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SLC23A1 polymorphism rs6596473 in the vitamin C transporter SVCT1 is associated with aggressive periodontitis

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Abstract

Aim: Identification of variants within genes *SLC23A1* and *SLC23A2* coding for vitamin C transporter proteins associated with aggressive (AgP) and chronic periodontitis (CP).

Material and Methods: Employment of three independent case–control samples of AgP (I. 283 cases, 979 controls; II. 417 cases, 1912 controls; III. 164 cases, 357 controls) and one sample of CP (1359 cases, 1296 controls).

Results: Stage 1: Among the tested single-nucleotide polymorphisms (SNPs), the rare allele (RA) of rs6596473 in *SLC23A1* showed nominal significant association with AgP ($p = 0.026$, odds ratio [OR] 1.26, and a highly similar minor allele frequency between different control panels. Stage 2: rs6596473 showed no significant association with AgP in the replication with the German and Dutch case–control samples. After pooling the German AgP populations (674 cases, 2891 controls) to significantly increase the statistical power (SP = 0.81), rs6596473 RA showed significant association with AgP prior to and upon adjustment with the covariates smoking and gender with $p_{adj} = 0.005$, OR = 1.35. Stage 3: RA of rs6596473 showed no significant association with severe CP.

Conclusion: SNP rs6596473 of *SLC23A1* is suggested to be associated with AgP. These results add to previous reports that vitamin C plays a role in the pathogenesis of periodontitis.

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Key words: association; periodontitis; *SLC23A1*; *SLC23A2*; single nucleotide polymorphism; vitamin C

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Vitamin C is considered by the WHO as an essential ingredient of the human diet that has a demonstrated effect in the disease aetiology of periodontitis (WHO 2003). Severe vitamin C deficiency may result in scurvy-related periodontitis (Woolfe et al. 1980) as well as necrotizing periodontitis (Melnick et al. 1988). Vitamin C is an essential nutrient in humans, and as a highly effective antioxidant, acts to lessen oxidative stress (Frei 1991). It further performs numerous other physiological functions in the human body, including the synthesis of collagen. For the latter, it is a specific electron donor for various enzymes that participate in collagen hydroxylation (Levine et al. 2011). Vitamin C is also found in high concentrations in leucocytes, and it has been hypothesized to contribute to the ability of these cells to react to inflammatory stimuli (Boxer et al. 1979).

The plasma and tissue concentrations of vitamin C in the organism are tightly controlled on various levels, such as by uptake, tissue accumulation, and renal reabsorption. Vitamin C is actively transported across the cell membranes by the sodium-dependent vitamin C transporters SVCT1 and SVCT2, encoded by the genes *SLC23A1* and *SLC23A2*, which map to chromosome 5q23 and 20p12, respectively (Stratakis et al. 2000). Notably, it was reported that vitamin C plasma levels were decreased in periodontitis patients compared to healthy controls (Väänänen et al. 1993, Amarasena et al. 2005, Amaliya et al. 2007, Chapple et al. 2007, Kuzmanova et al. 2012), which could not exclusively be explained by different vitamin C concentrations in the diet of the patients and healthy individuals (Kuzmanova et al. 2012). Allelic variation of *SLC23A1* and *SLC23A2* may influence vitamin C uptake and may correlate to different uptake efficacies of vitamin C transporters. In this context, Cahill & El-Sohemy (2009) showed that vitamin C serum levels were influenced by genetic variation in *SLC23A1* and that the strength of the correlation between dietary vitamin C and serum vitamin C was modified by genetic polymorphisms within *SLC23A1* and *SLC23A2*. A more recent large meta-analysis comprising >15,000 individuals showed association of *SLC23A1* single nucleotide polymorphisms (SNPs) with lower plasma vitamin C concentrations (Timpson et al. 2010). Furthermore, it was reported that genetic variation within *SLC23A1* and *SLC23A2* was associated with several cancers and conditions, such as lymphoma (Skibola et al. 2008), colorectal adenoma (Erichsen et al. 2008), gastric cancer (Wright et al. 2009), human papillomavirus associated head and neck cancer (Chen et al. 2009), glaucoma (Zanon-Moreno et al. 2011), and preterm birth (Erichsen et al. 2006), indicating pleiotropic effects of the genetic variability of these genes.

