Association Between XPD Lys751Gln and Asp312Asn Polymorphisms and Hepatocellular Carcinoma Risk
A Systematic Review And Meta-Analysis

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Abstract: Genetic polymorphisms of xeroderma pigmentosum group D (XPD) in the nucleotide excision repair pathway may influence cancer susceptibility by affecting the capacity for DNA repair. Studies investigating the association between XPD Lys751Gln and Asp312Asn polymorphisms and hepatocellular carcinoma (HCC) risk reported inconsistent results. The aim of this study was to quantitatively summarize the evidence for such an association.

Eligible studies were identified by searching electronic databases including PubMed, Embase, Cochrane library, and CBM, Chinese Biomedical Literature Database, for the period up to October 2014. The association of XPD Lys751Gln and Asp312Asn polymorphisms and HCC risk was assessed by odds ratios (ORs) together with their 95% confidence intervals (CIs).

Finally, a total of 11 studies with 4322 cases and 4970 controls were included for XPD Lys751Gln polymorphism and 6 studies with 2223 cases and 2441 controls were available for XPD Asp312Asn polymorphism. With respect to XPD Lys751Gln polymorphism, statistically significant increased HCC risk was found when all studies were pooled into the meta-analysis (Gln/Gln vs Lys/Lys: OR = 1.363, 95% CI 1.065–1.744, P = 0.004; Lys/Gln vs Lys/Lys: OR = 1.205, 95% CI 1.099–1.321, P = 0.000; Gln/Gln vs Lys/Lys vs Lys/Lys: OR = 1.300, 95% CI 1.141–1.480, P = 0.000). In subgroup analyses by ethnicity, source of control, Hardy–Weinberg equilibrium (HWE) in controls, hepatitis B virus (HBV) infection, and statistically significant increase of HCC risk was found in East Asians, population-based studies, studies deviating from HWE, and HBV-negative subjects. With respect to XPD Asp312Asn polymorphism, no significant association with HCC risk was found in the overall and subgroup analyses.

The results suggest that the XPD Lys751Gln polymorphism contributes to increased HCC susceptibility, especially in East Asian populations. Further, large and well-designed studies are required to validate this association.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third common cause of cancer mortality worldwide. The distribution of HCC is imbalanced throughout the world, with the highest incidence rates in East and Southeast Asia and Sub-Saharan Africa, and China alone accounted for >50% of all HCC malignancies. Etiologically, carcinogenesis of HCC is a complex, multistep, and multifactor process, in which many factors are implicated. As we know, chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), and obesity-related nonalcoholic steatohepatitis (NASH) are the most well-established environmental risk factors for HCC worldwide. However, only a fraction of lifelong HBsAg carriers and NASH subjects eventually develop HCC, and only 2.5% of HCV-infected individuals develop HCC later in life. The exact mechanism of hepatocarcinogenesis is still incompletely understood, and the risk factors for HCC still need to be further elucidated.

Human cancer can be initiated by DNA damage caused by endogenous and exogenous mutagens such as ionizing radiation, UV, viruses, and environmental chemical agents. Importantly, to counteract the deleterious consequences of DNA-damaging agents, humans have developed a set of complex DNA repair systems that as a whole take care of most of the insults inflicted on a cell’s vital genetic information. The DNA repair systems play critical roles in maintaining the functions of normal cells and genomic integrity through the reversal of the damaged DNA. Among the DNA repair systems, the nucleotide excision repair (NER) pathway constitutes the primary mechanism for removal of bulky adducts from DNA, and thus is an important part of the cellular defense against a large variety of structural unrelated DNA lesions generated by ionizing radiation and strong alkylating agents as well as lesions formed by endogenous DNA-damaging agents like viruses.

Xeroderma pigmentosum group D (XPD), also named excision repair cross-complimentary group 2, is 1/8 core genes (ie, ERCC1, XPA, XPB, XPC, XPD, XPE, XPF, and XPG) in the NER pathway of the DNA repair system. XPD maps to chromosome 19q13.3 and is composed of 23 exons. It codes for a DNA helicase subunit of the core transcription factor IIIH, which is essential for NER and transcription and plays a critical role in DNA repair and cellular responses to DNA damage.

The authors have no funding and conflicts of interest to disclose. Copyright © 2014 Wolters Kluwer Health, Inc. All rights reserved.

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DOI: 10.1097/MD.0000000000000330
role in transcription-coupled NER pathway. Single nucleotide polymorphisms (SNPs) in the exons of XPD may influence the protein activity, resulting in defects in the NER pathway and reduced DNA repair capacity. The Lys751Gln and Asp312Asn were the 2 most commonly studied SNPs in the coding region of the XPD gene. The XPD Lys751Gln polymorphism (rs13181) is characterized by an A to C substitution resulting in a lysine (Lys) to glutamine (Gln) amino acid exchange at position 751 in exon 23, whereas the Asp312Asn polymorphism (rs1799793) is characterized by a G to A transition causing an aspartic acid (Asp) to asparagine amino acid (Asn) exchange at position 312 in exon 10. Previous studies have demonstrated that the 2 polymorphisms were associated with lower DNA repair capacity and higher level of DNA adducts. Hence, it is biologically reasonable to hypothesize a potential relationship between the XPD Lys751Gln and Asp312Asn polymorphisms and HCC risk.

