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ORIGINAL ARTICLE

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A genome-wide association study meta-analysis in a European sample of stage III/IV grade C periodontitis patients ≤35 years of age identifies new risk loci

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Abstract

Aim: Few genome-wide association studies (GWAS) have been conducted for severe forms of periodontitis (stage III/IV grade C), and the number of known risk genes is scarce. To identify further genetic risk variants to improve the understanding of the

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disease aetiology, a GWAS meta-analysis in cases with a diagnosis at ≤35 years of age was performed.

Materials and Methods: Genotypes from German, Dutch and Spanish GWAS studies of III/IV-C periodontitis diagnosed at age ≤35 years were imputed using TopMed. After quality control, a meta-analysis was conducted on 8,666,460 variants in 1306 cases and 7817 controls with METAL. Variants were prioritized using FUMA for gene-based tests, functional annotation and a transcriptome-wide association study integrating eQTL data.

Results: The study identified a novel genome-wide significant association in the *FCER1G* gene ($p = 1.0 \times 10^{-9}$), which was previously suggestively associated with III/IV-C periodontitis. Six additional genes showed suggestive association with $p < 10^{-5}$, including the known risk gene *SIGLEC5*. *HMCN2* showed the second strongest association in this study ($p = 6.1 \times 10^{-8}$).

Conclusions: This study expands the set of known genetic loci for severe periodontitis with an age of onset ≤35 years. The putative functions ascribed to the associated genes highlight the significance of oral barrier tissue stability, wound healing and tissue regeneration in the aetiology of these periodontitis forms and suggest the importance of tissue regeneration in maintaining oral health.

KEYWORDS
aggressive periodontitis, genetic susceptibility, genome-wide association study, inflammation, wound healing

Clinical Relevance

Scientific rationale for study: Only a few genetic risk loci of stage III/IV grade C periodontitis are known, despite the high heritability of this disease. To identify further risk variants, a genome-wide association studies (GWAS) meta-analysis of III/IV cases with a diagnosis at ≤35 years of age was performed.

Principal findings: In the present meta-GWAS, FCER1G was identified as a novel genome-wide significant risk locus accompanied by six additional suggestive associations, with HMCN2 being the second most significant association. The known association of the known risk gene SIGLEC5 was validated.

Practical implications: The identified genetic risk loci emphasize the role of wound healing and tissue repair in the aetiology of severe periodontitis with an age of onset ≤35 years.

1 | INTRODUCTION

Periodontitis (PD) is a common inflammatory disease of the oral cavity characterized by dysbiotic plaque biofilms and the resorption of alveolar bone, making it a major cause of tooth loss in adults over 40 years of age (Chen, Zhong, et al., 2021; Eke et al., 2012; Marcenes et al., 2013). Stage III/IV grade C (III/IV-C) PD in particularly young individuals ≤35 years of age (formerly early onset and also designated as aggressive PD [AgP]) is a rare phenotype with a prevalence of around 0.1% in European Caucasians (Papapanou et al., 2018; Susin et al., 2014).

The pathogenesis of PD involves complex interactions between the oral microbiota and the oral mucosal tissue barrier in conjunction with the host's immune system, which is determined by both genetic factors and additional environmental risk factors such as smoking. Because of this complexity and the variability of each of these factors between individuals, PD manifests in different ways, including differences in severity, rate of tissue destruction and age at onset. Because the mucosal tissue barrier and host immune response largely determine an individual's predisposition to PD, knowledge of the genetic loci that carry genetic risk variants provides direct insight into the molecular mechanisms that protect or contribute to disease. In addition, they help to understand the causes that determine why some people develop the disease while others do not, despite often living in comparable environments and sharing lifestyles.

Several genome-wide association studies (GWAS) have been conducted to explore the genetic basis of PD, with 39 studies being listed in the GWAS catalogue so far (Buniello et al., 2019). However, only a few have specifically focused on stage III/IV grade C (III/IV-C) PD in

patients ≤35 years (de Coo et al., 2021; Freitag-Wolf et al., 2014, 2019, 2021; Munz et al., 2017, 2018, 2019; Schaefer et al., 2010). Because of limitations such as small sample sizes and the inclusion of disease phenotypes with low heritability, only a few genetic risk loci have been identified with sufficient statistical or experimental evidence (Schaefer, 2018).

