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RESEARCH ARTICLE

Genetic polymorphisms of the multidrug resistance 1 gene MDR1 and the risk of hepatocellular carcinoma

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Abstract A possible association between multiple drug resistance 1 gene (MDR1) polymorphisms and the risk of developing hepatocellular carcinoma (HCC) is currently under debate, and evidence from various epidemiological studies has yielded controversial results. To derive a more precise estimation of the association between MDR1 polymorphisms and HCC risk, the present meta-analysis was performed. A total of 8 studies containing 11 cohorts with 4407 cases and 4436 controls were included by systematic literature search of EMBASE, PubMed, Web of Science, and CNKI. All polymorphisms were classified as mutant/wild-type alleles. In particular, the variation type, functional impact, and protein domain location of the polymorphisms were assessed and used as stratified indicators. The pooled odds ratio (OR) with 95 % confidence interval (CI) was calculated to evaluate the association. Overall, our results suggested that the mutant alleles of the MDR1 gene were associated with a significantly increased risk for HCC under all genetic models (allelic model: OR=1.28, 95 % CI=1.20–1.36, $P<0.001$; dominant model: OR=1.27, 95 % CI=1.16–1.38, $P<0.001$; recessive model: OR=1.59, 95 % CI=1.36–1.85, $P<0.001$). Furthermore, increased risks for HCC were also revealed in stratified analyses by ethnicity, sample size, and quality scores of cohorts as well as variation type, functional impact, and protein domain location of polymorphisms. In conclusion, the present meta-analysis suggested that the presence of MDR1 mutant alleles might be a risk factor for HCC.

Keywords Gene polymorphism · Hepatocellular carcinoma · Meta-analysis · Multiple drug resistance 1 gene

Zhi-Chao Wang, Long-Zi Liu and Xin-Yang Liu contributed equally to this work.

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Introduction

Hepatocellular carcinoma (HCC) ranks as the fifth most frequent cancer and the second leading cause of cancer death globally [1]. In China, it is the second most common cancer, and Chinese cases represent half of the total HCC cases worldwide [2]. The high incidence of Chinese HCC is mainly attributed to the prevalence of hepatitis virus infection, especially hepatitis B virus (HBV). HBV infection and subsequent persistent inflammation result in the sequential development of chronic hepatitis, cirrhosis, and HCC [3]. Moreover, etiological studies have implicated that many host factors (including gender, age, and ethnicity), viral factors (including viral genotypes and replication levels), and environmental factors (including aflatoxin exposure, alcohol drinking, and tobacco

smoking) are involved in HCC susceptibility [4, 5]. In addition, there is increasing evidence indicating the association between genetic variants and the risk of HCC [6–8]. Among all the genetic factors, the single nucleotide polymorphisms (SNPs) are the most widely studied genetic variations.

Chronic inflammation caused by potential risk factors may result in hepatic accumulation of oxidative and other genotoxic products, possibly leading to the initiation of multistage hepatocarcinogenesis [9–11]. Biliary and sinusoidal clearance of such endogenous and exogenous carcinogenic substances is one of the most important protective functions of the liver. This function is largely achieved by a drug efflux transporter system that comprises mainly ATP-binding cassette transporter (ABC transporter) proteins [12, 13]. Therefore, polymorphisms of these proteins might compromise the efflux of mutagenic factors that could contribute to HCC.

Multiple drug resistance 1 gene (MDR1)-encoded P-glycoprotein (P-gp), one of the most important ABC transporters, is widely expressed in normal cells of various organs, including the liver, kidney, and brain [14–16]. MDR1 is extensively involved in the absorption and elimination of various harmful substances and also participates in regulating cell growth, differentiation, and death [17–19]. Of note, studies have indicated that SNPs in the MDR1 gene have an important impact on the expression and function of P-gp, therefore influencing susceptibility to numerous diseases, including HCC [20–26]. To date, more than 50 SNPs in the MDR1 gene have been documented [27, 28], and an association between these SNPs and the risk of HCC has been proposed; however, contradictory results have been reported and studies focused on one particular polymorphism are also limited [29–36]. Significant correlations of MDR1 polymorphisms and HCC risk were observed in several studies, whereas no significant associations were detected in others [29–36]. Therefore, we included all studies on association between MDR1 SNPs and the risk for HCC and classified the included SNPs as mutant/wild type. To precisely estimate the association of MDR1 mutant/wild-type alleles with HCC susceptibility, we performed the first meta-analysis on all eligible studies.

