Interleukin 1 beta (*IL1B*) Gene Polymorphisms Are Not Associated with Gastric Carcinogenesis in Germany

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Abstract. *Background: Polymorphisms of interleukin-1 beta gene (IL1B-511C>T) are considered as risk factor for gastric carcinogenesis, but conflicting data have been reported recently. Patients and Methods: The distribution of the four major IL1B variants (IL1B-3737C>T, -1464G>C, -511C>T, -31T>C) were analyzed in 116 and 142 patients with gastric cancer and 'high risk gastritis', respectively, as well as 94 healthy controls. Results: While identified frequencies of genotypes, haplotypes and haplotype pairs corresponded to those of other studies in Caucasians, none were significantly associated with the presence of gastric cancer or premalignant alterations. Conclusion: None of the four major polymorphisms is individually or in its haplotype configuration linked to the development of GC in this Caucasian population in Germany.*

Gastric cancer (GC) has a multifactorial etiology and develops in the majority (>97%) of patients sporadically (1). The strong association of GC with the infection of the stomach by the gram-negative bacterium *Helicobacter pylori* (*H. pylori*) led to the classification of this germ as definite carcinogen (class I) by the World Health Organization in 1994. *H. pylori* is currently regarded as the main risk factor for gastric carcinogenesis worldwide and GC eventually develops in 1-3% of all individuals infected with *H. pylori* (2-4). Besides this infection, other factors such as high intake of salt, nitrosamine-containing food, smoking, as well as gene polymorphisms play an important role for GC (3, 4).

The first cytokine polymorphism linked to the development of GC was reported by El-Omar *et al.* in 2001 (5). This polymorphism consisted of C-T and T-C transitions at positions –511 and –31 of the *IL1B* gene, respectively, as well as the presence of a penta-allelic tandem repeat in intron 2 of the IL-1 receptor antagonist gene (*IL1RN*) (5). The combination of both, *i.e.* the *IL1B-511T* haplotype and the allele 2 of the *IL1RN* gene (*IL1RN*2*) resulted in an additive effect leading to an odds ratio (OR) of 5.3 [95% confidence interval (CI): 1.9-14] for the likelihood of developing GC. In recent years, a complex situation has developed in the context of the role of *IL1B* gene polymorphisms for the development of GC. Although shortly after the initial discovery of the *IL1B* haplotypes linked to GC the majority of publications confirmed this finding, in recent years the number of reports opposing this association is increasing. To date, four meta-analyses have been published, of these, three identified a significant risk of *IL1B* polymorphisms, in particular for the *IL1B-511* C-T transition and the *IL1RN*2* allele for the development of GC (6-8), while one meta-analysis did not (9). Despite this discrepancy, the actual results of the four analyses are quite similar. While Kamangar *et al.* computed a non-significant relative risk of 1.07-1.16 for *IL1B-511* CT or TT allele *vs.* the wildtype *IL1B-511* CC in the overall study population (9), Vincenzi *et al.* reported a significant association with an OR of 1.23 (95% CI: 1.09-1.37) in 2008 (8). In agreement with the two other confirmative meta-analyses (6, 7), this study demonstrated that the increased risk for *IL1B* polymorphisms is restricted to Caucasians only (OR: 1.56; 95% CI: 1.32-1.84) and does not apply to Asians (OR: 1.0; 95% CI: 0.85-1.16). Furthermore, it was shown that the association is much stronger for GC of the intestinal type (OR: 1.76; 95% CI: 1.12-2.57) than for GC of diffuse type (OR: 1.16; 95% CI:

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Key Words: Gastric cancer, gene polymorphism, interleukin-1beta, IL-1, IL-1 receptor antagonist.

Table I. *Demographic data of patient groups. Statistical analysis was performed globally for all three groups; no pairwise tests were applied.*

	Controls (n=94)	GC (n=116)	HRG (n=142)	ANOVA (age) Chi-square test (*p*-value)
Age (mean±sd)	42.2±15.4	65.7±13.4	61.0±11.1	<0.001
Gender (male/female)	49/45	78/38	65/77	
	52.1%/47.9%	67.2%/32.8%	45.8%/54.2%	<0.001
H. pylori	50/44	69/47	70/72	
-positive / -negative	53.2%/46.8%	59.5%/40.5%	49.3%/50.7%	0.037
CagA	41/53	80/36	105/37	
-positive / -negative	43.6%/56.4%	69.0%/31.0%	73.9/26.1%	0.017
H. pylori or CagA	61/33	92/24	122/20	
-positive / -negative	64.9%/35.1%	79.3%/20.7%	85.9%/14.1%	0.008

GC, Gastric cancer; HRG, 'high risk gastritis'.