Conflict of interest and source of funding statement
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Regarding the onset and progression of periodontitis, a number of risk factors were identified, e.g. smoking (Grossi et al. 1995, Bergström et al. 2000), diabetes (Lalla et al. 2007a,b), stress (Hugoson et al. 2002), the (subgingival) presence of certain periodontal pathogens such as *Porphyromonas gingivalis*, *Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans* (Papapanou et al. 1997, Van der Velden et al. 2006), as well as genetic susceptibility factors (Schaefer et al. 2013). During the last decade, awareness for a potential role of nutrition as an additional putative risk factor of periodontitis has increased (Van der Velden et al. 2011).

In this study, we hypothesized that genetic variants of the genes *SLC23A1* and *SLC23A2* were associated with increased risk of periodontitis. Since early-onset and highly severe disease phenotypes such as aggressive periodontitis (AgP) are generally more attributed to the effects of genetic risk loci than more moderate and late-onset phenotypes such as chronic periodontitis (CP), for the genetic exploration of these genes, and for the subsequent replication of our findings, we used three independent analyses populations of the currently worldwide largest case–control sample of AgP. A large sample of severe CP cases was subsequently used to test the putative associations for their role in the aetiology of CP.

Material and Methods

Study populations

Cases and controls used in the explorative step were described before (Schaefer et al. 2010b). In brief, inclusion criteria for the AgP cases of this panel were ≥2 teeth with 50% alveolar bone loss, ≤35 years of age and parents and grandparents born in Germany ($n = 283$). The controls were population representative individuals from the region of Kiel, Germany ($n = 578$) and blood donors from the University-Clinic Schleswig-Holstein, Kiel, Germany ($n = 401$).

Cases and controls of the German replication panel were described recently (Schaefer et al. 2013). In brief, inclusion criteria for the AgP cases of this panel were ≥2 teeth with 30% bone loss, ≤35 years of age and parents and grandparents born in Germany ($n = 417$). The controls were population representative males from the region of Kiel, Germany ($n = 1360$) and periodontitis-free individuals from the region of Munich ($n = 552$). Cases and controls of the Dutch replication panel were also described before (Schaefer et al. 2010a). In brief, inclusion criteria for the Dutch AgP cases of this panel were ≥2 teeth with 50% bone loss and ≤35 years of age. The Dutch controls were free of AgP and were recruited at the Sanquin Bloodbank, Amsterdam, the Netherlands.

The CP cases of this study had ≥7 affected teeth with 50% bone loss, >35 years of age and have been described in Schaefer et al. (2013) ($n = 1359$). The controls of the CP case–control sample were provided by the Cooperative Health Research in the Region of Augsburg Study (KORA), Bavaria, Germany ($n = 506$) and from the German longevity collection [$n = 790$ [$n = 393$, 94–99 years of age; $n = 397$, ≥100 years of age); Nebel et al. 2011].

A description of the characteristics of the study populations is given in Table 1. The study was approved by each institute's own ethical review board and all participants who donated DNA gave a signed informed consent.

DNA isolation and genotyping

Genomic DNA was extracted from frozen blood samples. All DNA samples were quality controlled on agarose gels. For stage 1, DNA was genotyped with Affymetrix 500K genotyping arrays as described before (Schaefer et al. 2010b). Randomly selected participants of KORA were genotyped on the Affymetrix SNP 6.0 genotyping array as previously described (Marzi et al. 2010). SNP rs6596473 was subsequently genotyped on 384-well plates using TaqMan assay hCV310271 on the TaqMan genotyping system (Applied Biosystems, Foster City, CA, USA) with an automated platform (Hampe et al. 2001) as previously described (Schaefer et al. 2009). As hidden duplicates, two pairs of control DNA from CEPH Individual GM07057 were used on these plates.

Statistical analysis

The genotypes of the Affymetrix 500K Genotyping Arrays and the TaqMan assay were analysed using the software PLINK v2.049 (Purcell et al. 2007). In the GWAS data set, SNPs with a genotype call-rate <90% or a minor allele frequency (MAF) <5% were excluded. In the TaqMan replication, the call-rate was >95%. Significance of association with single-locus genotypes was assessed using χ^2 tests and Fisher's exact tests for allelic 2×2 and genotypic 2×3 contingency tables. All markers were tested for deviations from Hardy–Weinberg equilibrium in controls before inclusion into the analyses ($p > 0.05$). Logistic regression analysis was performed in the R statistical environment, version 2.8.1 (http://www.r-project.org). Significance was assessed by a Wald test and by a likelihood-ratio test. p values ≤0.05 were considered as nominally significant. We used Akaike's information criterion (AIC) to choose the model that best explained the underlying associations. HapMap CEU genotypes were selected from the International HapMap project (http://www.hapmap.org, NCBI build 36). Correction for simultaneous testing of independent SNP associations was performed with Bonferroni thresholds that corresponded to an uncorrected significance level of 0.05. Statistical power (SP) calculations were performed using PS Power and Sample Size Calculations software (Dupont & Plummer 1998).

