

Relationship of vitamin D receptor gene polymorphisms in *Helicobacter pylori* gastric patients

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Purpose: The present study aimed to investigate the association between the human *VDR* gene and *Helicobacter pylori* infection.

Patients and methods: A cross-sectional study was conducted on 208 adult patients with upper gastrointestinal symptoms. Gastric biopsy specimens were obtained from each patient for molecular DNA and histological examination. Patients were genotyped for *VDR* gene polymorphisms using polymerase chain reaction and restriction fragment length polymorphism analysis.

Results: The allelic and genotypic distribution analyses of the *FokI*, *ApaI* and *TaqI* polymorphisms of the *VDR* gene did not show distribution differences between *H. pylori*-positive and -negative groups. The genotype distribution observed for polymorphism *BsmI* deviated significantly from what was expected in a Hardy–Weinberg equilibrium test in the *H. pylori*-positive group ($\chi^2=29.048$, $p<0.001$). The distribution of *BsmI* genotypes differed significantly between the *H. pylori*-negative and *H. pylori*-positive groups ($p=0.0034$), where the frequency of the bb genotype increased among *H. pylori*-positive individuals compared with those without infection (63.25% versus 50.55%, respectively). Conversely, the *H. pylori*-negative group showed a Bb frequency that was 20.27% higher than in the infected group.

Conclusion: We identified a possible association between the *BsmI* polymorphism and infection by *H. pylori*. However, further research is required to clarify this relationship.

Keywords: vitamin D, VDR, polymorphism, *Helicobacter pylori* virulence factors, infectious disease, VDR SNPs, admixed population

Introduction

Helicobacter pylori is a spiral-shaped Gram-negative bacterium that, despite the adverse conditions in the human stomach, shows many mechanisms and specific virulence factors that affect the properties of the gastric mucosa and determine its adhesion and survival in such an environment.¹

Long-term colonization by *H. pylori*, with a posterior chronic gastritis induction, depends on the strains living in the gastric mucosa and how they interact with the host's immune system.² Moreover, human genetic polymorphisms also partly contribute to the susceptibility and clinical evolution of the infection. They can produce different expression levels of immune system mediators related to infection rates, *H. pylori* eradication, colonization by more virulent strains, gastric inflammation enhancement and cell damage.³

It was recently proposed that infection by *H. pylori* can induce an improvement in vitamin D receptor (VDR) expression in gastric epithelia. It was observed in vitro that the active form of vitamin D, $1\alpha,25$ -dihydroxyvitamin D₃, exhibits immune modulators

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properties against this pathogen, via stimulation of cathelicidin antimicrobial peptides and decreasing inflammatory cytokine and chemokine levels.⁴

The gene responsible for VDR expression is located on the 12q13.11 chromosome and comprises a region of >60 kb of DNA.⁵ From the single nucleotide polymorphisms (SNPs) of this gene that have been described so far, the restriction fragment length polymorphism (RFLP) for *FokI*, *BsmI*, *ApaI* and *TaqI* restriction enzymes is currently used in studies of genetic susceptibility to infectious diseases.^{6–8}

Of these variants, the *FokI* polymorphism (rs2228570; C>T), characterized by the substitution of thymine to cytosine (ATG to ACG), changes the protein structure of the VDR receptor, which in the presence of the mutant allele C or F produces a protein with 424 amino acids instead of 427 amino acids.¹⁰ In contrast to the *FokI* variant, the *TaqI* polymorphism (rs731236; T>C) does not alter the amino acid composition of the resulting protein when thymine is changed to cytosine (ATT to ATC). The remaining polymorphisms are located in the noncoding regions of the *VDR* gene, and guanine is changed to adenine (GCG to GCA) in the *BsmI* polymorphism (rs1544410; G>A) and cytosine is changed to adenine in the *ApaI* polymorphism (rs7975232, A>C).⁹

Even though findings are inconclusive regarding the exact role of these *VDR* gene variants, studies suggest effects of the *FokI*, located in exon 2, on VDR functionality and transcriptional activity of immune-specific transcription factors.^{10,11} It is also hypothesized that possible alterations in mRNA expression levels of the *VDR* gene are attributed to a 3' untranslated region (3'-UTR),¹² which includes the genetic variants observed in intron 8 of *BsmI* and *ApaI* sites and in exon 9 of the *TaqI* restriction enzyme.¹³

In this regard, this study aimed to investigate the link between *VDR* gene polymorphism in *H. pylori*-infected patients with gastric symptomatology. The study was used as a basis for comparing the distribution of genic frequencies of *VDR* gene polymorphisms described in a Brazilian population¹⁴ with that identified in the ethnic groups of the International HapMap Project.¹⁵

Patients and methods

Study population

A cross-sectional study was conducted with 208 outpatients (126 females and 82 males) aged 18 years or older (mean age \pm standard deviation 53.04 \pm 15.20 years) who underwent an upper gastrointestinal endoscopy between April 2011 to March 2012 at the University Hospital João de Barros Barreto (HUIBB), Belém, Para, Brazil.