Materials and methods

Literature search strategy and selection criteria

We performed a systematic review and meta-analysis in accordance with the guidelines of PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [37] (Additional file 1). We searched PubMed, Web of Science, EMBASE, and CNKI without language limitations till December 1, 2014. The following search algorithm was used: ("hepatocellular carcinoma" or "hepatocellular neoplasm" or "liver cancer" or "HCC") and ("multiple drug resistance 1 gene" or "MDR1" or "ABCB1" or "P-glycoprotein" or "P-pg") and ("polymorphism" or "variation" or "susceptibility"). Additionally, all the references cited in the included articles were hand-searched to identify potentially eligible studies.

Inclusion and exclusion criteria

We included studies that (i) evaluated MDR1 gene polymorphisms and HCC risk, (ii) designed as case-control studies, and (iii) provided data for calculating odds ratios (ORs) with corresponding 95 % confidence intervals (CIs). Studies were excluded if (i) no specific controls have been selected, like review, comment articles, and case-only studies; (ii) sufficient data to estimate ORs with 95 % CIs is lacking; and (iii) there is duplication of previous publications or samples.

Data extraction

Two reviewers (Z.C. W and L.Z. L) independently carried out the data extraction process using a predefined standard form. Disagreements were discussed and resolved with consensus. The following information were collected: first author's name, year of publication, ethnicity, number of patients and controls, age, gender, genotyping method, polymorphism site, exon location, variation type, amino acid alteration, and genotype frequency of cases and controls, respectively. All polymorphisms were classified as mutant/wild-type alleles to unify the statistical analyses.

When the mutant alleles of polymorphism site lead to a nonsynonymous change in the amino acid sequence, functional impacts of corresponding amino acid changes were estimated using online MutationAssessor [38]. The functional impacts were evaluated as neutral, low, or medium impacts according to the estimated potential impacts on the biological activity of MDR1. The locations of altered amino acids in the MDR1 structure were also assessed according to Uniprot.org (http://www.uniprot.org/uniprot/P08183; see details in Additional file 2). Allele frequencies were calculated from the corresponding genotype distributions if not given directly. For the studies that investigated different polymorphisms in one cohort [30, 32, 34], the datasets were recognized as independent studies. Study quality was assessed independently by Z.C. W. and L.Z. L using the 10-point scoring scale for quality of genetic association studies proposed by Clark and Baudouin [39].

Statistical methods

We estimated the HCC risk associated with mutant alleles (using 2 as a representative example) at different polymorphism sites compared with the wild-type alleles (using 1 as a representative example) under the following genotypic

models: allele (2 vs. 1); co-dominant (2/2 vs. 1/1, 2/2 vs. 1/2, and 1/2 vs. 1/1); dominant (2/2+1/2 vs. 1/1); and recessive (2/2 vs. 1/2+1/1). Pooled ORs with corresponding 95 % CIs were calculated to assess the strength of associations between the MDR1 polymorphisms and HCC risk. A P value below 0.05 was considered statistically significant.

Q tests and I^2 tests were performed to test the heterogeneity (indicated large heterogeneity) [40]. A fixed effects model was used when there was no heterogeneity ($P \geq 0.10$ and $I^2 \leq 50$ %); otherwise ($P<0.10$ and $I^2>50$ %), a random effects model was used [41]. Hardy-Weinberg equilibrium (HWE) in controls of each cohort was assessed using the online HWE calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) ($P<0.05$ indicated significant disequilibrium).