To increase the statistical power needed to identify further common risk variants, we performed a GWAS meta-analysis (de Coo et al., 2021; Munz et al., 2017), which included a total of 1339 cases of stage III/IV PD and 7916 controls of European genetic background in this study.

2 | MATERIALS AND METHODS

2.1 | PERIOGEN cohort

The PERIOGEN cohort comprises 442 analysed cases of PD cases of stages III/IV-C with an age of first diagnosis ≤35 years, recruited in Spain through the SEPA (Spanish Society of Periodontology) Research Network of Dental Clinics, and 1136 analysed controls from the Spanish National DNA Biobank (de Coo et al., 2021). Genotyping and quality control procedures are thoroughly described in the original study. Briefly, variants with calling rates <98%, MAF < 1%, failing the Hardy–Weinberg equilibrium (HWE) test and samples with <98% genotyping rate were discarded. Population stratification was addressed by excluding relatives after calculating the IBD (identity-by-descent) proportions and visually assessing the first 2 genetic principal components (PCs) (10 PCs were computed).

Genotypes were imputed with the TOPMed version r^2 reference panel (GRCh38) using the TOPMed Imputation Server. Post-imputation QC consisted of removal of SNPs with imputation R^2 < 30%, MAF < 1% and the restoration of original genotypes for non-imputed variants. A total of 1578 individuals and 8,949,488 genetic variants were included in the analyses.

2.1.1 | Phenotype definition

PD stage III/IV-C phenotype definition corresponded to the criteria that are designated as follows in the current classification: (1) localized stage III/IV-C PD (<30% of teeth involved) or molar/incisor pattern; (2) generalized stage III/IV-C PD (>30% teeth involved); and (3) no systemic disorder that has a major impact on the loss of periodontal tissues. All patients were ≤35 years old at diagnosis.

2.2 | AgP German and Dutch cohorts

2.2.1 | Quality control and imputation

The German cohort initially comprised 745 cases with aggressive PD (AgP) recruited across Germany by the Popgen biobank (Krawczak et al., 2006) and 4161 controls from North and West Germany gathered by the DOGS (Berger, 2012), HNR1-3 (Schmermund et al., 2002) and FoCus (Muller et al., 2015) studies. The Dutch cohort was composed of 174 AgP cases (Schaefer et al., 2015) and 2740 controls from the B-PROOF study (van Wijngaarden et al., 2011) recruited across the Netherlands. German and Dutch AgP case definitions correspond to the current classification as stage III/IV-C and were ≤35 years of age at the time of first diagnosis. Recruitment, genotyping and quality control procedures are described elsewhere (Munz et al., 2017). Genotyping call rate was set at 98%, AF threshold at <1% and p-value for deviation of the HWE at $<10^{-10}$. Assessment of population structure was conducted by estimating admixture proportions with ADMIXTURE, principal component analysis (PCA) and identity-by-descent estimation (see Supplementary Methods).

After quality control procedures, the German AgP sample included 692 cases and 4064 controls, whereas the Dutch AgP sample consisted of 172 cases and 2617 controls.

Genotyped cohorts were merged by common variants (565,928) and imputed with the TopMed reference panel in the TopMed Imputation Server. Variants with INFO score <0.3 and MAF < 1% were removed, after which 9,063,963 variants remained.

2.2.2 | Phenotype definition

German and Dutch AgP patients were defined by ≥2 teeth with ≥30% alveolar bone loss assessed in patients with a maximum age of 35 years, and were free of diabetes, as described elsewhere (Schaefer et al., 2015).

2.3 | Association analyses

Association analyses for each of the cohorts were done by fitting logistic mixed regression models using the SAIGEgds (Zheng & Davis, 2021) package in R, which implements the two-step mixed SAIGE (Zhou et al., 2018) model methodology and the SPA test. Baseline covariables included sex and the first 10 PCs.

2.4 | GWAS meta-analysis

The three European cohorts (PERIOGEN-Spain, AgP-Germany and AgP-Netherlands) were meta-analysed, forming a total sample of 1306 cases with AgP (all corresponding to stages III/IV-C and aged ≤35 years) and 7817 controls.

An inverse-variance weighting method as implemented in METAL (Willer et al., 2010) was used for the meta-analysis. Only variants present in the three studies were analysed, leaving a total of 8,666,460. Heterogeneity between studies was evaluated with Cochran's Q test and p-value inflation was assessed using quantile–quantile (Q–Q) plots and the genomic inflation factor λ.

