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ORIGINAL ARTICLE

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Sex-specific genetic factors affect the risk of early-onset periodontitis in Europeans

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Abstract

Aims: Various studies have reported that young European women are more likely to develop early-onset periodontitis compared to men. A potential explanation for the observed variations in sex and age of disease onset is the natural genetic variation within the autosomal genomes. We hypothesized that genotype-by-sex (G × S) interactions contribute to the increased prevalence and severity.

Materials and methods: Using the case-only design, we tested for differences in genetic effects between men and women in 896 North-West European early-onset cases, using imputed genotypes from the OmniExpress genotyping array. Population-representative 6823 controls were used to verify that the interacting variables G and S were uncorrelated in the general population.

Results: In total, 20 loci indicated G × S associations ($P < 0.0005$), 3 of which were previously suggested as risk genes for periodontitis (*ABLIM2*, *CDH13*, and *NELL1*). We also found independent G × S interactions of the related gene paralogs *MACROD1/FLRT1* (chr11) and *MACROD2/FLRT3* (chr20). G × S-associated SNPs at *CPEB4*, *CDH13*, *MACROD1*, and *MECOM* were genome-wide-associated with heel bone

mineral density (*CPEB4*, *MECOM*), waist-to-hip ratio (*CPEB4*, *MACROD1*), and blood pressure (*CPEB4*, *CDH13*).

Conclusions: Our results indicate that natural genetic variation affects the different heritability of periodontitis among sexes and suggest genes that contribute to inter-sex phenotypic variation in early-onset periodontitis.

KEYWORDS

alveolar bone loss, gene × sex interaction, genetic risk, heritability, inflammation

Clinical Relevance

Scientific rationale for study: Natural variation within the autosomal genomes likely has different heritability among sexes, which contributes to inter-sex phenotypic variation and affects metabolic and physiological traits. This implies that sex can be considered an "environmental" variable, which interacts with the genetic architecture and can modify both penetrance and expressivity.

Principal findings: We searched for genes that contribute to inter-sex phenotypic variation in early-onset periodontitis and found 20 loci that indicated G × S associations, 3 of which had been previously suggested as periodontitis risk genes.

Practical implications: Our results indicate that natural genetic variation affects the different heritability of periodontitis among sexes

1 | INTRODUCTION

Epidemiological studies consistently report higher prevalence rates of a progressing and early-onset phenotype of periodontitis in African populations compared with Europeans (M. Saxby, 1984; M. S. Saxby, 1987; Loe & Brown, 1991; Melvin et al., 1991; Susin & Albandar, 2005; Levin et al., 2006). However, studies that analysed differences in disease prevalence between the biological sexes and stratified the populations for ethnicity often reported that White women were more likely to have the disease compared with White men (Hormand & Frandsen, 1979; Loe & Brown, 1991). Specifically, in the United States, a female to male ratio of 4:1 for disease prevalence was reported in Europeans and 0.5:1 in Black people (Melvin et al., 1991). A study that analysed 156 juvenile patients with periodontitis (12–32 years of age) according to sex and age found a female to male ratio of 5.3:1 in the young patients, which decreased to 2.4:1 in older juveniles and 1.5:1 in post-juveniles (Hormand & Frandsen, 1979), concluding that the disease might have an earlier onset among females. Differences between the sexes had been thought to be mainly due to sex hormone levels, which differ between males and females, but natural variation within the autosomal genomes likely has a different heritability among sexes, which contributes to inter-sex phenotypic variation and affects metabolic and physiological traits (Ober et al., 2008). This implies that sex can be considered an "environmental" variable, which interacts with the genetic architecture and can modify both penetrance and expressivity of a wide variety of genetic traits, which are encoded not only by the sex chromosomes but also by the autosomes (Weiss et al., 2006). Sexual dimorphism is observed in the prevalence, course, and severity of many common human diseases. Sex differences in the endocrine and immune systems contribute to these observations, but recent studies have shown the interaction of sex with the specific genetic architecture that influences human phenotypes, including reproductive, physiological, and disease traits. It is likely that an underlying mechanism is the differential gene regulation in males and females, particularly in sex-steroid-responsive genes (Ober et al., 2008). Accordingly, many examples of autosome-wide genome–sex interaction exist (Traglia et al., 2017). The identification of the genetic variability that influences genome–sex interactions has the potential to leverage the understanding of molecular mechanisms that may differ by sex. We consider this as an important category for the diagnosis and treatment of oral diseases. In this study, we hypothesized that G × S interactions contribute to the increased prevalence and severity of the rare disease phenotype, namely severe early-onset periodontitis, in European women, and searched for sex-specific genetic effects by applying a logistic regression model to the case-only approach (Piegorsch et al., 1994), which provides a remarkable gain in power over classical case–control analyses. A requisite of the case-only design is that the disease is rare, with a prevalence <5% in the general population. Particularly for these disease phenotypes, the case-only design provides a clear advantage in terms of statistical power for gene × environment interaction studies compared to the case–control design. However, we verified the interacting variables alleles and sex to be uncorrelated in the general population in a sample of 6823 controls.

