RESEARCH ARTICLE

Lack of Association of the MDR1 C3435T Polymorphism with Susceptibility to Gastric Cancer and Peptic Ulcer: a Systemic Review and Meta-analysis

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Abstract

Background: The multidrug resistance 1 gene (MDR1) C3435T polymorphism has been demonstrated to influence the P-glycoprotein (P-gp) activity level which is related to inflammation and carcinogenesis. This meta-analysis was performed to estimate the association between the MDR1 C3435T polymorphism and the risk of gastric cancer (GC) and peptic ulcer (PU).

Materials and Methods: A literature search was conducted with PubMed, Embase and the Cochrane library up to November 2013. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of association. Data were analyzed using Review Manager (Version 5.2), and Stata package (version 12.0) for estimation of publication bias.

Results: Six case-control studies were included, of which five were for GC and two for PU. Overall, no evidence was found for any association between the MDR1 C3435T polymorphism and the susceptibility to GC and PU. In the stratified analysis by H. pylori infection status, stage and histology classification of GC, and PU type, there was still no significant association between them.

Conclusions: This meta-analysis suggested that the MDR1 C3435T polymorphism is not associated with susceptibility to GC and PU. Large and well-designed studies are warranted to validate our findings.

Keywords: Gastric cancer - peptic ulcer - gastric ulcer - duodenal ulcer - MDR1 - polymorphism - meta-analysis

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Introduction

Peptic ulcer (PU), including gastric ulcer (GU) and duodenal ulcer (DU), remains a relatively common condition worldwide with annual incidence up to 0.19% (Sung et al., 2009), and patients with GU significantly increase the chance of developing gastric cancer (GC). GC is not only the fourth most common type of cancer in males and fifth in females globally, but also the third leading cause of death due to cancer in 2010 (Jernal et al., 2011; Lozano et al., 2012). *Helicobacter pylori* (H. pylori) infection, which is strongly related to GC and PU, is now recognized as a worldwide problem. Some specific genotypes of H. pylori have been reported to be associated with GC and PU, such as vacA s1 genotype (Basiri et al., 2014). Besides the infection with H. pylori, some genetic factors also play important roles in the occurrence of GC and PU. Up to now, a wide range of gastrointestinal cancer susceptibility genes have been identified, such as murine double minute 2 (Mdm2) T309G, matrix metalloproteinase (MMP) gene, survivin gene -31G>C, epidermal growth factor (EGF) gene +61A>G, X-ray repair cross-complementing 1 (XRCC1) gene, Toll-like receptor 4 (TLR4) gene +896A/G, etc. (Song et al., 2013; Li et al., 2013; Liu et al., 2013; Piao et al., 2013; Wu et al., 2013; Zou et al., 2013). The multidrug resistance 1 gene (MDR1), also named ABCB1, was suggested to be a candidate gene (located at 7q21.1) for the pathogenesis of GC and PU (Sugimoto et al., 2008).

P-glycoprotein (P-gp) encoded by MDR1 is one of the most widely studied ATP-binding membrane transporters and expressed in normal cells of various organs such as intestine, liver, kidney, brain and placenta. Since it is involved in absorption and elimination of xenobiotics and drugs, it has a protective function in various cells and tissues/organs. Furthermore, P-gp probably plays a role in regulating cell death, differentiation and proliferation, as well as in immune response. With the decrease of P-gp activity level, inflammation and carcinogenesis may occur (Johnstone et al., 2000; Ho et al., 2003; Mizutani et al., 2008). C3435T SNP (rs1045642), one of the most popular MDR1 polymorphisms, is located in exon 26 as a silent mutation. C3435T encodes isoleucine and affects both in vitro and in vivo (Breier et al., 2005).

To date, many studies that investigated the association between the MDR1 C3435T polymorphism and risk of PU or GC have produced contradictory or inconclusive
results in that each study had limited sample size and was not enough to demonstrate the association (Tahara et al., 2007; Sugimoto et al., 2008; Sabahi et al., 2010; Chang et al., 2010; Tahara et al., 2011; Oliveira et al., 2012). In order to estimate the risk of MDR1 C3435T polymorphism associated with GC and PU, we carried out a meta-analysis on all eligible case-control studies.