Results

Explorative genetic analysis of *SLC23A1* and *SLC23A2*

In the first stage of the study, we analysed the genetic regions of *SLC23A1* and *SLC23A2* using Affymetrix 500K genotyping arrays and successfully genotyped 271 AgP cases and 946 healthy controls. On this array design, *SLC23A1* was covered by three SNPs and *SLC23A2* was covered by 24 SNPs. At the chromosomal region of *SLC23A1*, the rare alleles of two SNPs showed a nominal significant association under the allelic genetic model (Table 2). To reduce the

Table 1. Characteristics of patients of the study populations

	Explorative		Replication 1		Replication 2		Replication 3	
	German AgP patients (n = 283)	German AgP controls (n = 979)	German AgP patients (n = 417)	German AgP controls (n = 1912)	Dutch AgP patients (n = 164)	Dutch AgP controls (n = 357)	German CP patients (n = 1359)	German CP controls (n = 1296)
Mean age (at diagnosis)	30 (±5)	51 (±13)	28 (±5)	48 (±12)	31 (±4)	30 (±5)	45 (±11)	NA
Gender								
Male	100	498	176	1703	44	156	561	649
Female	180	448	204	189	120	198	731	647
Unknown status	3	33	37	20	0	3	67	0
Smoking habits								
Smokers*	169	421	214	1095	128	143	708	NA
Non-smokers	101	521	132	675	30	205	651	NA
Unknown status	13	37	71	142	6	9	0	NA
Subjects (N) with teeth ≥ 50% bone loss								
2–6 teeth	128	NA	94	NA	101	NA	0	NA
≥7 teeth	139	NA	54	NA	63	NA	1359	NA
Not calculated†	16		269		0		NA	NA

*Include current and former smokers.
†Not calc., the number of patients of whom full mouth radiographs were not available for the complete set of teeth.
NA, not applicable; CP, chronic periodontitis.

chances of testing false positive associations in the replication, which were caused by random fluctuations of allele frequencies in our control samples, we compared the allele frequencies of our North-West European control sample with those of the HapMap CEU reference population, which has a similar genetic background. Consistent without genotype results, SNP rs7448941 was found to be monomorphic as well as in the HapMap CEU reference population. Likewise, the allele frequencies of SNP rs6596473 were highly similar in our control sample and the HapMap CEU sample (MAF = 28.7% and 29.2% respectively), but were enriched in our case sample (MAF = 33.6%; Table 3). This indicated that the association of this SNP, with $p = 0.026$ and an odds ratio (OR) = 1.26 (95% confidence interval [95% CI]) = 1.03–1.53), was carried by the case sample. In addition, this SNP was previously reported to be associated with lymphoma (Skibola et al. 2008). Both findings let us select SNP rs6596473 for replication in the two additional AgP case–control samples of German and Dutch descent. The rare allele of the third SNP rs10063949 showed nominal significant association with $p = 0.036$, OR = 1.37 (95% CI = 1.02–1.85; Table 2). Comparison of the MAF of rs10063949 between our case sample and the HapMap CEU reference sample showed no difference in the MAF to the cases, with 36.3% and 36.2%, respectively (Table 3). For this reason, we did not select this SNP for replication.

At *SLC23A2*, the rare alleles of three SNPs showed nominal significant associations with AgP, which were rs16990309, rs1519860, and rs1715395 (Table 2). These SNPs all showed a MAF ≤10% in our German controls (Table 3). Because in our case–control sample, the SP was limited to detect a true positive association at low frequencies (SP = 0.31; given a MAF of 10% in the controls and an OR = 1.5, which was observed for the SNP associations), we did not select these SNPs for replication in our AgP replication samples, because of a high probability of false positive association findings.