2 | METHODS

2.1 | Study samples

We have previously described the cases in detail, which were formerly classified as aggressive periodontitis (AgP; Munz et al., 2017). In brief, 717 cases (302 men and 415 women; male to female ratio = 1:1.4)

TABLE 1 Association results of the G × S-associated loci with significant eQTLs on the nearest gene

Chr.	Lead SNP	*p*-Value lead SNP	Location, nearest gene	Strongest eQTL effect of lead-SNP, target gene, *p*-value (tissue)	GWAS lead SNP of other diseases or traits locate within the G × S-associated region (GWAS lead SNP of the specified diseases or traits also is the G × S associated SNP)
9	rs10982617	6.2×10^{-7}	Intronic, *DEC1*	*DEC1*, 1.5×10^{-19} (nerve)	None
9	rs11243957	6.4×10^{-6}	Intronic, *GFI1B*	*GFI1B*, 2.1×10^{-9} (testis)	Bipolar disorder (rs2905072), eosinophil, granulocyte counts, red cell distribution width (rs2073820)
11	rs475763	6.68×10^{-5}	Intergenic, *RPUSD4*	*RPUSD4*, 5.3×10^{-70} (blood)	Haemoglobin levels, white blood cell counts (rs2322192), estradiol plasma levels (rs10501858)
4	rs56400895	8.06×10^{-5}	Intronic, *ABLIM2*	*ABLIM2*, 6×10^{-8} (heart)	None
11	rs10833484	8.54×10^{-5}	Intronic, *NELL1*	*NELL1*, 4.5×10^{-22} (testis)	None
5	rs72812846	8.96×10^{-5}	Intronic, *CPEB4*	*CPEB4*, 9.8×10^{-198} (blood)	BMD (rs55646464, rs6861681, rs77822827)[a]
20	rs2423823	0.00012	Intronic, *MACROD2/FLRT3*	*MACROD2/FLRT3*, 0.000012 (kidney)	None
4	rs56007654	0.00023	<10 kb 5′ *CYTL1*	*CYTL1*, 2.3×10^{-47} (monocytes)	Lung function (rs6446312), height (rs6446315)
1	rs12739227	0.00024	Intergenic, *VAV3*	*VAV3*, 2.4×10^{-75} (blood)	none

[a]Waist-to-hip ratio (rs6897617, rs7705502, rs10516107, rs6861681); eosinophil counts (rs75049939); Crohn's disease (rs17695092, rs56163845, rs72812861); red blood cell count, systolic blood pressure (rs7705507, rs72812846), prion diseases, mean corpuscular volume, red cell distribution width.

TABLE 2 Association results of the G × S-associated loci with no significant eQTLs on the nearest gene

Chr.	Lead SNP	*p*-Value of lead SNP	Location, nearest gene	Strongest eQTL effect of lead SNP, target gene, *p*-value (tissue)	GWAS lead SNP of other specified diseases or traits locate within the G × S associated region (GWAS lead SNP of the specified diseases or traits also is the G × S-associated SNP)
1	rs7552089	7.81×10^{-7}	Intergenic, *PTCHD2*	*SLC39A3* $p = 5.7 \times 10^{-7}$ (macrophages)	None
2	rs11685689	7.23×10^{-6}	Intronic, *VIT*	None	BMI
12	rs1795818	1.85×10^{-5}	Exon, *GLYCAM1*	*PPP1R1A*, 1.8×10^{-11} (adipose)	None
16	rs403473	3.13×10^{-5}	Intronic, *CDH13*	*RP11-298D21.2*, 2.6×10^{-8} (brain)	Blood pressure (rs3096277), cognitive performance, IgG glycosyl
13	rs9514687	3.85×10^{-5}	Intronic, *FAM155A*	*EIF4E3*, 6.1×10^{-06} (liver)	None
20	rs79821641	5.62×10^{-5}	Intronic, *LOC101926889*	*ABHD12*, 2.7×10^{-09} (monocytes)	None
11	rs2701541	5.65×10^{-5}	Intronic, *MACROD1*, upstream *FLRT1*	*VEGFB*, 1.1×10^{-18} (heart)	Serum uric acid levels, intelligence, waist-to-hip ratio (rs11231693, rs11231694, rs2845885),triglycerides, HDL (rs11231698)
11	rs34150933	0.00020	<10 kb upstream *SLC37A4*	*VPS11*, 7.2×10^{-50} (nerve)	Post bronchodilator FEV1/FVC ratio, BMI, CAD, waist-to-hip ratio
3	rs75963875	8.17×10^{-5}	Intergenic, <30 kb upstream *MECOM*	None	COPD, BMD (rs13069567), cancer (rs75316749)
8	rs2630509	9.98×10^{-5}	Intronic, *CCDC26*	*PARP9*, 1.7×10^{-6} (liver)	Eosinophil counts
8	rs113586967	0.00020	Intergenic, *CSMD1*	*STX16*, 4.8×10^{-6} (macrophages)	Epstein–Barr virus, diabetic retinopathy

Abbreviations: BMI, body mass index; BMD, bone mineral density; CAD, coronary artery disease; COPD, chronic obstructory pulmonary disease.

were recruited across Germany by the biobank Popgen (University Hospital Schleswig-Holstein, Germany) and at the School of Dentistry of the Medical University Vienna, Austria. One-hundred and seventy-nine additional cases were collected at the Academic Centre for Dentistry Amsterdam (ACTA), The Netherlands (49 men, 130 women; male to female ratio: 1:2.7). The biological sex was taken as self-reported. The case sample had a median age of disease diagnosis of 31 years and consisted of patients with German family names, who were born and recruited in Germany and Austria, and of Dutch descent, determined by self-reports. The cases were diagnosed using full-mouth radiographs, and the selection criteria were as follows: >30% alveolar bone loss at >2 teeth, and ≤35 years of age. Genotypes were generated with the Omni Bead Chip (Illumina, USA) and imputed using the 1000 Genomes Phase 3 reference panel (build hg19/GRCh37).