After previous evaluations due to the presence of symptoms in the upper gastrointestinal tract, patients who showed dyspeptic disorders and who were subsequently diagnosed with chronic gastritis were considered for the study. Subjects who reported to have received anti-*Helicobacter* therapy within the last 6 months, used a proton pump inhibitor within the last 30 days, were pregnant, abused alcohol, used illicit drugs, or had an HIV infection were excluded.

Biological samples, epidemiological data and written informal consent were obtained from all patients in this study. All procedures performed in the study were approved by the Ethics Committee for Research Involving Human Subjects of HUIBB (protocol no. 1820/2010).

Description and laboratory evaluation

In total, two gastric antral biopsy specimens were taken from each patient. One fragment was fixed in 10% formalin and stained with hematoxylin–eosin for histological examination according to the Updated Sydney System.¹⁶ The other biopsy specimen was immersed in a saline solution (0.9%) ready for genomic DNA extraction using the phenol–chloroform method.¹⁷

A polymerase chain reaction (PCR) assay to detect *H. pylori* in gastric biopsy specimens was performed using gene-specific primers (P1 and P2) that amplified a 298 bp fragment present in all *H. pylori* strains.¹⁸ Expressions of *cagA* and *vacA* virulence genes were determined by multiplex PCR using a protocol adapted from the study of Chatopadhyay et al.¹⁹

VDR gene polymorphism genotyping was performed via PCR–RFLP using forward and reverse primers, DNA fragments, molecular size of PCR products and restriction endonucleases, as described previously.²⁰ PCR products were prepared in a 25 μ L reaction mixture containing 1 μ L genomic DNA, 2.5 mM dNTP, 0.5 μ L Taq DNA polymerase, 2.5 μ L of a 10 \times buffer, 1.5 mM MgCl₂ and 2.4 mM of each primer.

Figure 1 shows the cleavage patterns of the DNA fragments using a previously described technique. The genotype representation was denoted according to the presence (lower letter) or absence (capital letter) of the restriction site.

HapMap data

Allelic and haplotypic frequency comparisons of VDR polymorphisms based on individual ancestry were obtained from the HapMap database¹⁵ to represent three major continental populations: Asian, European and African. The study included a sample population of East Asian ancestries (ASN) comprising groups of Han Chinese in Beijing (CHB)

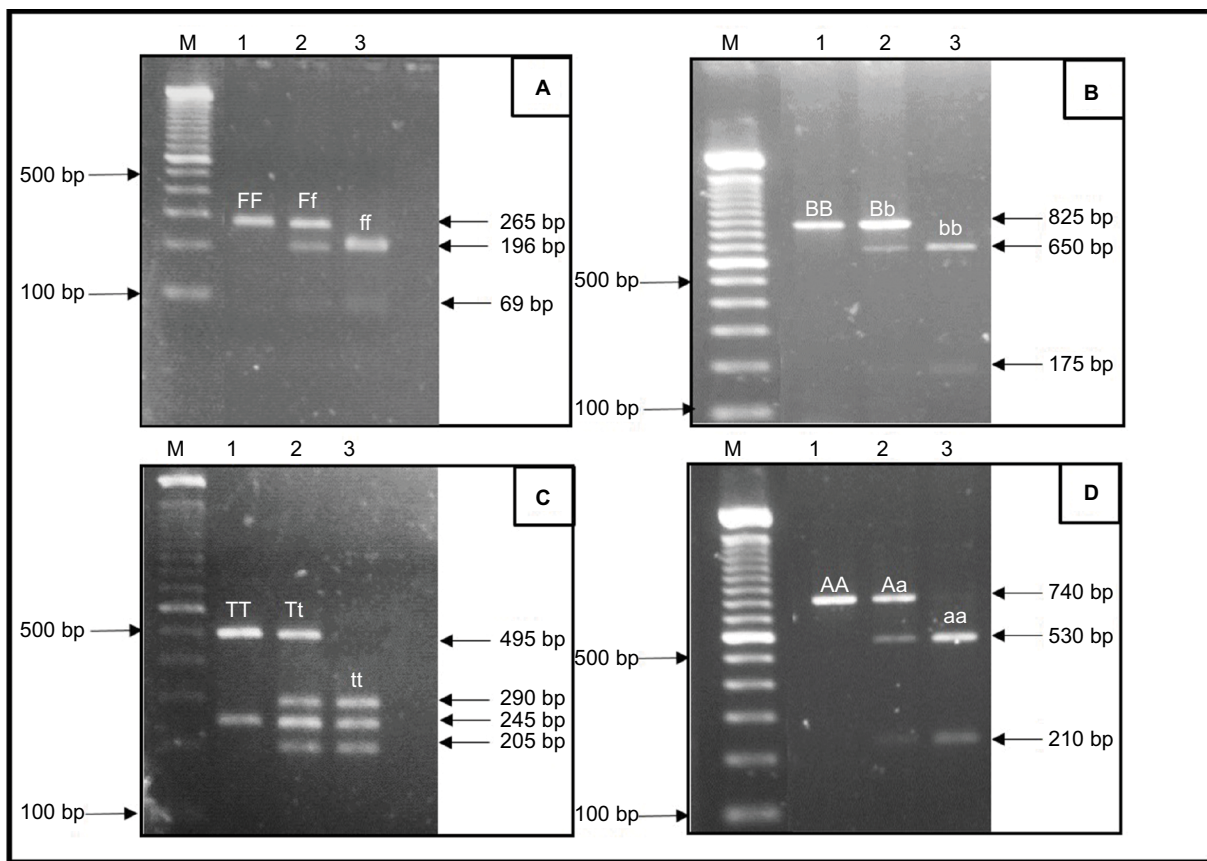


Figure 1 Representative RFLP pattern for the detection of VDR polymorphisms in agarose gel.