Table 2. Association statistics of the SNPs at *SLC23A1* and *SLC23A2,* which were analysed in the explorative study by Affymetrix 500K Genotyping arrays

Gene	SNP-ID	Affymetrix SNP-ID	Position (kb)	*p* value	OR (CI 95%)
SLC23A1	rs7448941	SNP_A-1816278	138,730	NA	NA
	rs6596473	SNP_A-2203296	138,739	**0.026**	1.26 (1.03–1.53)
	rs10063949	SNP_A-4248388	138,747	**0.036**	1.37 (1.02–1.85)
SLC23A2	rs6052935	SNP_A-4292979	4782	0.166	0.26 (0.03–2.01)
	rs16990301	SNP_A-1886829	4783	0.410	1.27 (0.72–2.24)
	rs16990309	SNP_A-2043017	4784	**0.016**	1.50 (1.08–2.08)
	rs6052943	SNP_A-1818498	4792	0.477	0.95 (0.69–1.19)
	rs6052946	SNP_A-1909273	4795	0.791	0.95 (0.62–1.43)
	rs6116571	SNP_A-4288712	4824	0.595	0.51 (0.02–12.45)
	rs6052961	SNP_A-2030662	4827	0.237	0.89 (0.73–1.08)
	rs1715377	SNP_A-4298680	4827	0.979	1.00 (0.75–1.34)
	rs1776964	SNP_A-1967515	4828	0.156	1.15 (0.95–1.38)
	rs1715382	SNP_A-2247126	4832	0.265	1.23 (0.85–1.77)
	rs4815754	SNP_A-2007242	4834	0.711	1.05 (0.81–1.37)
	rs1715385	SNP_A-1967516	4836	0.377	0.92 (0.76–1.11)
	rs1715365	SNP_A-2007243	4847	0.573	0.93 (0.71–1.21)
	rs1715367	SNP_A-4208767	4849	0.221	1.13 (0.93–1.36)
	rs1715372	SNP_A-1967517	4859	0.114	1.16 (0.96–1.40)
	rs6052972	SNP_A-2101841	4867	0.258	0.89 (0.73–1.09)
	rs6052974	SNP_A-1804179	4868	0.595	1.2 (0.1–29.0)
	rs1519860	SNP_A-1967518	4871	**0.014**	1.59 (1.09–2.30)
	rs1715395	SNP_A-2217474	4880	**0.042**	1.44 (1.01–2.06)
	rs16990455	SNP_A-1924621	4885	1.000	0.00 (0.00–nan)
	rs6053021	SNP_A-2029963	4921	0.662	1.05 (0.85–1.30)
	rs6139606	SNP_A-4294929	4925	0.635	1.05 (0.85–1.30)
	rs4815759	SNP_A-1791146	4928	0.759	0.97 (0.78–1.20)
	rs6084957	SNP_A-2021557	4929	0.790	0.97 (0.78–1.20)

NA, not applicable (monomorph); OR, odds ratios; CI, confidence interval; kb, kilobasepair; SNP, single nucleotide polymorphisms. Values in bold indicate SNPs that showed significant associations with AgP.

Replication

The original explorative German AgP cases were genotyped again with TaqMan assay (Table 4). Similarly as with Affymetrix 500K genotyping arrays, the MAF of rs6596473 was significantly more prevalent in cases ($p = 0.011$, OR = 1.42 [95% CI = 1.08–1.87]). Next we replicated the association of *SLC23A1* SNP rs6596473 in a second German AgP sample of 403 cases and 1912 controls. The association was not significant with a MAF = 32.6% in the cases and a MAF = 31.2% in the controls (Table 4). However, for the AgP cases of this sample we noticed the same trend towards enrichment of the minor allele and a reduction in the common allele. This was most obvious for the more frequent heterozygous individuals and for the homozygous carriers of the common allele (Table 4).

Likewise, replication in our Dutch AgP sample of 156 AgP cases and 357 healthy controls showed no significant association of the rare allele of this SNP. Comparison of the allele frequencies between the Dutch AgP cases and controls showed no trend towards enrichment of the minor allele or reduction in the common allele in this smallest case sample. We observed up to 10% difference in the frequencies of homozygous carriers of the common allele between the Dutch and German AgP cases and the controls (Table 4).

To increase the statistical power, we pooled the two German AgP case–control panels. The association signal was nominal significant in this pooled sample of 674 AgP cases and 2891 controls, with $p = 0.017$, OR = 1.23 (95% CI = 1.04–1.45; Table 4).