2.2 | Statistical analyses

The statistical interactions between a genotype (G), specified as the number of alleles (additive allelic model) of a given single nucleodide polymorphism (SNP), and the biological sex (S, dichotomized as male or female) on periodontitis were investigated in the same manner as recently described by our group (Freitag-Wolf et al., 2019). The most important characteristics briefly summarized are as follows: we used logistic regression models following the case-only approach (Piegorsch et al., 1994), which provides a remarkable gain in power over classical case–control analyses. For example, assuming that neither gene nor smoking main effects are present and an allele frequency of 0.1, with a sample size of 900 cases, the power to detect an interaction odds ratio (OR) of 1.6 is >80% (calculated with a two-sided Wald's test at $\alpha = 0.05$, QUANTO, http://hydra.usc.edu/gxe). The underlying idea of the case-only approach is described in Appendix S1. The necessary assumption that the disease has a prevalence <5% in the general population is fulfilled because the prevalence of the selected disease phenotype is <0.1%. The SNPs of the imputed genotype data set (79,780,573 SNPs) were tested for G × S interaction in the cases of our recent genome-wide association study (GWAS, Munz et al., 2017). We excluded the genotypes of the X chromosomes because the hemizygous genotypes of the X chromosomes in the male cases did not allow application of our statistical model. Multidimensional scaling showed minimal evidence for population stratification. We assessed that the interacting variables, namely alleles and sex, are uncorrelated in the general population by testing the association of G and S in the controls of this GWAS ($n = 6823$). For this test, we modelled the factor G as the independent variable and the factor S as the response in the logistic regression but now, however, in the group of controls. All SNPs with p values <0.05 (Wald's test) were excluded from further analysis to ensure the validity of the results from the case-only analysis. Thus, each significant association between G and S in the cases suggests an underlying G × S interaction. For these SNPs, sex-specific ORs with 95% confidence intervals (CIs) from the case–control comparison were estimated to assess their influence on periodontitis. Subsequently, from the output of the G × S analysis, we selected SNPs with the following criteria: no correlation of the interacting variables in the general population; minor allele frequency >1%; p-value for association = p-meta

chr	position	lead SNP	EA
1	11459017	rs7552089	C
1	108077102	rs12739227	T
2	37037127	rs11685689	G
3	168760144	rs75963875	T
4	5026810	rs56007654	T
4	8144692	rs56400895	C
5	173377636	rs72812846	A
8	5164460	rs113586967	A
8	130426284	rs2630509	T
9	117982239	rs10982617	T
9	135843525	rs11243957	A
11	63865672	rs2701541	A
11	118911039	rs34150933	C
11	21279094	rs10833484	C
11	126043383	rs475763	T
12	55002053	rs1795818	C
13	108084992	rs9514687	A
16	83767219	rs403473	G
20	14550380	rs2423823	G
20	25172759	rs79821641	G

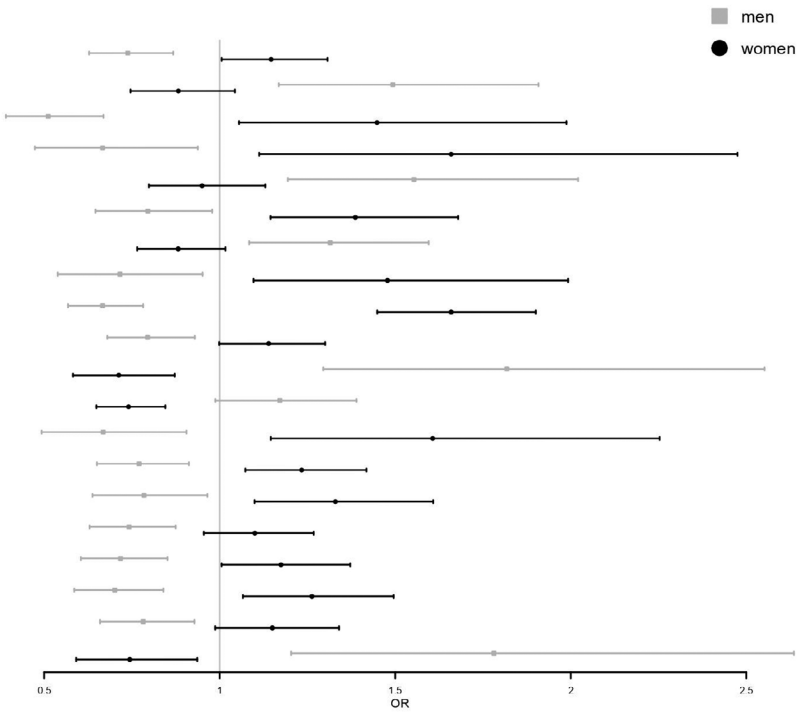

FIGURE 1 Main SNP effects for women and men for the G × S-associated loci. EA, effect allele, position in basepairs, Genome Assembly hg19