Over the last 2 decades, a number of epidemiological studies have been conducted to investigate the associations between XPD Lys751Gln and Asp312Asn polymorphisms and HCC risk, but the results remain inconsistent and underpowered. With respect to XPD Lys751Gln polymorphism, a meta-analysis by Zhang and Mou did not find any evidence of significant association between XPD Lys751Gln polymorphism and HCC risk in the overall populations and subgroup analysis. However, the evidence was limited because they failed to include all eligible studies in the meta-analysis. In addition, the source of heterogeneity was not extensively explored in this study. With respect to XPD Asp312Asn polymorphism, to the best of our knowledge, no meta-analyses on this issue have ever appeared. The exact relationship between genetic polymorphisms of XPD Lys751Gln and Asp312Asn polymorphisms and HCC risk was assessed by confidence interval (CI) or other information for estimating OR (95% CI); and the control group did not contain malignant tumor patients. Conference abstracts, review articles, meta-analyses, case reports, editorials, and letters were excluded.

Data Extraction

Data were extracted from each study by 2 authors independently (X.L. and Q.P) according to the selection criteria listed above. Decisions were compared and disagreements were resolved by consensus or by involving a third author (X.Q.). The following information was extracted from each study: first author, publication year, country of origin, ethnicity, genotyping method, source of control, matching variables, HCC diagnosis, total number of cases and controls, and genotype frequencies of cases and controls. When the genotype frequency or other important information was not reported, we contacted the author to get the relevant information by e-mail or telephone.

Statistical Analysis

The strength of the association between XPD Lys751Gln and Asp312Asn polymorphisms and HCC risk was assessed by ORs together with 95% CIs. The significance of the pooled ORs was determined by Z test, and the P values <0.05 were considered significant. We evaluated the XPD Lys751Gln and Asp312Asn polymorphisms and HCC risk using codominant, recessive, and dominant models.

The $\chi^2$-based $Q$ test was used to assess the statistical heterogeneity among studies. If the result of the $Q$ test was $P_Q < 0.10$, suggesting the existence of heterogeneity, the pooled ORs were calculated using the random-effects model (the DerSimonian and Laird method). Otherwise, when the result of the $Q$ test was $P_Q \geq 0.1$, indicating the absence of heterogeneity, the fixed-effects model was applied. Logistic metaregression and subgroup analyses were used to identify the sources of heterogeneity among studies. The following parameters were included as covariates in metaregression analysis: ethnicity (East Asians vs mixed/other), source of controls (hospital-based vs population-based studies), genotyping methods (polymerase chain reaction-restriction fragment length polymorphism vs no polymerase chain reaction-restriction fragment length polymorphism), Hardy–Weinberg equilibrium (HWE) in controls (Yes vs No). Subgroup analyses were performed according to ethnicity, source of control, HWE status, and HBV infection. Galbraith plot analysis was performed to further explore the source of heterogeneity.

Sensitivity analysis was performed by sequential exclusion of the individual studies to assess the robustness of the results. Begg funnel plot and Egger regression asymmetry test were performed to evaluate the publication bias. If the publication bias presented, the Duval and Tweedie’s nonparametric “trim and fill” method was applied to adjust for it. The HWE was evaluated by the goodness-of-fit $\chi^2$ test for the control groups of each study (significance set at $P = 0.05$, $P < 0.05$ was considered a departure from HWE). All analyses were performed using stata software, version 12.0 (Stata Corp, College Station, TX). All P values were 2-sided. To ensure the reliability and the accuracy of the results, 2 authors entered the data into the statistical software programs independently with the same results.

MATERIAL AND METHODS

Literature Search

We conducted a comprehensive literature search in PubMed, EMBASE, Cochrane library, and CBM, Chinese Biomedical Literature Database, up to October 01, 2014 using the following search strategy: (“hepatocellular carcinoma,” “HCC,” “liver cancer”) and (“Xeroderma pigmentosum complementation group D,” “XP-D,” “excision repair cross-complimentary group 2” or “ERCC2”). There was no restriction on sample size, language, population, or type of report. All eligible studies were retrieved and their references were checked for other relevant studies. In addition, we also used the “Related Articles” function in PubMed to search for other potential relevant articles. When several studies reported on the same or overlapping data, we chose the most recent or largest population. The meta-analysis was performed according to the proposal of Meta-Analysis of Observational Studies in Epidemiology group.

