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The Nonfunctional Allele TCRBV6S1B Is Strongly Associated with *Helicobacter pylori* Infection

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To determine genetic susceptibility factors for *Helicobacter pylori* infection, polymorphic T-cell receptor gene elements were investigated in 203 *H. pylori*-infected individuals and 180 uninfected individuals (controls). *H. pylori* infection is highly associated with individuals homozygous for the nonfunctional TCRBV6S1B element (odds ratio = 5.9; $\chi^2 = 13$; $P = 0.00032$; *P* value corrected for multiple comparisons [Bonferroni correction] = 0.00063).

Presentation of bacterial antigens and recognition via T lymphocytes play a central role in the immune response to most bacterial antigens, including *Helicobacter pylori* (1, 6). We investigated three highly polymorphic microsatellites, TCRBV6S7, TCRBV6S1, and TCRBV6S3 (3, 7), and correlated them with exonic polymorphisms of the T-cell receptor (4) for associated susceptibility to H. pylori infection.

A total of 383 unrelated, German individuals (ranging in age from 16 to 94 years; mean age, 59 years) undergoing gastroesophageal duodenoscopy for various clinical indications such as upper abdominal pain or noncardiac chest pain were included in this study after giving informed consent. Persons with a history of eradication therapy, acid-suppressive therapy within the last 4 weeks, evidence of malignancy, immunosuppression, or history of gastric surgery were excluded. *H. pylori* status was determined by rapid urease test, culture, and histology. At least two procedures had to yield positive results before subjects were considered infected. Individuals were considered *H. pylori* negative if all tests gave negative results. If one test result differed from the other two, serology was performed (Helicobacter pylori IgG ELISA kit; Medac, Hamburg, Germany). A total of 203 individuals were determined to be *H. pylori* positive and 180 individuals were determined to be *H. pylori* negative. In the *H. pylori*-positive group, 40 individuals suffered from gastric ulcer or had a history of gastric ulcer and 40 individuals had developed duodenal ulcer. There was no evidence for acute ulceration or history of ulcer disease in 123 individuals.

DNA preparation and microsatellite analysis were performed as described previously (8, 11). Haplotype frequencies were estimated by using ARLEQUIN software (standard deviation computed by 50 bootstraps) (14). Allele frequencies and estimated haplotype frequencies were compared by using a 2×2 contingency table and χ^2 statistics and were considered significantly different if the *P* values were <0.05. Differences in allele or genotype distribution between the infected cohort and control cohort were quantitated using odds ratios (OR). Only

TABLE 1. Distribution of allele frequencies of the microsatellites TCRBV6S7, TCRBV6S3, and TCRBV6S1 and the exonic polymorphism TCRBV6S1A/B in *H. pylori*-positive and -negative individuals[a]

Allele	Frequency (%) in:	
	H. pylori-positive cohort	*H. pylori*-negative cohort
TCRBV6S7 alleles[b]		
$(GT)_{27}$	0.5	0.3
$(GT)_{26}$	3.2	2.3
$(GT)_{25}$	16.9	18.2
$(GT)_{24}$	15.9	17.6
$(GT)_{23}$	9.7	7.7
$(GT)_{22}$	16.7	12.5
$(GT)_{21}$	7.7	6.3
$(GT)_{20}$	2.7	2.8
$(GT)_{19}$	0.0	0.3
$(GT)_{17}$	0.2	0.0
$(GT)_{16}$	0.5	2.0
$(GT)_{15}$	25.9	30.1
TCRBV6S3 alleles[c]		
$(GT)_{13}$	0.2	1.1
$(GT)_{12}$	10.3	9.2
$(GT)_{11}$	7.6	4.4
$(GT)_{10}$	0.0	0.0
$(GT)_{9}$	0.0	0.0
$(GT)_{8}$	81.9	85.3
TCRBV6S1 alleles[c]		
$(GT)_{13}$	11.8	8.6
$(GT)_{12}$	**21.7**[d]	**14.7**[d]
$(GT)_{11}$	0.2	0.6
$(GT)_{10}$	0.5	0.0
$(GT)_{9}$	**65.8**[e]	**76.1**[e]
Exonic polymorphism TCRBV6S1A/B[f]		
$BV6S1A(GT)_{9–11}$	66.5	76.7
$BV6S1B(GT)_{12\ or\ 13}$	**33.5**[g]	**23.3**[g]

[a] The boldface type indicates statistical significance.
[b] A total of 402 and 358 alleles were investigated for *H. pylori*-positive and -negative individuals, respectively.
[c] A total of 406 and 360 alleles were investigated for *H. pylori*-positive and -negative individuals, respectively.
[d] OR = 1.6; $\chi^2 = 6.14$; $P = 0.013$; $P_c = 0.04$ (three alleles tested).
[e] OR = 0.6; $\chi^2 = 9.6$; $P = 0.0017$; $P_c = 0.005$ (three alleles tested).
[f] The TCRBV6S1A/B alleles are shown in abbreviated form, without the TCR prefix.
[g] OR = 1.66; $\chi^2 = 9.6$; $P = 0.002$; $P_c = 0.004$ (two alleles tested).