<0.0005 and *p*-meta < *p*-Germany/Austria (Ger; <0.005) < *p*-The Netherlands (NL) (<0.05), or *p*-meta = <0.0005 and *p*-meta < *p*-Ger (<0.0005) < *p*-NL (<0.1). The rationale for this a priori defined disease-associated cut-off followed the criteria of the GWAS catalogue as being indicative of suggestive evidence of association with disease (https://www.ebi.ac.uk/gwas/docs/methods/criteria). Since our study sample was smaller than the average GWAS case samples (albeit with a more severe and early-onset disease phenotype), we slightly relaxed the significance threshold for not losing an interesting association as a false negative. The SNPs that passed these criteria were subsequently checked for deviation from Hardy–Weinberg equilibrium (HWE) in the controls and for the imputation quality (average maximal > posterior probability < 0.9). Each association between G and S in the cases suggests an underlying G × S interaction. For these SNPs, sex-specific ORs with 95% CIs from the case–control comparison were estimated to assess their influence on periodontitis. Possible heterogeneity of the samples from Ger and NL was accounted for by combining both test statistics as previously described (Freitag-Wolf et al., 2019). Data management and statistical analyses were conducted using GenAbel (impute2databel: gtool –M –g –s, ProbABEL v. 0.4.4 function palogistic) (Aulchenko et al., 2010), PLINK v2.049 (Purcell et al., 2007), and R version 3.6.2. The associated variants are located in non-coding regions, suggesting a regulatory role by modulating steps along the gene expression cascade. The most intuitive way to elucidate functional relationships between regulatory variants and genes is to investigate the correlation between variant occurrence and gene expression. The resulting data type is referred to as "expression quantitative trait locus" (eQTL) if a variant (i.e., the QTL) influences mRNA levels. eQTLs are only statistical correlations but indicate putative correlation of a regulatory variant with the target gene. This is useful to give a clue for the putative context of an associated variant. We used the software tool QTLizer (Munz et al., 2020) to integrate SNP associations with the functional and clinical relevance of eQTL mappings, and used eQTL data sets from 166 tissue-specific QTL data sets integrated in the web application of Qtlizer (http://genehopper.de/qtlizer), as previously described (Munz et al., 2017).

