

Cytokine gene polymorphisms and the risk of adenocarcinoma of the stomach in the European prospective investigation into cancer and nutrition (EPIC-EURGAST)

J. B. A. Crusius¹, F. Canzian^{2†}, G. Capellá^{3*}, A. S. Peña¹, G. Pera⁴, N. Sala^{3,4,5}, A. Agudo⁴, F. Rico^{3,5}, G. Del Giudice⁶, D. Palli⁷, M. Plebani⁸, H. Boeing⁹, H. B. Bueno-de-Mesquita¹⁰, F. Carneiro¹¹, V. Pala¹², V. E. Save¹³, P. Vineis^{14,15}, R. Tumino¹⁶, S. Panico¹⁷, G. Berglund¹⁸, J. Manjer¹⁹, R. Stenling²⁰, G. Hallmans²¹, C. Martínez²², M. Dorransoro²³, A. Barricarte²⁴, C. Navarro²⁵, J. R. Quirós²⁶, N. Allen²⁷, T. J. Key²⁷, S. Bingham²⁸, C. Caldas^{28,29}, J. Linseisen³⁰, R. Kaaks³⁰, K. Overvad³¹, A. Tjønneland³², F. C. Büchner¹⁰, P. H. M. Peeters³³, M. E. Numans³³, F. Clavel-Chapelon³⁴, A. Trichopoulou³⁵, E. Lund³⁶, M. Jenab², S. Rinaldi², P. Ferrari², E. Riboli¹⁴ & C. A. González⁴

¹Laboratory of Immunogenetics, Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands; ²International Agency for Research on Cancer, Lyon, France; ³Translational Research Laboratory; ⁴Department of Epidemiology, IDIBELL—Catalan Institute of Oncology, Barcelona; ⁵Center for Molecular and Medical Genetics, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL)—Institut de Recerca Oncològica, Barcelona, Spain; ⁶Immunobiological Research Institute of Siena (IRIS) Research Center, Chiron-Vaccines, Siena; ⁷Molecular and Nutritional Epidemiology Unit, Centro per lo Studio e la Prevenzione Oncologica, Scientific Institute of Tuscany, Florence; ⁸Department of Laboratory Medicine, Azienda Ospedaliera—Università di Padova, Padova, Italy; ⁹Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany; ¹⁰Center for Nutrition and Health, National Institute for Public Health and the Environment, Bilthoven, The Netherlands; ¹¹Institute of Pathology and Molecular Immunology of the University of Porto and Medical Faculty/H.S. Joao, Porto, Portugal; ¹²Epidemiology Unit, National Cancer Institute, Milan, Italy; ¹³Pathology Department, Addenbrooke's Hospital, Cambridge, UK; ¹⁴Department of Epidemiology and Public Health, Imperial College London, London, UK; ¹⁵Department of Biomedical Science, University of Torino, Turin; ¹⁶Cancer Registry Azienda Ospedaliera Civile-M.P. Arezzo, Ragusa; ¹⁷Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy; ¹⁸Department of Medicine, Lund University Malmö, Malmö; ¹⁹Department of Surgery, University Hospital, Malmö; ²⁰Department of Medical Biosciences, Pathology; ²¹Department of Nutritional Research, Umeå University, Sweden; ²²Andalusian School of Public Health, Granada; ²³Department of Public Health of Guipuzkoa, San Sebastian; ²⁴Public Health Institute, Navarra; ²⁵Department of Epidemiology, Health Council of Murcia; ²⁶Public Health and Health Planning Directorate, Asturias, Spain (Dirección General de Salud Pública, Consejería de Salud y Servicios Sanitarios Asturias, Spain); ²⁷Cancer Research UK, Epidemiology Unit, University of Oxford, Oxford; ²⁸Medical Research Council Dunn Human Nutrition Unit, Cambridge; ²⁹Cancer Genomics Program, Department of Oncology, University of Cambridge, Hutchison/MRC Research Center, Cambridge, UK; ³⁰Division of Clinical Epidemiology, German Cancer Research Center, Heidelberg, Germany; ³¹Department of Clinical Epidemiology, Aalborg Hospital, Aarhus University Hospital, Aalborg; ³²Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark; ³³Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, The Netherlands; ³⁴INSERM, U 521, Institut Gustave Roussy, Villejuif, France; ³⁵Department of Hygiene and Epidemiology, Medical School University of Athens, Athens, Greece; ³⁶Institute of Community Medicine, University of Tromsø, Tromsø, Norway

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Background: The relative contribution to gastric cancer (GC) risk of variants in genes that determine the inflammatory response remains mostly unknown and results from genotyping studies are inconsistent.

Patients and methods: A nested case–control study within the prospective European Prospective Investigation into Cancer and Nutrition cohort was carried out, including 248 gastric adenocarcinomas and 770 matched controls. Twenty common polymorphisms at cytokine genes [interleukin (*IL*)1A, *IL*1B, *IL*1RN, *IL*4, *IL*4R, *IL*6, *IL*8, *IL*10, *IL*12A, *IL*12B, lymphotoxin α and tumor necrosis factor (*TNF*)] were analyzed. Antibodies against *Helicobacter pylori* (Hp) and CagA were measured.

Results: *IL*1RN 2R/2R genotype [odds ratio (OR) 2.43; 95% confidence interval (CI) 1.19–4.96] and allele *IL*1RN Ex5–35C were associated with an increased risk of Hp(+) non-cardia GC. *IL*8 –251AA genotype was associated with a decreased risk of Hp(+) non-cardia GC (OR 0.51; 95% CI 0.32–0.81), mainly of the intestinal type. These associations were not modified

*Correspondence to: G. Capellá, Translational Research Laboratory, Catalan Institute of Oncology, Barcelona, Spain. Tel: +34-93-260-7952; Fax: +34-93-260-7466; E-mail: gcapella@iconcologia.net

†Present address: Genomic Epidemiology Group, Division of Molecular Genetic Epidemiology, German Cancer Research Center, Heidelberg, Germany

by CagA status. Carriers of *IL1B* -580C and *TNF* -487A alleles did not associate with an increased risk. A moderately increased risk of Hp(+) non-cardia GC for *IL4R* -29429T variant was observed (OR 1.74; 95% CI 1.15–2.63).