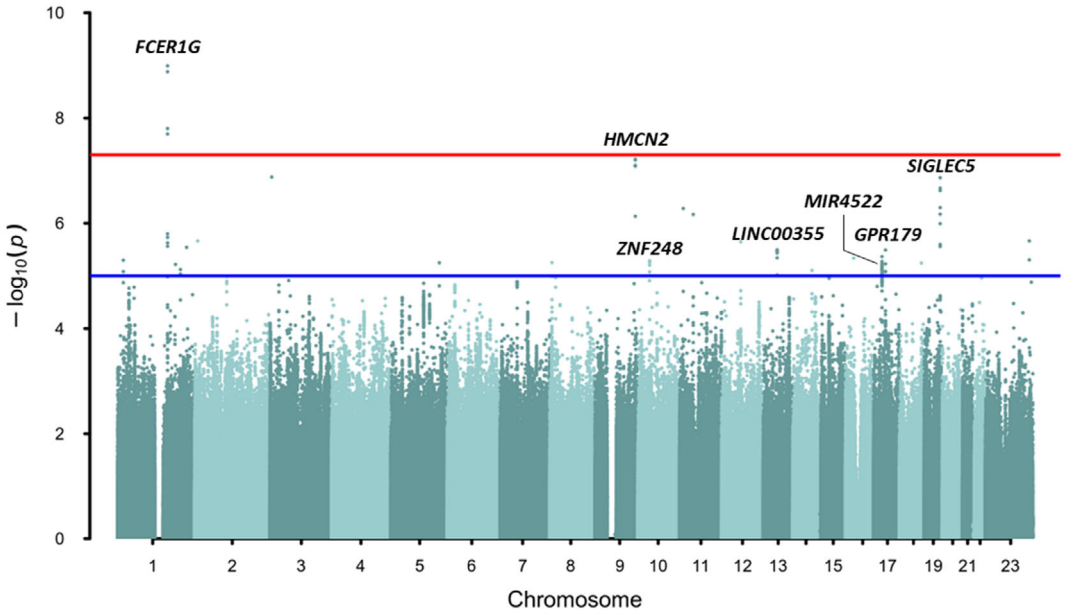

FIGURE 1 AgP genome-wide association studies meta-analysis Manhattan plot. In the *Y*-axis is $-\log_{10}$ (*p*-value), and in the *X*-axis is the chromosome number and relative position along the chromosome. Horizontal lines in blue and red correspond to suggestive (1×10^{-5}) and genome-wide (5×10^{-8}) *p*-value thresholds, respectively.

Definition of risk regions and variant annotation details can be found in Supplementary Methods.

2.5 | Transcriptome-wide association studies and colocalization

Functional roles of the prioritized variants were explored through their association with gene expression, for which a previously published pipeline was followed (Barbeira et al., 2021). First, tissue-agnostic analyses were conducted by running the transcriptome-wide association studies (TWAS) on every tissue. Statistical significance was then defined at $\alpha = 0.05$ according to the Benjamini–Hochberg FDR correction, accounting for 634.732 gene/tissue pairs. Afterwards, results from all the tissues were combined into a multi-tissue TWAS to improve power (Barbeira et al., 2021).

Then, to determine whether the two traits (gene expression and AgP) shared a single causal variant, COLOC (Giambartolomei et al., 2014) was run in each of the prioritized TWAS tissue-gene pairs.

Details from both methods, as well as gene prioritization, are described in Supplementary Methods.

3 | RESULTS

3.1 | GWAS meta-analysis

A GWAS meta-analysis on the AgP cases and controls of three European populations (German, Dutch and Spanish) was performed to determine shared genetic risk. A total of 1306 cases and 7817 controls were finally included in the analysis.

Quality control procedures and cohort descriptions are given in the Material and Methods section. Genotypes were imputed with the TOPMed reference panel, after which low-quality imputed variants were removed ($R^2 < 0.3$). Association testing was done within each cohort to control potential heterogeneity under logistic mixed models, which have been proven to control population stratification and case–control imbalances in GWAS studies (Zhou et al., 2018). Next, a meta-analysis using the inverse of the variance as weights and the overlapping variants (8,666,460) was conducted. Inflation was assessed with the genomic lambda ($\lambda = 1.014$) (Figure S1).

