no replication is available and no adjusting for the number of genetic models used in gene-set analysis was done. Although minimal, another limitation is the use of "reference" samples as controls, so some of the controls may have had AgP periodontitis (0.1% in European population). The quality of the controls could be improved by a specific periodontal examination in controls. Future studies are required to address these shortcomings.

In contrast, our study presents several strengths: high-quality genotyping and QC, samples with homogeneous ethnic background and selection of an extreme periodontitis phenotype (PIII/IV-C), highlighting the analysis of rare variants (MAF <1%), which contribute on complex diseases through modest-to-small effect sizes.

In summary, we performed the first GWAS for patients with stages III or IV, grade C periodontitis (rapid rate of progression) in a Spanish population. We did not identify SNPs that met the strict genome-wide statistical significance criteria. However, we identified eight markers with suggestive evidence of association with periodontitis and the previously reported association of *SIGLEC5* was confirmed. Nevertheless, we must be cautious with these results because our study is based on a single cohort and no replication is available. Our data might become more valuable with the pool of GWAS results and future meta-analyses, contributing to the knowledge of molecular and genetic mechanisms influencing stages III or IV, grade C periodontitis onset and progression. The results obtained and the absence of consistently associated genetic variants in different populations highlight the challenge posed by the study of the genetic basis of a common complex disease such as periodontitis.

ACKNOWLEDGEMENTS

We thank all the participants of the PerioGEN study, staff of CEGEN-PRB3-ISCIII for genotyping development (http://www.usc.es/cegen/) and all contributors from the SEPA Network of Research Clinics: Segundo Rodríguez Grandío (Clínica Dental Segundo Rodríguez Grandío, Vigo), Nuria Vallcorba (Clínica Vallcorba, Barcelona), Isabel Ramos (Clínica Blanco-Ramos, Santiago de Compostela), Alfonso Oteo (Clínica Periocentrum Segovia, Segovia), Cristina Serrano (Periodoncia Ciudad Real, Ciudad Real), Alejandro Esmatges Guasch (Clínica Esmatges Periodoncia, Lleida), Francisco Enrile (Clínica Enrile, Huelva), Leopoldo Mateos (Clínica Mateos, Sevilla), Roberto García Fernández (Clínica Miguel Ángel García Pérez e Hijos S.L, Palencia), Pablo Álvarez-Novoa (Clínica Álvarez-Novoa, Santander), Blas Noguerol (Clínica Noguerol, Granada), Ion Zabalegui (Clínica Dental Albia, Bilbao), José Blanco-Moreno (Clínica Blanco-Moreno, Oviedo), Ángel Alonso (Clínica Alonso, Salamanca), Ramón Lorenzo (Clínica Periocentrum Ávila, Ávila), Daniel Rodrigo (Clínica Periocentrum Guadalajara, Guadalajara), Pilar Batalla (Facultad de Odontología de la Universidad de Santiago de Compostela, Santiago de Compostela), Bettina Alonso (Facultad de Odontología Universidad Complutense de Madrid, Madrid), Javier Alández (Clínica Perio-Plénido, Madrid), Adrián Guerrero (Clínica Adrián Guerrero, Marbella), Ana Echeverría Manau (Clínica dental Echeverría, Barcelona), Julio Galván Guerrero (Clínica Galván - Recoletos cuatro, Valladolid), Antonio Luis Bujaldón (Clínica Periodontal Antonio Luis Bujaldón, Almería), Fernando Franch (Instituto Balear de Implantes y Periodoncia, Palma de Mallorca), Pedro Bullón (Facultad de Odontología Universidad de Sevilla, Sevilla) Jaime Alcaraz Sintes (Clínica Alcaraz, Alicante), Alberto Ortiz-Vigón (Clínica Ortiz-Vigón & Carnicero SLP, Bilbao), Joana Rosselló Alba (Consulta exclusiva de Periodoncia e Implantes Dra. Joana Rosselló Alba, Palma de Mallorca), José Nart Molina (Clínica universitaria de Odontología de la Universitat Internacional de Cataluña, Barcelona), Amador Álvarez García (Clínica Dental Amador de Cangas, Cangas), Agustín Casas (Clínica Plénido Dental-Alcorcón, Alcorcón), Antonio Liñares (Clínica de Periodoncia Antonio Liñares, A Coruña), Regina Izquierdo Fort (Clínica Izquierdo, Gandía), Cristina Carral (Clínica Flores Guillén, Toledo), Sebastián Fàbregues (Clínica Fabregues, Palma de Mallorca), Amparo Lorca (Clínica Perios Lorca-Salanova, Valencia) and Elena López Alvar (Clínica Guitián, Vigo). The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

CONFLICT OF INTEREST

The author JB reports grants from Manohay dental, during the conduct of the study. Grants from ITI, Intra-lock, Ticare, Straumann, personal fees from Straumann, outside the submitted work. The author DH reports Personal fees from Oral-B, Straumman, Klockner, Dexcel, Dentaid and Colgate, grants from Dentaid, Kulzer, outside the submitted work.