Conclusion: This prospective study confirms the association of *IL1RN* polymorphisms with the risk of non-cardia GC and indicates that *IL8* -251T>A may modify the risk for GC.

Key words: cytokine genes, gastric carcinoma, polymorphisms, severe chronic atrophic gastritis

introduction

A steady decline in the incidence of gastric cancer (GC) has been observed in most countries in the last decades. GC remains, however, the second most common cause of cancer death in the world [1]. Infection with *Helicobacter pylori* (Hp) is the strongest risk factor for non-cardia GC and chronic gastritis. Tobacco smoking is causally associated with GC while dietary factors are thought to have an important role in gastric carcinogenesis [2, 3]. Only <1% of Hp carriers will ever develop GC. Hp infection induces a chronic inflammation of the gastric mucosa that is intensified by the host inflammatory immune response by increasing cytokine levels.

Polymorphisms within regulatory and other functional regions of cytokine and cytokine receptor genes markedly influence cytokine expression and secretion profiles in response to infectious agents. Gene polymorphisms that modify the intensity of the inflammatory response may contribute to variations in GC risk [4]. Seminal studies by El-Omar et al. [5] pointed to an association between GC risk and polymorphisms in the interleukin (IL) 1 cluster genes (chromosome 2q13), particularly for non-cardia Hp(+) GC. Case-control studies carried out in different populations have, however, shown inconsistent results [6]. Three meta-analyses have recently reviewed this issue with inconclusive results [7–9].

Single-nucleotide polymorphisms (SNPs) in several other genes such as tumor necrosis factor (*TNF*) [10–13], *IL8* [14, 15], *HLA-DQB1* [16] and *IL12* [17, 18] and in genes encoding the anti-inflammatory cytokines *IL10* [11, 13, 18, 19] and *IL4* [19] have been associated with GC risk with controversial results. Carriage of multiple SNPs in *IL1B*, *IL1RN*, *IL10* and *TNF* seems to exert a synergistic increase in risk of GC when Hp infection is present [10, 11, 20].

The current study was conducted to examine the association between polymorphisms in the proinflammatory genes *IL1A*, *IL1B*, *IL6*, *IL8*, *IL12A*, *IL12B* and the major histocompatibility complex genes coding for lymphotoxin α (*LTA*) and *TNF*, as well as in the regulatory genes *IL1RN*, *IL10*, *IL4* and one of its receptors *IL4R*, with the risk of GC in a nested case-control study conducted within a large prospective study: the European Prospective Investigation into Cancer and Nutrition (EPIC) [21].

material and methods

the EPIC study

The EPIC cohort consists of 521 457 subjects (368 010 women and 153 447 men), mostly aged 35–70 years and recruited from 1992 to 1998 in 23 centers, in 10 European countries: Denmark, France, Greece, Germany, Italy, the Netherlands, Norway, Spain, Sweden and the UK. Eligible subjects were invited to participate in the study by mail or by personal contact [21]. Those who accepted signed an informed consent form. Diet questionnaire and blood samples were obtained. Follow-up is on the basis

of population cancer registries except in France, Germany and Greece, where it is mainly achieved by active contact with study subjects. Follow-up was completed from December 2000 to December 2002. All individuals included were Caucasian.

nested case-control study

Subjects were selected according to a nested case-control design. Prevalent GC cases ($n = 138$) and 2403 subjects lost to follow-up were excluded. Cases were all subjects newly diagnosed during the follow-up of cancer of the stomach, defined by code C16 of the International Statistical Classification of Diseases, 10th Revision. An independent panel of pathologists reviewed slides as well as pathology reports provided by each center [22]. Initially, 290 GC cases were identified; four cases of cancer located in gastric stump as well as 31 tumors other than adenocarcinoma were excluded. For each new incident case, up to four control subjects were randomly selected among cohort members alive and free of cancer at the time of diagnosis of the case, matched by center, gender, age (± 2.5 years) and date of blood collection (± 45 days). The set of controls ($n = 1125$) was used to describe the genotype frequencies and to compute Hardy-Weinberg equilibrium (HWE) tests and linkage disequilibrium (LD). The final population for GC risk assessment included 248 GC (location: 128 non-cardia, 72 cardia—including 16 in the gastroesophageal junction—and 48 unknown; histological type: 96 intestinal, 95 diffuse and 57 unknown) and 770 controls. Sixty of 128 (47%) non-cardia and 53 of 72 cardia carcinomas (74%) were males. Sixty-five percent of case-control sets were from central and northern Europe (UK, Sweden, Norway, Denmark, Germany and The Netherlands) and 35% from Mediterranean countries.

laboratory assays

genotyping analysis. Genomic DNA from cases and controls was extracted from a 0.5-ml aliquot of buffy coat, which had been kept frozen since blood extraction and processing [23]. Genes have been named according to the HUGO Gene Nomenclature committee (<http://www.genomic.unimelb.edu.au>). SNPs have been named according to the SNP500Cancer database (<http://snp500cancer.nci.nih.gov/home.cfm>) of the Cancer Genome Anatomy Project and have been identified according to the ID numbering of the dbSNP database of the NCBI (<http://www.ncbi.nlm.nih.gov/SNP>).

SNPs at *IL1B*, *IL4*, *IL6*, *IL8*, *IL10*, *IL12A* and *IL12B* and cytokine receptor *IL4R* genes (Table 1) were analyzed in an ABI 7900HT real-time PCR instrument (Applied Biosystems, Foster City, CA) at the International Agency for Research on Cancer (IARC) in Lyon with primers and probes as published in the SNP500Cancer database. Analysis of SNPs in *IL1A*, *IL1B*, *IL1RN*, *LTA* and *TNF* at the VU University Medical Center (VUMC) in Amsterdam took place in an ABI PRISM® 7000 Sequence detection system with primers and probes designed using Primer Express® software (version 2.0), except *TNF* -417G>A which has been analyzed according to Hampe et al. [24]. The intron 2 variable number of tandem repeats polymorphism in *IL1RN* was analyzed as described previously [5].