Next, we performed an association analysis with the most severe German AgP cases (≥7 affected teeth with 50% bone loss; $n = 193$) and the same German controls as were used in the pooled analysis ($n = 2891$). This analysis showed the most significant association, with $p = 0.015$, OR = 1.31 (95% CI = 1.05–1.62). The MAF in AgP cases were enriched compared to the controls with 36.3% and 30.4%, respectively (Table 4).

Covariate adjustment

We next tested the independence of the observed associations of the covariates smoking and gender in a logistic regression analysis. A large part of the controls of the German AgP replication sample consisted of males (71%); therefore, adjustment for the covariate gender would have introduced a bias to the analysis because gender was not an independent covariate in this sample. Thus, we adjusted the complete pooled German AgP sample only for the covariate smoking. Upon adjustment for smoking, the association was borderline significant under the recessive genetic model, with $p = 0.041$, OR = 1.21 (95% CI 1.01–1.44; Table 5). This genetic model was also suggested by the AIC to best explain the underlying association (Table 5). However, since gender generally attributes a large effect in genetic studies in periodontitis, we next adjusted for both the covariates smoking and gender with the pooled German AgP cases ($n = 688$ cases) and those German controls, which were sampled with no bias for

Table 3. Genotypes and allele frequencies of SNPs, which showed nominal significant association in the explorative analysis

Gene	SNP	Population	AgP cases					Controls				
			11 *n* (%)	12 *n* (%)	22 *n* (%)	MAF%	Total *n*	11 *n* (%)	12 *n* (%)	22 *n* (%)	MAF%	Total *n*
SLC23A1	rs10063949	German	112 (40.0)	133 (47.5)	35 (12.5)	36.3	280	239 (47.8)	202 (40.4)	59 (11.8)	32.0	500
		HapMap CEU*						37.5	52.7	9.8	36.2	224
	rs6596473	German	120 (42.4)	136 (48.1)	27 (9.5)	33.6	283	501 (51.2)	394 (40.3)	84 (8.6)	28.7	979
		HapMap CEU*						49.6	42.5	8.0	29.2	226
SLC23A2	rs16990309	German	177 (72.2)	66 (26.9)	2 (0.8)	14.3	245	388 (81.0)	86 (18.0)	4 (1.0)	10.0	478
		HapMap CEU*						77.7	20.5	1.8	12.1	224
	rs1519860	German	237 (84.9)	41 (14.7)	1 (0.4)	7.7	279	874 (90.1)	95 (9.8)	1 (0.1)	5.0	970
		HapMap CEU*						95.5	4.5	0	2.2	224
	rs1715395	German	237 (83.7)	46 (16.3)	0 (0)	8.1	283	868 (88.7)	109 (11.1)	2 (0.2)	5.8	979
		HapMap CEU*						94.7	3.5	1.8	1.7	226

*HapMap CEU (Utah residents with Northern and Western European ancestry, the genotype is given in percentage).
1, major allele; 2, minor allele; AgP, aggressive periodontitis; OR, odds ratios; N, number of subjects; MAF, minor allele frequency; SNP, single nucleotide polymorphisms.

Table 4. Association statistics of rs6596473 prior to covariate adjustment, genotypes and allele frequencies of the different case–control populations

Study population	*p* value	OR (95% CI)	AgP Cases					Controls				
			11 *n* (%)	12 *n* (%)	22 *n* (%)	MAF%	Total *n*	11 *n* (%)	12 *n* (%)	22 *n* (%)	MAF%	Total *n*
Explorative (German)	0.011*	1.42 (1.08–1.87)	115 (42.4)	128 (47.2)	28 (10.3)	33.9	271	501 (51.2)	394 (40.3)	84 (8.6)	28.7	979
Replication 1 (German)	n.s.	1.07 (0.91–1.26)	177 (43.9)	189 (46.9)	37 (9.2)	32.6	403†	899 (47.0)	832 (43.5)	181 (9.5)	31.2	1912
Replication 2 (Dutch)	n.s.	0.99 (0.74–1.32)	82 (52.6)	58 (37.2)	16 (10.3)	28.9	156	184 (51.5)	138 (38.7)	35 (9.8)	29.1	357
German pooled	0.017*	1.23 (1.04–1.45)	292 (43.3)	317 (47.0)	65 (9.6)	33.2	674	1400 (48.4)	1226 (42.4)	265 (9.2)	30.4	2891
German pooled most severe cases	0.015**	1.31 (1.05–1.62)	80 (41.5)	86 (44.6)	27 (14.0)	36.3	193‡	1400 (48.4)	1226 (42.4)	265 (9.2)	30.4	2891