Materials and Methods

Search strategy

All studies published in English that investigated the association between MDR1 C3435T polymorphism and the risk to PU or GC were identified by searching from PubMed, Embase and The Cochrane Library up to November 2013. The following search criteria were used: (“multidrug resistance 1 gene” or “MDR1” or “ABCB1”) AND (“gastric cancer” or “peptic ulcer” or “duodenal ulcer” or “gastric ulcer”) AND (“association” or “risk” or “susceptibility”). The search was restricted to humans. Other potential eligible studies were recognized by looking through the references listed in the retrieved articles or textbooks. Disagreements were resolved through discussion between the authors.

Inclusion and exclusion criteria

Studies met the following criteria were included in our meta-analysis: (a) case-control study focused on association between MDR1 C3435T polymorphism and risk to GC or PU, (b) contained available genotype frequency for both cases and controls, (c) all patients diagnosed with GC should be confirmed by pathological or histological examinations, (d) all patients diagnosed with PU had endoscopical and/or histological proofs, and (e) the H. pylori infection status was determined on the basis of histology, culture, urea breath test (UBT) or serum antibodies to H. pylori.

Studies were mainly excluded for the following reasons: (a) not a case-control study, (b) duplicated publications, (c) based on incomplete data, (d) not for human research, and (e) meta-analyses, letters, reviews or editorial articles.

Data extraction and quality assessment

Two of the authors extracted data independently according to the same standard. In the cases of conflicting, agreement was reached after a discussion. If conflicts still existed, an expert (Dong WG) would be invited to help make decisions. Following variables were collected from each study: the first author’s name, year of publication, country of origin, ethnicity, source of controls (population or hospital based controls), genotyping method, sample sizes of genotyped cases and controls, histological classification of GC, clinical stage of GC, and H. pylori infection status. Subjects of different ethnicity were categorized as Caucasian and Asian.

Statistical analysis

Meta-analysis was performed by using the Cochrane Collaboration RevMan 5.2 (Copenhagen, 2013) and Stata package version 12.0 (Stata Corporation, College Station, Texas). Before estimating effect of MDR1 C3435T polymorphism, the Hardy-Weinberg equilibrium (HWE) was calculated by employing a goodness-of-fit chi-square test for the control group of each study. HWE was accessed using Online software (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) with the significance set at a p value less than 0.05. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of association between the MDR1 C3435T polymorphism and GC and PU risk. The pooled ORs were performed for dominant model (TT+TC vs CC), co-dominant models (TT vs CC, TC vs CC), and recessive model (TT vs TC+CC). The degree of heterogeneity between the studies was estimated using Cochran’s Q-statistic with a p-value and F test, ranging from 0 to 100%, which represents the proportion of inter-study variability (Higgins et al., 2002; Zintzaras et al., 2005). When a significant Q-test (p<0.05) or F test (F>50%) indicates heterogeneity among studies, the random effects model (DerSimonian Laird method) would be employed for meta-analysis (DerSimonian et al., 1986). On the contrary, the fixed effects model (Mantel-Haenszel method) would be used (Mantel et al., 1959 [21]. Sensitivity analysis was also performed to assess the stability of the results by omitting each single study at one time, which reflects the influence of each study data set on the summary ORs. To test the publication bias, both funnel plots and Egger’s linear regression test were used (Begg et al., 1994; Egger et al., 1997).

Results

Study characteristics

The combined search yielded 27 references. A total of six articles were ultimately included. The flow chart of study selection was summarized in Figure 1. The publication year of involved studies ranged from 2007 to 2012. Overall, there were one study about both GC and PU, four about GC, and one about PU. Four of these studies were conducted in Asian populations and two were in Caucasian populations. The total number of GC cases and controls were 496 and 724, respectively, and 554 cases and 548 controls concerned PU. In three studies, GC was classified to diffuse and intestinal type according to Lauren’s classification (Lauren et al., 1965). Two studies also reported the tumor stages (Early stages include I, IIA, and IIB; Advanced stages include IIIA, IIIB, IIIIC, and IV). H. pylori infection status was reported for cases and controls in two studies. The distribution of genotypes (includes TT, TC, CC) among the controls of the studies was in agreement with HWE for most except
two studies (Sugimoto et al., 2008; Chang et al., 2010). All studies conducted a PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) assay to investigate the C3435T polymorphism of MDR1 except one (Chang et al., 2010), which used sequencing of PCR products. The detailed characteristics of the included studies were summarized in Table 1, 2.

