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SHORT COMMUNICATION

Polymorphisms *PSCA* rs2294008, *IL-4* rs2243250 and *MUC1* rs4072037 are associated with gastric cancer in a high risk population

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Abstract

Genetic variants are considered risk factors for gastric cancer. To date, 61 polymorphisms have been identified as associated with this disease. The aim of the present study was to analyze the association of some of those polymorphisms with GC in Chile. We performed a case-control study including 310 gastric cancer cases and 311 controls to assess the association of 36 single-nucleotide polymorphisms genotyped by Global Screening Array (GSA). Three polymorphisms was significantly associated: *PSCA* rs2294008 (allele model, OR = 1.49, 95%CI 1.17–1.88, $P = 1.08 \times 10–3$), *IL-4* rs2243250 (allele model, OR = 1.28, 95%CI 1.01–1.62, $P = 0.04$), and *MUC1* rs4072037 (allele model, OR = 0.78, 95%CI 0.61–0.99, P = 0.04).*PSCA* rs2294008, *IL-4* rs2243250 and *MUC1* rs4072037 are associated with gastric cancer in Chile. It suggests that those polymorphisms could be used as biomarkers to assess the genetic risk for this cancer outside of the previously studied populations, not only for East Asians and Caucasians populations.

Keywords Gastric cancer · Polymorphism · PSCA · IL-4 · MUC1

Introduction

Gastric cancer (GC) is the third cause of death related to cancer worldwide. Rates of incidence vary among countries, being higher in East Asia, Eastern Europe and South America, and lower in Africa, Oceania and North America [1]. Risk factors for GC include smoking, *Helicobacter pylori* infection, high intake of salty and smoked food, low consumption of fruits and vegetables and genetic factors [1]. Tian et al. [2] recently published a summary of meta-analyses assessing the contribution of genetic polymorphisms to GC risk, identifying a total of 61 polymorphisms associated with the disease with weak, moderate and strong level of evidence and 12 hits in genome-wide association studies (GWAS). Studies evaluating the association of those polymorphisms with GC are scarce in South America. In the present study we aimed to assess the association of some of those polymorphisms with GC in Chile.

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Materials and methods

Subjects

The study included 310 cases (202 men and 108 women) with gastric adenocarcinoma confirmed by a pathologist. The median age of diagnosis was 66 years (range 25–93). Patients were recruited between 2001 and 2018 at the time of surgical resection from the following hospitals in Santiago, Chile: Hospital Clínico de la Universidad de Chile and Biobanco de Tejidos y Fluidos de la Universidad de Chile (BTUCH), Hospital del Salvador, Hospital Barros Luco Trudeau, Hospital San Juan de Dios and Hospital Militar de Santiago. The control group included 311 individuals with no personal history of cancer (187 men and 124 women with a median age 53 years, range 18–82). This study was approved by the institutional review board of University of Chile School of Medicine (#045/2015) and was performed in accordance with the Declaration of Helsinki. All participants gave their written informed consent.

Genotyping

DNA was obtained from a blood sample collected in EDTA vacutainers and further cleaned using Monarch PCR and DNA cleanup columns (New England Biolabs, USA). Single nucleotide polymorphisms (SNPs) were genotyped by Infinium Global Screening Array-24 BeadChip (GSA) (Illumina, USA) according to the manufacturer's protocol at Erasmus MC, Netherlands. Quality control of genotype data was performed according to the guidelines in Anderson et al. [3]. The array contains 36 out of the 71 SNPs described by Tiang et al. [2]. rs3918242, rs763780, rs4986790, rs671 have an allele frequency < 0.05 in the control sample and were excluded from the analysis. Table 1 lists the 32 included SNPs.

Statistical analyses

The exact test was used to detect departures from the Hardy-Weinberg equilibrium (HWE). A set of 184,909 SNPs included in the array were used to conduct a principal component (PC) analysis as described in González-Hormazábal et al. [4]. Univariate (crude) and multivariate (adjusted) logistic regression models were used to assess the association of SNPs with GC, using sex, PC1 and PC2 (to correct for population stratification) as covariates. All statistical analyses were carried out using PLINK 1.9. A nominal P value <0.05 was considered statistically significant.