Genotypes were generated by the TaqMan assay hCV310271.
Genetic models giving the smallest *p* value: *allele positivity model [11]<–>[12 + 22]; **allele frequency difference model [1]<–>[2].
†From 417 German AgP cases 403 cases were successfully genotyped.
‡139 cases from exploration stage and 54 cases from replication 1 stage with ≥7 teeth with ≥50% bone loss.
1, major allele; 2, minor allele; AgP, aggressive periodontitis; OR, odds ratios; CI, confidence intervals; N, number of subjects; MAF, minor allele frequency; n.s., not significant.

gender ($n = 946$). In this analysis, again the recessive genetic model showed the smallest p value, with $p = 0.005$, OR = 1.35 (95% CI = 1.1–1.7). This genetic model was also suggested by the AIC to best explain the underlying association (Table 5). This association remained significant upon Bonferroni correction for multiple testing (seven independent tests).

We next adjusted the German AgP cases with the highest severity (≥7 teeth affected with >50% bone loss, $n = 193$) for gender and smoking, using the same controls as described in the previous paragraph ($n = 946$). In this analysis, the additive model showed a significant association of the rare allele with $p = 0.0213$ and a genetic effect of OR = 1.34 (95% CI = 1.04–1.71). The additive genetic model showed the smallest AIC values among the different genetic models tested, suggesting it best explained the observed association.

Validation of the rs6596473 association in severe chronic periodontitis

We subsequently tested the association of rs6596473 in a large independent case–control panel of severe CP patients of German descent ($n = 1359$ severe CP cases, 1296 independent geographically matched controls). SNP rs6596473 gave no statistical significant evidence for association with CP, with a minor trend towards enrichment of the rare allele of rs6596473 in the CP cases (MAF = 30.8%) compared to the controls (MAF = 29.1%; Table 6).

Discussion

At present, vitamin C is considered by the WHO as the most convincing evidence linking diet to periodontal disease (WHO 2003). Previous studies indicated that allelic variation of the vitamin C transporter genes *SLC23A1* and *SLC23A2* may influence vitamin C absorption and may be correlated to individual variation in vitamin C uptake efficiencies (Cahill & El-Sohemy 2009, Timpson et al. 2010). In this study, we tested the hypothesis that genetic variation of these genes might be associated with an increased risk of periodontitis. Thus, we applied a candidate gene approach towards our previous GWAS data set for exploration.

We observed a significant association of the rare C allele of SNP rs6596473 of *SLC23A1* with AgP in the German explorative case–control sample, as well as in the pooled German AgP sample and the sample that included the most severe cases only. No association was found in the Dutch and in the German replication samples alone. However, the latter and larger replication sample showed a trend towards enrichment of the rare allele in the cases. Given that the association of the rare allele of rs6596473 is not a type I error, the most likely explanation for the missing replication is a lack of SP. Likewise, in the Dutch AgP case–control sample we were able to reject the null hypothesis of no association with a probability of SP = 23%, given the observed MAF of 30% in the controls and an estimated genetic effect of OR = 1.3. In the larger German replication panel, the SP was higher (60%), but not sufficient. The increased SP in the German replication sample could explain the observed trend of enrichment of the rare allele in the cases. The fact that the German explorative panel showed a significant association of the rare C allele, albeit smaller than the German replication panel, might be explained by the more severe AgP phenotype of the exploration sample. In this sample, all cases had >50% bone loss at ≥2 affected teeth, whereas the inclusion criterion of the German replication sample was >30% bone loss at ≥2 affected teeth. This explanation was supported by the analysis of the most severe German AgP cases (>50% bone loss at ≥7 affected teeth, $n = 193$), which showed a positive association, although this sample was very limited in the number of cases. Further support for a true positive association is that the pooled German AgP sample, which had sufficient statistical power (SP = 0.81), showed a positive association. However, our analysis population was not large enough to replicate this finding in a sufficiently sized independent AgP case–control sample. Thus, the proposed association of SNP rs6596473 of *SLC23A1* should yet be regarded as suggestive. Clearly, the current findings need to be replicated in independent study populations; heterogeneity of the current study samples also limits conclusive evidence.