A minimum of 10 test DNAs were used to standardize all the genotyping protocols and are available upon request. The *IL1B* -580T>C SNP was analyzed at both centers and a 100% concordance rate was found. As quality control, 10% of the samples were reanalyzed for all SNPs. For the VUMC, concordance rate was 100% for seven of the eight polymorphisms analyzed and 99.2% for the remaining one.

Table 1. Frequency distribution of genotypes for cytokine gene polymorphisms in controls, in stomach, cardia and non-cardia adenocarcinoma cases

Gene	Polymorphism	Genotype	Control		Stomach		Cardia		Non-cardia	
			n	%	n	%	n	%	n	%
IL1A	Ex1+12C>T, 5' UTR, rs1800587, aka -889	TOTAL	1125		237		69		122	
		CC	555	49.3	112	47.3	38	55.1	52	42.6
		CT	465	41.3	104	43.9	27	39.1	58	47.5
		TT	105	9.3	21	8.9	4	5.8	12	9.8
IL1B	-580T>C, rs1143627, aka -31	TOTAL	1174		248		72		128	
		TT	548	46.7	109	44.0	30	41.7	59	46.1
		TC	485	41.3	109	44.0	30	41.7	55	43.0
		CC	141	12.0	30	12.1	12	16.7	14	10.9
IL1B	Ex5+14C>T, F105F, rs1143634, aka +3954	TOTAL	1125		237		69		122	
		CC	643	57.2	136	57.4	40	58.0	69	56.6
		CT	412	36.6	88	37.1	24	34.8	47	38.5
		TT	70	6.2	13	5.5	5	7.2	6	4.9
IL1RN	Ex5-35T>C, A60A, rs419598, aka +2018	TOTAL	1125		236		69		121	
		TT	608	54.0	104	44.1	34	49.3	54	44.6
		TC	425	37.8	105	44.5	28	40.6	52	43.0
		CC	92	8.2	27	11.4	7	10.1	15	12.4
IL1RN	IVS2 86-bp repeat, rs2234663	TOTAL	1144		244		72		124	
		2R/2R	90	7.9	29	11.9	8	11.1	16	12.9
		2R/3R	1	0.1	0	0.0	0	0.0	0	0.0
		2R/4R	422	36.9	104	42.6	27	37.5	51	41.1
		2R/5R	15	1.3	1	0.4	1	1.4	0	0.0
		3R/4R	6	0.5	1	0.4	1	1.4	0	0.0
		4R/4R	571	49.9	101	41.4	34	47.2	52	41.9
		4R/5R	36	3.1	8	3.3	1	1.4	5	4.0
		4R/6R	3	0.3	0	0.0	0	0.0	0	0.0
IL4	-588C>T, rs2243250, aka -524, -589, -590	TOTAL	1154		242		71		125	
		CC	824	71.4	159	65.7	44	62.0	83	66.4
		CT	305	26.4	76	31.4	24	33.8	39	31.2
		TT	25	2.2	7	2.9	3	4.2	3	2.4
IL4	Ex1-168C>T, 5' UTR, rs2070874, aka -34C>T	TOTAL	1160		243		70		127	
		GG	839	72.3	159	65.4	43	61.4	84	66.1
		GA	296	25.5	77	31.7	24	34.3	40	31.5
		AA	25	2.2	7	2.9	3	4.3	3	2.4
IL4R	Ex5+14A>G, I75F, rs1805010, aka I50V	TOTAL	1150		244		71		127	
		AA	352	30.6	71	29.1	21	29.6	37	29.1
		AG	549	47.7	134	54.9	39	54.9	69	54.3
		GG	249	21.7	39	16.0	11	15.5	21	16.5
IL4R	-29429C>T, rs2057768, aka -3223C>T	TOTAL	1107		235		67		123	
		CC	583	52.7	108	46.0	32	47.8	51	41.5
		CT	433	39.1	116	49.4	33	49.3	65	52.8
		TT	91	8.2	11	4.7	2	3.0	7	5.7
IL6	-236G>C, 5' UTR, rs1800795, aka -174	TOTAL	1138		243		70		126	
		GG	415	36.5	78	32.1	24	34.3	38	30.2
		GC	517	45.4	122	50.2	34	48.6	68	54.0
		CC	206	18.1	43	17.7	12	17.1	20	15.9
IL8	-251T>A, rs4073, aka -251	TOTAL	1139		236		68		124	
		TT	315	27.7	75	31.8	20	29.4	47	37.9
		TA	574	50.4	113	47.9	33	48.5	57	46.0
		AA	250	21.9	48	20.3	15	22.1	20	16.1
IL10	-1116A>G, rs1800896, aka -1082	TOTAL	1134		235		69		123	
		TT	340	30.0	54	23.0	19	27.5	26	21.1
		TC	526	46.4	131	55.7	37	53.6	70	56.9
		CC	268	23.6	50	21.3	13	18.8	27	22.0

Table 1. (Continued)