Results

The rs2294008 polymorphism, located in the prostate stem cell antigen (*PSCA*) gene, was significantly associated with GC (Table 1) under the dominant model but not statistically significant under the recessive model (Table 2). The second associated polymorphism was rs2243250 in the interleukin-4 (*IL-4*) gene. The association was statistically significant for the recessive model but not for the dominant model. The polymorphism rs4072037 of mucin-1 gene (*MUC1*) was also associated with GC under the allele model. Nevertheless, neither the dominant model nor the recessive model reached statistical significance.

Discussion

Sakamoto et al. [5] were the first to describe the rs2294008 polymorphism as associated with gastric cancer in a GWAS that included subjects from Japan and Korea. Subsequently, the same result was replicated in some GWAS involving individuals from Asia and in a study in Iceland [6]. Various case-control studies have been conducted to assess the role of rs2294008 in GC risk. Cui et al. [6] carried out a systematic synopsis and meta-analysis of studies published up to 2018 analyzing polymorphisms in *PSCA*. From this study, the meta-analysis of rs2294008, comprising 36.439 cases and 264.838 controls, gave an OR = 1.32 95%CI 1.27–1.39, $P = 5.1 \times 10^{-33}$, highlighting the association of this polymorphism with GC. Only seven out of the 42 published studies were conducted in Caucasians. One study [7] was carried out in South America and included 178 cases and 1057 controls from Venezuela (OR = 1.44, 95%CI 1.14–1.81). Therefore, our study contributes additional evidence for the role of rs2294008 polymorphism in GC in South America, and also suggests that rs2294008 confers risk of GC independent of ethnicity. Additional studies in other populations such as Afro-Americans are needed to confirm it.

PSCA encodes a glycosylphosphatidylinositol-anchored cell surface glycoprotein. The evidence suggests that PSCA contributes to cell cycle control and proliferation, regulates immune response, and therefore may link its functions with carcinogenesis; however the possible mechanism for promoting carcinogenesis remains obscure [8]. Data from an expression profile database [9] reveal that this gene is upregulated in prostate and bladder cancer, however is downregulated in GC. A recent study [10] showed that downregulation of PSCA increases the proliferation ability of GC cells in vitro and in vivo, while overexpression can reduce their proliferation in vitro. Sakamoto et al. [5], simultaneously finding that rs2294008 is associated with GC, found in a reporter luciferase assay that rs2294008-C allele increases

Table 1 Allele frequencies, genotype counts and association measures (odds ratio) under the allele model, for the analyzed polymorphisms