0.86-1.52) (6). The calculated OR between 1.3-1.8 was significantly lower than the initially reported relative risk of 2.8-9.0 (4, 10). Taking into consideration that several recent studies mostly with negative findings for a role of *IL1B* gene polymorphisms for GC and its premalignant lesions from European populations (11-14) have not been included in these meta-analyses, the hazard ratio is likely to be even lower. Potential explanations for these discrepant results have been comprehensively discussed in the four meta-analyses (6-9). Most important are differences in the study design (type of tumor, location, gender, age and *H. pylori* status, and ethnicity), technical flaws by genotyping and perhaps publication bias. Recently, Chen *et al*. comprehensively studied gene polymorphisms in the *IL1B* gene by sequencing and identified 20 individual single nucleotide polymorphisms (SNPs) within 12 kb of the gene analyzed (15). Four of these SNPs (positions: -3737, -1464, -511, -31) had a frequency of >4% and were further characterized with respect to functional and epidemiological aspects. Using transfected cell lines expressing these haplotypes, the authors quantified the transcriptional activity and demonstrated that the highest transcriptional activity of the proinflammatory *IL1B-511T* haplotype depends additionally on the haplotype compositions at positions *IL1B-3737* and *IL1B-1464*. If both sites revealed wildtype variations at -3737 (C) and -1464 (G), the transcriptional induction of the *IL1B-511T* allele was stronger than in the presence of alternative variations (-3737: T and/or -1464: C) (15). Since these additional sites have not been analyzed in all studies published so far, and no data for IL1B secretion in *ex vivo* tissue in context to these haplotypes are available, it is unclear whether the *in vitro* effect is relevant to humans. Taking into consideration the potential role of these new SNPs, we analyzed the distribution of these four alleles (-3737, -1464, -511, -31) in a study comprising 116 patients with GC, 94 controls and 142 patients with 'high risk gastritis'.

Patients and Methods

Study design. Patients with GC, histologically determined high risk gastritis and controls were recruited from the Clinic of Gastroenterology, Hepatology and Infectious Diseases of the Otto von Guericke University Magdeburg, Germany, between 1998 and 2008. From all participants, DNA for genotyping and serum samples for analyzing *H. pylori* status were available. GC was confirmed histopathologically. Patients with "high risk gastritis" were included from the Out-patient Department of our clinic, as well as from a clinical study aimed at the long-term follow-up of *H. pylori* infection initiated in 1998. Controls were recruited from clinical studies with healthy volunteers and from the Out-patient Department aimed at the diagnosis of dyspeptic symptoms. The inclusion criteria of both latter groups ('high risk gastritis', controls) were no history of neoplasia at any site and no history of gastrointestinal disease or surgery. Patients and healthy participants were examined by a physician and venous blood samples were taken for genotyping and serological studies. In total, 352 individuals (116 GC, 142 'high risk gastritis', 94 controls) were included. All were of Caucasian ethnicity. General characteristics of the three groups are illustrated in Table I. This study was carried out in accordance with the guidelines of the Ethics Committee of the University of Magdeburg.

Analysis of H. pylori status and histological characterization of patients with GC and "high risk gastritis". *H. pylori* status was serologically analyzed from all 352 participants by the determination of total anti-*H. pylori* IgG using anti-IgG ELISA (Pyloriset™ EIA-GIII; BAG, Lich, Germany) according to the manufacturer's protocol. The CagA status was analyzed serologically by the presence of anti-CagA-specific antibodies using ViraBlot test kit™ (Viramed Biotech AG, Lich, Germany) as described by the manufacturer.

Histological evaluation was performed for gastritis pattern using the updated Sydney system (16) and for GC type by the Laurén classification (17). Biopsies or surgical specimens were available from 98 out of 116 patients with GC and all 142 patients with high risk gastritis. "High risk gastritis" included pan-gastritis (similar inflammatory scores in antrum and corpus), corpus-predominant gastritis with or without the presence of gastric atrophy, and intestinal metaplasia either in antrum or corpus as proposed by Uemura *et al*. and Meinert *et al*. (18, 19).