Gene	Polymorphism	Genotype	Control		Stomach		Cardia		Non-cardia	
			n	%	n	%	n	%	n	%
IL10	-7334C>T, rs1800871, aka -819	TOTAL	1094		229		65		118	
		CC	636	58.1	145	63.3	41	63.1	73	61.9
		CT	378	34.6	72	31.4	20	30.8	39	33.1
		TT	80	7.3	12	5.2	4	6.2	6	5.1
IL10	-6653C>A, rs1800872, aka -592	TOTAL	1122		237		69		122	
		GG	642	57.2	148	62.4	44	63.8	73	59.8
		GT	397	35.4	78	32.9	21	30.4	44	36.1
		TT	83	7.4	11	4.6	4	5.8	5	4.1
IL12A	2204 bp 3' of STP A>G, 3' UTR, rs668998	TOTAL	1158		242		70		125	
		AA	358	30.9	80	33.1	23	32.9	38	30.4
		AG	560	48.4	120	49.6	39	55.7	60	48.0
		GG	240	20.7	42	17.4	8	11.4	27	21.6
IL12B	Ex8+159A>C, 3' UTR, rs3212227, aka A16974C	TOTAL	1060		230		65		120	
		TT	677	63.9	139	60.4	42	64.6	70	58.3
		TG	343	32.4	74	32.2	19	29.2	41	34.2
		GG	40	3.8	17	7.4	4	6.2	9	7.5
LTA	IVS1+90A>G, rs909253, aka +252	TOTAL	1126		235		69		120	
		AA	533	47.3	95	40.4	26	37.7	47	39.2
		AG	472	41.9	118	50.2	37	53.6	63	52.5
		GG	121	10.7	22	9.4	6	8.7	10	8.3
LTA	IVS1-82G>C, rs746868, aka +368	TOTAL	1124		237		69		122	
		GG	398	35.4	83	35.0	26	37.7	42	34.4
		CG	545	48.5	111	46.8	33	47.8	61	50.0
		CC	181	16.1	43	18.1	10	14.5	19	15.6
TNF	-417G>A, rs361525, aka -238	TOTAL	1123		235		69		120	
		GG	1004	89.4	218	92.8	64	92.8	113	94.2
		GA	114	10.2	16	6.8	5	7.2	6	5.0
		AA	5	0.4	1	0.4	0	0.0	1	0.8
TNF	-487G>A, rs1800629, aka -308	TOTAL	1125		236		69		121	
		GG	820	72.9	170	72.0	45	65.2	91	75.2
		GA	274	24.4	64	27.1	24	34.8	28	23.1
		AA	31	2.8	2	0.8	0	0.0	2	1.7

Hp antibodies. Quantification of anti-*Hp* antibodies and CagA antibodies in stored plasma sample (0.5 ml straw) was done by enzyme-linked immunosorbent assay as described elsewhere [25].

statistical methods. Each gene polymorphism was tested in controls to ensure the fitting with HWE. Multiple conditional logistic regression analyses were used for the analysis of associations between polymorphisms and GC, after adjusting for *Hp* infection, education, weight, height, physical activity at work and leisure time, tobacco smoking status (never, former and current), number of cigarettes by day (in current smokers only), intake of vegetables, fresh fruits, red and processed meat (in grams/day) and energy intake (kcal/day). Analyses were carried out initially under a codominant inheritance model (results not shown). Then simplified models were chosen: a dominant model—heterozygotes grouped with the homozygotes for the minor allele when both genotypes had a similar effect—or a recessive model—heterozygotes grouped with the homozygotes for the major allele. Reference genotype was defined as the homozygous more prevalent allele (wild type) in dominant models and as the homozygous wild type combined with the heterozygous genotype in recessive ones. The remaining genotypes were classified as variant. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for the variant compared with the reference category.

Pairwise LD for polymorphisms within the same gene or chromosomal region was measured using the metric r^2 . Haplotypes were inferred by use of PHASE 2.0 which implements a Bayesian algorithm to estimate the haplotype frequencies (<http://www.stat.washington.edu/stephens/software.html>). For each individual, the compatible haplotypes and their posterior probabilities were computed and coded with dummy indicator variables. *P* values for interaction were computed using the likelihood ratio test. The Wald statistic was used to test for homogeneity of risk.

results

The frequencies of the genotypes are shown in Table 1. All polymorphisms were in HWE among controls with the exception of *IL1B* -580T>C, *IL6* -236G>C, *IL10* -1116A>G and *IL10* -7334C>T (*P* values between 0.02 and 0.04). SNPs within *IL1RN* ($r^2 = 0.99$), *IL4* ($r^2 = 0.91$) and *IL10* ($r^2 = 1.00$ for *IL10* -7334C>T and *IL10* -6653C>A) genes were in strong LD. The other analyzed polymorphisms in the *IL1* cluster, *IL4R*, *IL10*, and *LTA/TNF*, were not in strong LD ($r^2 < 0.6$).

IL1B -580T>C and *IL1B* Ex5 + 14C>T were not associated with GC risk (Table 2). Two of the proinflammatory *IL1RN*