AUTHOR CONTRIBUTIONS

A de Coo contributed to design, contributed to acquisition, analysis and interpretation, and drafted manuscript; R Cruz contributed to conception and design, contributed to acquisition, analysis and interpretation, drafted manuscript and critically

revised manuscript; I Quintela contributed to conception and design, contributed to acquisition and critically revised manuscript; D Herrera contributed to design, contributed to interpretation and critically revised manuscript; M Sanz contributed to conception, contributed to interpretation and critically revised manuscript; P Diz, contributed to conception and design, drafted manuscript and critically revised manuscript; S Rodríguez Grandío, N Vallcorba, I Ramos, A Oteo, C Serrano, A Esmatges, F Enrile, L Mateos, R García, P Álvarez-Novoa, B Noguerol, I Zabalegui, J Blanco-Moreno, Á Alonso and R Lorenzo contributed to acquisition and critically revised manuscript; A Carracedo and J Blanco contributed to conception and design, and critically revised manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

ETHICS STATEMENT

This study was approved by the ethics commission of Research Ethics Committee of Santiago-Lugo, Galicia, Spain (number 2015/372, 25/06/2015). All participants joined this study voluntarily and provided oral and written informed consent. The project was conducted following the STROBE guidelines and according to ethical principles expressed in the Declaration of Helsinki.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Alicia de Coo https://orcid.org/0000-0002-7471-0254
Raquel Cruz https://orcid.org/0000-0002-6964-8898
David Herrera https://orcid.org/0000-0002-5554-2777
Mariano Sanz https://orcid.org/0000-0002-6293-5755
Pedro Diz https://orcid.org/0000-0002-1483-401X
Cristina Serrano https://orcid.org/0000-0001-6917-6416
Angel Carracedo https://orcid.org/0000-0003-1085-8986
Juan Blanco https://orcid.org/0000-0001-9251-513X

REFERENCES

Abusleme, L., Dupuy, A. K., Dutzan, N., Silva, N., Burleson, J. A., Strausbaugh, L. D., Gamonal, J., & Diaz, P. I. (2013). The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. *The ISME Journal*, *7*(5), 1016–1025. https://doi.org/10.1038/ismej.2012.174

Auer, P. L., & Lettre, G. (2015). Rare variant association studies: Considerations, challenges and opportunities. *Genome Medicine*, *7*(1), 16. https://doi.org/10.1186/s13073-015-0138-2

Aulchenko, Y. S., Ripke, S., Isaacs, A., & van Duijn, C. M. (2007). GenABEL: An R library for genome-wide association analysis. *Bioinformatics*, *23*(10), 1294–1296. https://doi.org/10.1093/bioinformatics/btm108

Bamford, R. A., Widagdo, J., Takamura, N., Eve, M., Anggono, V., & Oguro-Ando, A. (2020). The interaction between contactin and amyloid precursor protein and its role in Alzheimer's disease. *Neuroscience*, *424*, 184–202. https://doi.org/10.1016/j.neuroscience.2019.10.006

Bevilacqua, L., Navarra, C. O., Pirastu, N., Lenarda, R. D., Gasparini, P., & Robino, A. (2018). A genome-wide association study identifies an association between variants in EFCAB4B gene and periodontal disease in an Italian isolated population. *Journal of Periodontal Research*, *53*(6), 992–998. https://doi.org/10.1111/jre.12598

Bomba, L., Walter, K., & Soranzo, N. (2017). The impact of rare and low-frequency genetic variants in common disease. *Genome Biology*, *18*(1), 77. https://doi.org/10.1186/s13059-017-1212-4

Bouziane, A., Hamdoun, R., Abouqal, R., & Ennibi, O. (2020). Global Prevalence of aggressive periodontitis: A systematic review and meta-analysis. *Journal of Clinical Periodontology*, *47*, 406–428. https://doi.org/10.1111/jcpe.13266

