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ORIGINAL RESEARCH

Meta-analysis of the Association Between *PTPN11 G/A* Polymorphism at Intron 3 with Risk of Gastric Atrophy Among East Asians

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Abstract
Aim Inconsistency of reported associations of the *G/A* polymorphism (*rs2301756*) in the *PTPN11* gene and gastric atrophy prompted us to undertake a meta-analysis.
Materials and Methods We searched PubMed for published literature up to July 2013. Individual data from studies with case-control design were evaluated for the *PTPN11 G/A* polymorphism in *Helicobacter pylori* (−) (seronegative) and (+) (seropositive) subjects (four studies each, totaling 3,597 cases and 4,865 controls).
Results Associations of *PTPN11* polymorphism with gastric atrophy in *H. pylori* (−) and (+) subjects are more readily interpreted in the homozygous and recessive models given that the dominant codominant effects skirted null associations. Thus, homozygous and recessive effects indicated reduced risk [odds ratio (OR) 0.92–0.96, p=0.51–0.74], which is significant among *H. pylori* (+) subjects (OR 0.66–0.68, p= 0.04–0.05). Confined to the Japanese, reduced risk effects were unaltered in both groups, less protective among seronegative subjects (OR 0.85–0.86, p=0.71–0.73) than seropositive subjects with significance in the recessive model (OR 0.67, p=0.05). Sensitivity analysis demonstrated robustness of the seropositive findings, but probably not the seronegative results where homozygous and recessive pooled ORs were altered from protection to increased risk.
Conclusions Evidence of overall and subgroup decreased risks, strong in seropositive subjects, demonstrates protective effects of the *PTPN11 G/A* polymorphism from gastric atrophy.

Keywords *PTPN11* · *rs2301756* · Gastric cancer · Meta-analysis

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Introduction

Gastric atrophy is a well-established precursor to gastric cancer [1, 2] with *Helicobacter pylori* infection as a major risk factor for the latter [3, 4]. Severity of gastric damage has been attributed to *H. pylori* strains with *cytotoxin-associated gene A* (*CagA*) which, along with host proinflammatory genetic factors, are strongly associated with increased gastric adenocarcinoma risk [5]. Of the two major *CagA* subtypes [6], evidence has shown that infection with *CagA* (+) *H. pylori* associates with higher grades of gastric inflammation and is more virulent than the *CagA* (−) strains [7]. Because *CagA* (+) strains of *H. pylori* are more frequent than *CagA* (−) strains in East Asian populations, grade of gastric atrophy risk is higher in this ethnic group than in those with *CagA* (−) or Western *CagA* (+) strains [8]. Despite the higher risk for gastric atrophy among East Asians, however, only a fraction of subjects develop severe outcomes [9]. The *CagA* protein is translocated from the attached *H. pylori* into host gastric epithelial cells via the bacterial type IV secretion system and undergoes tyrosine phosphorylation in the host cells [10]. Tyrosine-phosphorylated *CagA* then acquires capability to interact with and deregulate Src homology 2 domain-

containing protein tyrosine phosphatase 2 (SHP-2), a bona fide oncoprotein [11]. Formation of *CagA*-SHP-2 complex induces abnormal proliferation and migration of gastric epithelial cells, consequently resulting in gastric atrophy and gastric carcinoma [12, 13]. In this *CagA*-dependent morphological transformation of gastric epithelial cells, SHP-2 plays a key role in intracellular signaling downstream of a number of growth factors, hormones, and cytokines [14]. Since SHP-2 closely interacts with the *CagA* protein, one could speculate that functional polymorphisms in the *protein tyrosine phosphatase*, *non-receptor type 11* (*PTPN11*) gene encoding SHP-2 may influence the degree of gastric atrophy and transformation to gastric cancer in *H. pylori*-infected subjects. The *PTPN11* gene is located on chromosome 12 q24.1 containing 16 exons. Several single nucleotide polymorphisms (SNPs) such as *rs11066322*, *rs11066320*, and *rs2301756* have been identified that influence SHP-2 activities. The *G*/*A* polymorphism (*rs2301756*) is located in the third intron with a *G*-to-*A* single nucleotide substitution 223-bp upstream of exon 4 in the *PTPN11* gene encoding SHP-2. While the biological function of this polymorphism has yet to be reported, frequencies of its alleles have been documented. The *A* allele in *rs2301756* is frequent among Caucasians (0.88 of 120 chromosomes), but not among the Japanese (0.18 of 902 chromosomes) and Chinese (0.08 of 48 chromosomes) [15]. In these two Asian ethnic groups, the high-risk *G* allele is dominant. This suggests high susceptibility of the Japanese and Chinese through *CagA* (+) *H. pylori* infection if we adhere to the hypothesis that the *G* allele confers stronger signals via the *CagA*-SHP-2 interaction [16]. Given the obvious difference in allele frequency between Asian and Western populations and high incidence of gastric cancer among the Japanese and Chinese, it could be that such ethnic differences may be determined partly by the *PTPN11* polymorphism [17]. Few studies have examined the genetic traits associated with a risk of gastric precancerous conditions which would potentially be of significance for preventing gastric cancer [16]. Reports of *A*/*A* genotype increased and reduced risks of gastric atrophy among the Chinese [18] and Japanese [9, 17, 19], respectively, which constitute a discrepancy of the findings for the *PTPN11* polymorphism. This prompted us to perform a meta-analysis, where we sought to determine the magnitude of effect of *rs2301756* in *PTPN11* with clinical outcomes of gastric atrophy in these East Asian populations.