This GWAS meta-analysis revealed a novel risk locus for AgP in the locus coding for *FCER1G* (Figure 1). Six sub-genome-wide risk regions were also identified with $p < 10^{-5}$ (Figure 1; Tables 1 and S1). The novel and the six sub genome-wide significant variants were mapped by position and by eQTL in FUMA, prioritizing a total of 20 genes (Table S2). Other annotations such as CADD scores, eQTL-mapped tissues and regulomeDB ranks for SNPs within the gene are shown in Table S3, whereas UniProt annotations and target drugs are shown in Table S4.

rs2070902, the lead variant in the *FCER1G* locus in chromosome 1, is an intron variant with an associated 1.38-fold increased risk (95% CI: 1.23–1.51, $p = 1.02 \times 10^{-9}$) of developing AgP for the T allele. Eight genes were linked to this lead variant, either by position or by eQTL mapping. In fact, rs2070902 variant is an eQTL associated with an increased expression of NADH:ubiquinone oxidoreductase core subunit S2 (*NDUFS2*), protoporphyrinogen oxidase (*PPOX*), β-1,4-galactosyltransferase 3 (*B4GALT3*), ADAM metallopeptidase with thrombospondin type 1 motif 4 (*ADAMTS4*)

and *FCER1G* in cultured fibroblasts (Table S3, https://gtexportal.org/home/snp/rs2070902).

Within *HMNC2*, rs10988663 is a rare variant (with an allele frequency of 1% in European groups) leading the locus in chromosome 9. Although not achieving genome-wide significance, this locus is a potentially relevant risk factor with the T allele carrying an associated risk of 3.32-fold (95% CI: 2.15–5.14, $p = 6.07 \times 10^{-8}$). As rs2070902 was not an eQTL in GTEx v8, LDlink was queried and rs7028773 was identified as a variant in close LD ($r^2 = 0.798$). rs7028773 is associated with decreased expression of *HMNC2* in ovary tissue (NES = −1.3). This SNP was not mapped to any gene by positional mapping in FUMA, as it uses the Ensemble build 85 annotation. However, it maps to the 5′UTR region of the *HMCN2* gene.

A gene-based analysis achieved significant associations only at *FCER1G* and *SIGLEC5* with $p = 2.3 \times 10^{-9}$ and $p = 7.4 \times 10^{-8}$, respectively (Table S5).

Regional plots for the main risk signals (*FCER1G*, *HMCN2* and *SIGLEC5*) are shown in Figure S3.

3.2 | TWAS analyses and colocalization

Tissue-agnostic TWAS were conducted using the meta-analysis summary statistics and the MASH-R models trained with expression data from the GTEx V8 tissues. Gene expression was predicted and tested for association with PrediXcan in each of the tissues. The prioritized gene-tissue pairs are shown in Table S6.

For each pair, colocalization analyses between AgP and gene expression were performed to reduce potential false positives (Table S7). Sensitivity analyses are shown in Figure S3. *SIGLEC5* colocalized in stomach with the highest probability of sharing a single causal variant (PPH4 = 0.99) and robust results. Colocalization was

TABLE 1 Lead independent variants with $p < 1 \times 10^{-5}$ in the genome-wide association studies meta-analysis of aggressive periodontitis (AgP) of three European cohorts (Germany, Netherlands and Spain).

SNP	Chr:Pos	EA	NEA	*p*-Value	OR (95% CI)	GENE	EAF cases	EAF controls
rs2070902	1:161217875	T	C	1.02E−09	1.38 (1.24–1.52)	*FCER1G*	0.30	0.23
rs10988663	9:130265816	T	G	6.07E−08	3.32 (2.15–5.14)	*HMNC2*	0.02	0.01
rs150956098	10:37884551	G	A	5.19E−06	1.69 (1.35–2.13)	*ZNF248*	0.05	0.03
rs75527084	13:64244614	C	T	3.20E−06	0.44 (0.31–0.62)	*LINC00355*	0.01	0.02
rs7224672	17:27221716	G	A	4.31E−06	1.29 (1.16–1.45)	*MIR4522*	0.23	0.20
rs72832278	17:38334544	A	G	3.23E−06	0.63 (0.52–0.77)	*GPR179*	0.04	0.05
rs11084094	19:51616293	C	T	1.36E−07	1.27 (1.16–1.38)	*SIGLEC5*	0.53	0.48

Abbreviations: CI, confidence interval; chr:pos, chromosome and position (hg38); EA, effect allele; NEA, non-effect allele; OR, odds ratio.