Selection Criteria

We reviewed titles and abstracts of all citations and retrieved studies. The following inclusion criteria were used for literature selection: case–control studies that evaluated the association between XPD Lys751Gln and Asp312Asn polymorphisms and HCC risk; used an unrelated case–control design; presented an odds ratio (OR) together with 95% confidence interval (CI) or other information for estimating OR (95% CI); and the control group did not contain malignant tumor patients. Conference abstracts, review articles, meta-analyses, case reports, editorials, and letters were excluded.
RESULTS

Study Characteristics

Based on our search strategy, 34 records were found, but only 14 full-text studies were preliminarily identified for further detailed evaluation after screening the titles and abstracts. According to the exclusion criteria, 3 studies were excluded including 1 presenting insufficient data for calculating OR and 95% CI,26 1 was a review,27 and 1 was a meta-analysis.15 Manual search of references cited in the retrieval studies identified 1 additional study.28 As a result, a total of 12 relevant studies (1 was a dissertation by a postgraduate student) met the inclusion criteria for the meta-analysis.16–20,22–34 The main characteristics of the studies were presented in Table 1. Among them, 11 studies with 4322 cases and 4970 controls were available for XPD Asp312Asn polymorphism and 6 studies with 2223 cases and 2441 controls were available for XPD Lys751Gln polymorphism; 6/6 studies were conducted on East Asians for the XPD Asp312Asn polymorphism. One study in the present meta-analysis did not provide definite criteria for HCC diagnosis.33

The genotype distributions of the controls in 2 studies were inconsistent with HWE for the XPD Lys751Gln polymorphism and HCC risk when all eligible studies were pooled into the meta-analysis (Gln/Gln vs Lys/Lys: OR = 1.363, 95% CI 1.065–1.744, P = 0.014; Lys/Gln vs Lys/Lys: OR = 1.205, 95% CI 1.099–1.321, P = 0.000; Gln/Gln + Lys/Gln vs Lys/Lys: OR = 1.300, 95% CI 1.141–1.480, P = 0.000). In subgroup analysis by ethnicity, statistically significant increased HCC risk was found when all studies were stratified by ethnicity, statistically significant increased HCC risk was found in East Asians (Gln/Gln vs Lys/Lys: OR = 1.372, 95% CI 1.058–1.777, P = 0.017; Lys/Gln vs Lys/Lys: OR = 1.199, 95% CI 1.093–1.316, P = 0.000; Gln/Gln + Lys/Gln vs Lys/Lys: OR = 1.294, 95% CI 1.131–1.481, P = 0.000; Figure 1) but not in mixed/other populations. In subgroup analysis according to source of control, significantly increased HCC risk was found in population-based studies (Lys/Gln vs Lys/Lys: OR = 1.353, 95% CI 1.181–1.551, P = 0.000; Gln/Gln + Lys/Gln vs Lys/Lys: OR = 1.323, 95% CI 1.168–1.498, P = 0.000), but not in hospital-based studies. When stratified by HWE in controls, significantly increased HCC risk was observed in studies consistent with HWE (Lys/Gln vs Lys/Lys: OR = 1.328, 95% CI 1.164–1.516, P = 0.000; Gln/Gln + Lys/Gln vs Lys/Lys: OR = 1.295, 95% CI 1.147–1.463, P = 0.000) but not in studies inconsistent with HWE. In subgroup analysis according to HBV infection, significantly increased HCC risk was identified in HBV-positive patients (Lys/Gln vs Lys/Lys: OR = 1.502, 95% CI 1.094–2.157, P = 0.007; Gln/Gln + Lys/Gln vs Lys/Lys: OR = 1.431, 95% CI 1.046–2.099, P = 0.013) but not in HBV-negative subjects.

Table 3 lists the main results of meta-analysis of XPD Asp312Asn polymorphism and HCC risk. There was no evidence of significant association between the XPD Asp312Asn polymorphism and HCC risk when all eligible studies were pooled into the meta-analysis (Asn/Asn vs Asp/Asp: OR = 0.959, 95% CI 0.761–1.208, P = 0.720; Asp/Asn vs Asp/Asp: OR = 1.048, 95% CI 0.920–1.195, P = 0.479; Asp/Asn + Asp/Asn vs Asp/Asp: OR = 1.031, 95% CI 0.913–1.163, P = 0.625; Asn/Asn vs Asp/Asn + Asp/Asp: OR = 0.925, 95% CI 0.660–1.295, P = 0.648). In subgroup analyses by ethnicity, source of controls, HWE in controls, and HBV infection, statistically significant association was also not detected in all subgroups (Figure 2).

Heterogeneity Analysis

With respect to XPD Lys751Gln polymorphism, statistically significant between-study heterogeneity was found in the pooled analyses of total eligible studies (Gln/Gln vs Lys/Lys: P = 0.007; Gln/Gln + Lys/Gln vs Lys/Lys: P = 0.052; Gln/Gln vs Lys/Gln + Lys/Lys: P = 0.020; Table 2). To investigate the sources of heterogeneity, we performed metaregression and subgroup analyses. Metaregression revealed that the ethnicity, source of controls, genotyping methods, and HWE in controls were not effect modifiers. Subsequently, we performed subgroup analyses by ethnicity, source of control, and HBV infection. However, heterogeneity still existed in East Asians, hospital-based studies, and studies consistent with HWE (Table 2). To further explore the source of heterogeneity, we performed Galbraith plot analysis to identify the outlier that might contribute to the heterogeneity. The results indicated that the study by Jiang et al10 was the outlier in the overall populations (Figure 3). All P values in the overall populations, East Asians, hospital-based studies, and studies consistent with HWE were >0.10 after excluding the study by Jiang et al.20 Interestingly, the significance of the pooled ORs for the XPD Lys751Gln polymorphism and HCC risk in the overall populations, East Asians, hospital-based studies, and studies consistent with HWE did not change after omitting this study.