Notes: (A) *FokI*: the FF (265 bp), Ff (265 bp, 196 bp and 69 bp) and ff (196 bp and 69 bp) genotypes. (B) *BsmI*: the BB (825 bp), Bb (825 bp, 650 bp and 175 bp) and bb (650 bp and 175 bp) genotypes. (C) *TaqI*: the TT (495 bp and 245 bp), Tt (495 bp, 290 bp, 245 bp and 205 bp) and tt (290 bp, 245 bp and 205 bp) genotypes. (D) *ApaI*: the AA (740 bp), Aa (740 bp, 530 bp and 210 bp) and aa (530 bp and 210 bp) genotypes. M, molecular marker 100 bp ladder.

Abbreviations: RFLP, restriction fragment length polymorphism; VDR, vitamin D receptor.

and Japanese, Tokyo (JPT); Utah residents of Northern and Western European origin (CEU) and Sub-Saharan Africans from Yoruba, Ibadan, Nigeria (YRI).

Statistical analysis

Genotypic and allelic frequencies were compared between the *H. pylori*-negative and -positive groups using the Mantel–Haenszel chi-square test with an adjusted odds ratio (OR) for the covariates of sex and age. Hardy–Weinberg equilibrium (HWE) was also determined to the genotype frequencies of VDR polymorphisms. The variation in genotype proportions and a histopathological analysis of the *BsmI* polymorphism in the groups studied were compared by a binomial test. All analyses were performed using the statistical software program BioEstat 5.0²¹ with a 5% level of statistical significance.

Pairwise linkage disequilibrium (LD) between VDR polymorphisms was computed using Haploview program v.4.2.²² Online SHESi software was used to calculate the

haplotype frequencies,²³ and genetic distances were estimated for each population pair by Wright's *F* statistics (F_{ST}) using Arlequin v. 3.01.²⁴

Results

Of the 208 patients enrolled in the present study, the mean age at which *H. pylori* was diagnosed as positive was 51.38±15.22 years, while the mean age of the *H. pylori*-negative group was 55.18±14.98 years. Overall, 60.58% (126/208) of patients were females and 90.86% (189/208) were categorized as negroids (those with brown or black skin).

H. pylori infection was detected in 56.25% (117/208) of patients with upper gastrointestinal symptoms, 72.65% (85/117) of whom were colonized by CagA+/VacA s1m1 virulent strains. In relation to demographic and epidemiological aspects that may be considered a risk factor for *H. pylori* infection, no significant differences were observed between

H. pylori-negative and *H. pylori*-positive groups in terms of sex, age, comorbidities and lifestyle (smoking, alcohol use and consumption of fruits).

It should be emphasized that in relation to ancestral family history based on their self-reported ethnicity, a similar distribution was observed between the study groups relative to the ethnic groups of African, European and Native American origin.

Analysis of allelic and genotypic distributions of the *FokI*, *Apal* and *TaqI* polymorphisms in the *VDR* gene (Table 1) showed that there were no statistically significant differences between *H. pylori*-infected and -uninfected patients. However, concerning the *BsmI* polymorphism, the frequency of the bb genotype was higher in *H. pylori*-positive patients compared with those without an *H. pylori* infection (63.25%

versus 50.55%, respectively). Conversely, the Bb genotype was more common in the *H. pylori*-negative group than in the *H. pylori*-positive group (37.26% versus 17.09%, respectively), and an OR of 0.28 suggests a protective effect of this genotype. No significant sex difference in genotypic distribution was found for polymorphism in the *VDR* gene.

Additionally, the chi-square test of homogeneity revealed that the allelic frequencies of *BsmI* did not differ significantly between the *H. pylori*-negative and -positive groups and the b allele was the most prevalent in both samples, showing that these groups were extracted from the same population.

A significant deviation by HWE was found only in the genotypic distribution of the *BsmI* SNP identified in the *H. pylori*-positive group ($\chi^2=29.048$, $p<0.001$). In this sense, the

Table 1 Genotypic and allelic frequencies of VDR gene polymorphisms in *H. pylori*-infected and -uninfected patients from Belém, Pará, Brazil