Association between MDR1 C3435T polymorphism and gastric cancer and peptic ulcer

Five studies reported the association between MDR1 C3435T polymorphism and susceptibility to GC. Overall, there was no significant difference in genotype C3435T distribution between GC and control (TT+TC vs CC: OR=0.85, 95% CI: 0.65-1.10, p=0.15; TT vs TC+CC: OR=0.68, 95% CI: 0.28-1.62, p<0.0001; TC vs CC: OR=0.66, 95% CI: 0.32-1.38, p=0.003; TT vs CC: OR=1.02, 95% CI: 0.77-1.35, p=0.31). In the subgroup analysis by H. pylori infection status, there was no significant association in each model, and so was it in the subgroup analysis by ethnicity, stage of GC, and histological classification of GC (Table 3, Figure 2).

Only two studies reported the association between C3435T polymorphism and susceptibility to PU, all patients came from Asian population. Overall, no association was found between C3435T polymorphism and susceptibility to PU in four models (TT+TC vs CC: OR=1.04, 95% CI: 0.79-1.37, p=0.63; TT vs TC+CC: OR=1.36, 95% CI: 0.98-1.90, p=0.31; TT vs CC: OR=1.32, 95% CI: 0.90-1.92, p=0.32; TC vs CC: OR=0.96, 95% CI: 0.70-1.34, p=0.50; TT vs CC: OR=0.78, 95% CI: 0.56-1.09, p=0.11; TT vs TC+CC: OR=0.90, 95% CI: 0.64-1.27, p=0.56; TC vs CC: OR=1.44, 95% CI: 0.89-2.34, p=0.14). In the subgroup analysis by H. pylori infection status, only one study (Chang et al., 2010) reported no significant association in each model, and so was it in the subgroup analysis by ethnicity and histological classification of GC (Table 3, Figure 2).
95% CI: 0.71-1.28, \( p = 0.82 \). In the subgroup analysis of GU and DU, the similar results were observed (Table 3, Figure 3).

Sensitivity analysis
To assess the influence of each individual study on the pooled ORs for GC, the sensitivity analysis was performed by omitting each study from meta-analysis sequentially. The results suggested that no single study affected the pooled ORs qualitatively. When deleting the two studies deviated from HWE, no significant association was observed. It suggested that the results of this meta-analysis were stable. For the peptic ulcer, as there were only two studies included, we didn’t perform the sensitivity analysis.

Publication bias
The Begg’s funnel plot and Egger’s test were conducted to assess publication bias among the studies selected for this meta-analysis. As for GC, the shape of...
No Association of MDR1 C3435T Polymorphism with Gastric Cancer and Peptic Ulcer: a Meta-analysis

The MDR1 C3435T polymorphism has been demonstrated for its role in regulating the P-glycoprotein (P-gp) activity level which is related to the inflammation and carcinogenesis (Johnstone et al., 2000; Ho et al., 2003; Mizutani et al., 2008). P-gp functions as a transmembrane efflux pump which has an ability to protect the organism against toxic xenobiotic agents and environmental carcinogens (Breier et al., 2005; Yuan et al., 2008). In 2000, Hoffmeyer et al. (2000) first reported that healthy individuals with the MDR1 3435TT genotype had lower intestinal expression of P-gp and higher intestinal uptake of the oral digoxin which is a P-gp substrate. Larsen et al. (2007) found that the wild-type C allele of the synonymous polymorphism conferred a higher P-gp activity by increasing duodenal MDR1 mRNA and P-gp levels. Markova et al. (2006) also showed C3435 allele carriers might be more effective to anti-inflammation of glucocorticoid than non-carriers. It was also supported that MDR1 C3435T polymorphism may contribute to individual susceptibility to breast cancer, colorectal cancer, and inflammatory bowel disease, respectively (Annese et al., 2006; Wang et al., 2013; Zhang et al., 2013). As for the association between MDR1 C3435T polymorphism and GC or PU, the results of all available studies are controversial. Tahara et al. (2011) reported that the 3435T carrier was significantly associated with a higher degree of neutrophil infiltration in H. pylori-positive subjects. Sabahi et al. (2010) suggested that the polymorphic homozygote (T/T) genotype showed a significant association with the incidence of gastric cancer. However, there were also some reports that found no association between MDR1 C3435T polymorphism and risk to GC and PU (Sugimoto et al., 2008; Oliveira et al., 2012).