Table II. *Products of primer extension reaction for IL1B-511/-31 and RFLP analysis of IL1B-1464/-3737.*

SNP (MS)	Sequence primer	M (Da)	Genotypes
h*IL1B*_SNP-511	5'-Ctgcaattgacagagagctcc-3'	6415.2	
rs: 16944	5'-CtgcaattgacagagagctccddC-3'	6687.4	C
	5'-Ctgcaattgacagagagctcc TddG-3'	7032.6	T
h*IL1B*_SNP-31	5'-Tcctacttctgcttttgaaagc-3'	6651.4	
rs: 1143627	5'-Tcctacttctgcttttgaaagc TddA-3'	7252.8	T
	5'-TcctacttctgcttttgaaagcddC-3'	6923.6	C
SNP (RFLP)	Primer, size of the fragment	Enzymes	Fragments
h*IL1B*_SNP-1464	5'-ccc-tcg-tgt-ctc-aaa-tac-ttg-c-3'	*StyI*	CC: 127 bp, 36 bp
rs: 1143623	5'-gca-cag-tgc-ctg-gca-tgt-ac-3'		CT: 163 bp, 127 bp, 36 bp
	163 bp		TT: 163 bp
h*IL1B*_SNP-3737	5'-gcc-ctc-ctt-gtt-cta-gac-cag-gga-gga-gac-tgg-aat-g-3'	*DrdI*	GG: 108bp, 35 bp
rs: 4848306	5'-atc-gct-tcc-act-tcc-ttt-atg-gca-tc-3'		GC: 143 bp, 108 bp, 35 bp
	143 bp		CC: 143 bp

Laboratory methods. DNA was isolated from peripheral blood using QIA-BLOOD-mini kit™ (Qiagen, Hilden, Germany) as described by the manufacturer. The polymorphisms at positions –511 and –31 of the *IL1B* gene were studied by restriction fragment length polymorphism (RFLP) analysis as described (20) or by primer extension and mass-spectrometry. PCR products, obtained by genomic PCR utilizing the two following primers: 5'-att-ttc-tcc-tca-gag-gct-cc-3' and 5'-aag-aga-atc-cca-gag-cag-cc-3', were purified using the Genopure Purification Kit for Double-Stranded DNA™ (Bruker Daltonics, Germany) according to the instructions of the manufacturer. The purified PCR product was extended in 10 µl reaction mixture containing 200 µM dTTP, 200 µM ddCTP, ddATP, ddGTP, 20 and 40 pmol extension primers IL1B-511, IL1B-31, respectively, 1 U Thermo Sequenase™ (Amersham Biosciences, Germany), Thermosequenase™ reaction buffer and 5 µl PCR product using standard cycling conditions. Purified extension products and residual primers were purified again, eluted and subsequently subjected to matrix-assisted laser desorption and ionization – time-of-flight mass spectrometry (MALDI-TOF-MS). One µl of matrix solution (3-hydroxypicolinic acid, 10 mg/ml and dibasic ammonium citrate, 1 mg/ml in water) was applied on an AnchorChip™ 600 sample target (Bruker Daltonics, Germany) and allowed to dry at room temperature. Subsequently, 1 µl of purified extension product was added to matrix spots and dried at room temperature. The sample target was introduced into a Reflex III MALDI-TOF mass spectrometer (Bruker Daltonics), operated in linear mode. Measurements were performed at 19 kV acceleration voltage with pulsed ion extraction. Spectra acquisition was carried out using the XACQ 4.0 software (Bruker Daltonics). The annotation of the spectra was performed using XMASS software (Table II).

Polymorphisms at positions *IL1B-3737G/C* and *IL1B-1464C/T* were analyzed by RFLP of amplified genomic fragments. Genomic PCR was performed using primer pairs identified in Table II applying standard conditions (annealing temperature of 52˚C). PCR products were subsequently digested by either StyI or DrdI (both enzymes NEB, Frankfurt/Main, Germany) and genotypes were assigned based on fragment patterns as illustrated in Table II.

