Published in final edited form as:
Mutat Res. 2011 ; 728(1-2): 67–79. doi:10.1016/j.mrrev.2011.06.002.

Candidate Gene Studies in Gallbladder Cancer: A Systematic Review and Meta-Analysis

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Abstract

Gallbladder cancer (GBC) is the most frequent biliary tract malignancy. Wide variations in GBC incidence and familial and epidemiological data suggest involvement of a genetic component in its etiopathogenesis. A systematic review of genetic association studies in GBC was performed by applying a meta-analysis approach and systematically reviewing PubMed database using appropriate terms. Odds ratios (ORs) and 95% confidence intervals (CIs) were appropriately derived for each gene–disease association using fixed and random effect models. Meta-regression with population size and genotyping method was also performed. Study quality was assessed using a 10-point scoring system designed from published guidelines. Following a review of 44 published manuscripts and one unpublished report, 80 candidate gene variants and 173 polymorphisms were analysed among 1046 cases and 2310 controls. Majority of studies were of intermediate quality. Four polymorphisms with > 3 separate studies were included in the meta-analysis [*OGG1* (rs1052133), *TP53* (rs1042522), *CYP1A1* (rs1048943) and *GSTM1* Null polymorphism]. The meta-analysis demonstrated no significant associations of any of the above polymorphisms with GBC susceptibility. To conclude, existing candidate gene studies in GBC susceptibility have so far been insufficient to confirm any association. Future research should focus on a more comprehensive approach utilizing potential gene–gene, gene–environment interactions and high-risk haplotypes.

Keywords

Gallbladder cancer; Polymorphism; Review; Cancer; Meta-analysis

1. Introduction

Gallbladder cancer (GBC) is the most frequent biliary tract malignancy and the fifth most common malignant neoplasm of the digestive tract [1]. Despite recent advances in the diagnosis and management of gastrointestinal cancers, GBC remains a challenging tumour

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Conflict of interest
The authors declare that there are no conflicts of interest.

with a poor overall prognosis. Many of these tumors are not resectable at the time of presentation, and the 5-year survival rate is < 10% in most of the reported series [2]. GBC incidence varies greatly throughout the world, highest being in Native American and South American populations, and people from Poland and Northern India. Between 17000 and 18000 new cases of GBC are diagnosed in India each year, and the annual death rate is almost comparable [3]. The frequency increases with age and reaches to peak during the seventh and eighth decades of life [4]. GBC incidence also shows striking gender bias and affect females 2–3 times more frequently than males [4].

Several epidemiological studies have been conducted to estimate the effects of environmental factors on GBC risk to elucidate the large ethnic variations in risk [5-6]. Yet, no single environmental factor has persistently been linked with GBC risk. It has been observed that Indian migrants to different countries have a higher risk of acquiring GBC as compared to the respective native populations [7]. Thus, the wide geographical, ethnic and interindividual variations observed in the incidence of GBC suggest the involvement of a genetic component in its etiopathogenesis.

The discovery of common genetic polymorphisms in human DNA has led to the publication of a large number of association studies in GBC [8-30]. However, a number of contradictory findings have been reported, and in several cases it has proved difficult to reproduce initial results. Also due to the high false positive associations, the possibility of a true association is well dependent on the quality of the studies concerned [31]. The major flaw with the individual studies is small sample sizes and therefore insufficient statistical power to detect positive associations and incapability to demonstrate the absence of an association. This necessitates the use of meta-analysis to provide an integrative approach by pooling the results of independent analyzes, thereby increasing statistical power and resolution [32-35]. Thus, to get a better insight in the GBC pathogenesis, the present study was undertaken to explore the role of candidate gene polymorphisms in GBC susceptibility by applying a meta-analytical approach and systematically reviewing the available data.