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TABLE 2. TCRBV6S1A/B genotype frequencies in *H. pylori*-positive and -negative individuals[a]

TCRBV6S1A/B	Frequency (%)[b] in:	
	H. pylori-positive individuals (n = 203)	*H. pylori*-negative individuals (controls) (n = 180)
AA	44.4	55.6
AB	43.8	42.2
BB	**11.8**[c]	**2.2**[c]

[a] The boldface type indicates statistical significance.
[b] n, number of individuals investigated.
[c] OR = 5.9; χ^2 = 13; P = 0.00032; P_c = 0.00063 (two genotypes tested).

alleles or haplotypes with a frequency of >2% were tested, because rare alleles are unlikely to contribute significantly to the pathogenesis of the disease. P values were corrected for the number of comparisons made (Bonferroni correction) (P_c), namely, the number of alleles tested. All genetic markers followed Hardy-Weinberg equilibrium (GENEPOP software [12]).

TCRBV6S1 is located between the TCRBV6S7 and TCRBV6S3 elements. No significant differences in allele frequencies of TCRBV6S7 and TCRBV6S3 in infected and noninfected individuals were observed (Table 1). However, the frequency of allele TCRBV6S1$(GT)_9$ is significantly lower in *H. pylori*-positive individuals (OR = 0.6; χ^2 = 9.6; P = 0.0017; P_c = 0.005) (Table 1). The frequency of allele TCRBV6S1$(GT)_{12}$ is higher in infected individuals than in uninfected controls (OR = 1.6; χ^2 = 6.14; P = 0.013; P_c = 0.04). This microsatellite marker is in strong linkage disequilibrium with exonic polymorphisms in TCRBV6S1 and allows indirect genotyping of three distinct alleles. One allele is functional (TCRBV6S1A), and two are nonfunctional, operationally combined as TCRBV6S1B (4). The allele frequency of this nonfunctional allele TCRBV6S1B in *H. pylori*-positive individuals differed significantly from that in uninfected controls (Table 1) (OR = 1.66; χ^2 = 9.6; P = 0.002; P_c = 0.004). Individuals homozygous for the nonfunctional allele accounted for most of this effect. Some of the affected individuals (11.8%) were homozygous for the TCRBV6S1B allele compared to 2.2% of the controls (OR = 5.9; χ^2 = 13; P = 0.00032; P_c = 0.00063) (Table 2). A previous study (10) has shown that individuals homozygous for the null allele do not express TCRBV6S1B mRNA in the peripheral blood cells. This is explained by the death of T cells with nonproductive T-cell receptor rearrangements. The possibility of spontaneous resolution of *H. pylori* infection in humans has been suggested (2) and requires a specific type of immune response that depends on the nature of the pre-existing and induced T-cell repertoire (9, 15). We assume that individuals lacking T cells with the functional TCRBV6S1 element (genotype TCRBV6S1B/B) are not able to eliminate *H. pylori* efficiently and may have a higher risk for chronic infection that may finally result in ulcer disease. However, the clinical outcome of *H. pylori* infection (diagnosis of gastric or duodenal ulcer or history of ulcer disease) did not correlate with the genotype TCRBV6S1B/B in our investigated cohort (Table 3). Therefore, the assessment of T-cell response by donors with transient infections would be very helpful in evaluating the genetic background of the immune response in these hosts and the composition of the T-cell repertoire.

TABLE 3. Clinical outcome of *H. pylori*-positive patients and T cells with a functional TCRBV6S1 element (genotypes TCRBV6S1A/A and -A/B) and a nonfunctional TCRBV6S1 element

Clinical outcome of *H. pylori*-positive individuals (n = 203)[a,b]	Frequency (%) of TCRBV6S1 element	
	Functional	Nonfunctional
Duodenal ulcer (n = 40)	90.0	10.0
Gastric ulcer (n = 40)	90.0	10.0
Chronic gastritis without ulcer (n = 123)	87.0	13.0

[a] n, number of genotypes investigated.
[b] Chi-square analysis (2 × 3 table): df = 2; χ^2 = 0.43; P = 0.81.