The analysis of the variable numbers of tandem repeats in intron 2 in the *IL1RN* gene was performed by genomic PCR and agarose gel electrophoresis using the following primers: 5'-ctc-agc-aac-act-cct-at-3' and 5'-tcc-tgg-tct-gca-ggt-aa-3'. The different alleles were identified by the size of the PCR fragments (allele 1: 410 bp, allele 2: 240 bp, allele 3: 500 bp, allele 4: 325 bp and allele 5: 595 bp). For statistical analysis, the frequencies of allele 2 were compared to the combined presence of all other alleles, collectively identified as 'L' for 'long repeats'.

Statistical analysis. Age is shown as means and standard deviations, and was analyzed by unpaired ANOVA test. Categorical data (*e.g.* gender, genotype distributions) are presented as frequencies; comparisons were performed using Chi-square or by Fisher's exact test. Here, global comparisons of all geno- and haplotypes among the three groups were performed. Additionally, the distribution of individual genotypes (*e.g.* CC *vs.* CT+TT) were analyzed among the three groups. Since none of the tests revealed significant results, the Bonferroni adjustment was not applied to correct for multiple groups. For all the analyses, a two-sided p-value <0.05 was considered to be significant. For statistical analysis, SPSS Version 16.0 (SPSS, Chicago, IL, USA) was used. The relative risks for mutations were studied by different models. The recessive and dominant model compares wild-type+heterozygous *vs.* homozygous+wild-type *vs.* heterozygous+homozygous, respectively.

Results

Age and gender distribution differed significantly among the three groups analyzed (Table II). Patients with gastric cancer had the highest proportion of males and were 5 and 13 years older than individuals with 'high risk gastritis' and controls, respectively. The serological *H. pylori* status was different among the three groups. Considering the presence of anti-*H. pylori* IgG or anti-CagA antibodies as indicative for positive *H. pylori* status resulted in the detection of a recent or former

Table III. *Distribution of genotypes among patients groups. Genotype frequencies were analyzed globally (italic and bold-marked p-values), and subsequently for each genotype vs. the remaining two genotypes.*

	Total (n=352)	Controls (n=94)	GC (n=116)	HRG (n=142)	Chi-square test (*p*-value)
IL1B -3737					0.447
CC	113 (32.1%)	27 (28.7%)	37 (31.9%)	49 (34.5%)	0.647
CT	168 (47.7%)	50 (53.2%)	50 (43.1%)	68 (47.9%)	0.346
TT	71 (20.2%)	17 (18.1%)	29 (25.0%)	25 (17.6%)	0.284
C allele	394 (56.0%)	104 (55.3%)	124 (53.4%)	166 (58.5%)	0.512
T allele	310 (44.0%)	84 (44.7%)	108 (46.6%)	118 (41.5%)	
IL1B -1464					0.585
GG	200 (56.8%)	53 (56.4%)	63 (54.3%)	84 (59.2%)	0.733
GC	133 (37.8%)	38 (40.4%)	47 (40.5%)	48 (33.8%)	0.448
CC	19 (5.4%)	3 (3.2%)	6 (5.2%)	10 (7.0%)	0.436
G allele	533 (75.7%)	144 (76.6%)	173 (74.6%)	216 (76.1%)	0.877
C allele	171 (24.3%)	44 (23.4%)	59 (25.4%)	68 (23.9%)	
IL1B -511					0.963
CC	169 (48.0%)	43 (45.8%)	58 (50.0%)	68 (47.9%)	0.828
CT	143 (40.6%)	41 (43.6%)	45 (38.8%)	57 (40.1%)	0.769
TT	40 (11.4%)	10 (10.6%)	13 (11.2%)	17 (12.0%)	0.949
C allele	481 (68.3%)	127 (67.5%)	161 (69.4%)	193 (68.0%)	0.908
T allele	223 (31.7%)	61 (32.5%)	71 (30.6%)	91 (32.0%)	
IL1B-31					0.957
TT	170 (48.3%)	44 (46.8%)	58 (50.0%)	68 (47.9%)	0.892
TC	141 (40.1%)	40 (42.6%)	44 (37.9%)	57 (40.1%)	0.793
CC	41 (11.6%)	10 (10.6%)	14 (12.1%)	17 (12.0%)	0.938
T allele	481 (68.3%)	128 (68.1%)	160 (69.0%)	193 (68.0%)	0.808
C allele	223 (31.7%)	60 (31.9%)	72 (31.0%)	91 (32.0%)	
IL1RN					0.754
L/L	203 (57.6%)	56 (59.6%)	61 (52.6%)	86 (60.6%)	0.396
L/2	121 (34.4%)	31 (33.0%)	45 (38.8%)	45 (31.7%)	0.463
2/2	28 (8.0%)	7 (7.4%)	10 (8.6%)	11 (7.7%)	0.946
L allele	527 (74.9%)	143 (76.1%)	167 (72.0%)	217 (76.4%)	0.466
2 allele	177 (25.1%)	45 (23.9%)	65 (28.0%)	67 (23.6%)	

GC, Gastric cancer; HRG, 'high risk gastritis'.