rsID	Gene	Tested allele	Tested allele frequency in controls	HWE P value	Genotype counts in cases[a]	Genotype counts in controls[a]	OR (95% CI)[b]	*P* value	OR (95% CI)[c]	P value
rs4072037	*MUC1*	G	0.34	0.90	27/123/158	37/139/135	0.78 (0.61–0.99)	0.04	0.78 (0.61–0.99)	0.04
rs763110	*FASL*	T	0.27	0.67	35/113/161	21/126/162	1.12 (0.88–1.43)	0.36	1.15 (0.90–1.48)	0.25
rs1801282	*PPARG*	G	0.11	0.40	2/50/257	2/65/244	0.76 (0.52–1.11)	0.16	0.77 (0.52–1.13)	0.19
rs13361707	*PPKAA1*	T	0.25	0.36	13/109/186	16/124/170	0.83 (0.63–1.08)	0.17	0.84 (0.64–1.10)	0.21
rs2243250	*IL-4*	T	0.30	0.50	47/129/133	26/137/147	1.28 (1.01–1.62)	0.04	1.28 (1.01–1.62)	0.04
rs2275913	*IL-17A*	A	0.21	0.32	17/87/203	11/111/189	0.90 (0.69–1.19)	0.47	0.93 (0.70–1.23)	0.60
rs2294008	*PSCA*	T	0.53	0.91	110/162/35	87/156/67	1.49 (1.17–1.88)	1.08×10^{-3}	1.45 (1.14–1.84)	2.22×10^{-3}
rs3781264	*PLCE1*	C	0.20	0.72	13/102/195	13/97/199	1.05 (0.79–1.38)	0.75	1.09 (0.82–1.44)	0.56
rs2274223	*PLCE1*	G	0.26	0.87	15/112/182	15/103/190	1.08 (0.83–1.42)	0.56	1.21 (0.85–1.47)	0.41
rs751402	*ERCC5*	T	0.30	0.10	27/135/144	34/117/155	1.03 (0.81–1.31)	0.81	1.04 (0.81–1.32)	0.78
rs1801133	*MTHFR*	T	0.44	0.57	63/155/91	63/148/100	1.06 (0.85–1.32)	0.62	1.04 (0.83–1.30)	0.74
rs20417	*COX2*	C	0.18	1.00	15/100/191	10/93/207	1.21 (0.91–1.60)	0.19	1.27 (0.95–1.69)	0.10
rs689466	*COX2*	G	0.29	0.27	34/116/157	30/120/160	1.04 (0.82–1.32)	0.73	1.03 (0.81–1.30)	0.83
rs16944	*IL-1B*	G	0.46	0.43	65/148/97	63/161/85	0.94 (0.75–1.17)	0.57	0.98 (0.78–1.23)	0.87
rs12693932	*CASP8*	C	0.37	1.00	39/141/128	43/145/122	0.93 (0.74–1.17)	0.53	0.95 (0.75–1.20)	0.66
rs1800734	*MLH1*	A	0.31	1.00	27/124/154	29/132/149	0.93 (0.73–1.19)	0.58	0.96 (0.75–1.22)	0.72
rs9820958	*ZBTB20*	A	0.17	0.42	8/99/201	11/83/217	1.13 (0.84–1.52)	0.41	1.10 (0.82–1.48)	0.51
rs4073	*IL-8*	A	0.35	0.80	38/126/143	38/136/132	0.93 (0.74–1.17)	0.53	0.94 (0.74–1.18)	0.58
rs4444903	*EGF*	A	0.46	0.65	70/142/97	69/149/91	0.97 (0.78–1.21)	0.78	0.97 (0.78–1.22)	0.82
rs1799724	*TNFα*	T	0.27	0.77	23/115/161	22/113/161	1.02 (0.79–1.32)	0.88	0.97 (0.75–1.26)	0.83
rs2234767	*FAS*	A	0.16	0.04	3/71/236	3/94/214	0.72 (0.51–1.01)	0.05	0.74 (0.53–1.03)	0.08
rs1695	*GSTP1*	G	0.42	0.56	60/149/99	53/157/100	1.05 (0.84–1.32)	0.66	1.07 (0.85–1.34)	0.58
rs11568818	*MMP7*	G	0.31	0.51	29/115/163	33/129/149	0.87 (0.68–1.10)	0.24	0.88 (0.69–1.12)	0.31
rs2366150	*HOTAIR*	A	0.39	0.81	40/146/122	48/146/117	0.91 (0.72–1.15)	0.42	0.91 (0.72–1.14)	0.41
rs873601	*ERCC5*	G	0.36	1.00	39/129/127	38/138/122	0.97 (0.76–1.22)	0.77	0.94 (0.74–1.19)	0.61
rs2275007	*APE1*	G	0.44	0.82	59/138/109	59/156/96	0.92 (0.73–1.14)	0.44	0.94 (0.75–1.17)	0.57
rs1800566	*NADPH*	T	0.37	0.33	44/148/118	46/136/128	1.06 (0.84–1.33)	0.64	1.08 (0.86–1.36)	0.52
rs1042522	*TP53*	C	0.24	0.22	16/97/195	14/123/173	0.82 (0.63–1.07)	0.15	0.84 (0.64–1.10)	0.21
rs1800470	*TGF-β1*	C	0.50	0.82	68/160/71	74/156/76	0.99 (0.79–1.25)	0.95	0.97 (0.77–1.22)	0.80
rs1799782	*XRCC1*	T	0.12	0.59	6/69/234	3/68/240	1.12 (0.80–1.57)	0.52	1.10 (0.78–1.54)	0.59
rs13181	*ERCC2*	G	0.23	0.63	19/106/184	14/114/183	1.03 (0.79–1.34)	0.84	1.06 (0.81–1.38)	0.67
rs1799793	*ERCC2*	A	0.22	1.00	16/88/194	13/100/180	0.92 (0.70–1.22)	0.57	0.96 (0.73–1.28)	0.79