In addition, the sources of heterogeneity were investigated by subgroup analysis based on the following: ethnicity, sample sizes (no. of cases ≤1000 or >1000), quality scores (scores >7 or ≤7), variation type (synonymous or nonsynonymous), functional impact (neutral/low or medium), positions in coding sequences (cytoplasmic or transmembrane), and positions in the functional protein domains (ABC transmembrane type 1 or ABC transporter). Sensitivity analyses were performed by deletion of a single dataset each time to assess the impact of the individual dataset to the pooled OR. Begg's funnel plots [42] and Egger's linear regression tests [43] were performed to evaluate publication bias. All P values were two-sided, and $P<0.05$ was considered statistically significant. Statistical analyses were conducted using Validating Review Manager Version 5.1 (Copenhagen, The Nordic Cochrane Centre, The Cochrane Collaboration, 2011) and STATA 11.0 (StataCorp LP, College Station, TX, USA).

Results

Characteristics of the studies and polymorphisms

The database search yielded 81 relevant studies. After excluding 73 studies according to the exclusion criteria (Additional file 3), a total of 8 studies that consisted of 11 cohorts with 4407 cases and 4436 controls [29–36] was enrolled. The characteristics of the included studies are shown in Table 1. All 11 cohorts selected healthy individuals as control, and all cohorts were consistent with the Hardy-Weinberg equilibrium except for Minoru F-2 cohort focused on the 3435C>T polymorphism of MDR1 [30].

Furthermore, different polymorphisms of the included studies are listed in Table 2 with exonic location. All polymorphisms were classified as mutant/wild-type alleles. In particular, the corresponding variation type, amino acid alteration with related functional impact, feature key, calculated positions in the coding sequence, and positions in the protein functional domain are also shown (Table 2).

Meta-analysis

In the overall analyses of all 11 cohorts, a significant association was detected under all genetic models, suggesting that the mutant alleles were associated with an increased HCC risk (Fig. 1 and Table 3): (i) allelic model, 2 vs. 1: OR=1.28, 95 % CI 1.20–1.36, $P<0.001$; (ii) dominant model, 2/2+1/2 vs. 1/1: OR=1.27, 95 % CI 1.16–1.38, $P<0.001$; (iii) co-dominant model, 2/2 vs. 1/1: OR=1.81, 95 % CI 1.58–2.07, $P<0.001$; (iv) co-dominant model, 2/2 vs. 1/2: OR=1.49, 95 % CI 1.30–1.70, $P<0.001$; (v) co-dominant model, 1/2 vs. 1/1: OR=1.15, 95 % CI 1.05–1.26, $P=0.002$; and (vi) recessive model, 2/2 vs. 1/2+1/1: OR=1.59, 95 % CI 1.36–1.85, $P<0.001$. No significant heterogeneity among studies was observed.

Subgroup analyses

Subgroup analyses were performed by stratifying of ethnicity, sample size, quality score, variation type, functional impact, position in the coding sequence, and protein functional domain. Of the 11 cohorts, there were only 2 datasets for the Japanese subgroup [30], medium functional impact subgroup [32, 34], and positions in the transmembrane region subgroup [32, 34], respectively. Therefore meta-analyses were not performed in these subgroups.

In the subgroup analyses by ethnicity, a significant association was observed in the Chinese subgroup under the allelic model (OR=1.28, 95 % CI 1.20–1.36, $P<0.00001$; Table 4). Though meta-analyses could not be conducted in the Japanese subgroup, it was found that neither dataset exhibited significant results (Minoru F-1, 2010: OR=1.23, 95 % CI 0.74, 2.07; $P=0.43$; Minoru F-2, 2010: OR=1.61, 95 % CI 0.95, 2.72; $P=0.72$). Likewise, subgroup analyses were performed according to the sample size and quality score and the results indicated significantly increased risks for HCC irrespective of size and quality (Table 4).