VDR/SNPs	<i>H. pylori</i> infection		χ^2 (p-value)	OR (CI 95%)	OR ^a (CI 95%)
	Positive	Negative			
	n=117 (%)	n=91 (%)			
<i>FokI</i>					
Genotypes					
FF	56 (47.86)	41 (45.05)	0.2061 (0.9021)	1.00 ^b	1.00 ^b
Ff	45 (38.46)	36 (39.56)		0.92 (0.50–1.66)	0.81 (0.44–1.52)
ff	16 (13.68)	14 (15.38)		0.18 (0.37–1.90)	1.04 (0.44–2.42)
Alleles					
F	157 (67.09)	118 (64.84)	0.2331 (0.6292)	1.00 ^b	
f	77 (32.91)	64 (35.16)		0.90 (0.60–1.36)	
<i>BsmI</i>					
Genotypes					
BB	23 (19.66)	11 (12.09)	11.325 (0.0034)	1.00 ^b	1.00 ^b
Bb	20 (17.09)	34 (37.36)		0.28 (0.11–0.70)	0.29 (0.11–0.72)
bb	74 (63.25)	46 (50.55)		0.77 (0.34–1.72)	0.70 (0.31–1.59)
Alleles					
B	66 (28.21)	56 (30.77)	0.3247 (0.5687)	1.00 ^b	
b	168 (71.79)	126 (69.23)		1.13 (0.74–1.73)	
<i>Apal</i>					
Genotypes					
AA	47 (40.17)	37 (40.66)	0.4525 (0.7974)	1.00 ^b	1.00 ^b
Aa	48 (41.03)	40 (43.96)		0.94 (0.52–1.72)	0.91 (0.49–1.68)
aa	22 (18.80)	14 (15.38)		1.24 (0.56–2.74)	1.23 (0.53–2.83)
Alleles					
A	142 (60.68)	114 (62.64)	0.1650 (0.2331)	1.00 ^b	
a	92 (39.32)	68 (37.36)		1.09 (0.73–1.62)	
<i>TaqI</i>					
Genotypes					
TT	70 (59.83)	53 (58.24)	0.0766 (0.9624)	1.00 ^b	1.00 ^b
Tt	39 (33.33)	32 (35.16)		0.92 (0.51–1.66)	0.95 (0.52–1.73)
tt	8 (6.84)	6 (6.59)		1.01 (0.33–3.09)	1.00 (0.52–1.73)
Alleles					
T	179 (76.50)	138 (75.82)	0.0254 (0.8732)	1.00 ^b	
t	55 (23.50)	44 (24.18)		0.96 (0.61–1.52)	

Notes: ^aAge and sex-adjusted OR. ^b1.00=reference group used in the analysis.

Abbreviations: VDR, vitamin D receptor; *H. pylori*, *Helicobacter pylori*; SNP, single nucleotide polymorphism; OR, odds ratio.

results shown in Figure 2 indicate a very strong significant difference in the distribution of genotype frequencies of *BsmI* SNP between patients infected by virulent strains and those infected by non-virulent strains or uninfected patients. The frequency of the BB+Bb genotype was elevated in *H. pylori*-negative patients infected by non-virulent *H. pylori* strains. In contrast, the bb genotype was more prevalent in patients infected by virulent *H. pylori* strains.

As shown in Table 2, infection with virulent *H. pylori* strains and a high degree of neutrophil activity in the gastric mucosa were less frequently observed in patients with the bb genotype compared with subjects carrying the BB/Bb genotype, while no significant difference was observed among patients in the *H. pylori*-negative group and among those infected with non-virulent strains of *H. pylori*.

The 3'-UTR *VDR* SNPs (*BsmI*, *ApaI* and *TaqI*) did not exhibit significant LD with each other. No LD was also observed between *FokI* and other polymorphisms tested.

Table 3 shows the allelic and haplotypic frequencies of *VDR* polymorphisms in infected and noninfected *H. pylori* patients in relation to data from the literature for the Brazilian population¹⁴ and HapMap population.¹⁵ Comparative analysis revealed differences in the allelic distribution of *FokI* polymorphism estimated according to relative populations from Europe and Africa. Indeed, the F allele of the *FokI* polymorphism was more common in the African group than in the Belém population. Similarly, differences were observed in the allelic frequencies of the 3'-UTR *VDR* polymorphisms (*BsmI*, *ApaI* and *TaqI*). The Belém population was genetically more distant from the Asian groups than the European and African populations, but the allelic frequencies of *TaqI* polymorphism in the studied population were distinguished from the Brazilian population.

Table 4 summarizes the results of this comparative analysis among different interpopulation pairs, based on the frequencies of the four SNPs of the *VDR* gene. In general,