H. pylori infection in 79 and 87% of patients with GC and "high risk gastritis", respectively and 65% of controls (*p*-value=0.008).

Individual analysis of genotype frequencies at positions -3737/-1464/-511/-31 of *IL1B* and *IL1RN*2* genotype revealed a similar distribution among all three groups without significant differences. In order to confirm the negative finding of global comparisons, sub analyses for each genotype *vs*. the remaining two genotypes at all five loci were performed with identical results (Table III). The known genetic linkage between *IL1B-511* and *IL1B-31* genotype was confirmed in 348 out of 352 individuals (98.9%). The combined frequency of heterozygous and homozygous carriers of the *ILRN*2* allele was increased in GC (47.0%) compared to controls (41.1%) and RG (39.4%) but without statistical significance (p=0.39, Table III).

Based on haplotype composition reported by Chen *et al.* (15), we stratified the genotype information to the corresponding haplotypes, which was based on the four investigated sites. In total, 695 (98.5%) were uniquely classified as one of the four major haplotypes, while only 9 samples (1.5%) could not be assigned to one of these four major haplotypes (Table IV). The higher allele frequency of *IL1RN*2* in GC compared to controls and RG (28% *vs*. 24%) did not reach significance (Table IV). The calculation of relative risks for the different haplotypes (with/without *IL1RN* status) was performed using dominant and recessive models using *IL1B* haplotype 1 (*IL1B-373: T, -1464: G, -511: C, -31: T*) with *IL1RN*L* allele as reference. None of the genetic models applied revealed significant hazard risks for any *IL1B* haplotype/*IL1RN* combination for the presence of GC or RG (data not shown).

Taking into consideration the recently identified functional role of *IL1B* haplotype pairs, subjects were stratified by the presence of 'high-, 'intermediate- and 'low-expressing haplotype pairs' as reported by Rogus *et al.* (21). Neither their distribution (Table V) nor the calculated OR (data not shown) revealed significant associations of 'high expressing *IL1B* haplotype pairs' to GC and 'high risk gastritis'.

Table IV. *Distribution of haplotypes among patient groups.*

IL1B haplotypes (-3737/-1464/ -511/-31)	Total (n=704)	Controls (n=188)	GC (n=232)	HRG (n=284)	Chi-square test (*p*-value)
H1 (T/G/C/T)	319 (45.3%)	84 (44.7%)	104 (44.8%)	131 (46.1%)	0.938
H2 (C/C/T/C)	165 (23.4%)	44 (23.4%)	56 (24.1%)	65 (22.9%)	0.946
H3 (C/G/C/T)	159 (22.6%)	43 (22.9%)	54 (23.3%)	62 (21.8%)	0.921
H4 (C/G/T/C)	52 (7.4%)	16 (8.5%)	12 (5.2%)	24 (8.5%)	0.289
5 (rare haplotypes)	9 (1.3 %)	1 (0.5%)	6 (2.6%)	2 (0.7%)	0.095

GC, Gastric cancer; HRG, 'high risk gastritis'.

Table V. *Haplotype pair analysis among patient groups.*

IL1B haplotype pairs	Total (n=352)	Controls (n=94)	GC (n=116)	HRG (n=142)	Chi-square test (*p*-value)
'High haplotype pairs' (H3/H3 + H1/H1 + H1/H3)	167 (47.4%)	42 (44.7%)	57 (49.1%)	68 (47.9%)	0.841
'Low haplotype pairs' (H2/H2 + H1/H4 + H1/H2)	108 (30.7%)	33 (35.1%)	32 (27.6%)	43 (30.3%)	0.843
'Intermediate haplotype pairs' (all remaining combinations)	77 (21.9%)	19 (20.2%)	27 (23.3%)	31 (21.8%)	0.568

GC, Gastric cancer; HRG, 'high risk gastritis'.