Moreover, to draw a preliminary conclusion, we also carried out a systematic quality review of the published literature on genetic association studies in GBC. This was accomplished by strictly following the various guidelines and checklists that have been published on the conduct of genetic association studies in complex diseases [36-39]. Using the criteria as described by Clark et al [40], we analyzed each publication to highlight the quality issues in the conduct and interpretation of these studies and also to establish if the existing data supports any polymorphism to be conclusively associated with GBC.

2. Material and Methods

2.1 Literature Search

Studies were selected by searching PubMed for articles listed from 2000 until March 2011 using the strategy outlined below:

1. Restriction fragment length polymorphism/ OR genetic polymorphism/ OR single nucleotide polymorphism/ OR DNA polymorphism/ OR genetic variation
2. Genetic Association/
3. Single Nucleotide Polymorphism/ OR SNP
4. Gallbladder cancer/ OR carcinoma of gallbladder/ OR GBC
5. 1 OR 2 OR 3
6. 4 AND 5

7. Limit 6 to (Human and English language)

Titles and abstracts of articles found were screened, and full texts of articles of interest were evaluated. In addition, articles and bibliographies were hand searched for further suitable papers. We included only original articles that reported associations between GBC and a particular human gene polymorphisms or variability in a case-control or population-based study and were available in English. Case reports and series, reviews and other publications, such as editorials and animal studies were excluded from the analysis.

2.2 Data extraction

From each study, information like: author, year of publication, country of origin, cancer type, ethnicity, number of cases and controls, source of control groups (study design) and genotyping method, was extracted. Ethnic groups were categorized as Asian, European and Mixed. We also checked for HWE in control subjects among all publications.

2.3 Scoring Analysis

The analysis was based on a 10-point scoring sheet adapted from Clark et al [40] which is based on the criteria implemented from published recommendations on the evaluation of the quality of genetic association studies [36-39]. The categories in scoring system used for assessing study quality are summarized in Table 1. The criteria were scored as 1 if present or 0 if absent. Studies were scored as “good” if the score was 8 to 10, “fair” if the score was 5 to 7 and “poor” if the score was <4.

In some cases, same data was reported in more than one publication, in which case these secondary studies were not included in the meta-analysis. In some studies part of the data had already been reported elsewhere, therefore, only the novel data were included.

2.4 Meta analysis

In the present meta-analysis, we investigated the potential association between those polymorphisms for which there were atleast 3 studies in gallbladder cancer risk. A χ^2 test with one degree of freedom was performed in controls to examine deviation from Hardy-Weinberg equilibrium. Analysis between the heterozygote versus wild homozygote, the variant homozygote versus wild homozygote and also in dominant and recessive models was done to estimate cancer risk. For each statistically significant association observed, we estimated the false positive report probability (FPRP) as described by Wacholder et al [41]. Meta-regression analyses were performed by ethnicity, population size, minor allele frequency and genotyping method. A multivariate analysis was performed using the mixed model framework to handle heterogeneity in risks across studies and to adjust genotypic estimates for the effects of ethnicity. This approach can include study effects as either being fixed or random. Between-study heterogeneity was evaluated with a χ^2-based *Q*-test among the studies. Heterogeneity was considered significant when $p < 0.05$. In case of no significant heterogeneity, point estimates and 95% CI (confidence interval) were estimated using the fixed effect model (Mantel-Haenszel), otherwise, random effects model (DerSimonian Laird) was employed. The significance of overall odds ratio (OR) was determined by the *Z*-test. Publication bias was assessed by Begg’s funnel plot and Egger’s linear regression test with $p < 0.05$ being considered statistically significant [42]. In order to assess the stability of the results, sensitivity analyses were performed. Each study in turn was removed from the total, and the remaining were reanalyzed. Moreover, analysis was also performed, excluding studies whose allele frequencies in controls exhibited significant deviation from the Hardy-Weinberg equilibrium (HWE), given that the deviation may denote bias [43]. The type I error rate was set at 0.05. All the *p* values were two sided and all

the statistical tests were performed using the Comprehensive Meta-analysis software (Version 2.0, BIOSTAT, Englewood, NJ).