We showed the association of SNP rs6596473 to be independent of the covariates smoking and gender. The adjustment for smoking alone, for which we were able to use a larger control sample, showed a larger p value compared to the adjustment for smoking and gender. It could be that the larger control sample revealed a bias in the smaller control sample. However, because the effect of gender is considered large in periodontitis, it most likely acted as a confounder in the logistic regression analysis that considered smoking alone and as a consequence stratified the analysis despite of the larger control sample. Thus, we consider the reported p value and OR of the pooled German AgP sample, that was adjusted to smoking and gender, as best to describe the association of rs6596473 with AgP.

Table 5. Association statistics of rs6596473 upon covariate adjustment

Covariate	Study population	Additive genetic model			Recessive genetic model			Controls (n)	Cases (n)
		p	OR (95% CI)	AIC	P (rec)	OR (95% CI)	AIC		
Smoking	German (pooled)	0.099	1.12 (0.98–1.28)	3127	**0.041**	1.21 (1.01–1.44)	3125	2891	674
Smoking, gender	German (pooled)	**0.021**	1.21 (1.03–1.43)	1975	**0.005**	1.35 (1.10–1.67)	1973	946	674
Smoking, gender	German (most severe cases)	**0.021**	1.34 (1.04–1.71)	922	0.058	1.38 (0.99–1.94)	924	946	193

Additive genetic model analysed by Armitage Test for trend, recessive genetic model [11 + 12]<–>[22].
AIC, Akaike's information criterion; OR, odds ratios; CI, confidence intervals.
Values in bold indicate SNPs that showed significant associations with AgP.

Table 6. Association statistics of rs6596473 in the case–control sample of CP

Study population	p (Allelic)	OR (95% CI)	CP Cases					Controls				
			11 *n* (%)	12 *n* (%)	22 *n* (%)	MAF (%)	Total *n*	11 *n* (%)	12 *n* (%)	22 *n* (%)	MAF%	Total *n*
German CP	n.s.	1.08 (0.96–1.22)	658 (48.4)	565 (41.6)	136 (10.0)	30.8	1359	654 (50.5)	529 (40.8)	113 (8.7)	29.1	1296

1, major allele; 2, minor allele; CP, chronic periodontitis; OR, odds ratios; CI, confidence intervals; N, number of subjects; MAF, minor allele frequency; n.s., not significant.

The AIC values in the sub-population analysis of the most severe cases, proposed an allelic or additive genetic model to best explain the observed association, but a recessive model for the pooled and on average less severe cases. This implies that in the most severe cases, the effect is observable in both heterozygous and homozygous individuals, whereas the effect is observable in the less severe cases in homozygous carriers of the risk allele only. On the basis of this observation we hypothesize that the effect of the risk allele rather may contribute to the severity in the course of aggressive periodontitis, once it is established by other factors.

The intention of this study was to elucidate, whether the investigated genes carry genetic variants that are associated with increased disease susceptibility. We did not comprehensively investigate the genetic regions of the vitamin C transporter genes *SLC23A1* and *SLC23A2*. Thus, we have no information if other risk variants show similar or stronger associations with the disease. Furthermore, we did not replicate the associations of the low frequent SNPs of *SLC23A2*, which were nominally significant in the explorative analysis. Thus, we have no information whether *SLC23A2* carries putative risk alleles of periodontitis.

We conclude that SNP rs6596473 of *SLC23A1* is associated with aggressive periodontitis and that our study points to the relevance of vitamin C in oral health. Lower vitamin C levels have been observed in several studies on periodontitis and it is clear that vitamin C, as an essential nutrient, plays a crucial role in the pathogenesis of this disease (reviewed by Van der Velden et al. 2011). One of the explanations for lower vitamin C in periodontitis might be related to the genetic differences in transporter genes between patients and controls, such as suggested in this study. Future research will be focused on the role of vitamin C in the extent and severity of periodontitis.

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Clinical Relevance

Scientific rationale for the study: Studies showed that on average, individuals with periodontitis have lower plasma concentrations of vitamin C. Vitamin C is actively transported across membranes by the two sodium-dependent vitamin C transporter proteins SVCT1 and SVCT2, which are encoded by the genes *SLC23A1* and *SLC23A2*. Genetic variants might be associated with increased disease risk.
Principal findings: We identified single nucleotide polymorphism rs6596473 in *SLC23A1* to be associated with aggressive periodontitis prior to and upon adjustment to the covariates smoking and gender in a German population.
Practical implications: rs6596473 is associated with aggressive periodontitis. This association points to the relevance of vitamin C in oral health.