Table 2. Risk of stomach, cardia and non-cardia adenocarcinoma by genotype

Gene	Polymorphism region	Stomach				Cardia				Non-cardia			
		n ^a	OR	95% CI	P value	n ^a	OR	95% CI	P value	n ^a	OR	95% CI	P value
<i>IL1A</i>	Ex1+12C>T	122	1.05	0.78–1.41	0.76	29	0.76	0.44–1.33	0.34	69	1.23	0.82–1.86	0.32
<i>IL1B</i> ^b	–580T>C	136	1.12	0.83–1.49	0.46	40	0.97	0.55–1.70	0.92	68	1.12	0.75–1.68	0.57
<i>IL1B</i>	Ex5+14C>T	99	1.00	0.74–1.34	0.98	28	1.04	0.59–1.83	0.89	52	1.01	0.66–1.53	0.97
<i>IL1RN</i>	Ex5–35T>C	128	1.45	1.08–1.93	0.01	33	0.96	0.56–1.66	0.89	65	1.52	1.00–2.29	0.05
<i>IL1RN</i>	IVS2 86-bp repeat (2R carriers)	130	1.40	1.06–1.87	0.02	34	0.98	0.58–1.67	0.95	65	1.43	0.95–2.14	0.09
<i>IL1RN</i>	IVS2 86-bp repeat (2R/2R)	28	1.77	1.10–2.85	0.02	7	1.24	0.50–3.07	0.64	16	2.02	1.05–3.91	0.04
<i>IL1RN</i>	IVS2 86-bp repeat (4R/4R)	100	0.71	0.53–0.95	0.02	33	1.11	0.65–1.90	0.69	52	0.65	0.43–0.98	0.04
<i>IL4</i>	–588C>T	82	1.26	0.92–1.72	0.15	26	1.36	0.77–2.40	0.29	42	1.16	0.76–1.79	0.49
<i>IL4</i>	Ex1–168C>T	83	1.35	0.99–1.84	0.06	26	1.55	0.88–2.76	0.13	43	1.21	0.79–1.85	0.37
<i>IL4R</i>	Ex5+14A>G	171	1.22	0.89–1.68	0.22	49	1.42	0.76–2.67	0.27	89	1.19	0.77–1.85	0.44
<i>IL4R</i>	–29429C>T	125	1.34	1.00–1.80	0.05	34	1.14	0.63–2.06	0.66	71	1.74	1.15–2.63	0.01
<i>IL6</i> ^b	–236G>C	160	1.14	0.82–1.57	0.43	43	0.77	0.43–1.39	0.39	86	1.30	0.83–2.06	0.25
<i>IL8</i>	–251T>A	156	0.76	0.56–1.05	0.09	45	0.84	0.47–1.50	0.55	75	0.57	0.37–0.87	0.01
<i>IL10</i> ^b	–1116A>G	177	1.47	1.05–2.07	0.03	47	1.15	0.64–2.09	0.64	96	1.59	0.97–2.60	0.07
<i>IL10</i> ^b	–7334C>T	82	0.78	0.57–1.07	0.13	22	0.73	0.41–1.30	0.28	45	0.88	0.56–1.36	0.56
<i>IL10</i>	–6653C>A	86	0.82	0.60–1.10	0.19	23	0.69	0.39–1.21	0.20	48	0.99	0.65–1.51	0.96
<i>IL12A</i>	2204 bp 3' of STP A>G	158	0.86	0.63–1.17	0.34	45	0.94	0.53–1.68	0.84	85	0.94	0.61–1.45	0.78
<i>IL12B</i>	Ex8+159A>C	90	1.23	0.89–1.69	0.21	23	1.44	0.78–2.65	0.24	49	1.30	0.85–2.01	0.23
<i>LTA</i>	IVS1+90A>G	136	1.31	0.97–1.77	0.08	41	1.31	0.75–2.30	0.34	71	1.36	0.89–2.10	0.16
<i>LTA</i>	IVS1–82G>C	153	1.03	0.76–1.41	0.83	42	0.90	0.49–1.65	0.74	80	1.07	0.70–1.64	0.76
<i>TNF</i>	–417G>A	16	0.58	0.33–1.02	0.06	4	0.62	0.20–1.93	0.41	7	0.45	0.20–1.02	0.06
<i>TNF</i>	–487G>A	63	0.98	0.70–1.37	0.90	22	1.08	0.60–1.95	0.80	29	0.78	0.48–1.28	0.33

All single-nucleotide polymorphism effects computed using dominant inheritance models. IL, interleukin; LTA, lymphotoxin α ; TNF, tumor necrosis factor; OR, odds ratio; CI confidence interval. Significant odds ratios with 95% confidence intervals are indicated in bold. Alternative phrasing: bold face numbers denote statistically significant associations.

^aNumber of cases in the effect category.

^bPolymorphisms that were not in Hardy–Weinberg equilibrium.

alleles (2R of the IVS2 repeat and Ex5–35C) were associated with a significantly increased risk of GC that was restricted to non-cardia neoplasm (OR 2.02; 95% CI 1.05–3.91 for the 2R/2R genotype) (Table 2). We observed that these positive associations with alleles *IL1RN* 2R and *IL1RN* Ex5–35C were limited to Hp-positive subjects (Table 3). Genotype *IL1RN* 2R/2R seems to be associated with both histological types although association was only significant for the diffuse type (Table 4).

The proinflammatory allele *LTA* IVS1+90G showed modest not significant association with GC risk (Table 2). Also, allele *LTA* IVS1+90G was associated with the diffuse type ($P = 0.04$) (Table 4) especially in Hp-positive cases. On the other hand, carriers of allele *TNF* –487A, associated with higher TNF- α production, did not associate with an increased GC risk (Table 2).

Allele *IL8* –251A was associated with a significant reduced risk of non-cardia GC (OR 0.57; 95% CI 0.37–0.87). This negative association was restricted to the Hp-positive group, irrespective of CagA status (Table 3), and to intestinal-type carcinomas ($P = 0.01$) (Table 4).

Some significant associations between *IL4R*, *IL4* alleles (especially for *IL4R* –29429T allele) and GC were found (Tables 2 and 3) but did not follow consistent patterns. Also, a significant increase of GC risk for *IL10* –1116A>G was observed that was not confirmed when considering non-cardia cancer exclusively (Table 2). Finally, it must be emphasized that

no association with any polymorphism was observed when cardia carcinomas were separately considered (data not shown). We did not find any additional association with the other polymorphisms analyzed.

Haplotype analysis on polymorphisms in *IL10*; *IL1A*, *IL1B* and *IL1RN*; *TNF* and *LTA* either confirmed or reinforced the information provided by each polymorphism analyzed individually (Table 5). Thus, from the five haplotypes with frequencies >5% defined by the four SNPs analyzed in the *IL1A* (Ex1+12C>T), *IL1B* (Ex5+14T>C and –580T>C) and *IL1RN* (Ex5–35T>C) genes at 2q13, only those carrying the C allele of *IL1RN* Ex5–35T>C were found to increase the risk of non-cardia GC.