Clayton, D. G., Walker, N. M., Smyth, D. J., Pask, R., Cooper, J. D., Maier, L. M., Smink, L. J., Lam, A. C., Ovington, N. R., Stevens, H. E., Nutland, S., Howson, J. M. M., Faham, M., Moorhead, M., Jones, H. B., Falkowski, M., Hardenbol, P., Willis, T. D., & Todd, J. A. (2005). Population structure, differential bias and genomic control in a large-scale, case-control association study. *Nature Genetics*, *37*(11), 1243–1246. http://dx.doi.org/10.1038/ng1653

Delaneau, O., Marchini, J., & Zagury, J. F. (2012). A linear complexity phasing method for thousands of genomes. *Nature Methods*, *9*(2), 179–181. https://doi.org/10.1038/nmeth.1785

Divaris, K., Monda, K. L., North, K. E., Olshan, A. F., Lange, E. M., Moss, K., Barros, S. P., Beck, J. D., & Offenbacher, S. (2012). Genome-wide association study of periodontal pathogen colonization. *Journal of Dental Research*, *91*(7 suppl), S21–S28. https://doi.org/10.1177/0022034512447951

Divaris, K., Monda, K. L., North, K. E., Olshan, A. F., Reynolds, L. M., Hsueh, W.-C., Lange, E. M., Moss, K., Barros, S. P., Weyant, R. J., Liu, Y., Newman, A. B., Beck, J. D., & Offenbacher, S. (2013). Exploring the genetic basis of chronic periodontitis: a genome-wide association study. *Human Molecular Genetics*, *22*(11), 2312–2324. https://doi.org/10.1093/hmg/ddt065

DNA Genotek. (2018). Laboratory protocol for manual purification of DNA from 0.5 mL of sample. (PD-PR-006).

Dominy, S. S., Lynch, C., Ermini, F., Benedyk, M., Marczyk, A., Konradi, A., Nguyen, M., Haditsch, U., Raha, D., Griffin, C. & Potempa, J. (2019). Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. *Science Advances*, *5*(1), eaau3333. https://doi.org/10.1126/sciadv.aau3333

Dupont, W. D., & Plummer, W. D. (1998). Power and sample size calculations for studies involving linear regression. *Controlled Clinical Trials*, *19*(6), 589–601. https://doi.org/10.1016/S0197-2456(98)00037-3

Feng, P., Wang, X., Casado, P. L., Küchler, E. C., Deeley, K., Noel, J., Kimm, H., Kim, J.-H., Haas, A. N., Quinelato, V., Bonato, L. L., Granjeiro, J. M., Susin, C., & Vieira, A. R. (2014). Genome wide association scan for chronic periodontitis implicates novel locus. *BMC Oral Health*, *14*(1), 84. https://doi.org/10.1186/1472-6831-14-84

Freitag-Wolf, S., Dommisch, H., Graetz, C., Jockel-Schneider, Y., Harks, I., Staufenbiel, I., Meyle, J., Eickholz, P., Noack, B., Bruckmann, C., Gieger, C., Jepsen, S., Lieb, W., Schreiber, S., König, I. R. & Schaefer, A. S. (2014). Genome-wide exploration identifies sex-specific genetic effects of alleles upstream NPY to increase the risk of severe periodontitis in men. *Journal of Clinical Periodontology*, *41*(12), 1115–1121. https://doi.org/10.1111/jcpe.12317

González, J. R., Carrasco, J. L., Dudbridge, F., Armengol, L., Estivill, X., & Moreno, V. (2008). Maximizing association statistics over genetic models. *Genetic Epidemiology*, *32*(3), 246–254. http://dx.doi.org/10.1002/gepi.20299

Hong, K.-W., Shin, M.-S., Ahn, Y.-B., Lee, H.-J., & Kim, H.-D. (2015). Genomewide association study on chronic periodontitis in Korean population: Results from the Yangpyeong health cohort. *Journal of Clinical Periodontology*, *42*(8), 703–710. https://doi.org/10.1111/jcpe.12437

Howie, B. N., Donnelly, P., & Marchini, J. (2009). A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genetics*, *5*(6), e1000529. https://doi.org/10.1371/journal.pgen.1000529

Ionita-Laza, I., Lee, S., Makarov, V., Buxbaum, J. D., & Lin, X. (2013). Sequence Kernel association tests for the combined effect of rare and common variants. *The American Journal of Human Genetics*, *92*(6), 841–853. https://doi.org/10.1016/j.ajhg.2013.04.015

Kassebaum, N. J., Bernabé, E., Dahiya, M., Bhandari, B., Murray, C. J. L., & Marcenes, W. (2014). Global burden of severe periodontitis in 1990–2010: A systematic review and meta-regression. *Journal of Dental Research*, *93*(11), 1045–1053. https://doi.org/10.1177/0022034514552491

Krämer, A., Green, J., Pollard, J. Jr, & Tugendreich, S. (2014). Causal analysis approaches in ingenuity pathway analysis. *Bioinformatics*, *30*(4), 523–530.