Materials and Methods

Literature Search

Figure 1 outlines the steps we took to search for literature. Using the terms "polymorphism" and "gastric," we had two search strategies with two additional terms: (i) "PTPN11" and (ii) "SHP-2" in MEDLINE using PubMed for association studies as of July 2013. Studies were eligible if they had genotypic data with a case-control design. The number of citations yielded by the first search strategy was 19 (*PTPN11*) and 7 from the second (*SHP-2*). The seven were all found in the 19 citations. Thus, focusing on the first search, we screened 11 reviews and functional papers. To further evaluate the remaining eight papers, abstracts and then full texts of the articles were retrieved from which four were excluded for various reasons. Paucity of data addressing gastric cancer confined our analysis to gastric atrophy. The remaining four papers were eventually included in the meta-analysis [9, 17–19].

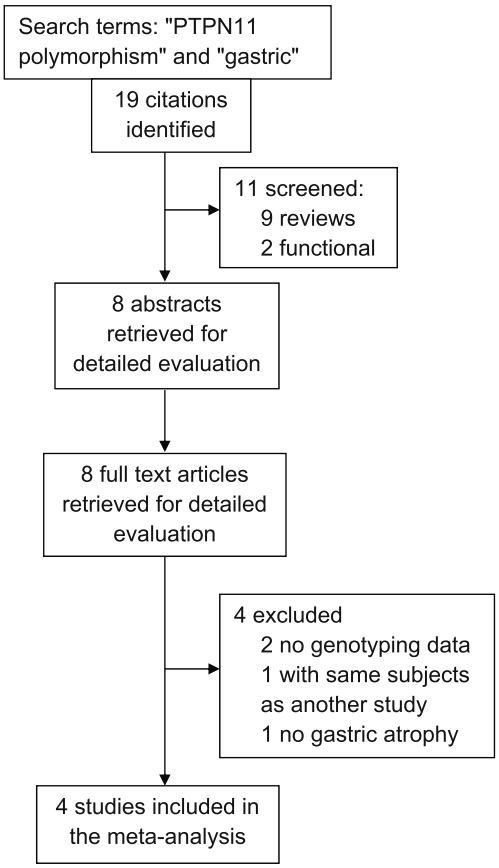

Fig. 1 Flowchart of literature search

Data Extraction and Power Calculations

Two investigators independently extracted data and reached consensus on all the items. The following information was obtained from each publication: first author's name, publication year, country of origin, dominant ancestry of the study populations, genotype data, and number of cases and controls. We also calculated frequencies of the variant allele, deviations of controls from the Hardy-Weinberg Equilibrium (HWE), as

well as statistical power of each study. Assuming an odds ratio (OR) of 1.5 at a genotypic risk level of α=0.05 (two-sided), power was considered adequate at ≥80 %.

Meta-analysis

Risks (ORs) of gastric atrophy of the *PTPN11 G/A* polymorphism were estimated for each study, and then, overall and subgroup summary effects were obtained. We analyzed data among *H. pylori* (−) (seronegative) and (+) (seropositive) subjects separately. We estimated the OR and 95 % confidence interval of association with the variant *AA* genotype compared with the wild-type *GG* genotype. To address the importance of heterozygous genotype, we evaluated recessive (*GG* vs. *GA+AA*), dominant (*GG+GA* vs. *AA*), and codominant (*A* vs. *G*) effects. To compare the effects on the same baseline, we used raw data to calculate pooled ORs which were obtained using either the fixed [20] (in the absence of heterogeneity) or random [21] (in its presence) effects models [22]. Given the low power of this test [23], significance threshold was set at p=0.10. Heterogeneity between studies was estimated using the chi-based Q test, was explored using subgroup analysis [22] with ethnicity (Japanese) as variable, and was quantified with the I^2 statistic which measures a degree of inconsistency among studies [24]. Sensitivity analysis was used to test for robustness of the summary effects. Here, influence of each study on the pooled ORs was examined by repeating the meta-analysis omitting each study one at a time [25]. Data were analyzed using Review Manager 4.3 and SigmaStat 2.03. Significance was set at a p value of ≤0.05 throughout the study except in heterogeneity estimation. We treated the *H. pylori* (−) and (+) studies independent of each other. Thus, we did not investigate a publication bias because of the low sensitivity of qualitative and quantitative tests when the number of studies is lower than 10 [26].