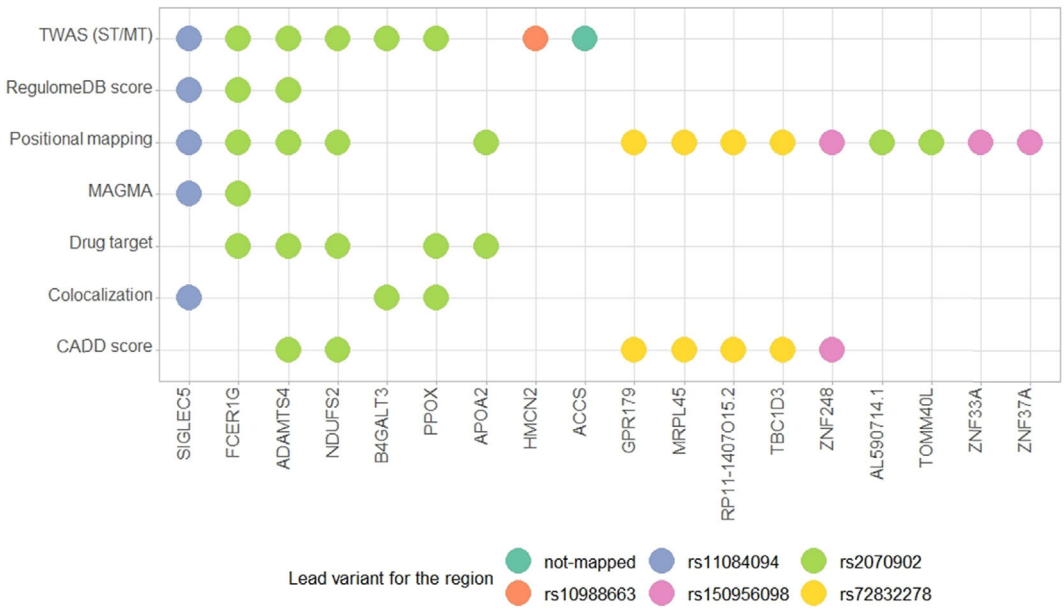

FIGURE 2 Prioritizing gene strategies for each AgP genome-wide association studies (GWAS) meta-analysis lead variant. Each dot represents which strategy prioritized each of the genes, which are sorted along the *X*-axis by rank. The colour scale maps each risk region to the corresponding gene through the GWAS lead variant, except for ACCS, which was not seen in the GWAS meta-analysis but showed significance in the transcriptome-wide association studies. The choice of using the lead variant is just for simplicity and does not imply its direct involvement in the strategy.

also obtained with expression of this gene in the aortic artery, left atrial appendage and sun-exposed skin. As for the risk region in chromosome 1, the best result was obtained with *B4GALT3* in mammary tissue with a PPH4 of 0.93, whereas in fibroblasts there was colocalization with *PPOX* (PPH4 = 0.88). No further genes colocalized according to our criteria, but it should be noted that COLOC assumes a single shared causal variant.

Results from each of the tissues were combined into a multi-tissue test using MultiXcan (Table S8). Significant associations were found for *NR1I3*, *FCER1G*, *B4GALT3*, *SIGLEC5*, *NDUFS2* and *ADAMTS4*.

3.3 | Gene prioritization

Gene prioritization strategies pointed out a total of 22 genes within the seven risk regions (Figure 2), leveraging information from several databases as well as gene expression. Although TWAS and colocalization are not independent, there are situations in which TWAS associations capture a correlation between traits without a shared causal variant (Hukku et al., 2022); thus, separate categories were maintained.

SIGLEC5 was the first, most frequent gene for the region in chromosome 19. It was associated at the gene level. Furthermore, it was mapped by positional mapping and gene expression data through TWAS analyses and colocalization results. The highest RegulomeDB rank score was achieved by rs3829655 (1f: eQTL/ca1TL + TF binding/chromatin accessibility peak).