The four SNPs studied in the *TNF/LTA* region encompass the five haplotypes previously reported [26]. The low prevalence of the haplotype AGGA carrying the *TNF* –417A allele precludes drawing conclusions. We also explored the effect of combinations of proinflammatory genotypes [11] and found that the OR did not increase progressively with increasing number of genotypes (data not shown).

discussion

This is the second [12] and the largest prospective study in healthy volunteers from Western countries evaluating the association between individual susceptibility in cytokine genes

Table 3. Risk of non-cardia adenocarcinoma by genotype among subjects positive and negative for antibodies against *Helicobacter pylori* (Hp) and CagA^a

Gene	Polymorphism	Non-cardia				Hp+				Hp-				CagA+				CagA-				P for interaction					
		n ^b	OR	95% CI	P value	n ^b	OR	95% CI	P value	n ^b	OR	95% CI	P value	n ^b	OR	95% CI	P value	n ^b	OR	95% CI	P value	n ^b	OR	95% CI	P value	n ^b	OR
<i>IL1RN</i>	Ex5-35T>C	6	0.64	0.17-2.48	0.52	59	1.62	1.03-2.54	0.04	0.38	9	2.52	0.70-9.13	0.16	50	1.60	0.96-2.67	0.07	0.51								
<i>IL1RN</i>	IVS2 86-bp repeat	6	0.64	0.17-2.48	0.52	59	1.49	0.96-2.32	0.08	0.56	9	2.26	0.64-7.98	0.21	50	1.48	0.89-2.44	0.13	0.46								
	(2R carriers)																										
<i>IL1RN</i>	IVS2 86-bp repeat (2R/2R)	1	0.44	0.05-4.16	0.47	15	2.43	1.19-4.96	0.02	0.32	2	4.28	0.48-38.5	0.20	13	2.01	0.90-4.49	0.09	0.45								
<i>IL1RN</i>	VNTR rs2234663 (4R/4R)	5	1.17	0.31-4.38	0.82	47	0.66	0.42-1.03	0.07	0.83	5	0.47	0.13-1.68	0.24	42	0.69	0.41-1.14	0.14	0.50								
<i>IL4</i>	Ex1-168C>T	2	0.43	0.08-2.38	0.34	41	1.30	0.82-2.05	0.27	0.22	6	2.46	0.55-11.1	0.24	35	1.31	0.77-2.23	0.33	0.64								
<i>IL4R</i>	Ex5+14A>G	11	10.3	0.96-111	0.05	78	0.92	0.57-1.48	0.73	0.08	6	0.25	0.06-1.04	0.06	72	1.01	0.58-1.76	0.98	0.10								
<i>IL4R</i>	-29429C>T	8	2.54	0.62-10.4	0.19	63	1.75	1.11-2.75	0.02	0.81	5	0.91	0.24-3.49	0.90	58	1.68	1.01-2.80	0.05	0.42								
<i>IL8</i>	-251T>A	9	1.15	0.25-5.32	0.85	66	0.51	0.32-0.81	0.01	0.26	7	0.14	0.03-0.70	0.02	59	0.55	0.32-0.93	0.03	0.40								
<i>IL12A</i>	2204 bp 3' of STP A>G	5	0.37	0.09-1.51	0.16	80	1.08	0.66-1.76	0.75	0.08	9	0.67	0.16-2.81	0.58	71	1.20	0.69-2.08	0.52	0.37								
<i>IL12B</i>	Ex8+159A>C	4	1.04	0.27-4.05	0.95	45	1.31	0.82-2.09	0.25	0.37	3	0.53	0.11-2.49	0.42	42	1.30	0.77-2.20	0.33	0.60								
<i>LTA</i>	IVS1+90A>G	10	5.95	1.03-34.2	0.05	61	1.18	0.74-1.88	0.49	0.07	8	1.74	0.45-6.76	0.43	53	1.13	0.66-1.92	0.66	0.70								

All single-nucleotide polymorphism effects computed using dominant inheritance models. P for interaction computed using likelihood ratio test. OR, odds ratio; CI, confidence interval; IL, interleukin; LTA, lymphotoxin α ; VNTR, variable number of tandem repeats. Significant odds ratios with 95% confidence intervals are indicated in bold.

^aUnconditional logistic regression adjusted by sex, center, age and date of blood extraction.

^bNumber of cases in the effect category.

and GC risk in Caucasian European populations. We have found that proinflammatory IL1 receptor antagonist genotypes *IL1RN* 2R/2R and *IL1RN* Ex5-35C/C, adequate surrogate markers of *IL1* gene cluster, were significantly associated with an increased risk of non-cardia adenocarcinoma, apparently restricted to Hp-positive cases.

Our results add to the current controversy about the role of these polymorphisms in GC risk, recently reported in three meta-analyses [7-9]. The first [7] concluded that allele *IL1B* -1060T (aka *IL1B* -511T)—but not the *IL1B* -580C (*IL1B* -31C) allele in near complete LD—and allele *IL1RN* 2R were significantly associated with GC risk in Caucasians but not in Asians, where these alleles are rare. This association was more evident for non-cardia neoplasm mainly of the intestinal type. A second meta-analysis [8] observed no association between *IL1B* or *IL1RN* polymorphisms and GC risk, even in studies conducted in Western countries. The third [9] largest meta-analysis concluded that *IL1B* -1060 and *IL1RN* gene polymorphisms are associated with an increased GC risk but this association was less evident when only good quality epidemiological studies were considered.

IL1B encodes IL1B, a potent proinflammatory cytokine and a powerful inhibitor of gastric acid secretion that is believed to play a major role in the inflammatory response to Hp infection. *IL1RN* encodes the endogenous IL-1 receptor antagonist, a regulatory cytokine that competitively binds to type I IL-1 receptors. The *IL1B* -1060TT genotype (and therefore also *IL1B* -580CC) and the *IL1RN* 2R allele are associated with increased gastric mucosal levels of IL-1 β [27]. Carriage of allele *IL1B* -1060T and *IL1RN* 2R/2R genotype has been found to be associated with an increased risk of developing a hypochlorhydric response to Hp [5] and considered as proinflammatory. In line with previous studies [5-8], we did not observe an association between *IL1B* -580T>C SNP and GC risk. It must be emphasized that this allele was in HWE equilibrium among controls as previously reported [11], a fact that cannot be attributed to genotyping errors.