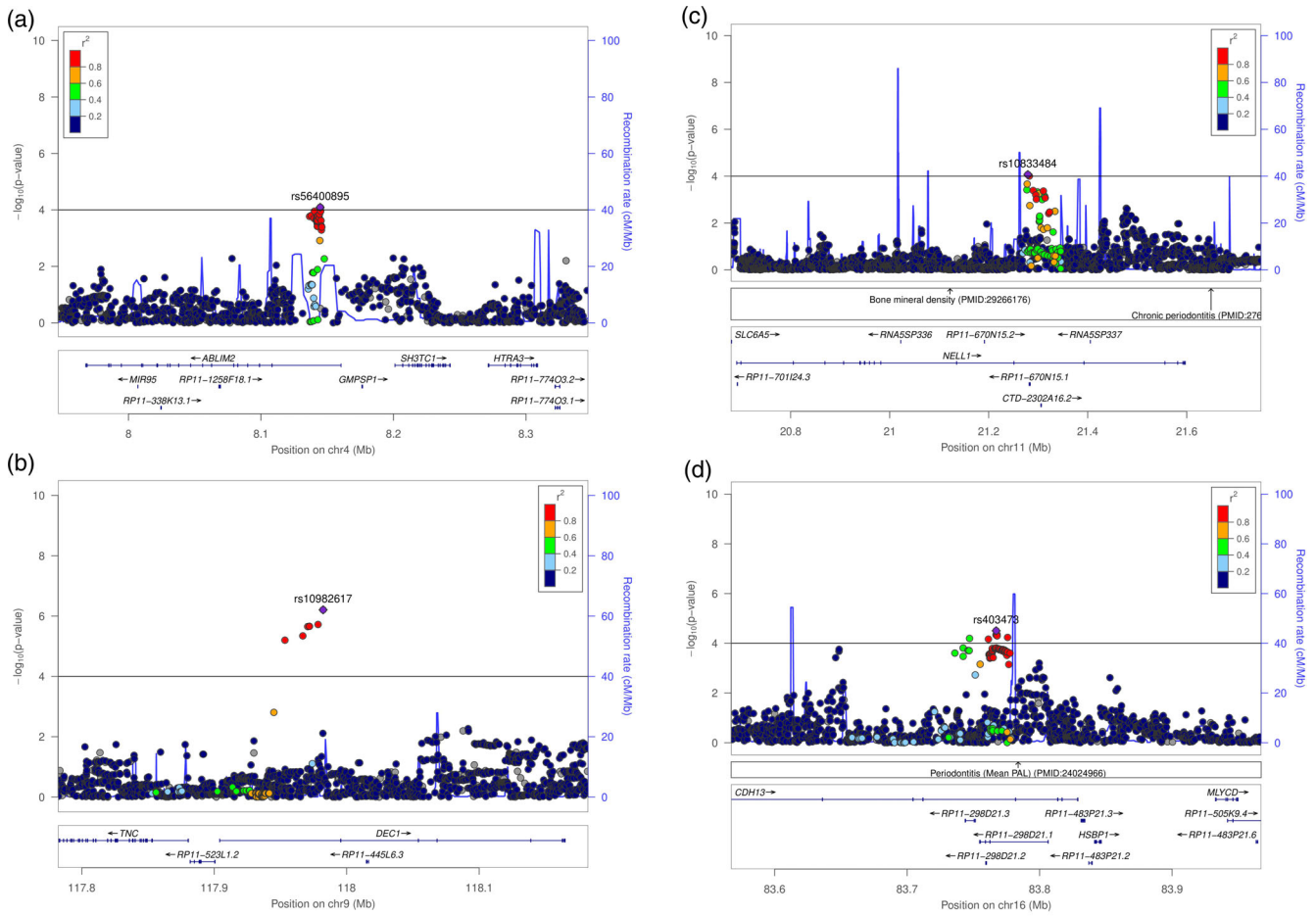

FIGURE 2 Regional association plots for the G × S-associated loci that were previously described to have a role in the aetiology of periodontitis. The associated haplotype blocks are aligned to the chromosomal positions. The G × S-associated SNPs were plotted with the *p* values of the interaction analysis. The SNPs that showed no G × S association at a *p*-value <0.05 were plotted with the *p* values of the case–control genome-wide association analysis. The horizontal line indicates the assigned G × S significance threshold of $p = 1 \times 10^{-4}$. The middle panel shows the positions for GWAS lead SNPs of other diseases and traits. (a) *ABLIM*, (b) *DEC1*, (c) *NELL1*, (d) *CDH13* (regional association plots of the other loci are shown in Figure 3 and Figure S1)

3 | RESULTS

3.1 | Genotype × sex interaction analysis

The G × S interaction analysis included 896 cases (545 women, 351 men), and the lead variants at 20 loci passed the pre-assigned cut-off for suggestive associations (Tables 1 and 2; Figure 1). For these SNPs, sex-specific ORs with 95% CIs from the case–control comparison were estimated to assess their influence on periodontitis (Table S1). Genotypes and minor allele frequencies are given in Table S2. Of the most significant SNPs at each of the 20 regions (lead SNPs), 11 were located in introns of protein-coding genes, 1 was in the intron of a long noncoding RNA, and 2 were <10 kb of the transcription start site of protein-coding genes. SNP rs10982617, located in an intron of *DEC1* (deleted in oesophageal cancer 1), showed the smallest *p*-value of 6.2×10^{-7}. Notably, we found two independent suggestive associations for the genes *MACROD1/FLRT1* (chr11) and *MACROD2/FLRT3* (chr20). Regional association plots for the G × S interacting loci are shown in Figures 2 and 3 and Figure S1.

SNPs that are located in introns, gene promoters, or intergenic regions presumably have regulatory effects on gene expression. Often, these effects act *in cis* on the nearest gene, and a strong cis-eQTL is a useful indicator for the target gene of the putative regulatory SNP. For this reason, we searched public sources of eQTL mappings to identify eQTL effects of the associated haplotype blocks (lead SNPs and variants in linkage disequilibrium with $r^2 > 0.8$; Tables S3 and S4). The most significant eQTLs were reported *in cis* for 9 of the 20 associated loci (*VAV3*, *CYTL1*, *ABLIM2*, *CPEB4*, *DEC1*, *GFI1B*, *NELL1*, *RPUSD4*, *MACROD2/FLRT3*; Table 1). The eQTL on *CPEB4* showed the smallest *p*-value of 9.8×10^{-198} (rs72812846). No cis-eQTLs were reported for the associated SNPs that were located on the introns or close to the promoter of