For the risk region in chromosome 1, *FCER1G* and *ADAMTS4* were the first-ranked genes. Both were mapped by position and achieved a significant association in TWAS analyses. Maximum RegulomeDB rank scores were 1f for *FCER1G* in rs2070902 and 1f for ADAMTS4 in rs4233366. In addition, *FCER1G* was significant in the gene-based analysis, and a variant in LD with the *ADAMTS4* lead variant had a CADD score of 15.6 (rs33941127). The Drugbank and Therapeutic Target Database (TTD) reported Tinzaparin and US9206139, 5, respectively, as drugs targeting *ADAMTS4*. For *FCER1G*, TTD and Drugbank reported benzylpenicilloyl polylysine.

HMCN2 was prioritized only by TWAS analyses, but as discussed previously, the lead variant rs10988663 can be mapped to this gene by position.

4 | DISCUSSION

In this meta-analysis of three different GWAS on AgP in individuals ≤35 years of age (having stage III/IV severity and grade C), an association of rs2070901 within the *FCER1G* gene at a genome-wide significance level of $p < 5 \times 10^{-8}$ was identified. This SNP was earlier found to be suggestively associated with $p < 10^{-5}$ in a previous GWAS of AgP (Munz et al., 2017). The current increased statistical power allowed this association to gain genome-wide significance.

In the present study, no further variants reached genome-wide significance. However, six variants gained suggestive association with $p < 10^{-5}$, with the second strongest being within *HMCN2*. This association narrowly missed genome-wide significance and was not linked to PD before, therefore representing a new suggestive risk locus. The previously reported association within *SIGLEC5* showed the third strongest association signal.

FCER1G encodes an adapter protein that contains an immunoreceptor tyrosine-based activation motif (ITAM) involved in several signalling pathways, and it predominantly mediates allergic inflammatory signalling in mast cells. Moreover, it is a constitutive component of the interleukin-3 receptor complex, is involved in neutrophil and platelet activation and may function cooperatively with other activating receptors. Interestingly, *FCER1G* was among the top up-regulated genes involved in granulocyte chemotaxis in wounded mouse skin (Gharbia et al., 2023). Additionally, studies have demonstrated that ITAM-containing Fc receptor common gamma subunits are required for the maintenance of bone homeostasis through activating signals for osteoclastogenesis in mice (Koga et al., 2004) and that FcεRI-mediated activation in transfected rat basophil leukaemia cells is inhibited by *SIGLEC5* (Avril et al., 2005). Apart from being the most significant single-variant association, the strongest gene-based association also mapped to *FCER1G*.

Furthermore, the rare allele of the best associated SNP rs2070902, which is located in the first intron of *FCER1G*, is associated with increased expression of several genes in cultured fibroblasts of the GTEx panel. Apart from *FCER1G* itself, *NDUFS2*, *PPOX*, *ADAMTS4* and *B4GALT3* show differential expression depending on the rs2070902 alleles. In the present study, the strongest tissue TWAS signal referred to *FCER1G*, where a significant association of the GWAS signals with differential expression in the mucosal barrier organ vagina was observed. Like the gingiva, the vagina is a barrier organ. These interfaces between the body and the bacterial environment face comparable challenges in maintaining barrier stability, implying similar expression patterns in both organs. *FCER1G* also exhibited highly significant results in the multi-tissue TWAS. The eQTL effects of the lead SNP rs2070902 on the expression of *B4GALT3* and *ADAMTS4* are of special interest regarding the function of these genes in the context of periodontitis. *B4GALT3* encodes a galactosyltransferase involved in the synthesis of complex-type N-linked oligosaccharides in glycoproteins and plays a role in keratin and keratan sulfate (KS) metabolism. KS is one of the major glycosaminoglycans (GAGs) in periodontal tissues, attached to the core proteins fibromodulin and lumican, collagen-binding, leucine-rich proteoglycans that are known to regulate collagen fibrillogenesis (Y. Chen, Guan, et al., 2021). These have been shown to play a role in wound re-epithelialization and connective tissue regeneration during wound healing of the oral mucosa (Honardoust et al., 2008). Remarkably, these GAGs also play an important role in wound healing in atherosclerosis (Hultgardh-Nilsson et al., 2015). Concurrently, *ADAMTS4*, another gene with eQTL association of the *FCER1G* lead SNP rs2070902, encodes a protease that is responsible for the degradation of aggrecan, a large proteoglycan with attached KS chains, being one