In addition to the typical standards used above, we classified these cohorts by the variation type, impacts on protein function, and positions in the coding sequence of MDR1 polymorphisms. In the subgroup analyses of variation type, both synonymous and nonsynonymous subgroups revealed significant results for increasing HCC risk. Interestingly, larger ORs were observed in the nonsynonymous subgroup compared with the synonymous subgroup (nonsynonymous subgroup: OR=1.31, 95 % CI 1.21–1.41, $P<0.00001$; synonymous subgroup: OR=1.22, 95 % CI 1.08–1.37, $P=0.001$). Subgroup analyses according to the estimated functional impacts (FI) indicated that even neutral/low FI resulted in a significantly higher risk for HCC (neutral/low FI subgroup: OR=1.23, 95 % CI 1.12–1.35, $P<0.00001$). However, the cohorts with a medium FI revealed higher ORs (Gao J-2, 2013: OR=1.65, 95 % CI 1.32–2.05, $P<0.0001$; Li XF, 2013: OR=1.33, 95 % CI 1.13–1.57, $P<0.001$). Positions in different coding

Table 1 Characteristics of the studies and cohorts included in the meta-analysis

Name of studies	Country	Ethnicity	Type of case/control	Genotyping method	Quality scores	Age				Male/female ratio		Variant site	Genotype frequency of case/control			HWE
						Case		Control		Case	Control		1/1	1/2	2/2	
						Mean	SD	Mean	SD							
Chen Y [29]	China	Chinese	HCC[a]/HP	PCR-RFLP	6	55.8	14.7	54.5	13.9	91/9	90/10	2677G>T/A	18/19	56/53	26/28	0.492
Minoru F-1 [30]	Japan	Japanese	HCC[b]/HP	PCR-SSCP	8	70	7	–	–	43/15	61	2677G>T/A	12/16	29/30	17/15	0.900
Minoru F-2 [30]	Japan	Japanese	HCC[b]/HP	PCR-SSCP	8	70	7	–	–	43/15	61	3435C>T	16/14	29/39	13/8	0.023
Ren YQ [31]	China	Chinese	HCC[a]/HP	CRS-PCR	7	58.7	11.3	55.8	15.6	512/177	499/181	4125A>C	299/312	289/303	101/65	0.487
Gao J-1 [32]	China	Chinese	HCC[a]/HP	CRS-PCR	7	57.9	13.7	53.5	14.9	278/75	269/66	335T>C	141/172	150/128	62/35	0.132
Gao J-2 [32]	China	Chinese	HCC[a]/HP	CRS-PCR	7	57.9	13.7	53.5	14.9	278/75	269/66	3073A>C	116/155	158/139	79/41	0.261
Rui J [33]	China	Chinese	HCC[a]/HP	MALDI-TOF-MS	8	46	–	48	–	95/14	90/19	1236C>T	19/22	54/48	36/39	0.310
Yang D-1 [34]	China	Chinese	HCC[a]/HP	CRS-PCR	8	59.2	14.3	58.3	15.3	418/287	429/297	159G>T	312/342	298/308	95/76	0.591
Yang D-2 [34]	China	Chinese	HCC[a]/HP	CRS-PCR	8	59.2	14.3	58.3	15.3	418/287	429/297	1465C>T	294/367	306/292	105/67	0.420
Li XF [35]	China	Chinese	HCC[a]/HP	CRS-PCR	8	58.6	14.5	59.1	13.5	409/236	445/213	3751G>A	283/325	271/286	91/47	0.136
Wan YY [36]	China	Chinese	HCC[a]/HP	CRS-PCR	8	57.7	13.2	58.6	14.2	399/233	435/210	1564A>T	278/311	266/276	88/58	0.772
Total										4407	4436		1788/2055	1906/1902	713/479	

1/1, 1/2, and 2/2 represent wild homozygous genotype, wild/mutant heterozygous genotype, and mutant homozygous genotype, respectively