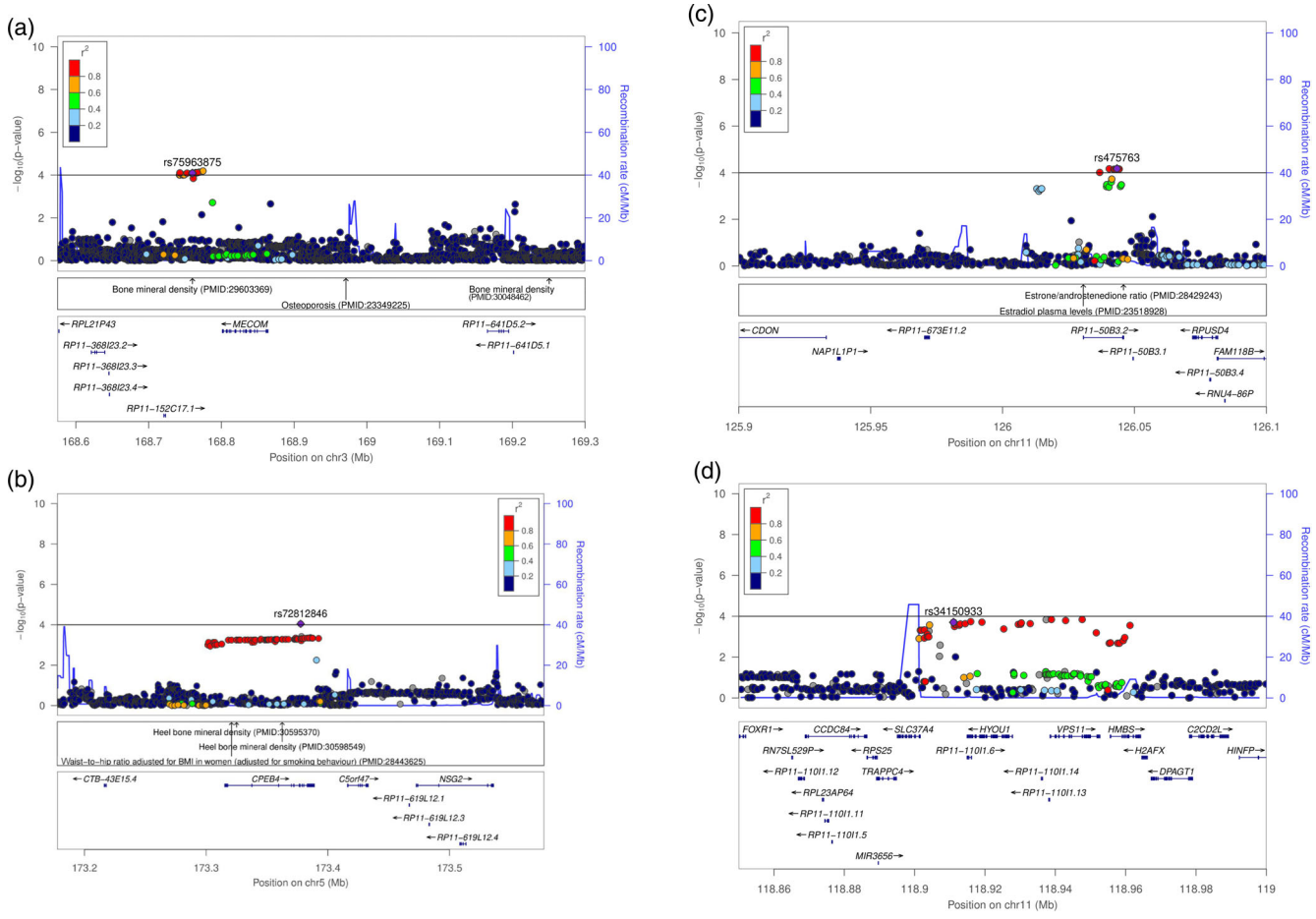

FIGURE 3 Regional association plots for the G × S-associated regions that were associated with periodontitis and heel bone mineral density, sex hormones, and waist-to-hip ratio. The associated haplotype blocks are aligned to the chromosomal positions. The G × S-associated SNPs were plotted with the *p* values of the interaction analysis. The SNPs that showed no G × S association at a *p*-value <0.05 were plotted with the *p* values of the case–control genome-wide association analysis. The horizontal line indicates the assigned G × S significance threshold of $p = 1 \times 10^{-4}$. The middle panel shows the positions for GWAS lead SNPs of other diseases and traits. (a) *MECOM* (BMD), (b) *CPEB4* (BMD, WHR), (c) *RPUSD4* (sex hormones), (d) *SLC37A4* (WHR), (e) *MACROD1* (WHR), (f) *MACROD2* (BMD). Pleiotropic SNP associations were reported for *MECOM* (PD, BMD), *CPEB4* (PD, BMD, WHR), and *MACROD1* (PD, WHR), see Table 1. BMD = bone mineral density, WHR, waist-hip-ratio. Regional association plots of the other loci are shown in Figure 2 and Figure S1

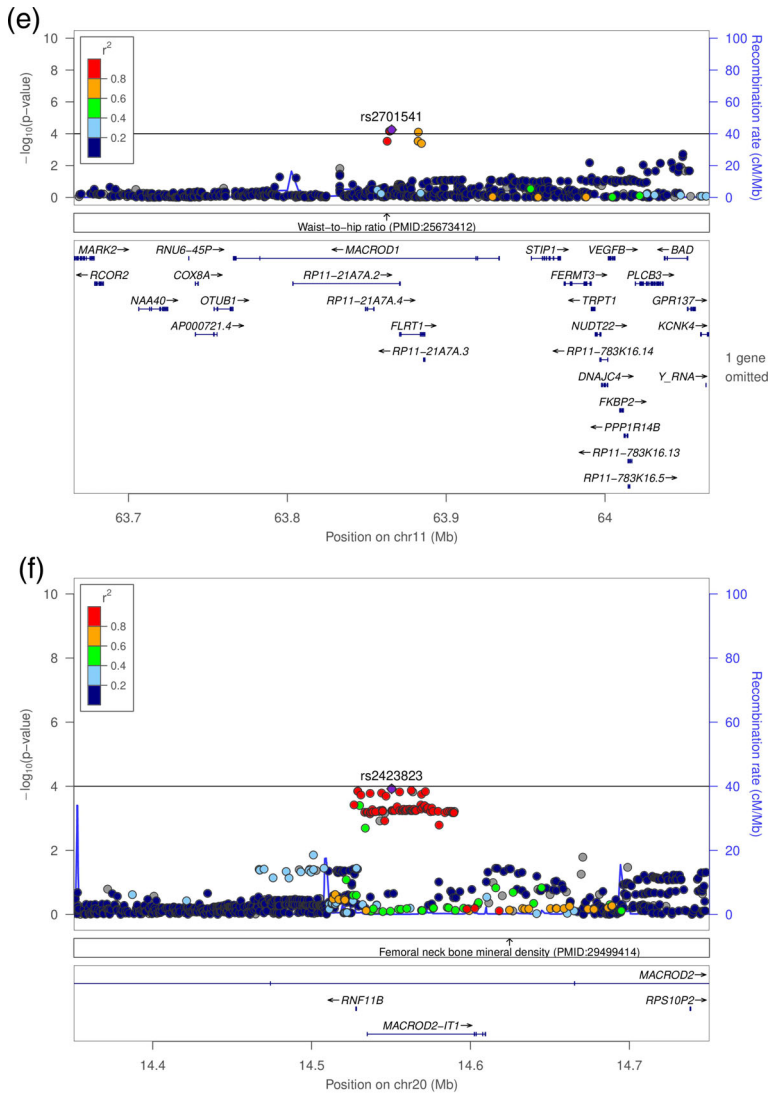

FIGURE 3 (Continued)

the genes *VIT*, *MECOM*, *CCDC26*, *SLC37A4*, *GLYCAM1*, *FAM155A*, *CDH13*, and *MACROD1/FLRT1* (Table 2).