of the major structural components of cartilage. Because of the different eQTLs, the target gene of this association is so far unclear and requires a more detailed investigation. Although speculative, the associated variants at *FCER1G* may mediate the disease risk through impaired regulation of the gene itself, possibly affecting wound healing or bone homeostasis, or, in addition or independently in different cell types, they regulate the expression of *B4GALT3* and/or *ADAMTS4*, with putative consequences for periodontal tissue repair via the KS synthesis pathway. Of note, while TWAS analyses can be a useful tool to link gene expression to a trait, recent work has raised concerns about the inflation of *p*-values (de Leeuw et al., 2023; Liang et al., 2023). Whereas early forms of severe periodontitis are not expected to be highly polygenic, caution is still required when considering these results.

The second strongest association is located in *HMCN2*. Hemicentins are a family of highly conserved secreted extracellular matrix proteins with only two members, HMCN1 and HMCN2 (Vogel & Hedgecock, 2001). The proteins exhibit the von Willebrand A and Nidogen G2 domains as well as immunoglobulin, EGF-like and Fibulin-type modules, typically characteristic of extracellular proteins involved in cell–cell adhesion (Argraves et al., 2003; Whittaker & Hynes, 2002). Hemicentins represent the largest among matricellular proteins. These connect the extracellular matrix and basement membrane with each other or with cell surface proteins and confer tissue-specific properties to the basement membrane. Specifically, matricellular proteins are up-regulated during tissue regeneration and wound healing, remodulating interactions between cells and the intracellular matrix (Midwood et al., 2004). Although the exact function of *HMCN2* in mammals is unclear, this suggests a role for *HMCN2* in tissue regeneration, a process that also includes wound healing. Likewise, it was shown that hemicentins are found in the pericellular extracellular matrix of several epithelial cells and blood vessels in mice, suggesting that they may contribute to the structure of adhesive and flexible attachments between epithelial cells, especially in tissues exposed to substantial mechanical strain (Xu et al., 2007). Furthermore, hemicentins were found to play a crucial role in tissue organization, assembling at the cleavage furrow of dividing cells and guiding cytokinesis in preimplantation mouse embryos. Interestingly, rs7028773, which is in strong LD with *HMCN2* lead SNP rs10988663, is associated with decreased expression with *HMCN2* in ovary tissue, one of the most dynamic human organs, with constant follicular growth and regression (Fan et al., 2019). Notably, in a study aimed at identifying causative mutations for severe adolescent-onset periodontitis, a highly deleterious missense mutation (rs1404827864, CADD = 29, gnomAD genomes European MAF = 0.00003) within *HMCN1* was detected by whole exome sequencing. This mutation was found in two affected children with stage III/IV-C periodontitis that was diagnosed at adolescent age (Richter et al., 2022), but not in a healthy sibling. Taken together, it is conceivable that *HMCN2* is an important factor in human tissue (re-)modelling and may play a role in periodontal tissue repair and wound healing.

In the present study, the established association of PD with variants within *SIGLEC5* was validated, with the strongest association for rs11084094 mapping to an ENCODE candidate cis-regulatory element with putative enhancer function in the last intron of the gene. Together with *FCER1G*, *SIGLEC5* was the only locus that showed significant association in the gene-based analysis with MAGMA. Indeed, in our meta-GWAS, eight SNPs within *SIGLEC5* passed the pre-assigned significance threshold of $p < 1 \times 10^{-5}$, among which the lead SNP rs4284742 from (Munz et al., 2017) associated with stage III, grade C periodontitis and the lead SNPs rs12461706 (Shungin et al., 2019) associated with less progressive and less early onset forms. Functional studies suggest a functional role for rs4284742 and rs11084095, which impair binding of MAF bZIP transcription factor B and ETS transcription factor ERG, respectively (Mueller et al., 2022). rs11084095 was also among the associated variants in the present meta-GWAS. Furthermore, within *SIGLEC5*, a deleterious rare frameshift mutation (rs149243374, CADD = 24.0, ExAc EUR MAF = 0.00455) was found by whole exome sequencing in a family of adolescent stage III, grade C periodontitis (Richter et al., 2022).