Histological type of GC was intestinal, diffuse and mixed type in 45, 42 and 11 patients, respectively. In order to compare both major histological types of GC (intestinal and diffuse), the 11 patients with mixed type tumors were excluded from subanalysis. As illustrated in Table VI, GC of the intestinal type was significantly associated with the presence of atrophy, intestinal metaplasia and differentiation, but exhibited similar rates for *H. pylori* and CagA status compared to diffuse type of GC. Neither the distribution of genotypes (Table VI) nor that of haplotypes and haplotype pairs (data not shown) differed between both histological types. Analyzing the distribution of genotypes in relation to histomorphological parameters, a borderline significance between *IL1B-3737*1* allele and the presence of intestinal metaplasia was noted (55.6% *vs*. 50% for intestinal metaplasia-positive *vs*. -negative, $p=0.046$). This association was further confirmed in the haplotype analysis demonstrating that individuals with *IL1B* haplotype 3 (C/G/C/T), see table III) had a higher prevalence of intestinal metaplasia (89.7%) compared to those with haplotypes 1, 2 and 4 (36.4 to 67.5%, $p=0.004$).

Table VI. *Subanalysis of patients with GC in relation to histological type. Genotype frequencies were analyzed globally (italic and bold-marked p-values), and subsequently for each genotype vs. the remaining two genotypes.*

Parameter	Intestinal (n=45)	Diffuse (n=42)	Chi-square test (*p*-value)
H. pylori-positive	37 (82.2%)	32 (76.2%)	0.60
and CagA-positive	30 (66.7%)	29 (69.0%)	0.82
Intestinal metaplasia	37 (82.2%)	25 (59.5%)	0.032
Gastric atrophy	27 (60.0%)	8 (19.0%)	<0.001
Differentiation			
Grade 1	7 (15.6%)	3 (7.1%)	<0.001
Grade 2	21 (46.7%)	5 (11.9%)	
Grade 3	17 (37.7%)	29 (69.1%)	
Grade 4	0	5 (11.9%)	
IL1B-3737			0.464
CC	13 (28.9%)	17 (40.5%)	0.256
CT	20 (44.4%)	14 (33.3%)	0.289
TT	12 (26.7%)	11 (26.2%)	0.960
IL1B-1464			0.743
GG	24 (53.3%)	24 (57.1%)	0.721
GC	17 (37.8%)	16 (38.1%)	1.000
CC	4 (8.9%)	2 (4.8%)	0.448
IL1B-511			0.731
CC	24 (53.3%)	20 (47.6%)	0.594
CT	16 (35.6%)	15 (35.7%)	1.000
TT	5 (11.1%)	7 (16.7%)	0.453
IL1B-31			0.845
TT	24 (53.3%)	20 (47.6%)	0.594
TC	15 (33.3%	15 (35.7%)	0.815
CC	6 (13.4%)	7 (16.7%)	0.603
IL1RN			0.624
L/L	25 (55.5%)	19 (45.2%)	0.336
L/2	16 (35.6%)	18 (42.9%)	0.486
2/2	4 (8.9%)	5 (11.9%)	0.644
L allele	66 (73.3%)	56 (66.7%)	
2 allele	24 (26.7%)	28 (33.3%)	0.387

Discussion

Our finding that gene polymorphisms of the *IL1B* locus do not play a major role in the pathogenesis of gastric inflammation and GC in German patients of Caucasian ethnicity adds another piece of the puzzle to the ongoing debate about the role of genomic susceptibility for these diseases. Taking advantage of recently published studies demonstrating an important role of haplotype context for the functional relevance of *IL1B* promoter polymorphisms (15, 21), we studied for the first time the role of these haplotypes in clinical diagnoses, namely 'high risk gastritis', a premalignant condition of gastric carcinogenesis (1, 18, 19) as well as in GC itself. While all studies reviewed in the