Liu, J., Wang, X., Zheng, M., & Luan, Q. (2018). Lipopolysaccharide from Porphyromonas gingivalis promotes autophagy of human gingival fibroblasts through the PI3K/Akt/mTOR signaling pathway. *Life Sciences*, *211*, 133–139. http://dx.doi.org/10.1016/j.lfs.2018.09.023

Manolio, T. A. (2010). Genomewide association studies and assessment of the risk of disease. *The New England Journal of Medicine*, *363*(2), 166–176. https://doi.org/10.1056/NEJMra0905980

Munz, M., Chen, H., Jockel-Schneider, Y., Adam, K., Hoffman, P., Berger, K., Kocher, T., Meyle, J., Eickholz, P., Doerfer, C., Laudes, M., Uitterlinden, A., Lieb, W., Franke, A., Schreiber, S., Offenbacher, S., Divaris, K., Bruckmann, C., Loos, B. G., … Schäefer, A. S. (2017). A haplotype block downstream of plasminogen is associated with chronic and aggressive periodontitis. *Journal of Clinical Periodontology*, *44*(10), 962–970. https://doi.org/10.1111/jcpe.12749

Munz, M., Richter, G. M., Loos, B. G., Jepsen, S., Divaris, K., Offenbacher, S., Teumer, A., Holtfreter, B., Kocher, T., Bruckmann, C., Jockel-Schneider, Y., Graetz, C., Ahmad, I., Staufenbiel, I., van der Velde, N., Uitterlinden, A. G., de Groot, L. C. P. G. M., Wellmann, J., Berger, K., … Schaefer, A. S. (2018). Meta-analysis of genome-wide association studies of aggressive and chronic periodontitis identifies two novel risk loci. *European Journal of Human Genetics*, *27*, 102–113. https://doi.org/10.1038/s41431-018-0265-5

Munz, M., Willenborg, C., Richter, G. M., Jockel-Schneider, Y., Graetz, C., Staufenbiel, I., Wellmann, J., Berger, K., Krone, B., Hoffmann, P., van der Velde, N., Uitterlinden, A. G., de Groot, L. C. P. G. M., Sawalha, A. H., Direskeneli, H., Saruhan-Direskeneli, G., Guzeldemir-Akcakanat, E., Keceli, H. G., Laudes, M., … Schaefer, A. S. (2017). A genome-wide association study identifies nucleotide variants at SIGLEC5 and DEFA1A3 as risk loci for periodontitis. *Human Molecular Genetics*, *26*(13), 2577–2588. https://doi.org/10.1093/hmg/ddx151

Nashef, A., Matthias, M., Weiss, E., Loos, B. G., Jepsen, S., van der Velde, N., Uitterlinden, A. G., Wellmann, J., Berger, K., Hoffmann, P., Laudes, M., Lieb, W., Franke, A., Dommisch, H., Schäfer, A., Houri-Haddad, Y., & Iraqi, F. A. (2020). Translation of mouse model to human gives insights into periodontitis etiology. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-61819-0

Nibali, L., Di Iorio, A., Tu, Y.-K., & Vieira, A. R. (2017). Host genetics role in the pathogenesis of periodontal disease and caries. *Journal of Clinical Periodontology*, *44*, S52–S78. https://doi.org/10.1111/jcpe.12639

Offenbacher, S., Divaris, K., Barros, S. P., Moss, K. L., Marchesan, J. T., Morelli, T., Zhang, S., Kim, S., Sun, L. U., Beck, J. D., Laudes, M., Munz, M., Schaefer, A. S., & North, K. E. (2016). Genome-wide association study of biologically informed periodontal complex traits offers novel insights into the genetic basis of periodontal disease. *Human Molecular Genetics*, *25*(10), 2113–2129. https://doi.org/10.1093/hmg/ddw069