Associations of proinflammatory alleles with specific histological types remain controversial [5, 11, 26]. While our results indicate that this association may be similar for both histological types, the small size of these subgroups precludes drawing conclusions. In our cohort, Hp infection increases the risk of non-cardia GC by 2- to 3-fold [25]. However, the simultaneous association between the *IL1B* proinflammatory allele and CagA-positive strains did not identify a population at high risk, thus failing to replicate previous studies [20].

Our study points to a positive association between allele *LTA* IVS1+90G and GC risk, mainly for diffuse type positive for Hp. Previously, carriers of allele *TNF* -487A (aka -308A) were associated with an increase in risk of non-cardia GC [11] in both anatomical subtypes [10]. We have not been able to reproduce these findings in our European study, in line with recent studies in Caucasian [11] and non-Caucasian populations [13, 18, 28]. The low (<1%) frequency of the AA genotype of *TNF* -417G>A (aka -238G>A) SNP and its tagged protective *TNF/LTA* haplotype precluded drawing any conclusion with respect to GC risk.

Promoter allele *IL8*-251A has been associated with an increased production of IL8 [12, 29]. Previous studies exploring

Table 4. Risk of diffuse and intestinal non-cardia gastric adenocarcinoma by genotype (overall and among Hp infected)

Gene	Polymorphism	Diffuse								Intestinal							
		Overall ^a				Hp ^b				Overall ^a				Hp ^b			
		n ^c	OR	95% CI	P value	n ^c	OR	95% CI	P value	n ^c	OR	95% CI	P value	n ^c	OR	95% CI	P value
<i>IL1RN</i>	Ex5-35T>C, rs419598	49	1.41	0.89-2.22	0.14	44	1.41	0.84-2.38	0.19	52	1.37	0.84-2.25	0.21	43	1.38	0.79-2.41	0.25
<i>IL1RN</i>	IVS 86-bp repeat (ref: 4R/4R)	40	Ref			36	Ref			35	Ref			28	Ref		
	(2R/3R+2R/5R+3R/4R+4R/5R+4R/6R)	4	0.77	0.24-2.49	0.66	3	0.60	0.16-2.19	0.44	4	0.82	0.26-2.59	0.74	4	0.83	0.25-2.75	0.77
	(2R/4R)	36	1.18	0.72-1.94	0.51	33	1.18	0.67-2.08	0.56	42	1.31	0.79-2.18	0.29	35	1.33	0.75-2.38	0.33
	(2R/2R)	11	2.63	1.14-6.06	0.02	9	2.47	0.96-6.35	0.06	13	1.91	0.88-4.13	0.10	11	2.38	0.98-5.75	0.06
<i>IL1RN</i>	IVS2 86-bp repeat (2R carriers)	47	1.27	0.81-2.00	0.30	42	1.29	0.77-2.16	0.34	55	1.38	0.86-2.21	0.18	46	1.40	0.82-2.38	0.22
<i>IL1RN</i>	IVS2 86-bp repeat (2R/2R)	11	2.50	1.13-5.52	0.02	9	2.39	0.96-5.94	0.06	13	1.68	0.81-3.45	0.16	11	2.08	0.92-4.72	0.08
<i>IL1RN</i>	IVS2 86-bp repeat (4R/4R)	40	0.78	0.49-1.24	0.29	36	0.80	0.48-1.35	0.41	35	0.74	0.46-1.19	0.21	28	0.71	0.42-1.23	0.22
<i>IL4</i>	Ex1-168C>T	30	1.20	0.72-2.00	0.49	27	1.09	0.62-1.90	0.77	34	1.56	0.96-2.55	0.07	30	1.86	1.06-3.27	0.03
<i>IL4R</i>	Ex5+14A>G	70	1.51	0.89-2.55	0.13	64	1.49	0.82-2.71	0.19	60	0.90	0.54-1.50	0.69	48	0.78	0.45-1.37	0.39
<i>IL4R</i>	-29429C>T	50	1.49	0.93-2.41	0.10	44	1.53	0.88-2.63	0.13	45	1.16	0.71-1.90	0.55	36	1.14	0.66-1.98	0.63
<i>IL8</i>	-251T>A	62	0.98	0.58-1.65	0.94	54	0.97	0.54-1.74	0.92	56	0.52	0.32-0.86	0.01	46	0.45	0.25-0.79	0.01
<i>LTA</i>	IVS1+90A>G	56	1.61	0.97-2.66	0.07	50	1.79	1.02-3.16	0.04	47	0.96	0.59-1.54	0.86	36	0.73	0.42-1.28	0.28
<i>TNF</i>	-417G>A	10	1.08	0.52-2.28	0.83	9	1.11	0.47-2.63	0.81	2	0.15	0.03-0.66	0.01	1	0.08	0.01-0.65	0.02

All single-nucleotide polymorphism effects computed using dominant inheritance models. Hp, *Helicobacter pylori*; OR, odds ratio; CI, confidence interval; IL, interleukin; LTA, lymphotoxin α; TNF, tumor necrosis factor. Significant odds ratios with 95% confidence intervals are indicated in bold.

^aConditional logistic regression, adjusted by Hp infection. Matched by sex, center, age and date of blood extraction.

^bUnconditional logistic regression adjusted by sex, center, age and date of blood extraction.

^cNumber of cases in the effect category.