four meta-analyses (6-9) as well as recently published work (13, 14, 22, 23) have solely analyzed single polymorphisms (mostly *IL1B-511C>T* or *IL1B-31 T>C*) in the context of gastrointestinal diseases, in this study, the potential association of the four major *IL1B* haplotypes were prospectively investigated. The importance of haplotype configuration for the secretion of IL1B was recently demonstrated by Rogus *et al*. who extended their previous findings on different transcriptional initiation rates of the four haplotypes (15). Based on the four major haplotypes, the possible composite genotypes of the *IL1B* promoter haplotypes (expressed as haplotype pairs) were analyzed in relation to IL1B secretion (21). In this study, it was demonstrated for the first time that in human tissue specimens (gingival fluid, and peripheral mononuclear cells), IL1B levels differ significantly among the various haplotype configurations. Notably, the differences observed between the 'high-secreting haplotype' B3/B3 (correspond to H3 in this study) and 'lowest-secreting haplotype' B1/B2 (H1/H2) was about 75% in the gingival tissue fluid, while the corresponding difference was almost 5-fold for isolated mononuclear cells with these haplotype pairs (21). Furthermore, the authors revealed that three of the 'high-IL1B secreting' haplotype pairs were associated with elevated serum levels of C-reactive protein (CRP), while two others were not. Although it is unclear whether this finding reflects a regulatory link between IL1B and CRP production in hepatocytes, or merely an association of two unrelated phenomena it clearly underlines the complexity between defined genotypes and functional variability. In the context of gastric carcinogenesis, the *IL1B* alleles *IL1B-511T* and *IL1B-31C* were initially proposed to represent risk factors for Caucasians (1, 5). However, more recent research has shown that the numbers of positive and negative associations between these alleles and the presence of GC is balanced, and the hazard ratios of *IL1B-511C>T* for developing GC is dependent on the different meta-analyses between 1.2 to 1.6 for Caucasians only (6-9), but with a substantial heterogeneity among European countries. While positive associations were reported from Poland (5, 24) and Portugal (10), studies from Sweden (13) and Italy (12) did not find similar associations, questioning a major pathophysiological role of *IL1B* polymorphisms for gastric carcinogenesis (25). As yet, the potential linkage between *IL1B* polymorphisms and GC in German patients of Caucasian ethnicity was investigated in 88 patients with early GC only, demonstrating a significant association for the presence the *IL1RN*2* allele, but not for *IL1B-511/-31* (26). Rad *et al*. reported significant linkage between *IL1B-511/-31* and *IL1RN*2* and the degree of inflammation, mucosal gene expression and histomorphological alterations (atrophy, intestinal metaplasia) in patients with *H. pylori* infection (20, 27). However, the proposed association between *IL1* gene polymorphisms and premalignant changes was not verified in a large epidemiological study comprising 534 serologically defined cases of chronic atrophic gastritis (22). Gao *et al*. demonstrated an inverse association between proinflammatory genotypes *IL10-819CC* and *IL1RN 9589TT* and *H. pylori* seropositivity, but was unable to find an association with *IL1B-511 T* allele (22).

Taking into consideration these four previous studies from Germany, this study has the advantage of being the first to investigate patients with premalignant alterations ('high risk gastritis') and GC in relation to the *IL1B* locus in German individuals with Caucasian ethnicity and applying the extended analysis of haplotypes. Neither for the *IL1B* loci investigated nor for the *IL1RN* genotypes were significant associations identified, supporting the hypothesis that *IL1B*-associated polymorphisms do not play a predominant role in gastric carcinogenesis. The negative findings of the subanalysis between the two histological types of GC imply further that this finding includes both the intestinal and diffuse type of GC. Taking into consideration the similar haplotype distribution of our 352 patients with the reported frequencies of 900 Caucasians (15, 21), as well as the almost complete linkage disequilibrium between *IL1B-511* and *IL1B-31*, major technical flaws in genotyping can be ruled out. A potential limitation of our study is the rather broad definition of 'high risk gastritis' that was based on the work of Uemura *et al*. (18) and Meining *et al*. (19). Furthermore, controls did not undergo endoscopic evaluation, hence individual cases of 'high risk gastritis' in this group cannot be ruled out. Based on epidemiological data (22) and our own unpublished data, the frequency of chronic atrophic gastritis among patients with dyspeptic symptoms in Germany is rather low and in the range of 3-7%, therefore making it unlikely to affect the conclusion of this study.

In summary, we report that polymorphisms of the *IL1B (-3737/-1464/-511/-31)* and *IL1RN*2* alleles do not play a role in gastric carcinogenesis in Germans with Caucasian ethnicity, and therefore cannot be considered as reliable biomarker for risk stratification.

Disclosure Statement

None of the Authors has a conflict of interest with respect to the study.

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