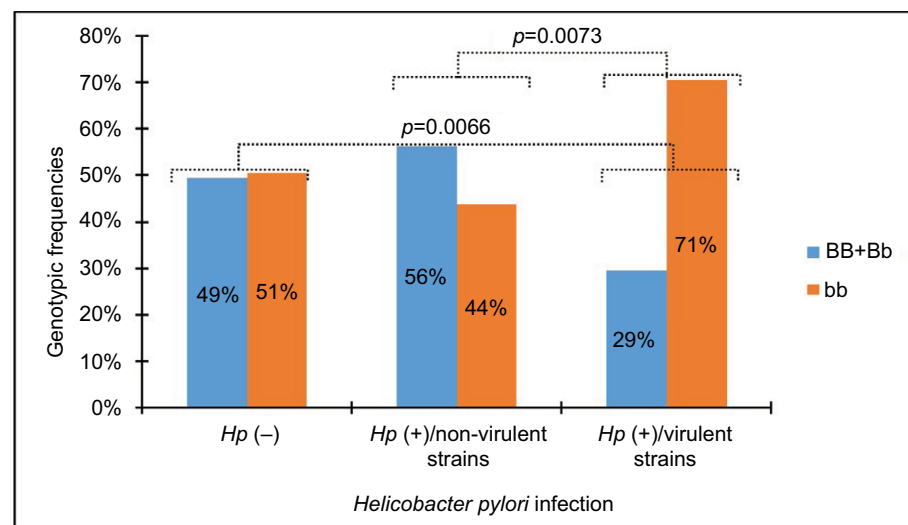


Figure 2 Genotypic frequencies of the *BsmI* VDR gene polymorphism in *Helicobacter pylori*-infected and -uninfected patients.

Abbreviations: VDR, vitamin D receptor; *Hp*, *Helicobacter pylori*.

Table 2 Genotypic distribution frequencies of the *BsmI* VDR gene polymorphism in *H. pylori*-infected and -uninfected patients according to the strong degree of neutrophil polymorphs activity in gastric mucosa

<i>H. pylori</i> infection	Genotypes	Strong degree of neutrophil polymorphic activity		<i>p</i> -value	<i>Z</i> -value
		n	%		
Virulent strains	bb	27/60	45.00	0.055	1.596
	BB+Bb	16/25	64.00		
Non-virulent strains	bb	6/14	42.86	0.187	0.891
	BB+Bb	5/18	27.78		
Negative	bb	3/46	6.25	0.220	0.773
	BB+Bb	5/45	11.11		

Abbreviations: VDR, vitamin D receptor; *H. pylori*, *Helicobacter pylori*.

significant differences in F -values indicate the level of genetic differentiation resulting from this comparison.

Discussion

In the present study, a significant difference in genotypic distribution of the SNP *BsmI* between *H. pylori*-positive and -negative samples was observed. Basically, these findings showed a significant deviation in genotype frequency of SNP *BsmI* from that of the *VDR* gene, which was determined by a high bb genotype frequency in the *H. pylori*-positive group colonized by a virulent-type CagA/VacAs1m1 strain.

Evidence of an elevated frequency of the *BsmI* SNP bb in patients with gastritis, compared with that observed in patients carrying other genotypes (BB/Bb), suggests that this can be another additive factor in the interaction with *H. pylori* virulent strains, which encodes the CagA protein, conferring a higher risk for gastric cancer.^{25,26} In general,

CagA shows the ability to bind several kinds of vital proteins, causing dysregulation of multiple classic signaling pathways by complex molecular mechanisms, especially target genes transcribed to promote tumorigenesis,²⁶ with the possible involvement of VDR expression.

It is a fact that immune modulator properties of the active vitamin D are at least partially mediated by VDR,²⁷ since the vitamin D metabolites are involved in antimicrobial activity induction and they also have anti-inflammatory effects,^{28,29} thereby inhibiting the Th1/Th17 immune response.³⁰

Another relevant aspect is that *H. pylori* virulent strains can induce higher inflammatory reactions in the gastric mucosa. Previous studies have reported that virulent strains increased the secretion of proinflammatory cytokines that are chemotactic for neutrophils and mononuclear cells, resulting in intense infiltration of these immune cells. Therefore, they are related to more severe pathologies such as ulcer and gastric cancer.^{31,32}

It is worth mentioning that in the present study, *H. pylori* infection diagnosed via molecular PCR showed 58% prevalence in patients with chronic gastritis. Similarly, a predominance of virulent type I (CagA+/s1m1) strains that reached 73% in the infected group was verified. Vinagre et al³³ also reported similar findings using data from the same population.

During infection, *H. pylori* can persist for long periods in the mucosa, because it can neutralize gastric acid through strong activity of its urease enzymes, which hydrolyze urea and produce a large amount of ammonia and carbon dioxide that increase gastric pH³⁴ and the enzyme γ -glutamyl transpeptidase, which supports its growth and survival in the gastric mucosa.³⁵ It has been observed that both mechanisms can be inhibited by vitamin D administration.³⁶

Table 3 Comparison of allelic frequencies of VDR gene polymorphisms between the study population and the HapMap populations

Population	SNP (allele)			
	<i>FokI</i> (F)	<i>BsmI</i> (b)	<i>Apal</i> (a)	<i>TaqI</i> (T)
Belém	0.661	0.707	0.385	0.762
Hp+	0.671	0.718	0.393	0.765
Hp-	0.648	0.692	0.374	0.758
BRZ	0.674	0.605	0.460	0.627
CEU	0.525	0.525	0.424	0.526
YRI	0.833	0.712	0.375	0.750
ASN	0.646	0.921	0.645	0.933

Notes: Belém, total surveyed patients; Hp+, *H. pylori* positive; Hp-, *H. pylori* negative; Brazil, Brazilian population (BRZ) described by Lins et al;¹⁴ CEU, Utah residents of Northern and Western European origin; YRI, Sub-Saharan Africans from Yoruba, Ibadan, Nigeria; ASN, Han Chinese in Beijing (CHB) and Japanese, Tokyo (JPT).