With respect to XPD Asp312Asn polymorphism, the P value of the Q test was <0.10 in the recessive model in the overall populations (Asn/Asn vs Asp/Asn + Asp/Asp: P = 0.097; Table 3), indicating statistically significant heterogeneity among studies. Metaregression analysis revealed that the ethnicity, source of control, genotyping methods, and HWE in controls were not effect modifiers. Subgroup analyses indicated that the heterogeneity was evident in East Asians, hospital-based studies, and studies consistent with HWE. Galbraith plot analysis suggested that the study by Zeng et al19 was the outlier and the major source of the heterogeneity (Figure 4). The P values were >0.10 after excluding the study by Zeng et al19 in the overall populations, East Asians, hospital-based studies, and studies consistent with HWE. However, the significance of the pooled ORs for the XPD Asp312Asn polymorphism and HCC risk in the overall populations, East Asians, hospital-based studies, and studies consistent with HWE were also not changed by excluding this study.

Sensitivity Analysis

Sensitivity analysis was performed by sequential omission of individual studies for both the XPD Lys751Gln and Asp312Asn polymorphisms. For analyses of pooling >3 studies, the significance of ORs was not materially influenced by omitting any single study. For the XPD Lys751Gln polymorphism, sensitivity analysis was further performed by excluding the studies by Yao et al17 and Long et al31 in which the control populations were significantly deviated from HWE. The significance of all ORs was not altered after excluding the 2
### TABLE 1. Characteristics of the Included Studies

| First Author | Year | Country | Ethnicity | Genotyping Methods | Source of Control | Matched Variables | Sample Size (Case/Control) | HCC Diagnosis | HWE (P Value) |
|--------------|------|---------|-----------|--------------------|-------------------|-------------------|-----------------------------|---------------|--------------|
| **XPD Lys751Gln** |      |         |           |                    |                   |                   |                             |               |              |
| Wu           | 2014 | China   | East Asian| MALDI-TOF          | HB                | Age, sex, and smoking | 218/277            | Biopsy, imaging, or imaging combined with AFP, all cases were confirmed histologically | 0.068         |
| Yao          | 2014 | China   | East Asian| TaqMan            | HB                | Age, sex, ethnicity, HBV and HCV infection | 1486/1996          | Histopathology | <0.0001      |
| Gulnaz       | 2013 | Pakistan| Mixed/other| PCR-RFLP          | HB                | Sex, smoking, and drinking | 50/74              | Imaging or imaging combined with AFP | 0.115         |
| Guo          | 2012 | China   | East Asian| PCR-CTPP          | PB                | Age and sex          | 410/410            | Histology | 0.158         |
| Chen         | 2005 | Taiwan  | East Asian| TaqMan            | PB                | Age and sex          | 570/381            | Biopsy, imaging, or imaging combined with AFP | 0.346         |
| Long         | 2009 | China   | East Asian| TaqMan            | PB                | Age, sex, ethnicity, HBV and HCV infection | 618/712            | Histopathology | <0.0001      |
| Zeng         | 2009 | China   | East Asian| TaqMan            | HB                | Age, sex, ethnicity | 300/312            | Imaging or imaging combined with AFP; all cases were confirmed histopathologically | 0.244         |
| Xu           | 2004 | China   | East Asian| PCR-RFLP          | HB                | Age, sex, residential area | 70/136            | Pathology | 0.135         |
| Cui          | 2010 | China   | East Asian| PCR-RFLP          | PB                | Age and sex          | 94/111             | NR | 0.478         |
| Jiang        | 2012 | China   | East Asian| PCR-RFLP          | HB                | Age, sex, and drinking | 76/80             | Pathology | 0.054         |
| Xie          | 2007 | China   | East Asian| PCR-RFLP          | PB                | Age, sex, and drinking | 430/481            | Biopsy, imaging, or imaging combined with AFP | 0.062         |
| **XPD Asp312Asn** |      |         |           |                    |                   |                   |                             |               |              |
| Wu           | 2014 | China   | East Asian| MALDI-TOF          | HB                | Age, sex, and smoking | 218/277            | Biopsy, imaging, or imaging combined with AFP; all cases were confirmed histologically | <0.0001       |
| Guo          | 2012 | China   | East Asian| PCR-CTPP          | PB                | Age and sex          | 410/410            | Histology | <0.0001       |
| Yuan         | 2012 | China   | East Asian| Sequencing        | HB                | Age, sex, drinking, and smoking | 252/250          | Postsurgical pathology | 0.461         |
| Long         | 2009 | China   | East Asian| TaqMan            | PB                | Age, sex, ethnicity, HBV and HCV infection | 618/712            | Histopathology | 0.067         |
| Zeng         | 2009 | China   | East Asian| TaqMan            | HB                | Age, sex, and ethnicity | 300/312            | Imaging, or imaging combined with AFP; all cases were confirmed histopathologically | 0.133         |
| Xie          | 2007 | China   | East Asian| PCR-RFLP          | PB                | Age, sex, and drinking | 425/480            | Biopsy, imaging, or imaging combined with AFP | 0.407         |