HCC hepatocellular carcinoma, *HP* healthy people, *CHC* chronic hepatitis C, *CHB* chronic hepatitis B, "–" unclear, *PCR-RFLP* polymerase chain reaction-restriction fragment length polymorphism, *PCR-SSCP* polymerase chain reaction-single-strand conformation polymorphism, *CRS-PCR* created restriction site-polymerase chain reaction, *MALDI-TOF-MS* matrix-assisted laser desorption ionization time-of-flight mass spectrometry

[a] Hepatitis B-related HCC

[b] Hepatitis C-related HCC

Table 2 Characteristics of the MDR1 polymorphisms included in the meta-analysis

Studies	Polymorphism site	Exon location	Variation type	A.A. alteration	FI[a]	FI score[a]	Feature key	P. location description[b]	P. function description[b]
Chen Y [29]	2677G>T/A	Exon 21	Nonsynonymous	S893A	Neutral	−0.98	Topological domain	Cytoplasmic	ABC transmembrane type 1
				S893T	Low	1.66	Topological domain	Cytoplasmic	ABC transmembrane type 1
Minoru F-1 [30]	2677G>T/A	Exon 21	Nonsynonymous	S893A	Neutral	−0.98	Topological domain	Cytoplasmic	ABC transmembrane type 1
				S893T	Low	1.66	Topological domain	Cytoplasmic	ABC transmembrane type 1
Minoru F-2 [30]	3435C>T	Exon 26	Synonymous	–	–	–	Topological domain	Cytoplasmic	ABC transporter
Ren YQ [31]	4125A>C	Exon 28	Nonsynonymous	E1211A	Low	1.805	Topological domain	Cytoplasmic	ABC transporter
Gao J-1 [32]	335T>C	5′-UTR	Noncoding	–	–	–	–	–	–
Gao J-2 [32]	3073A>C	Exon 22	Nonsynonymous	L860F	Medium	2.715	Transmembrane	Helical	ABC transmembrane type 1
Rui J [33]	1236C>T	Exon 12	Synonymous	–	–	–	Topological domain	Cytoplasmic	ABC transporter
Yang D-1 [34]	159G>T	Exon 5	Synonymous	–	–	–	Transmembrane	Helical	ABC transmembrane type 1
Yang D-2 [34]	1465C>T	Exon 14	Nonsynonymous	R489C	Medium	1.97	Topological domain	Cytoplasmic	ABC transporter
Li XF [35]	3751G>A	Exon 28	Nonsynonymous	V1251I	Neutral	−0.365	Topological domain	Cytoplasmic	ABC transporter
Wan YY [36]	1564A>T	Exon 15	Nonsynonymous	T522S	Low	1.42	Topological domain	Cytoplasmic	ABC transporter

A.A. amino acid, *FI* functional impact, *ABC* ATP-binding cassette

[a] The functional impact is evaluated using online MutationAssessor.org

[b] Location of SNP in the protein structure is assessed by Uniprot.org online service