In a previous G × S interaction study with 329 AgP cases and 983 controls and 287,224 unimputed SNPs (Affymetrix 500 K Arrays), we reported G × S interactions upstream of the gene neuropeptide Y (NPY; Freitag-Wolf et al., 2014). In the current case-only study with only partly overlapping cases, the lead SNP rs198712 showed association with $p = 0.01$.

3.2 | Pleiotropic context of the associated genes with other diseases and traits

In general, most disease-associated SNPs show pleiotropic effects. To classify the functional context of the associated genes, we analysed the NHGRI-EBI Catalogue of published GWASs (GWAS Catalogue) for genome-wide associations of these genes with other diseases and traits (Buniello et al., 2019). For most G × S-associated genes, as indicated by *cis*-eQTLs of the associated SNPs, numerous associations with other diseases were reported (Table 1 and 2, Tables S3 and S4). Some of the G × S-associated SNPs were also GWAS lead SNPs of other diseases and traits. Of these, G × S-associated SNPs at the gene *CPEB4* had the highest number of shared associations and were associated with heel bone mineral density, waist-to-hip ratio, Crohn's disease, systolic blood pressure, and blood cells counts. Other genes with G × S interacting SNPs that had also been reported to be GWAS-lead SNPs were *MECOM* (heel bone mineral density, cancer), *MACROD1* (waist-to-hip ratio, triglycerides, HDL), and *CDH13* (blood pressure).

Out of the nine G × S associations with significant cis-eQTL effects, the genes *ABLIM2* (Freitag-Wolf et al., 2019), *CDH13* (Teumer et al., 2013), and *NELL1* (Sanders et al., 2017) had been reported earlier to be associated with periodontitis. Associations with bone mineral density, a measure for osteoporosis risk, were reported for the genes *VAV3*, *CYTL1*, *CCDC26*, *MACROD1/FLRT1*, and *MACROD2/FLRT3*. *FAM155A* had a reported association with vitamin D levels.

4 | DISCUSSION

In this G × S interaction study of early-onset phenotypes of periodontitis in post-juveniles and young adults, we report 20 loci which carry specific alleles that modify disease susceptibility and expressivity of periodontitis in interaction with the variable sex. For women, to a larger part, the common alleles of the associated SNPs showed a risk-increasing effect. This is in line with epidemiological observations that European women have a higher rate of prevalence of periodontitis than men. All G × S-associated alleles showed statistically opposite effects. Effects with different magnitudes in the same direction very likely also exist, but they are expected to be smaller compared to opposite directions. Accordingly, the detection of same-direction effects is because they would require huge sample sizes, or extremely large effect sizes which are unlikely for a complex disease. Given the relatively small sample size, the discovery of same-direction effects are unlikely in our study. Importantly, opposite-direction effects are biologically plausible and are commonly observed in humans and across different classes and orders of animals (Sullivan et al., 2009; Seney et al., 2018; Camus et al., 2019).

The most significant association is located on the gene encoding for the transcription factor DEC1. For this gene, a function in the pathophysiology of periodontitis has been suggested (Hu et al., 2015). Furthermore, we re-discovered associations with the genes *ABLIM2*, *CDH13*, and *NELL1*, which were previously suggested to be associated with periodontitis (Teumer et al., 2013; Sanders et al., 2017; Freitag-Wolf et al., 2019).

Integration of eQTL effects with the associated variants suggested additional genes involved in the pathophysiology of periodontitis. The most significant eQTL showed a *cis* effect on the cytoplasmic polyadenylation element binding protein CPEB4. CPEs are found within the 3′ UTRs of mRNAs and function as docking sites for CPEBs, which repress or activate translation. CPEB4 activates translation by adding poly(A) tails to mRNAs to its target mRNA, for example, tissue plasminogen activator (Ortiz-Zapater et al., 2011). Genetic associations with periodontitis and plasminogen have been described earlier (Schaefer et al., 2015; Munz et al., 2019). Likewise, plasminogen knock-out mice develop highly progressive periodontitis (Sulniute et al., 2011). Furthermore, G × S-associated SNPs at *CPEB4* showed shared associations with waist-to-hip ratio (rs6897617, rs7705502, rs10516107, rs6861681), blood pressure (rs7705507, rs72812846), and bone mineral density (rs55646464, rs6861681, rs77822827). Notably, also rs13069567 at *MECOM* was associated in the G × S analysis and heel bone mineral density, which is a measure of osteoporosis. This bone disease is diagnosed when the skeletal bones lose bone density by resorbing too much and/or generating too little bone mass. Similar to early-onset periodontitis, the prevalence of osteoporosis varies between women and men and between different ethnicities (Cauley, 2011). European women are significantly more likely to develop osteoporosis than European men, or women of other ethnic backgrounds.