Gln = glutamine, HB = hospital-based studies, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HWE = Hardy–Weinberg equilibrium in control population, Lys = lysine, MALDI-TOF = matrix assisted laser desorption ionization-time of flight, NR = not reported, PB = population-based studies, PCR-CTPP = polymerase chain reaction with the confronting 2-pair primer, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, XPD = xeroderma pigmentosum group D.
| Analysis          | No. of Studies | OR (95% CI) | \( P/P_h \) | OR (95% CI) | \( P/P_h \) | OR (95% CI) | \( P/P_h \) | OR (95% CI) | \( P/P_h \) |
|-------------------|----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| **Overall**       | 11             | 1.363 (1.065–1.744) | 0.014/0.007 | 1.205 (1.099–1.321) | 0.000/0.182 | 1.300 (1.141–1.480) | 0.000/0.052 | 1.233 (0.988–1.538) | 0.063/0.020 |
| **Ethnicity**     |                |             |             |             |             |             |             |             |             |
| East Asian        | 10             | 1.372 (1.058–1.777) | 0.017/0.004 | 1.199 (1.093–1.316) | 0.000/0.164 | 1.294 (1.131–1.481) | 0.000/0.038 | 1.247 (0.989–1.575) | 0.062/0.013 |
| Mixed/other       | 1              | 1.267 (0.445–3.604) | 0.658/—     | 1.742 (0.789–3.847) | 0.170/—     | 1.583 (0.766–3.274) | 0.215/—     | 1.984 (0.371–2.613) | 0.974/—     |
| **Source of control** |              |             |             |             |             |             |             |             |             |
| HB                | 6              | 1.182 (0.789–1.770) | 0.417/0.122 | 1.093 (0.964–1.239) | 0.164/0.238 | 1.311 (0.952–1.633) | 0.116/0.078 | 1.057 (0.736–1.520) | 0.763/0.025 |
| PB                | 5              | 1.511 (0.993–2.375) | 0.056/0.148 | 1.353 (1.181–1.551) | 0.000/0.756 | 1.323 (1.168–1.498) | 0.000/0.179 | 1.105 (0.825–1.414) | 0.211/0.114 |
| **HWE in controls** |              |             |             |             |             |             |             |             |             |
| Yes               | 9              | 1.372 (0.952–1.979) | 0.090/0.10  | 1.328 (1.164–1.516) | 0.000/0.761 | 1.295 (1.147–1.463) | 0.000/0.718 | 1.207 (0.865–1.684) | 0.269/0.023 |
| No                | 2              | 1.438 (0.978–1.969) | 0.124/0.272 | 1.161 (0.851–1.584) | 0.347/0.229 | 1.259 (0.927–1.708) | 0.140/0.216 | 1.313 (0.937–1.716) | 0.206/0.253 |
| **HBV infection** |                |             |             |             |             |             |             |             |             |
| Positive          | 4              | 1.278 (0.932–2.411) | 0.204/0.115 | 1.502 (1.094–2.157) | 0.007/0.236 | 1.431 (1.046–2.099) | 0.013/0.437 | 1.375 (0.791–2.436) | 0.472/0.372 |
| Negative          | 2              | 0.997 (0.412–3.143) | 0.643/0.268 | 1.239 (0.376–2.887) | 0.436/0.197 | 1.174 (0.395–2.794) | 0.511/0.254 | 1.074 (0.679–2.798) | 0.365/0.258 |

CI = confidence interval, Gln = glutamine, HB = hospital-based studies, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, Lys = lysine, OR = odds ratio, PB = population-based studies, \( P_h \) = \( P \) values of \( Q \)-test for heterogeneity test, HWE = Hardy–Weinberg equilibrium, XPD = xeroderma pigmentosum group D.
studies. For the XPD Asp312Asn polymorphism, sensitivity analysis was also performed by excluding those 2 studies by Wu et al\(^\text{16}\) and Guo et al\(^\text{18}\) in which the control populations were inconsistent with HWE, and the significance of all ORs was also not altered.

Publication Bias

Begg funnel plot and Egger test were used to access the publication bias of literatures. The shape of funnel plots did not reveal any evidence of obvious asymmetry (Figure 5), and the \( p \) values of the Egger tests for both the XPD Lys751Gln and Asp312Asn polymorphisms were all >0.05, providing statistical evidence of the funnel plots’ symmetry. The results suggested that publication bias did not exist in this study.

DISCUSSION

NER pathway of the DNA repair systems is the primary mechanism for the removal of bulky adducts from DNA, and thus is an important part of the cellular defense against a large variety of structural unrelated DNA lesions.\(^\text{35,36}\) XPD is one of the most important proteins in NER and closely associated with NER pathway coordination by interacting with most components of the NER short-patch pathway. Genetic polymorphisms and mutations in XPD may influence the protein activity, resulting in defects in the NER pathway and reduced DNA repair capacity;\(^\text{77}\) thus, modulating cancer susceptibility including HCC. To date, emerging epidemiological studies have evaluated the association of XPD Lys751Gln and Asp312Asn polymorphisms with HCC risk, but the results remain controversial and underpowered. Therefore, we performed this meta-analysis including all available studies to provide the most comprehensive assessment of the associations. The results based on 12 studies suggested that the XPD Lys751Gln polymorphism was significantly associated with increased HCC risk. However, our data did not support a genetic association of the XPD Asp312Asn polymorphism with HCC susceptibility.