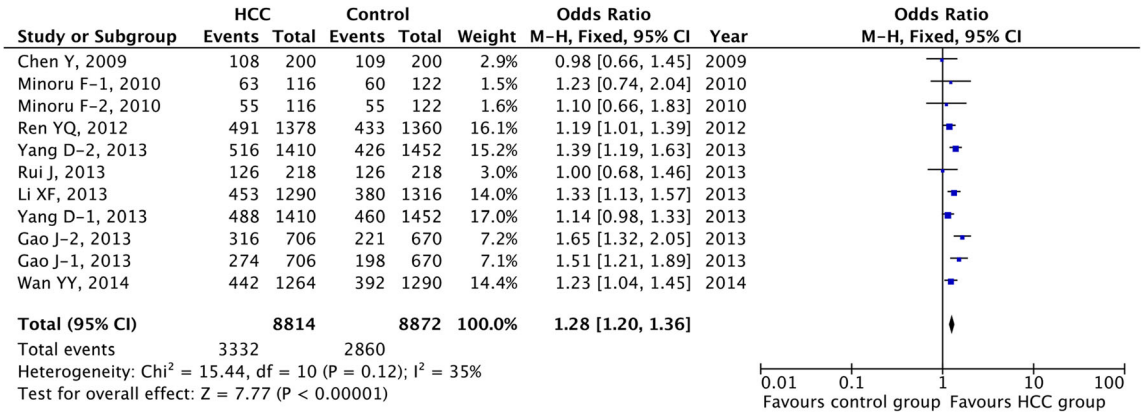

Fig. 1 Forest plot presenting the meta-analysis of MDR1 polymorphisms and the susceptibility to hepatocellular carcinoma under the allelic model in all cohorts. The *horizontal lines* represent 95 % confidence intervals for estimating the outcome of the mutant allele versus the wild-type allele in the meta-analysis. *Blue squares* denote overall estimates of the effects

sequence subgroup analyses revealed that cytoplasmic polymorphisms correlated with a significantly higher HCC risk (cytoplasmic subgroup: OR=1.28, 95 % CI 1.19–1.37; $P<0.00001$), whereas transmembrane polymorphisms exhibited site-specific results (Gao J-2, 2013: OR=1.65, 95 % CI 1.32–2.05, $P<0.0001$; Yang D-1, 2013: OR=1.65, 95 % CI 0.98–1.33, $P=0.10$). Positions in different protein functional domain subgroup analyses indicated that only polymorphisms in the ABC transporter domain rather than the ABC transmembrane type 1 domain significantly increased HCC risk (ABC transporter subgroup: OR=1.28, 95 % CI 1.18–1.38, $P<0.0001$; ABC transmembrane type 1 subgroup: OR=1.21, 95 % CI 0.98–1.49, $P=0.07$). The results indicated that polymorphisms positioned in the cytoplasmic coding sequence and ABC transporter domain play a vital role in hepatocarcinogenesis.

Sensitivity analyses and publication bias

One cohort was excluded at each time to assess the influence of the individual dataset on the overall results. The overall significance was not altered when any single cohort was deleted, suggesting that the results were robust. Begg's funnel plots and Egger's tests were conducted to assess the publication bias for the available datasets. The funnel plots were symmetrical (Fig. 2), indicating that there were no publication biases in the studies of MDR1 polymorphisms (Egger's test, $P=0.640$).

Discussion

HCC is a common malignancy resulting from a complex interaction between environmental and genetic factors. With high interest in gene susceptibility to carcinogenesis, increasing efforts have been devoted to the study of genetic variants and HCC risk. Human MDR1 is physiologically widely expressed in the liver, kidney, colon, adrenal gland, and blood-brain barrier [28]. MDR1 plays an important role in protecting organs from xenobiotics or toxins, which can cause chronic inflammation that is thought to be an etiological factor for many diseases, including HCC. Several studies have digged into the association between MDR1 polymorphisms and HCC risk; however, controversial results exist. Hence, we performed the first meta-

Table 3 Allelic and genotypic meta-analysis of the MDR1 polymorphism in all cohorts under alternative genetic models

Allele/genotype	HCC	Control	HCC vs. control			Heterogeneity	
			OR	CI	*P*	I^2 (%)	*P*
2 vs. 1	8814	8872	1.28	[1.20, 1.36]	<0.001	35	0.12
2/2 vs. 1/1	2501	2592	1.81	[1.58, 2.07]	<0.001	15	0.31
2/2 vs. 1/2	2619	2381	1.49	[1.30, 1.70]	<0.001	10	0.35
1/2 vs. 1/1	3694	3957	1.15	[1.05, 1.26]	0.002	2	0.42
2/2+1/2 vs. 1/1	4407	4436	1.27	[1.16, 1.38]	<0.001	20	0.25
2/2 vs. 1/2+1/1	4407	4436	1.59	[1.36, 1.85]	<0.001	26	0.19

1/1, 1/2, and 2/2 represent wild homozygous genotype, wild/mutant heterozygous genotype, and mutant homozygous genotype, respectively