HWE Hardy-Weinberg Equilibrium, *OR* Odds ratio, *CI* Confidence interval. rs2275007 is proxy of rs1760944 ($r^2 = 0.95$), rs2366150 is proxy of rs920778 ($r^2 = 0.92$), rs12693932 is proxy of rs3834129 ($r^2 = 0.90$), rs9820958 is proxy of rs9841504 ($r^2 = 0.84$), rs2366150 is proxy of rs920778 ($r^2 = 0.92$), and rs9820958 is proxy of rs9841504 ($r^2 = 0.84$)

[a]aa/Aa/AA, being "a" the tested allele

[b]Crude

[c]Adjusted for sex, principal component (PC) 1 and PC2

Table 2 Association of rs2294008 (*PSCA*), rs2243250 (*IL-4*) and rs4072037 (*MUC1*) with gastric cancer under the dominant and recessive models

Model	rs2294008 OR (95%CI)	rs2243250 OR (95%CI)	rs4072037 OR (95%CI)
Dominant			
Crude	2.14 (1.38–3.34), $P=0.0008$	1.19 (0.87–1.64), $P=0.27$	0.72 (0.53–1.00), P = 0.05
Adjusted	2.09 (1.33–3.28), $P=0.0013$	1.19 (0.86–1.64), $P=0.30$	0.72 (0.53–1.00), $P=0.05$
Recessive			
Crude	1.43 (1.02–2.01), $P=0.039$	1.96 (1.18–3.26), P = 0.009	0.71 (0.42–1.20), $P=0.20$
Adjusted	1.39 (0.99–1.96), $P=0.057$	1.97 (1.18–3.29), $P=0.009$	0.72 (0.42–1.25), $P=0.24$

HWE Hardy-Weinberg Equilibrium, *OR* Odds ratio, *CI* Confidence interval

the reporter activity while rs2294008-T allele (associated with GC risk) reduces it. Together, those findings indicate that these polymorphisms may be functional.

In the synopsis of Tian et al. [2], *IL-4* rs2243250 was classified as having moderate cumulative epidemiological evidence. Nevertheless, the evidence is contradictory. A later meta-analysis [11] did not observe a significant association with GC, overall and stratified by ethnicity. A posterior study by Martinez-Campos et al. [12] on 125 GC patients and 125 controls from Mexico did not find statistically significant association with GC (OR = 1.69, 95%CI 0.82–3.48, $P=0.07$), possibly due to the small sample size. Interestingly, rs2243250-T allele has a high frequency in East Asian populations (0.78), low among Caucasians (0.17) and intermediate in Admixed Americans (0.37). This polymorphism is located at the promoter of *IL-4* gene, and the TT genotype is associated with increased *IL-4* expression in CD4+ cells [13]. Evidence from functional studies suggest a role of IL-4 in tumor biology. In fact, its expression is high in tumor versus normal tissue ($P=4.02\times10^{-4}$) [9].

A recently published meta-analysis [14] gives additional evidence for a relationship of rs4072037-G (*MUC1*) allele with GC as a protective allele in both Asian and Caucasian populations. There is evidence that rs4072037 is a functional polymorphism. The polymorphism is located at exon 2, and creates an alternative splicing site, leading to a difference of 9 amino acids in the signal peptide region of the encoded protein [15].

In conclusion, our study found that *PSCA* rs2294008, *IL-4* rs2243250 and *MUC1* rs4072037 are associated with GC in Chile. This work represents an advance in biomedical science because our results suggest that those polymorphisms could be used as biomarkers to assess the genetic risk for this cancer outside of the previously studied populations, not only for East Asians and Caucasians populations.

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Author contributions PG-H conceived, designed the analysis, performed statistical analyses and wrote the draft manuscript; RR-O and RAV performed statistical analyses; MM, MB, JSt, RP, HV, EL, HC, JSu, LAQ, NMV and ZB collected samples and clinicopathological data, MM cured and analyzed clinicopathological data, DP, LJ and VGC: Performed and contributed to laboratory procedures; PG-H supervised. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the institutional review board of University of Chile School of Medicine (#045/2015).

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