2.5 Hardy–Weinberg equilibrium correction

For evaluating impact of HWE-deviated studies on point estimates in genotype-based contrasts, ORs were corrected by using the HWE-predicted genotype counts in the control instead of the observed counts, as suggested by Trikalinos et al [44]; thereafter, they were included in the sensitivity analysis.

2.6 Assessment of cumulative evidence

To each nominally statistically significant association in meta-analysis, we applied the "Venice criteria" [45] to assess the credibility of the evidence. According to this criterion, each meta-analyzed association was graded based on the basis of amount of evidence, extent of replication and protection from bias. Grades of A, B, or C were assigned for each of the above-mentioned criteria. Associations with three A grades assigned were considered to have strong epidemiological credibility; associations with a grade of B but for which all other grades were B or greater were considered to have moderate credibility; any association that received a grade of C were considered to have weak credibility.

3. Results

The PubMed search returned 52 unique articles of which 44 articles met the established inclusion criteria and were included in the analysis. Overall, these 44 articles investigated 80 candidate genes and 173 different polymorphisms in association with GBC. Fourteen studies investigated the role of gene-gene interactions in GBC susceptibility. Of the 80 genes studied, twenty five were involved in inflammatory pathway, eleven in DNA repair, twenty three in metabolic pathway, fifteen in hormonal pathway, two in signalling pathway and angiogenesis and one each in apoptosis and DNA methylation. One study was on miRNA and GBC association. Thirteen studies reported combination genotypes of polymorphisms and their association with GBC. One unpublished report from our lab analyzed three polymorphisms of *ESR1* and one polymorphism of *PROGINS*.

Validated genotyping methods were used in all studies, which were TaqMan assay (in ten studies), restriction fragment length polymorphisms (RFLP, in thirty three studies) and automated fragment analysis in one study (Table 2). However, blinding of investigators involved in genotyping on the case/control status of the participants was reported in only fourteen studies. Moreover, performing a random double check to detect potential genotyping errors was mentioned in thirty eight studies. Four of the studies did not report information on genotyping [25, 30, 46-47]. Genotype frequencies were consistent with Hardy-Weinberg equilibrium (HWE) in all of the studies except one [47]. Some studies/ polymorphisms were not included in meta-analysis due to redundant information or missing data. All study populations were of mixed gender except in one study, Tsuchiya et al. [48]. All studies were retrospective case-control studies.

The quality of studies was generally mediocre; mean quality score was 7.11/10 (range 4 to 9). Sixteen studies were scored as "good", 27 were "mediocre," while only one was of "poor" quality. Primarily, studies were deficient in the areas of blinding, reproducibility, power calculations and independent replication. The studies were also divided into year of publication. The average score of genetic association studies in GBC, expressed as a percentage of total possible scores from 0 to 1, with standard error of the mean was plotted against the year of publication. There was an evidence of a trend towards improvement over time with R^2=0.69 (logarithmic trendline).

3.1 Meta-analysis of the association between the studied polymorphisms and GBC

The summary of meta-analysis for the candidate gene polymorphisms with GBC is shown in Table 3. Some of the studies were excluded from analysis due to limited available data (Table S1). The overall OR of the association between the variant allele of all the polymorphisms analyzed and GBC was 1.14 (95% CI = 1.06–1.24, P = 0.001). Among the candidate gene studies, *XPC* (rs2228000), *ERCC2* (rs1799793), *MSH2* (rs2303426), *OGG1* (rs2072668), *XRCC1* (rs25487), *CR1* (rs2274567), IL-*1RN*, *PTGS2* (rs689466), *IL1B* (rs16944), *EGF* (rs4444903), *KRAS* Gln25His, *NAT2*, *GSTT1*, *ESR1* (rs9340799) and *CYP7A1* (rs3808607) showed significant association with GBC (Table 3).