Results

Characteristics of the Studies

Table 1 summarizes features of the included articles, three Japanese [9, 17, 19] and one Chinese [18]. All four studies were homogeneous, clinically and methodologically, where gastric atrophy was assessed by measuring serum pepsinogen levels. *H. pylori* status was determined with the serology test in three studies [9, 18, 19] and the *IgG* antibody test in one study [17]. Although one Japanese study was done in Brazil, lifestyle of the Japanese Brazilians was reported to have remained unchanged [17]. Of the four studies, two were statistically adequate with more than 90 % power, assuming an alpha level of 0.05. None of the controls in the *H. pylori* (−) studies deviated from HWE, but one did [17] in the *H. pylori* (+).

Summary Effects

Table 2 summarizes the pooled effects of our meta-analysis. In both *H. pylori* (−) and (+), the dominant and codominant findings in the overall and subgroup analyses skirted the null association (OR 0.95–1.03, p=0.52–0.96). Presence of readily interpretable associations was thus confined mainly to the homozygous and recessive models. Indication of overall reduced risk was significant among seropositive subjects (OR 0.66–0.68, p=0.04–0.05), but not in the seropositive analysis (OR 0.92–0.93, p=0.69–0.74). For the Japanese, effects were still protective less so among *H. pylori* (−) subjects (OR 0.85–0.86, p=0.71–0.73) than in the seropositive analysis with its recessive significance (OR 0.67, p=0.05). Of the 16 comparisons, in which tests for heterogeneity were applied, most (87.5 %) were non-heterogeneous, with the remaining two being moderately heterogeneous ($p_{\text{heterogeneity}}$=0.09, I^2= 58 %). Of the 14 non-heterogeneous summary effects, majority (57.1 %) had zero heterogeneity (I^2=0 %).

Table 1 Characteristics of the studies associating *PTPN11 GA* (*rs2301756*) polymorphism in gastric atrophy

First author	Publication year	Ethnicity	Genotyping method	*H. pylori* (−) seronegative						*H. pylori* (+) seropositive					
				1,812 cases/2,774 controls						1,785 cases/2,091 controls					
				Case (*n*)	Control (*n*)	Total (*n*)	Power (α=0.05, OR=1.5)	maf	HWE	Case (*n*)	Control (*n*)	Total (*n*)	Power (α=0.05, OR=1.5)	maf	HWE
Hishida [19]	2009	Japanese	PCR-CTPP	583	1,636	2,219	98.6	0.17	0.67	583	937	1,520	96.6	0.17	0.94
Goto [9]	2006	Japanese	PCR-CTPP	202	203	405	51.9	0.20	0.70	202	248	450	55.8	0.16	0.70
Kawai [17]	2006	Japanese	PCR-CTPP	918	471	1,389	94.1	0.19	0.34	918	447	1,365	93.4	0.20	0.04
Jiang [18]	2012	Chinese	Taqman	109	464	573	46.7	0.14	0.65	82	459	541	38.4	0.14	0.68

PCR-CTPP polymerase chain reaction with confronting two-pair primers, *maf* minor allele frequency, *HWE* Hardy-Weinberg equilibrium

Table 2 Summary effects of *PTPN11 GA* (*rs2301756*) polymorphism in gastric atrophy

	Case/control (*n*)	No.	Homozygous			Recessive			Dominant			Codominant		
			OR (95 % CI) *p* value	p_{het}	I^2	OR (95 % CI) *p* value	p_{het}	I^2	OR (95 % CI) *p* value	p_{het}	I^2	OR (95 % CI) *p* value	p_{het}	I^2
H. pylori (−)														
Overall	1,812/2,774	4	0.92 (0.60–1.40) 0.69	0.14	46	0.93 (0.61–1.42) 0.74	0.14	45	0.95 (0.82–1.10) 0.52	0.47	0	0.96 (0.84–1.09) 0.51	0.28	22
Japanese	1,703/2,310	3	0.85 (0.37–1.95) 0.71	0.09^R	58	0.86 (0.38–1.96) 0.73	0.09^R	58	0.97 (0.83–1.13) 0.70	0.38	0	0.98 (0.85–1.12) 0.72	0.23	32
H. pylori (+)														
Overall	1,785/2,091	4	*0.68 (0.46–1.00) 0.05*	0.45	0	*0.66 (0.45–0.98) 0.04*	0.44	0	1.02 (0.88–1.18) 0.78	0.95	0	0.97 (0.86–1.10) 0.66	0.93	0
Japanese	1,703/1,632	3	0.69 (0.46–1.02) 0.06	0.27	23	*0.67 (0.45–0.99) 0.05*	0.26	25	1.03 (0.89–1.20) 0.67	1.00	0	0.98 (0.86–1.12) 0.77	0.91	0