HCC hepatocellular carcinoma, *CI* confidence interval, *OR* odds ratio

Table 4 Subgroup meta-analyses of the association between *MDR1* polymorphisms and hepatocellular carcinoma risk under the allele model

Comparison	No. of datasets	Case	Control	OR (95 % CI)	*P* for OR	*P* for heterogeneity	I^2 (%)	Model
Overall	11	8814	8872	1.28 (1.20, 1.36)	*<0.00001*	0.12	35	Fixed
Ethnicity								
Chinese	9	8582	8628	1.28 (1.17, 1.41)	*<0.00001*	0.06	47	Random
Japanese[a]	2	–	–	–	–	–	–	–
Sample size								
>1000	5	6752	6870	1.25 (1.17, 1.34)	*<0.00001*	0.39	3	Fixed
≤1000	6	2062	2002	1.29 (1.06, 1.56)	*0.01*	0.08	49	Random
Quality score								
>7	7	5824	5972	1.25 (1.16, 1.35)	*<0.00001*	0.51	0	Fixed
≤7	4	2990	2990	1.34 (1.09, 1.64)	*0.005*	0.03	68	Random
Variation type								
Synonymous	3	2450	2462	1.22 (1.08, 1.37)	*0.001*	0.14	45	Fixed
Nonsynonymous	7	6364	6410	1.31 (1.21, 1.41)	*<0.00001*	0.17	33	Fixed
Functional impact[b]								
Neutral/low	5	4248	4288	1.23 (1.12, 1.35)	*<0.00001*	0.66	0	Fixed
Medium[a]	2	–	–	–	–	–	–	–
Protein structure location[c]								
Cytoplasmic	8	6698	6750	1.28 (1.19, 1.37)	*<0.00001*	0.41	3	Fixed
Transmembrane[a]	2	–	–	–	–	–	–	–
Protein functional domain[c]								
ABC transmembrane type 1	5	2650	2662	1.21 (0.98, 1.49)	0.07	0.04	60	Random
ABC transporter	5	5458	5540	1.28 (1.18, 1.38)	*<0.00001*	0.60	0	Fixed

OR odds ratio, *CI* confidence interval, *ABC* ATP-binding cassette

[a] The subgroup is not suitable for meta-analysis for insufficient datasets

[b] The functional impact is evaluated using online MutationAssessor.org

[c] Location of SNP in the protein structure is assessed by Uniprot.org online service

analysis to clarify the inconsistency among available studies. This meta-analysis included 8 studies (11 cohorts) with 4407 HCC patients and 4436 controls. The sample size is nearly ten times that of the largest cohort included.

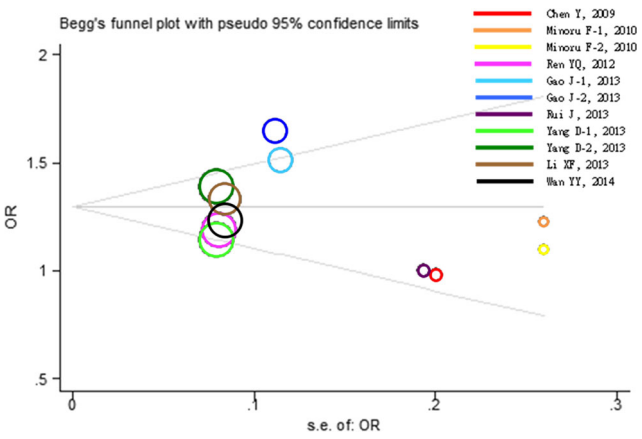

Fig. 2 Begg's funnel plot of pseudo 95 % confidence limits. Evaluation of publication bias for the association of MDR1 polymorphisms with hepatocellular carcinoma risk in all cohorts. *OR* odds ratio, *s.e.* standard error