Abbreviations: VDR, vitamin D receptor; SNP, single nucleotide polymorphism; *H. pylori*, *Helicobacter pylori*.

Table 4 Locus-by-locus comparison of frequencies of fixation index (F_{ST}) values between different pairwise Brazilian and HapMap populations

Pairwise population	<i>FokI</i>		<i>BsmI</i>		<i>Apal</i>		<i>TaqI</i>	
	F_{ST}	p	F_{ST}	p	F_{ST}	p	F_{ST}	p
Hp+/Hp-	-0.009	0.782	-0.008	0.709	-0.009	0.754	-0.009	0.718
Hp+/BRZ	-0.007	0.909	0.021	0.045	0.002	0.218	0.040	0.009
Hp+/CEU	0.039	0.009	0.070	0.000	-0.007	0.754	0.118	0.000
Hp+/YRI	0.062	0.000	0.008	1.000	-0.007	0.800	-0.006	0.636
Hp+/ASN	-0.006	0.545	0.125	0.000	0.112	0.000	0.096	0.000
Hp-/BRZ	-0.007	0.645	-0.008	0.182	0.007	0.264	0.031	0.018
Hp-/CEU	0.026	0.045	0.049	0.018	-0.004	0.527	0.103	0.000
Hp-/YRI	0.078	0.000	-0.008	0.709	-0.009	1.000	-0.009	0.836
Hp-/ASN	-0.009	0.727	0.158	0.000	0.129	0.000	0.112	0.000

Notes: Hp+, *H. pylori* positive; Hp-, *H. pylori* negative; Brazil, Brazilian population (BRZ) described by Lins et al;¹⁴ CEU, Utah residents of Northern and Western European origin; YRI, Sub-Saharan Africans from Yoruba, Ibadan, Nigeria; ASN, Han Chinese in Beijing (CHB) and Japanese, Tokyo (JPT).

Abbreviation: *H. pylori*, *Helicobacter pylori*.

Particularly notable was the reduced neutrophil activity in the gastric mucosa observed among the patients infected by virulent strains of *H. pylori* and carrying the bb genotype, allowing bacterial development and persistent colonization of the stomach. This probably reflects the immunosuppressive role of vitamin D on the inhibition of neutrophil chemotaxis and host inflammatory response induced by CagA-positive *H. pylori*, suggesting that the *BsmI* polymorphism may be a marker of the cellular effect of vitamin D. These findings are consistent with the results reported by Guo et al,⁴ who observed a significant positive correlation between the chronic inflammation score, VDR mRNA expression and cathelicidin levels in *H. pylori*-infected patients.

It has been detected that expression of the cathelicidin-peptide antimicrobial complex,³⁷ the product that is enzymatically cleaved by proteinase 3 to produce the antimicrobial agent cathelicidin (LL37),³⁸ is more abundant in neutrophils and epithelial cells.^{39,40} The bactericidal activity of cathelicidin is mediated by its ability to bind to phosphatidylglycerol monolayer and disrupt the bacterial cell wall.⁴¹ The antibacterial activity of vitamin D₃ and its metabolites was investigated recently in *H. pylori* cultures. In that experiment, the authors noticed that intact forms of 25-hydroxyvitamin D₃ and 1 α ,25-dihydroxyvitamin D₃ significantly inhibited the *H. pylori* proliferation in vitro.⁴²

In this sense, our results showed an elevated frequency of the *BsmI* SNP BB/Bb genotypes in patients infected by CagA-negative *H. pylori*, supporting the hypothesis that there are VDR gene variants associated with different types of immunomodulatory effects. Furthermore, the *BsmI* B allele was reported to be significantly associated with the reduced risk of many types of cancer in a previous meta-analysis.^{43,44} Therefore, it is intriguing to speculate on the reason for the increased susceptibility to gastric risk presented by individuals infected by CagA-positive *H. pylori* and therefore having a less active VDR.

On the other hand, it is not clear whether the *BsmI* polymorphism has an effect on the expression level and activity of the translated VDR protein.^{45,46} In addition, there is controversy in the genetic association studies concerning the role of VDR polymorphism in diseases that can be assigned due to variation in LD patterns in different ethnic groups.¹²

These divergences strengthen the need to characterize the immunogenetic profile of patients carrying a set of variants that influence their genetic predisposition to *H. pylori* infection. In this respect, it is appropriate to evaluate the allelic and genotypic frequencies of VDR polymorphism in different ethnic groups, mainly because the population

of Belém has a mixed racial origin and presents with a high prevalence of *H. pylori* infection. Thus, the *F*-statistics values revealed significant differences in the allele frequencies of VDR SNPs between the population studied and other ethnic groups reported in the literature.¹⁵ These differences might be the result of the mix of different racial groups, given that the population of Belém is highly distinctive in terms of genetic origin.⁴⁷

On the other hand, it must be emphasized that infectious diseases, such as *H. pylori* infection, result from a complex interaction between genes and environmental and social factors. These interactions are crucial for the evolutionary processes and genetic composition of a population, which probably contributed (together with the ethnic influences) to the variation in the allelic frequencies observed in the study population.