Cis-eQTLs also indicated *GFI1B*, *CYTL1*, *VAV3*, and *MACROD2/FLRT3* as candidate genes of the G × S associations. *GFI1B* has an important role in the production of thrombocytes (Monteferrario et al., 2014). CYTL1 (cytokine-like 1) is a positive regulator of chondrogenic differentiation (Kim et al., 2007). *VAV3* (Vav-3 guanine nucleotide exchange factor) was identified to be significantly differentially expressed in inflamed gingival tissue of periodontitis patients with fold change 1.66, $p = 3.59 \times 10^{-14}$ (Kebschull et al., 2014). It is assumed to be essential for osteoclast activation and bone density, because *VAV3*-knock-out mice show increased bone density and protection from bone loss induced by systemic bone resorption stimuli (Yu et al., 2016).

The associated region on chromosome 20 is located in intron 4 of the gene *MACROD2* (MACRO domain-containing 2) and upstream of the gene *FLRT3* (fibronectin leucine-rich transmembrane protein 3), nested in anti-sense orientation within intron 3 of *MACROD2*. Notably, our G × S interaction study additionally identified an association with intron 3 of gene *MACROD1* on chromosome 11. Again, nested in anti-sense orientation in intron 3 of *MACROD1* locates the gene *FLRT1*, which shares 55% amino acid sequence identity with *FLRT3*. The function of *FLRT1* is not yet characterized. However, *MACROD1* binds to the androgen receptor and amplifies its transactivation function in response to androgen. Androgen receptor activation seems to be an important factor in the regulation of bone homeostasis. In a rat model of ligature-induced periodontitis, it was shown that inhibition of androgen receptors significantly increased radiographic bone loss in females (Steffens et al., 2019). In the context of G × S associations, we note that our study identified associations at *MACROD1*, which are shared with GWAS lead SNPs for waist-to-hip ratio (rs11231693, rs11231694, rs2845885), HDL, and triglycerides (rs11231698). Waist-to-hip ratio was shown to be associated with estradiol concentrations in young women (Mondragon-Ceballos et al., 2015) and with Parkinson's disease (PD) but in both sexes (Meisel et al., 2019).

In a previous study, we reported a G × S association with a haplotype block upstream of the gene *NPY* with $p = 4 \times 10^{-6}$ (rs198712; Freitag-Wolf et al., 2014). Highlighting this region as a likely susceptibility locus of periodontitis, a GWAS reported SNP associations downstream from NPY with severe periodontitis (Divaris et al., 2013). In the current study, this G × S-associated haplotype block showed association with $p = 0.01$ (rs198712). The weaker association signal may be due to the less severe phenotype in the current study compared to the earlier one (>50% instead of 30% bone loss at >2 teeth; ≤35 years of age). Alternatively, the strong association of NPY in the previous study could be caused by the Winner's curse bias.

The limitation of the current study was the sample size, which was small compared to current GWAS standards. By using the case-only design, we strengthened the power to test our hypothesis. However, although we gained power, the genome-wide significance threshold could not be passed with our size-limited analysis sample. In this situation, replication is the gold standard of an association study. Here, it was not possible to replicate the findings with genotypes from an independent second case sample of the same disease phenotype because such a sample does not exist. Randomly splitting our sample into an explorative and a replication cohort would decrease the statistical power and correspondingly increase the likelihood of false positives selected for replication. Although the low number of SNPs that is tested in a replication of a split sample substantially lowers the significance threshold for the correction of multiple testing, and thus

increases the likelihood for a successful statistical replication, it would not give new information compared to that obtained from the full sample. Thus, for the benefit of reducing the discovery of false positives, we refrained from splitting the cases in an explorative and replication sample to allow Bonferroni correction of few selected SNPs in the replication. We think that the increased power allowed the discovery of true positive findings, which is indicated by the fact that out of the 20 selected loci that suggested G × S interaction within the entire set of >20,000 protein-coding genes that we investigated in this study, three loci had previously been reported to have associations with periodontitis (*ABLIM2*, *CDH13*, *NELL1*). Furthermore, we identified independent suggestive associations with the genes *MACROD1/FLRT1* and *MACROD2/FLRT3*, which are located on the different chromosomes 11 and 20, respectively. The independent associations of, among others, the related genes *MACROD1/FLRT1* and *MACROD2/FLRT3*, and of *MACROD1*, which has an established role in oestrogen signalling, suggest that specific genes harbour common alleles that affect the different heritability among sexes. We conclude that the identified genes contribute to inter-sex phenotypic variation in early-onset periodontitis.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

All authors contributed to drafting and revising the work critically, gave final approval of the published version, and are accountable for all aspects of the work. Sandra Freitag-Wolf and Arne S. Schaefer contributed to the conception and design of the work, data acquisition, analysis, and interpretation of data; Matthias Munz contributed to the conception and design of the work, data acquisition, analysis, and interpretation of data; Olaf Junge contributed to the analysis and interpretation of data; and Christian Graetz, Corinna Bruckmann, Ingmar Staufenbiel, Yvonne Jockel-Schneider, Wolfgang Lieb, Andre Franke, Bruno G. Loos, Søren Jepsen, and Henrik Dommisch contributed to data acquisition for the work.

ETHICS STATEMENT

The study was approved by the institutional review boards of the Christian-Albrechts University, Kiel. Participants' completion of the questionnaires constituted informed consent.

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