XPD is an important DNA repair gene in the NER pathway. XPD Lys751Gln polymorphism is located at position 751 in exon 23 of the XPD gene with an A to C substitution leading to a Lys to Gln amino acid exchange. Inherited functional polymorphisms in exons of the XPD gene may influence the protein function, resulting in differences of the individual NER and DNA repair capacity that may affect the susceptibility to cancers. It has been reported that the Gln allele of the Lys751Gln polymorphism within the XPD gene was associated with higher DNA adduct levels and lower DNA repair efficiency.\(^\text{35,38}\) Therefore, it is biologically plausible that the XPD Lys751Gln polymorphism plays a critical role in hepatocarcinogenesis. This hypothesis was confirmed by our meta-analysis.

In the present study, we observed that the XPD Lys751Gln polymorphism presented a risk factor for HCC in East Asians, but not in mixed/other populations. When we excluded the study by Zhang and Mou,\(^\text{12}\) which was shown as an outlier in Galbraith plot analysis, statistically significant increased HCC risk was still found in the overall populations and East Asians but not in the mixed/other populations. The inconsistent results among diverse ethnicities demonstrated different effects of the XPD Lys751Gln polymorphism on HCC risk in different ethnic genetic backgrounds. Nevertheless, because of the small number of eligible studies among mixed/other populations, the observed association between the XPD Lys751Gln polymorphism and HCC risk in mixed/other populations is likely to be caused by chance because a study with small sample sizes may have insufficient statistical power to detect a slight effect or may have generated a fluctuated estimation. In this study, there was only 1 study for mixed/other populations concerning the XPD.

\begin{table}[h]
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\begin{tabular}{|l|c|c|c|}
\hline
Study ID & OR (95%) & % Weight & P-value
\hline
East Asian & 1.39 (0.97, 1.98) & 8.51 & 0.21
Wu (2014) & 1.09 (0.95, 1.25) & 18.80 & 0.52
Yao (2014) & 1.52 (1.16, 2.01) & 11.45 & 0.005
Guo (2012) & 1.05 (0.80, 1.36) & 12.02 & 0.52
Chen (2005) & 1.49 (1.20, 1.85) & 14.25 & 0.005
Long (2009) & 1.14 (0.82, 1.58) & 9.44 & 0.49
Zeng (2009) & 1.57 (0.88, 2.82) & 4.10 & 0.005
Xu (2004) & 1.28 (0.72, 2.30) & 4.10 & 0.49
Cui (2012) & 2.86 (1.49, 5.49) & 3.39 & 0.005
Jiang (2012) & 1.17 (0.88, 1.56) & 11.12 & 0.49
Xie (2007) & 1.29 (1.13, 1.48) & 97.18 & 0.005
Subtotal (I-squared = 49.3%, \( p = 0.038 \)) & 1.58 (0.77, 3.27) & 2.82 & 0.49
Mixed/other & 1.58 (0.77, 3.27) & 2.82 & 0.49
Guinaz (2013) & 1.57 (0.88, 2.82) & 4.10 & 0.005
Overall (I-squared = 45.0%, \( p = 0.052 \)) & 1.30 (1.14, 1.48) & 100.00 & 0.005
\hline
\end{tabular}
\caption{Forest plots of XPD Lys751Gln polymorphism and HCC risk in subgroup analysis by ethnicity using a random-effects model (dominant model Gln/Gln + Lys/Gln vs. Lys/Lys). Gln = glutamine, HCC = hepatocellular carcinoma, Lys = lysine, OR = odds ratio, XPD = xeroderma pigmentosum group D.}
\end{table}
TABLE 3. Meta-Analysis of XPD Asp312Asn Polymorphism and HCC Risk