Table 5. Risk of cardia, non-cardia, diffuse, and intestinal adenocarcinoma by haplotype

Genes	Haplotype ^a	% in controls	Cardia			Non-cardia			Diffuse			Intestinal				
			OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value		
<i>IL10</i> (-7334C>T; -6653C>A; -1116A>G)	111	28.3	Ref			Ref			Ref			Ref				
	112	46.8	1.00	0.64-1.57	1.00	1.00	0.71-1.40	0.99	1.14	0.77-1.69	0.53	1.00	0.67-1.47	0.98		
	221	24.9	0.75	0.44-1.30	0.31	0.89	0.59-1.32	0.55	0.87	0.54-1.40	0.57	0.81	0.52-1.28	0.37		
<i>IL1A</i> Ex1+12T>C; <i>IL1B</i> Ex5+14C>T; <i>IL1B</i> -580T>C; <i>IL1RN</i> Ex5-35T>C	1111	32.1	Ref			Ref			Ref			Ref				
	1112	7.1	0.84	0.37-1.92	0.68	1.89	1.10-3.25	0.02	1.39	0.75-2.57	0.30	1.11	0.57-2.16	0.75		
	1121	13.7	0.89	0.49-1.64	0.71	1.23	0.76-2.02	0.40	0.96	0.55-1.67	0.89	1.02	0.58-1.81	0.94		
	1122	14.0	0.94	0.52-1.70	0.83	1.53	0.97-2.41	0.07	1.28	0.76-2.17	0.35	1.31	0.81-2.14	0.27		
	2211	17.3	0.96	0.53-1.75	0.89	1.32	0.86-2.05	0.21	0.90	0.54-1.50	0.69	1.10	0.66-1.83	0.72		
	Other ^b		0.97	0.52-1.80	0.91	1.31	0.85-2.01	0.22	1.00	0.60-1.66	0.99	1.41	0.85-2.34	0.18		
<i>LTA</i> (IVS1+90A>G; IVS1-82G>C) + <i>TNF</i> (-487G>A; -417G>A)	1111	22.5	Ref			Ref			Ref			Ref				
	1112	5.5	0.47	0.12-1.75	0.26	0.57	0.26-1.28	0.17	1.36	0.62-3.00	0.45	0.16	0.04-0.67	0.01		
	1211	40.3	1.04	0.61-1.76	0.90	0.99	0.67-1.46	0.97	1.11	0.70-1.76	0.66	0.91	0.58-1.42	0.68		
	2111	16.8	1.06	0.57-2.01	0.85	1.18	0.75-1.86	0.48	1.34	0.78-2.29	0.29	0.91	0.53-1.57	0.74		
	2121	14.9	1.01	0.53-1.92	0.98	0.81	0.48-1.35	0.41	1.39	0.78-2.46	0.26	0.70	0.39-1.27	0.24		
<i>IL4R</i> (-29429C>T; Ex5+14A>G)	11	53.4	Ref			Ref			Ref			Ref				
	12	18.6	1.08	0.62-1.88	0.78	0.74	0.49-1.12	0.16	0.89	0.56-1.42	0.63	0.83	0.52-1.32	0.43		
	21	1.2	0.19	0.01-3.45	0.26	1.56	0.46-5.27	0.48	1.13	0.26-4.87	0.87	1.29	0.30-5.57	0.73		
	22	26.9	1.01	0.64-1.59	0.97	1.14	0.82-1.58	0.44	1.26	0.85-1.85	0.25	0.88	0.60-1.30	0.52		

Individuals with no information for all the single-nucleotide polymorphisms used to estimate the haplotypes have been excluded. OR, odds ratio; CI, confidence interval; IL, interleukin; LTA, lymphotoxin α; TNF, tumor necrosis factor. Significant odds ratios with 95% confidence intervals are indicated in bold.

^aHaplotypes with low uncertainty levels formed by major (1) and minor (2) alleles.

^bHaplotypes with frequencies <5% and/or high uncertainty levels.

IL8 –251A and GC risk yielded contradictory results [12, 14, 29, 30]. The association of allele *IL8* –251A with a decreased risk of Hp-positive non-cardia GC is in line with a previous Asian study [30].

In contrast with the proinflammatory alleles, no clear association of anti-inflammatory alleles and GC risk was observed. In accordance with another study [11], we detected no association between SNP *IL4* –588C>T and GC risk, for which inconsistent results were found in an Asian population [19]. Also, a positive association with the –29429 variant of the *IL4R* gene was observed. This is paradoxical, since the variant, by inducing an increased response to IL4 [18, 31], should be associated with a decreased GC risk. Finally, we cannot rule out a role of *IL10* 1116G allele but both the lack of HWE and the modest degree of association preclude drawing more definitive conclusions.

Previous studies have indicated that considering simultaneously multiple proinflammatory genotypes will incrementally increase the risk of non-cardia GC [10, 11]. We examined the effect of similar combinations of proinflammatory genotypes and found, as in another cohort study [11], that the OR did not increase progressively with increasing number of genotypes. Altogether, our results indicate that assessment of polymorphisms on inflammatory genes may not be useful for the identification of high-risk populations in the clinical setting.

Our study design has several advantages. It was carried out in a highly homogeneous Caucasian population. Most of the cases were validated by a panel of expert pathologists. Its prospective nature allows Hp serology to be accurately determined in healthy subjects before the onset of the disease. An additional advantage is that our healthy controls are not affected by diseases that may be associated with polymorphisms of interest.

On the other hand, this study has limitations. Although its statistical power for GC analysis remains among the highest reported so far (80% power at the 5% significance level to detect main effects of genotypes with a frequency between 5% and 10% in controls for an OR of 1.5), the number of cases is low for subtypes analyses. Since many tests were carried out, some false-positive results may be expected. However, it must be considered that all the gene polymorphisms analyzed were included because there was *a priori* hypothesis about its potential relationship with the disease. Thus, each test could be considered, to some extent, independent. For this reason, we decided not to apply any correction for multiple testing.

Our results strongly support the role of genetic variability of *IL1RN* gene, or in other genes in strong LD with it in the risk of non-cardia GC in Caucasians, mainly in those Hp+. Similarly, it also indicates that *IL8*, and maybe *IL4R*, variants may modify the risk for GC. The inconsistency of the results reported so far indicates, however, that the influence of genetic variations in the intensity of the inflammatory response may be more modest than initially expected and/or that the most relevant genes have not been identified yet. Larger studies that take into account simultaneously the different environmental and life-style factors potentially involved in gastric carcinogenesis are needed to better explore gene–environmental interactions as well as the role of novel candidate genes.

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