The present meta-analysis included 6 case-control studies, including 496 cases and 724 controls for GC analysis, and 554 cases and 548 controls for PU. As for GC analysis, there was no significant association between MDR1 C3435T polymorphism and the risk of GC in four genetic models. Although we omitted the two studies inconsistent with HWE and deleted two studies in Caucasian population to avoid the influence of HWE or ethnicity, respectively, there was still no significant association for both. Since H. pylori infection status was detected in two studies, we stratified the subjects into two groups, H. pylori positive and H. pylori negative. However, stratified analysis also indicated no association between MDR1 C3435T and the susceptibility to GC under all genetic models. In the subgroup analysis by stage and histological classification, similar results were found. For PU analysis, no matter in overall analysis or in subgroup analysis by GU and DU, no evidence showed that MDR1 C3435T polymorphism was associated with the risk to PU, GU or DU respectively. The sensitivity analysis didn’t show any significance. As for publication bias, Begg’s funnel plots revealed no asymmetry, and the Egger’s test suggested no publication bias among the studies of GC.

Some limitations of this meta-analysis should be addressed. Firstly, GC and PU are complex diseases with a multifactorial etiology, so the contributing pathogenetic role of lifestyle and drugs intake should also be considered. The existence of gene-environment and

Figure 4. Begg’s Funnel Plot for Publication Bias. Each point represents a separate study for the indicated association. logOR, natural logarithm of OR. Horizontal line, mean effect size. A) dominant model. B) recessive model. C) TT vs CC. D) TC vs CC.

Discussion

The MDR1 C3435T polymorphism has been demonstrated for its role in regulating the P-glycoprotein (P-gp) activity level which is related to the inflammation and carcinogenesis (Johnstone et al., 2000; Ho et al., 2003; Mizutani et al., 2008). P-gp functions as a transmembrane efflux pump which has an ability to protect the organism against toxic xenobiotic agents and environmental carcinogens (Breier et al., 2005; Yuan et al., 2008). In 2000, Hoffmeyer et al. (2000) first reported that healthy individuals with the MDR1 3435TT genotype had lower intestinal expression of P-gp and higher intestinal uptake of the oral digoxin which is a P-gp substrate. Larsen et al. (2007) found that the wild-type C allele of the synonymous polymorphism conferred a higher P-gp activity by increasing duodenal MDR1 mRNA and P-gp levels. Markova et al. (2006) also showed C3435 allele carriers might be more effective to anti-inflammation of glucocorticoid than non-carriers. It was also supported that MDR1 C3435T polymorphism may contribute to individual susceptibility to breast cancer, colorectal cancer, and inflammatory bowel disease, respectively (Annese et al., 2006; Wang et al., 2013; Zhang et al., 2013). As for the association between MDR1 C3435T polymorphism and GC or PU, the results of all available studies are controversial. Tahara et al. (2011) reported that the 3435T carrier was significantly associated with
gene-gene interactions may explain the discrepancy of results obtained in individual genetic association studies. Secondly, there are only two studies of peptic ulcer included in our studies, which will reduce the statistical potency. Thirdly, two studies for GC and one study for PU are not consistent with HWE, which counted for 40 percent and 50 percent, respectively. When we omitted these studies, the size of left studies was so small, so it’s difficult to retrieve dependable results. Fourthly, ages, ratio of males and females, the source of controls and so on, were not matched well that may influence the results greatly. Although we tried to stratified subjects by age or others, there were not sufficient data. Fifthly, the genotyping methods are not identical for all the investigations in selected studies. Different methods may have different results for the same sample. In spite of these, our meta-analysis also had some advantages. This may be the first meta-analysis to assess the association between MDR1 C3435T and risk to PU. And this may be also the first analysis to take H. pylori infection status and GC classification into consideration. Since our sensitivity analysis indicated that there were no significant changes, the pooled ORs are likely to be reliable.

In summary, this meta-analysis supported evidence that MDR1 C3435T polymorphism may have no association with risk of GC and PU. In the subgroup analysis by ethnicity, H. pylori infection status, stage and histological classification of GC, and type of PU, we obtained the similar results. Because of the above limitation, standardized unbiased genotyping methods, well-matched controls and cases, and containing more subjects should be introduced in future studies. Also, case-control studies that investigate gene-gene and gene-environment interactions may also help to further elucidate the molecular and genetic epidemiology of cancer predisposition.

References


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