The fixed effects model was used unless otherwise indicated by superscript letter R where we used the random effects model. Values in italics are significant ($p \leq 0.05$)

No. number of studies, *OR* odds ratio, *CI* confidence interval, p_{het} *p* value for heterogeneity

Sensitivity Analysis

Table 3 summarizes effects of sensitivity analysis which altered the direction of association among seronegative subjects only. This change is attributed to the study of Goto et al. [9] both in overall and subgroup analyses. Change in I^2 values to 0 % after its omission demonstrates this study to be responsible for presence of heterogeneity (I^2=45–58 %).

Discussion

Based on a total sample size of 9,519 subjects (4,417 cases and 5,102 controls), this meta-analysis presents evidence of homozygous and recessive associations of the A allele between *rs2301756 PTPN11* and gastric atrophy. Besides homogeneity and significance of the overall protective associations among seropositive subjects, these effects were robust. Thus, seropositive subjects were more protected than their seronegative counterparts.

Two explanations have been proposed to explain the association between *rs2301756* and gastric atrophy [17]. The first is that rs2301756 is itself functional. Location of the *G*/*A* SNP 223-bp upstream of exon 4 could probably cause a different splicing, which may reduce SHP-2 activity. The other explanation is a linkage to a functional polymorphism at the promoter region or at the coding region, which could influence SHP-2 activity. In fact, most SNPs at minor allele frequency >0.05 in *PTPN11* gene in the Japanese are in absolute linkage disequilibrium (LD) (D'= 1 and r^2=1) or complete LD (D'=1 and r^2<1) to each other [19]. The *PTPN11 G*/*A rs2301756* is in complete LD to another *PTPN11 G*/*A* SNP at intron 10 (*rs12229892*) [27], supporting the hypothesis that the *PTPN11* polymorphism is functional. If this polymorphism is functional or linked to a functional one, association could be biologically explained by the difference in strength of signal transduction through the *CagA*-SHP-2 complex [16]. Subgroup analysis by ethnicity reflected our overall findings, lending reliability of the results. This could be due to the methodological similarities (genotyping approaches) of the component

Table 3 Sensitivity analysis showing the study that affected a change of direction of associations among seronegative subjects

GM	Original summary effects				Omitted study	Resulting pooled OR			
	OR	95 % CI	I^2	Direction of association		OR	95 % CI	I^2	Effect of study omission
Overall									
H	0.92	0.60–1.40	46	Reduced risk	Goto [9]	1.07	0.68–1.67	0	Increased risk
R	0.93	0.61–1.42	45	Reduced risk	Goto [9]	1.08	0.69–1.68	0	Increased risk
Japanese									
H	0.85	0.37–1.95	58	Reduced risk	Goto [9]	1.14	0.72–1.82	0	Increased risk

GM genetic model, *H* homozygous, *R* recessive, *OR* odds ratio, *CI* confidence interval

studies as well as adequate statistical power to find an association [17, 19].

Limitations of our study are the following: (i) we were unable to fully explore the effects of *PTPN11* with gastric cancer because only one study provided a full genotypic data set [18], and (ii) instability of the reduced risk effects among seronegative subjects was shown by sensitivity treatment to be less reliable. Despite these limitations, our meta-analysis has the following strengths: (i) controls were either healthy or cancer-free; (ii) controls were matched to cases in four (67 %) of the six studies in terms of age, sex, and residency; and (iii) reduced risk effects among seropositive subjects were shown to be robust, which is therefore reliable. These clinical and epidemiological features minimize selection bias as well as non-differential misclassification bias because the issue of different risks in the control groups of developing gastric cancer has been modulated. Furthermore, these statistical features add to the strengths of our study: (i) that majority (67 %) of the Japanese studies had high statistical power rendering chance effects less likely and (ii) that majority (84.4 %) of the 32 comparisons were performed with the fixed effects model which indicated a lack of variance among the studies and, hence, were similar enough to be compared.

Conclusion

To our knowledge, this is the first meta-analysis that investigates associations of the *PTPN11 G/A* polymorphism with gastric atrophy risk among East Asians. With this methodological approach, we hope to have contributed to (i) examining a genetic trait involved in gastric precancerous conditions and (ii) establishing effective ways of early detection and individualized prevention of gastric cancer. Our results provided evidence of associations of this polymorphism with a risk of gastric atrophy but require more studies for confirmation.

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Conflict of Interest The authors have no conflicts of interest to declare.

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