TABLE 4. Selected haplotype frequencies of TCRBV6S1/BV6S3 microsatellites in *H. pylori*-positive and -negative individuals[a]

TCRBV6S1/BV6S3 allele[b]	Haplotype frequency (%) (± SD) in:	
	H. pylori-positive individuals (n = 406)[a]	*H. pylori*-negative controls (n = 358)
$6S1(GT)_{13}/6S3(GT)_8$	8.3 (±1.46)	6.5 (±1.86)
$6S1(GT)_{12}/6S3(GT)_{11}$	4.9 (±1.00)	3.5 (±1.19)
$6S1(GT)_{12}/6S3(GT)_8$	**15.0**[d] ***(±1.31)***	**8.6**[d] **(±1.29)**
$6S1(GT)_9/6S3(GT)_{12}$	6.2 (±1.24)	5.5 (±1.16)
$6S1(GT)_9/6S3(GT)_8$	**57.9**[e] **(±2.33)**	**69.7**[e] **(±2.37)**

[a] Only haplotypes with a frequency of >2% are shown. The boldface type indicates statistical significance.
[b] The TCRBV6S1/BV6S3 alleles are shown in abbreviated form below, without the TCRB and BV prefixes, respectively.
[c] n, number of alleles investigated.
[d] OR = 1.87; χ^2 = 7.3; P = 0.007; P_c = 0.034 (five alleles tested).
[e] OR = 0.59; χ^2 = 11.7; P = 0.00062; P_c = 0.0031 (five alleles tested).

Our data suggest that this infectious disease is strongly associated with specific constellations in the TCRBV region. Table 4 shows haplotype frequencies of TCRBV6S1/BV6S3 microsatellites in *H. pylori*-infected persons and uninfected controls. The frequency of haplotype TCRBV6S1$(GT)_9$/BV6S3 $(GT)_8$ in infected individuals is significantly lower than in *H. pylori*-negative controls (OR = 0.59; χ^2 = 11.7; P = 0.00062; P_c = 0.0031), and the haplotype TCRBV6S1$(GT)_{12}$/BV6S3 $(GT)_8$ is more prevalent in *H. pylori*-positive individuals (OR = 1.87; χ^2 = 7.3; P = 0.007; P_c = 0.034). The distribution of the haplotypes TCRBV6S1/BV6S7 is shown in Table 5. The haplotype TCRBV6S1$(GT)_{12}$/BV6S7$(GT)_{22}$ is significantly associated with *H. pylori* infection (OR = 2.47; χ^2 = 8.8; P =

TABLE 5. Selected haplotype frequencies of TCRBV6S1/BV6S7 microsatellites in *H. pylori*-positive and -negative individuals[a]

BV6S1/BV6S7 allele[b]	Haplotype frequency (%) (±SD) in:	
	H. pylori-positive individuals (n = 398)[a]	*H. pylori*-negative controls (n = 346)
$6S1(GT)_{13}/6S7(GT)_{21}$	6.3 (±1.48)	5.1 (±1.10)
$6S1(GT)_{13}/6S7(GT)_{20}$	2.5 (±0.96)	2.1 (±0.66)
$6S1(GT)_{12}/6S7(GT)_{22}$	**10.1**[d] **(±1.42)**	**4.4**[d] **(±1.14)**
$6S1(GT)_{12}/6S7(GT)_{15}$	6.1 (±1.29)	6.0 (±1.47)
$6S1(GT)_9/6S7(GT)_{26}$	2.7 (±0.73)	2.0 (±0.77)
$6S1(GT)_9/6S7(GT)_{25}$	14.6 (±2.07)	17.3 (±1.58)
$6S1(GT)_9/6S7(GT)_{24}$	14.1 (±1.71)	17.6 (±2.23)
$6S1(GT)_9/6S7(GT)_{23}$	7.1 (±1.12)	5.8 (±1.24)
$6S1(GT)_9/6S7(GT)_{22}$	5.6 (±1.12)	7.4 (±1.61)
$6S1(GT)_9/6S7(GT)_{15}$	19.9[d] (±2.15)	23.5[d] (±2.39)

[a] Only haplotypes with a frequency of >2% are shown. The boldface type indicates statistical significance.
[b] The TCRBV6S1/BV6S7 alleles are shown in abbreviated form below, without the TCRB and BV prefixes, respectively.
[c] n, Number of alleles investigated.
[d] OR = 2.47; χ^2 = 8.8; P = 0.00296; P_c = 0.03 (10 alleles tested).

0.00296; $P_c = 0.03$). Taken together, the extended haplotype TCRBV6S7$(GT)_{22}$/BV6S1$(GT)_{12}$/BV6S3$(GT)_8$ is associated with *H. pylori* infection. Thus, an additional TCRBV element may be associated with the disease in a second group of patients. Certain alleles of the relevant gene should be much more strongly associated to *H. pylori* infection than the association shown here for certain haplotypes. The haplotype TCRBV6S1$(GT)_9$/BV6S3$(GT)_8$ was more frequent in uninfected controls, which could imply that a haplotype offers some protection from the disease. This haplotype, however, does not extend to the TCRBV6S7 element. Therefore, other functionally relevant polymorphisms in this region could be responsible for an elevated risk in another subset of patients. In the chromosomal region of TCRBV6S1, several TCRBV elements are located (13) and biallelic polymorphisms resulting in amino acid substitutions have been demonstrated for certain elements (5). Thus, additional polymorphisms in this region will be investigated to determine if they play a role in the immune response to *H. pylori*.

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