One limitation of our study was that for the Spanish samples, no information on smoking was available. Smoking is an important environmental confounding factor influencing the disease risk of periodontitis. According to combined data from the National Health and Nutrition Examination Survey for the years 2009–2012 (Eke et al., 2015), among adults aged 30 and older, 19% of current smokers had severe chronic PD according to the case definitions provided by the Centers for Disease Control and Prevention and the American Academy of Periodontology, while only 6% of non-smokers met the same criteria. Additionally, there is substantial evidence indicating that smokers in general experience more severe PD, including increased bone and tooth loss, as recently reviewed by Nociti et al. (2015). Smoking thus modifies disease risk and to some extent genetic effects (Freitag-Wolf et al., 2019), and should be accounted for in the statistical analyses, if possible. However, it is noteworthy that early onset forms have a stronger and better established heritable component, being less affected by environmental risk factors such as smoking. In our previous GWAS that included the German and Dutch samples, available smoking information allowed us to adjust for smoking status (Munz et al., 2017). Here, at the top significant loci, *p*-values were consistently only marginally lower in the smoking-adjusted analysis than in the unadjusted analysis: for example, for rs4284742 at the *SIGLEC5*, $p_{adj} = 3.7 \times 10^{-6}$, compared to $p_{unadj} = 3.6 \times 10^{-6}$. These results suggest that integrating information about smoking improves statistical power and makes true genetic findings more likely to be detected. Consequently, in the absence of this information, false-negative errors may occur more easily, but not false-positive errors. Moreover, it should be noted that regarding the greater heritable component, these effects appear to be rather small in early onset forms.

Although this study represents the largest combined GWAS of stage III/IV-C periodontitis with an age of first diagnosis ≤35 years to date, comprising 1306 cases in total, another limitation is its still relatively small sample size, as GWAS conducted for other complex diseases utilize hundreds of thousands of cases (e.g., >180,000 cases for coronary artery disease; Aragam et al., 2022). Regarding the high

heritability of the phenotype analysed in this study, which exceeds that of common complex diseases such as coronary artery disease, larger genetic effects can be assumed, which can be detected with smaller sample sizes. Likewise, in the current study, for the first time, an association of *FCER1G* variants at a genome-wide significance level was shown. Additionally, we found a novel suggestive susceptibility gene, *HMCN2*, which only marginally missed the genome-wide significance level.

5 | CONCLUSION

With this study, a further genome-wide association with severe early onset and aggressive periodontitis, having a stage III/IV severity and grade C, was identified. Additionally, this study provides novel evidence for suggestive association of *HMCN2* with this disease phenotype. The functions of the implicated genes underscore the importance of oral barrier tissue stability in severe periodontitis forms with an age of onset ≤35 years and may also imply the importance of tissue regeneration and remodelling in maintaining oral health aetiology.

AUTHOR CONTRIBUTIONS

Silvia Diz de Almeida, Gesa M. Richter, and Alicia de Coo contributed to data analysis, interpretation, and drafting of the manuscript. Søren Jepsen, Ines Kapferer-Seebacher, Henrik Dommisch, Klaus Berger, Matthias Laudes, Wolfgang Lieb, Bruno G. Loos, Nathalie van der Velde, Natasja van Schoor, Lisette de Groot, PerioGEN Cohort Group, Juan Blanco, and Angel Carracedo contributed to data acquisition. Raquel Cruz and Arne S. Schaefer contributed to conception and design, data acquisition, analysis and interpretation and drafting of the manuscript. All authors revised the work critically, gave final approval of the published version, and are accountable for all aspects of the work.

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CONFLICT OF INTEREST STATEMENT

Juan Blanco reports grants from Manohay Dental, during the conduct of the PerioGEN study, and grants from ITI, Intra-lock, Ticare, Straumann, and personal fees from Straumann, outside the submitted work. Henrik Dommisch reports personal fees from Oral-B, Straumman, Klockner, Dexcel, Dentaid and Colgate, and grants from Dentaid and Kulzer, outside the submitted work.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The PerioGEN study was approved by the ethics commission of the Research Ethics Committee of Santiago-Lugo, Galicia, Spain (number 2015/372, 25 June 2015). The GWAS of the German and Dutch samples were approved by the ethics commission of the medical faculty of the Christian-Albrechts-Universität zu Kiel, Germany (Vote for cases: A 156/03, Vote for controls: B 231/98). 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.

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SUPPORTING INFORMATION

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