In view of the reported results, there are some limitations, which should be considered with respect to the possible effects of population substructure, which can result in false-positive associations. The other critical point is that moderate sample sizes affect statistical power, making it difficult to evaluate the haplotypic interactions in a *H. pylori* infection. Contradictory data in genetic association studies might also be owing to differences in environmental exposure risk patterns and the combination of susceptibility variants, as previously reported.⁴⁸

Thus, considering such limitations, a careful approach must be adopted in the interpretation of these findings, and more extensive investigations at the molecular level should be conducted to elucidate the relationship between VDR gene polymorphisms and pathogenesis of *H. pylori* infection, mainly because of the absence of data on this subject, making it impossible to perform a comparative analysis. Independent studies must be conducted to validate the relationship between VDR gene polymorphisms and the pathogenesis of *H. pylori* infection.

Conclusion

We identified a possible association between the *BsmI* polymorphism and infection by *H. pylori*. However, further research is required to clarify this relationship.

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Author contributions

All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

- Yang I, Nell S, Suerbaum S. Survival in hostile territory: the microbiota of the stomach. *FEMS Microbiol Rev*. 2013;37(5):736–761.
- Rhee KH, Park JS, Cho MJ. *Helicobacter pylori*: bacterial strategy for incipient stage and persistent colonization in human gastric niches. *Yonsei Med J*. 2014;55(6):1453–1466.
- Kusters JG, van Vliet AHM, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. *Clin Microbiol Rev*. 2006;19(3):449–490.
- Guo LH, Chen WG, Zhu HT, et al. *Helicobacter pylori* induces increased expression of the vitamin D receptor in immune responses. *Helicobacter*. 2014;19(1):37–47.
- Crofts LA, Hancock MS, Morrison NA, Eisman JA. Multiple promoters direct the tissue-specific expression of novel N-terminal variant human vitamin D receptor gene transcripts. *Proc Natl Acad Sci U S A*. 1998;95(18):10529–10534.
- Alagarasu K, Honap T, Mulay AP, Bachal RV, Shah PS, Cecilia D. Association of vitamin D receptor gene polymorphisms with clinical outcomes of dengue virus infection. *Hum Immunol*. 2012;73(11):1194–1199.
- Sortica VA, Cunha MG, Ohnishi MDO, et al. IL1B, IL4R, IL12RB1 and TNF gene polymorphisms are associated with *Plasmodium vivax* malaria in Brazil. *Malar J*. 2012;11:409.
- Salimi S, Farajian-Mashhadi F, Alavi-Naini R, Talebian G, Narooie-Nejad M. Association between vitamin D receptor polymorphisms and haplotypes with pulmonary tuberculosis. *Biomed Rep*. 2015;3(2):189–194.
- Sherry ST, Ward MH, Kholodov M, et al. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res*. 2001;29:308–311.
- Colin EM, Weel A, Uitterlinden AG, et al. Consequences of vitamin D receptor gene polymorphisms for growth inhibition of cultured human peripheral blood mononuclear cells by 1,25-dihydroxyvitamin D-3. *Clin Endocrinol (Oxf)*. 2000;52(2):211–216.
- Jurutka PW, Remus LS, Whitfield GK, et al. The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. *Mol Endocrinol*. 2000;14(3):401–420.
- Morrison NA, Qi JC, Tokita A, et al. Prediction of bone-density from vitamin-d receptor alleles. *Nature*. 1994;367(6460):284–287.
- Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. *Gene*. 2004;338(2):143–156.
- Lins TC, Vieira RG, Grattapaglia D, Pereira RW. Population analysis of vitamin D receptor polymorphisms and the role of genetic ancestry in an admixed population. *Genet Mol Biol*. 2011;34(3):377–385.
- International HapMap Consortium. The International HapMap Project. *Nature*. 2003;426(6968):789–796.
- Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis – the updated Sydney system. *Am J Surg Pathol*. 1996;20(10):1161–1181.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988;16(3):1215.
- Hammar M, Tyszkiewicz T, Wadstrom T, et al. Rapid detection of *Helicobacter-pylori* in gastric biopsy material by polymerase chain-reaction. *J Clin Microbiol*. 1992;30(1):54–58.
- Chattopadhyay S, Patra R, Ramamurthy T, et al. Multiplex PCR assay for rapid detection and genotyping of *Helicobacter pylori* directly from biopsy specimens. *J Clin Microbiol*. 2004;42(6):2821–2824.
- Pani MA, Knapp M, Donner H, et al. Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans. *Diabetes*. 2000;49(3):504–507.
- Ayres M, Ayres M Jr, Ayres DL, dos Santos AS. *Bioestat 5.0: Aplicações estatísticas nas áreas das ciências biológicas e médicas*. [Bioestat 5.0: Statistical applications in the areas of biological and medical sciences] 3th ed. Belém: MCT-CNPq; 2005:364. Portuguese.
- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005;21(2):263–265.
- Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res*. 2005;15(2):97–98.
- Excoffier L, Laval G, Schneider S. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol Bioinform Online*. 2005;1:47–50.
- Hatakeyama M. *Helicobacter pylori* CagA and gastric cancer: a paradigm for hit-and-run carcinogenesis. *Cell Host Microbe*. 2014;15(3):306–316.
- Yong X, Tang B, Bo-Sheng L, et al. *Helicobacter pylori* virulence factor CagA promoter tumorigenesis of gastric cancer via multiple signaling pathways. *Cell Commun Signal*. 2015;13:30.
- White JH. Vitamin D metabolism and signaling in the immune system. *Rev Endocr Metab Disord*. 2012;13(1):21–29.
- Youssef DA, Miller CW, El-Abbassi AM, et al. Antimicrobial implications of vitamin D. *Dermatoendocrinol*. 2011;3(4):220–229.
- Mangin M, Sinha R, Fincher K. Inflammation and vitamin D: the infection connection. *Inflamm Res*. 2014;63(10):803–819.
- Cantorna MT, Snyder L, Lin YD, et al. Vitamin D and 1,25(OH)(2)D regulation of T cells. *Nutrients*. 2015;7(4):3011–3021.
- Montecucco C, Papini E, de Bernard M, Zoratti M. Molecular and cellular activities of *Helicobacter pylori* pathogenic factors. *FEBS Lett*. 1999;452(1–2):16–21.
- Nogueira C, Figueiredo C, Carneiro F, et al. *Helicobacter pylori* genotypes may determine gastric histopathology. *Am J Pathol*. 2001;158(2):647–654.
- Vinagre RM, Corvelo TC, Arnaud VC, Leite AC, Barile KA, Martins LC. Determination of strains of *Helicobacter pylori* and of polymorphism in the interleukin-8 gene in patients with stomach cancer. *Arq Gastroenterol*. 2011;48(1):46–51.
- Hazell SL, Lee A. *Campylobacter pyloridis*, urease, hydrogen-ion back diffusion, and gastric-ulcers. *Lancet*. 1986;2(8497):15–17.
- Chevalier C, Thiberge JM, Ferrero RL, et al. Essential role of *Helicobacter pylori* gamma-glutamyltranspeptidase for the colonization of the gastric mucosa of mice. *Mol Microbiol*. 1999;31(5):1359–1372.
- Kawaura A, Takeda E, Tanida N, et al. Inhibitory effect of long term 1 alpha-hydroxyvitamin D3 administration on *Helicobacter pylori* infection. *J Clin Biochem Nutr*. 2006;38(2):103–106.
- Martineau AR, Wilkinson KA, Newton SM, et al. IFN-gamma- and TNF-independent vitamin D-inducible human suppression of mycobacteria: the role of cathelicidin LL-37. *J Immunol*. 2007;178(11):7190–7198.
- Sorensen OE, Follin P, Johnsen AH, et al. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. *Blood*. 2001;97(12):3951–3959.
- Risso A. Leukocyte antimicrobial peptides: multifunctional effector molecules of innate immunity. *J Leukoc Biol*. 2000;68(6):785–792.
- van Wetering S, Tjabringa GS, Hiemstra PS. Interactions between neutrophil-derived antimicrobial peptides and airway epithelial cells. *J Leukoc Biol*. 2005;77(4):444–450.
- Neville F, Cahuzac M, Konovalov O, et al. Lipid headgroup discrimination by antimicrobial peptide LL-37: insight into mechanism of action. *Biophys J*. 2006;90(4):1275–1287.