| Analysis | No. of Studies | Homozygote (Asn/Asn vs Asp/Asp) | Heterozygote (Asp/Asn vs Asp/Asp) | Dominant Model (Asn/Asn + Asp/Asn vs Asp/Asp) | Recessive Model (Asn/Asn vs Asp/Asn + Asp/Asp) |
|----------|----------------|---------------------------------|----------------------------------|-----------------------------------------------|-----------------------------------------------|
|          |                | OR (95% CI)                      | OR (95% CI)                      | OR (95% CI)                                   | OR (95% CI)                                   |
| Overall  | 6              | 0.959 (0.761–1.208)              | 0.720/0.105                     | 1.048 (0.920–1.195)                           | 0.479/0.629                                   |
|          |                |                                  |                                  | 1.031 (0.913–1.163)                           | 0.625/0.663                                   |
|          |                |                                  |                                  | 0.925 (0.660–1.295)                           | 0.648/0.097                                   |
| Ethnicity |                |                                  |                                  | 0.959 (0.761–1.208)                           | 0.720/0.105                                   |
| East Asian | 6              |                                  |                                  | 1.048 (0.920–1.195)                           | 0.479/0.629                                   |
|          |                |                                  |                                  | 1.031 (0.913–1.163)                           | 0.625/0.663                                   |
|          |                |                                  |                                  | 0.925 (0.660–1.295)                           | 0.648/0.097                                   |
| Source of control | |                                  |                                  | 0.959 (0.761–1.208)                           | 0.720/0.105                                   |
| HB       | 3              | 0.759 (0.319–1.806)              | 0.533/0.038                     | 1.115 (0.892–1.395)                           | 0.340/0.373                                   |
|          |                |                                  |                                  | 1.057 (0.858–1.303)                           | 0.603/0.562                                   |
|          |                |                                  |                                  | 0.741 (0.302–1.817)                           | 0.513/0.028                                   |
| PB       | 3              | 1.027 (0.782–1.349)              | 0.846/0.362                     | 1.015 (0.863–1.193)                           | 0.856/0.593                                   |
|          |                |                                  |                                  | 1.018 (0.877–1.181)                           | 0.818/0.368                                   |
|          |                |                                  |                                  | 1.019 (0.780–1.332)                           | 0.890/0.438                                   |
| HWE in controls | |                                  |                                  | 0.959 (0.761–1.208)                           | 0.720/0.105                                   |
| Yes      | 4              | 0.853 (0.500–1.454)              | 0.558/0.050                     | 1.056 (0.907–1.229)                           | 0.484/0.384                                   |
|          |                |                                  |                                  | 1.030 (0.893–1.187)                           | 0.689/0.514                                   |
|          |                |                                  |                                  | 0.841 (0.489–1.444)                           | 0.529/0.042                                   |
| No       | 2              | 1.050 (0.709–1.555)              | 0.808/0.297                     | 1.028 (0.795–1.328)                           | 0.835/0.535                                   |
|          |                |                                  |                                  | 1.034 (0.821–1.302)                           | 0.778/0.331                                   |
|          |                |                                  |                                  | 1.042 (0.708–1.535)                           | 0.834/0.342                                   |
| HBV infection | |                                  |                                  | 0.959 (0.761–1.208)                           | 0.720/0.105                                   |
| Positive | 2              | 1.276 (0.812–1.994)              | 0.274/0.293                     | 1.077 (0.623–2.167)                           | 0.298/0.376                                   |
|          |                |                                  |                                  | 1.249 (0.828–1.885)                           | 0.290/0.342                                   |
|          |                |                                  |                                  | 1.137 (0.725–1.796)                           | 0.359/0.497                                   |
| Negative | 2              | 0.982 (0.375–1.991)              | 0.476/0.481                     | 0.823 (0.398–1.877)                           | 0.608/0.474                                   |
|          |                |                                  |                                  | 0.861 (0.452–1.639)                           | 0.649/0.531                                   |
|          |                |                                  |                                  | 0.958 (0.466–1.872)                           | 0.697/0.389                                   |

Asn = asparagine amino acid, Asp = aspartic acid, CI = confidence interval, HB = hospital-based studies, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HWE = Hardy–Weinberg equilibrium, OR = odds ratio, PB = population-based studies, P_h = P values of Q-test for heterogeneity test, XPD = xeroderma pigmentosum group D.
Lys751Gln polymorphism on HCC risk. Therefore, the results from the mixed/other populations should be interpreted with caution.

It was reported that hospital-based studies have inherent selection bias because of the fact that the controls just represent a sample of ill-defined reference population and may not be representative of the study population or the general population, particularly when the genotypes under investigation were associated with the disease-related conditions that the hospital-based controls may have. Selecting of proper and representative population-based controls is important in eliminating biases in such genetic association studies. Therefore, we performed subgroup analysis stratified by source of controls. The results revealed that the XPD Lys751Gln polymorphism was associated with an increased HCC risk in population-based studies, but not in hospital-based studies. Therefore, a methodologically preferable design, such as using a proper and representative population-based high quality study, is of great importance in case–control studies.

Evidence has demonstrated that studies disobeyed the law of HWE may be as a result of genetic reasons including nonrandom mating, or the alleles reflect recent mutations that have not reached equilibrium, as well as methodological reasons including biased selection of subjects from the population or genotyping errors. Because of reasons of disequilibrium, the results of genetic association studies might be unreliable when the distributions of genotypes in the control groups deviate from HWE. In the present study, the genotype distributions of the controls in 2 studies were inconsistent with HWE for the XPD Lys751Gln polymorphism. Therefore, we performed subgroup analysis according to HWE in controls.

| Study ID | OR (95%) | Weight |
|----------|----------|--------|
| Wu (2014) | 0.82 (0.43, 1.55) | 16.03 |
| Yuan (2012) | 1.57 (0.72, 3.43) | 12.50 |
| Zeng (2009) | 0.25 (0.08, 0.76) | 4.35 |
| Subtotal (I-squared = 72.0%, p = 0.028) | 0.74 (0.30, 1.82) | 35.96 |
| Guo (2012) | 1.21 (0.74, 1.97) | 20.96 |
| Long (2009) | 1.06 (0.72, 1.56) | 25.29 |
| Xie (2007) | 0.74 (0.41, 1.32) | 17.79 |
| Subtotal (I-squared = 0.0%, p = 0.438) | 1.02 (0.78, 1.34) | 64.04 |
| Overall (I-squared = 46.4%, p = 0.097) | 0.92 (0.66, 1.29) | 100.00 |

NOTE: weights are from random effects analysis.