Consistent with the biological function of MDR1, wild-type MDR1 was found to be protective against HCC development [27]. The positive association of a mutant allele in MDR1 polymorphisms among all cohorts was detected under allelic and all genotypic models, suggesting that mutant alleles of the MDR1 gene significantly increased HCC risk. In the Chinese subgroup, a positive association of a mutant allele and increased HCC risk was also observed. Meanwhile, subgroup analyses revealed a significant association in both cohorts with large and small sample sizes. However, the I^2 for a large sample size cohort (3 %) was substantially smaller than the I^2 for a small sample size cohort (49 %). Likewise, the I^2 for the high-quality score cohorts and low-quality score cohorts exhibited the same trend as the large/small sample size cohorts. These results indicated that the heterogeneity might be caused by small sample sizes and low-quality scores.

Unlike traditional meta-analysis for gene polymorphisms, the present meta-analysis analyzed multiple polymorphism sites classified as mutant or wild type to explore impacts of

MDR1 on HCC risk as a whole, and we included variation type, estimated functional impact, positions in the coding sequence, and the protein functional domain as subgroup stratifiers to assess their site-related influences on HCC risk. In the variation type subgroup analyses, both synonymous and nonsynonymous cohorts indicated that mutant alleles significantly correlated with HCC risk. The effect of nonsynonymous variations was largely due to the alteration of protein structures leading to functional changes in MDR1, thereby causing toxin accumulation in hepatocytes. In contrast, the effect of synonymous variations may partly be due to impacts on transcriptional and translational processes [44, 45]. Using online MutationAssessor, we calculated the estimated functional impact of different SNPs, and subsequent functional impact subgroup analyses indicated that even neutral/low functional impacts had significant results. A medium functional impact may also have significant findings based on individual datasets. Whether the medium functional impact subgroup exhibited greater significance could not be assessed because of the limited data available. Further investigations on medium functional impact SNPs will be of great value. In protein structure location-based subgroup analyses, we found that cytoplasmic variations significantly increased HCC risk. The transmembrane subgroup only contained two cohorts, making them unsuitable for meta-analysis. Interestingly, we noted that the two cohorts that investigated transmembrane SNPs reported contradictory results, and the larger sample size one reached a negative conclusion. Therefore, we cautiously conclude that SNPs in the cytoplasmic region, rather than in the transmembrane region of MDR1, exhibit a significant association with increased HCC susceptibility. A possible explanation is that the cytoplasmic region exhibits multiple biological activity domains that are responsible for downstream signaling pathway activation, whereas the transmembrane region is only an anchor for MDR1 and appears to be less important. This hypothesis was further validated by protein functional domain subgroup analyses, which indicated that the ABC transporter subgroup exhibits a significant association ($P<0.00001$), whereas the ABC transmembrane type 1 subgroup exhibits no association with HCC risk.

There are some limitations of our meta-analysis that should be noticed in interpreting the results. First, the ethnicity of the majority of cohorts was Chinese. There were insufficient data for a meta-analysis of Japanese subgroups. Considering that the SNPs in different populations varied with respect to both sites and frequency, we must be cautious in our conclusions. Second, some potential confounding factors that might have biased this finding could not be assessed due to insufficient data, such as age, tobacco smoking, alcohol drinking, and medicine usage. Third, MDR1 possesses at least 50 different SNPs, but sufficient data were available for only 10 SNPs in the present meta-analysis and the impact of a specific SNP on HCC risk cannot be assessed using available data. At least 40 different SNPs and more studies focused on a specific SNP in a larger HCC cohort require further investigation with respect to their association with HCC risk.

In conclusion, this study is the first meta-analysis to summarize the association between MDR1 gene polymorphisms and HCC susceptibility. The results indicated that mutant alleles of MDR1 significantly increase HCC risk. Interestingly, we also, for the first time, revealed that only the SNPs in the cytoplasmic region significantly correlated with increased HCC risk, which requires further experimental confirmation. Further large studies with multi-ethnic groups, high-quality scores, and different ethnic populations will be necessary to further validate our conclusions.

Conflicts of interest None

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