42. Hosoda K, Shimomura H, Wanibuchi K, et al. Identification and characterization of a vitamin D3 decomposition product bactericidal against *Helicobacter pylori*. *Sci Rep*. 2015;5:8860.
43. Raimondi S, Pasquali E, Gnagnarella P, et al. *BsmI* polymorphism of vitamin D receptor gene and cancer risk: a comprehensive meta-analysis. *Mutat Res*. 2014;769:17–34.
44. Xu YQ, He BS, Pan YQ, et al. Systematic review and meta-analysis on vitamin D receptor polymorphisms and cancer risk. *Tumour Biol*. 2014;35(5):4153–4169.
45. Luo XY, Yang MH, Wu FX, et al. Vitamin D receptor gene *BsmI* polymorphism B allele, but not BB genotype, is associated with systemic lupus erythematosus in a Han Chinese population. *Lupus*. 2012;21(1):53–59.
46. Selvaraj P, Prabhu AS, Harishankar M, Alagarasu K. Plasma 1,25 dihydroxy vitamin D₃ level and expression of vitamin D receptor and cathelicidin in pulmonary tuberculosis. *J Clin Immunol*. 2009;29(4):4470–4478.
47. Santos NPC, Ribeiro-Rodrigues EM, Ribeiro-dos-Santos AKC, et al. Assessing individual interethnic admixture and population substructure using a 48-insertion-deletion (INSEL) ancestry-informative marker (AIM) panel. *Hum Mutat*. 2010;31(2):184–190.
48. Kraft P, Hunter D. Integrating epidemiology and genetic association: the challenge of gene-environment interaction. *Philos Trans R Soc Lond B Biol Sci*. 2005;360(1460):1609–1616.

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