Papapanou, P. N., Sanz, M., Buduneli, N., Dietrich, T., Feres, M., Fine, D. H., Flemmig, T. F., Garcia, R., Giannobile, W. V., Graziani, F., Greenwell, H., Herrera, D., Kao, R. T., Kebschull, M., Kinane, D. F., Kirkwood, K. L., Kocher, T., Kornman, K. S., Kumar, P. S., … Tonetti, M. S. (2018). Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *Journal of Clinical Periodontology*, *45*(Suppl 2), S162–S170. https://doi.org/10.1111/jcpe.12946

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., de Bakker, P. I. W., Daly, M. J., & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *The American Journal of Human Genetics*, *81*(3), 559–575. https://doi.org/10.1086/519795

R Core Team. (2008). R. A language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved January 29, 2018, from https://www.r-project.org/

Rhodin, K., Divaris, K., North, K. E., Barros, S. P., Moss, K., Beck, J. D., & Offenbacher, S. (2014). Chronic periodontitis genome-wide association studies. *Journal of Dental Research*, *93*(9), 882–890. https://doi.org/10.1177/0022034514544506

Sanders, A. E., Sofer, T., Wong, Q., Kerr, K. F., Agler, C., Shaffer, J. R., Beck, J. D., Offenbacher, S., Salazar, C. R., North, K. E., Marazita, M. L., Laurie, C. C., Singer, R. H., Cai, J., Finlayson, T. L., & Divaris, K. (2017). Chronic periodontitis genome-wide association study in the Hispanic community health study / study of Latinos. *Journal of Dental Research*, *96*(1), 64–72. https://doi.org/10.1177/0022034516664509

Schaefer, A. S. (2018). Genetics of periodontitis: Discovery, biology, and clinical impact. *Periodontology 2000*, *78*(1), 162–173. https://doi.org/10.1111/prd.12232

Schaefer, A. S., Bochenek, G., Jochens, A., Ellinghaus, D., Dommisch, H., Güzeldemir-Akçakanat, E., Graetz, C., Harks, I., Jockel-Schneider, Y., Weinspach, K., Meyle, J., Eickholz, P., Linden, G. J., Cine, N., Nohutcu, R., Weiss, E., Houri-Haddad, Y., Iraqi, F., Folwaczny, M., Noack, B. & Schreiber, S. (2015). Genetic evidence for PLASMINOGEN as a shared genetic risk factor of coronary artery disease and periodontitis clinical perspective. *Circulation: Cardiovascular Genetics*, *8*(1), 159–167. https://doi.org/10.1161/CIRCGENETICS.114.000554

Schaefer, A. S., Richter, G. M., Nothnagel, M., Manke, T., Dommisch, H., Jacobs, G., Arlt, A., Rosenstiel, P., Noack, B., Groessner-Schreiber, B., Jepsen, S., Loos, B. G., & Schreiber, S. (2010). A genome-wide association study identifies GLT6D1 as a susceptibility locus for periodontitis. *Human Molecular Genetics*, *19*(3), 553–562. https://doi.org/10.1093/hmg/ddp508

Shaffer, J. R., Polk, D. E., Wang, X., Feingold, E., Weeks, D. E., Lee, M.-K., Cuenco, K. T., Weyant, R. J., Crout, R. J., McNeil, D. W., & Marazita, M. L. (2014). Genome-wide association study of periodontal health measured by probing depth in adults ages 18–49 years. *G3: Genes|genomes|genetics*, *4*(2), 307–314. https://doi.org/10.1534/g3.113.008755

Shimizu, S., Momozawa, Y., Takahashi, A., Nagasawa, T., Ashikawa, K., Terada, Y., Izumi, Y., Kobayashi, H., Tsuji, M., Kubo, M., & Furuichi, Y. (2015). A genome-wide association study of periodontitis in a Japanese population. *Journal of Dental Research*, *94*(4), 555–561. https://doi.org/10.1177/0022034515570315

Teumer, A., Holtfreter, B., Völker, U., Petersmann, A., Nauck, M., Biffar, R., Völzke, H., Kroemer, H. K., Meisel, P., Homuth, G., & Kocher, T. (2013). Genome-wide association study of chronic periodontitis in a general German population. *Journal of Clinical Periodontology*, *40*(11), 977–985. https://doi.org/10.1111/jcpe.12154

Tonetti, M. S., Greenwell, H., & Kornman, K. S. (2018). Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *Journal of Clinical Periodontology*, *45*, S149–S161. https://doi.org/10.1111/jcpe.12945

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: de Coo A, Cruz R, Quintela I, et al. Genome-wide association study of stage III/IV grade C periodontitis (former aggressive periodontitis) in a Spanish population. *J Clin Periodontol*. 2021;00:1–11. https://doi.org/10.1111/jcpe.13460