FIGURE 2. Forest plots of XPD Asp312Asn polymorphism and HCC risk in subgroup analysis by source of controls using a random-effects model (recessive model Asn/Asn vs Asp/Asn + Asp/Asp). Asn = asparagine amino acid, Asp = aspartic acid, HB = hospital-based studies, HCC = hepatocellular carcinoma, OR = odds ratio, PB = population-based studies, XPD = xeroderma pigmentosum group D.

FIGURE 3. Galbraith plots of XPD Lys751Gln polymorphism and HCC risk (dominant model Gln/Gln + Lys/Gln vs Lys/Lys). The study by Jiang et al20 was spotted as the outlier. Gln = glutamine, glutamine, HCC = hepatocellular carcinoma, Lys = lysine, XPD = xeroderma pigmentosum group D.

FIGURE 4. Galbraith plots of XPD Asp312Asn polymorphism and HCC risk (recessive model Asn/Asn vs Asp/Asn + Asp/Asp). The study by Zeng et al19 was spotted as the outlier. Asn = asparagine asparagine amino acid, Asp = aspartic acid, HCC = hepatocellular carcinoma, XPD = xeroderma pigmentosum group D.

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When excluding the studies that were inconsistent with HWE, the results were persistent and robust, suggesting that this factor probably had little effect on the overall estimates.

Hepatocarcinogenesis has been found to be closely correlated with chronic HBV or HCV infection, and in fact, a certain proportion of chronically infected HBV and HCV individuals will develop HCC. HBV belongs to a family of DNA viruses called hepadnaviruses. The oncogenic potential of HBV has been attributed to its ability to integrate into host cellular DNA, which impairs the genomic integrity and activates neighboring cellular genes directly to offer a selective growth advantage to liver cells. In addition, the production of hepatitis B x protein can serve as a transactivator on various cellular genes for cell growth and tumorogenesis. Therefore, there might be differences in carcinogenetic mechanisms of HCC between the HBV-positive patients and HBV-negative subjects. In the present study, we found that the XPD Lys751Gln polymorphism was significantly associated with increased HCC risk in HBV-positive patients but not in HBV-negative subgroup, which was consistent with the studies conducted by Long et al.\(^{31}\) and Xu et al.\(^{32}\) The possible mechanism for the preferentially increased HCC risk of XPD Lys751Gln polymorphism in HBV-positive patients is that the DNA repair functions of the XPD have been reduced by the XPD Lys751Gln polymorphism, making them more vulnerable to cancer development. However, the hypotheses need to be proved in future studies.

The major concern in a sound meta-analysis is the degree of heterogeneity that exists between studies because heterogeneous data are prone to produce misleading results, and finding the sources of heterogeneity is one of the most important goals in a meta-analysis.\(^{42}\) In this study, statistically significant between-study heterogeneity was observed in the pooled analyses of total eligible studies. To identify the sources of heterogeneity, we performed subgroup analyses and metaregression. Subgroup analyses indicated that the heterogeneity was still evident in most of the subgroups for both the XPD Lys751Gln and Asp312Asn polymorphisms. Metaregression analysis revealed that none of the investigated variables was the source of heterogeneity. When excluding the study by Jiang et al.\(^{20}\) for the XPD Lys751Gln polymorphism and the study by Zeng et al.\(^{17}\) for the XPD Asp312Asn polymorphism, all \(P_h\) values in the overall populations and subgroup analyses were >0.10. Interestingly, the summary ORs for the XPD Lys751Gln and Asp312Asn polymorphisms in different comparison models in the overall population and subgroup analyses were not materially changed by excluding the outlier studies, suggesting that our results were robust and reliable.

Despite our efforts to perform a comprehensive analysis, limitations in this study should be acknowledged. First, in subgroup analysis by ethnicity, the included studies regarded only East Asians and mixed/other populations. Data concerning other ethnicities such as whites and Africans were not found. Therefore, additional studies are needed to evaluate the effect of this functional polymorphism on HCC risk in different ethnicities. Second, the controls in the eligible studies were not uniformly defined. Although the controls were mainly selected from healthy populations, some had benign diseases such as liver cirrhosis, HBsAg positivity, and so on. Therefore, non-differential misclassification bias was possible because these studies may have included the control populations who have different risks of developing HCC. Third, our results were based on unadjusted estimates. We did not perform analysis adjusted for other covariates such as sex, age, drinking and smoking status, HBV and HCV carrier status, environmental factors, and so on because of the unavailable original data in the eligible studies.

Despite the limitations, the results suggest that the XPD Lys751Gln polymorphism contributes to increased HCC susceptibility, especially in East Asians. Further, large and well-designed studies are required to validate this association.

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