3.2.1 DNA repair pathway genes—The *OGG1* Ser326Cys polymorphism was analyzed in 3 studies [49-51] with 671 cases and 1176 controls and was found to be associated with increased risk of GBC although the association was not statistically significant (OR=1.76, 95% CI=0.63-4.91, CysCys vs. SerSer; Table 4). Significant heterogeneity was observed (Q=8.748, P=0.013, I^2=77.14%, CC vs. SS; Table 4). Three studies comprising 154 cases and 348 controls evaluated a possible association between TP53 rs1042522 polymorphism and risk of GBC [48, 52-53]. No association was observed (OR=1.28, 95% CI=0.68-2.42, P=0.441; Table 4) and there was no heterogeneity (Q=0.623, P=0.732, I^2=0%). In a study of 334 GBC cases, Jiao et al. [54] examined the association of polymorphisms in *XPC* gene, involved in nucleotide excision repair pathway (NER). Individuals with the T allele of *XPC* Ala499Val polymorphism possessed greater risk of GBC (OR=1.40). Srivastava et al. [49] found significant association with the variant alleles of *ERCC2*, *MSH2* and *OGG1* gene polymorphisms. No significant associations were found with other genes involved with DNA repair pathway.

3.2.2 Hormone pathway genes—Five polymorphisms of *ESR1* gene have been investigated relative to the risk of GBC. Two of the studies, including one unpublished report from our lab, looked at *ESR1* IVS1-397T>C and Ex4-122G>C polymorphisms using 480 cases and 957 controls [55]. They found no significant association of these polymorphisms with GBC risk (OR=1.46, 95% CI=1.00-2.12 and OR=0.88, 95% CI=0.60-1.29, respectively; Table 3). However, one unpublished report from our lab analyzing potential association of *ESR1* IVS1-351A>G polymorphism and GBC risk found 2.5 fold increased risk of GBC in multivariable analysis (95% CI=1.29-4.85; Table 3). None of the polymorphisms in other genes analyzed in hormone pathway were found to be significantly associated with GBC risk in published reports (Table 3).

3.2.3 Inflammatory pathway genes—Eight different studies examined a total of 73 polymorphisms in twenty five genes involved in inflammatory pathways. Due to limited information, we were able to incorporate only 18 polymorphisms in our analysis. The *IL1B* rs16944 polymorphism was investigated in two studies, of which only Vishnoi et al. [26] found a significant association. Srivastava et al. [17] found GA+AA carriers of *PTGS2* −1195G>A polymorphism were significantly associated with increased risk of GBC (OR=2.12). Other polymorphisms of inflammatory pathway which were found to be associated with GBC were *CR1* His1208Arg, *IL1RN* 86-bp VNTR and *EGF* +61A>G. Hsing et al. [20], in a population of 237 GBC patients found that the T allele (CT+TT genotype) of the *VEGF* rs3025039 polymorphism conferred 0.70 fold reduced risk of GBC (95%, CI= 0.50–0.97). This remained true after adjustment for smoking, drinking, BMI, gallstones, as well as correction for multiple comparisons. Also *IL10* TC genotype of −7334T>C polymorphism was found to be associated with a reduced risk of gallbladder cancer (OR=0.69) [20].

3.2.4 Metabolic pathway genes—Only two polymorphisms reported by Pandey et al. [24] (*NAT2*) and Srivastava et al. [56] (*CYP7A1* −204 A>C) reported significant association with GBC in multivariable analysis (OR= 3.40 and 2.05, respectively). The association of *CYP1A1* rs1048943 polymorphism and GBC risk was evaluated in 4 studies that comprised 391 cases and 1085 controls [18, 48, 52-53]. No association between the risk for GBC and *CYP1A1* rs1048943 polymorphism was observed (OR=0.72, 95% CI=0.42-1.22, *P*=0.221; Table 4). There was no heterogeneity observed among the studies (*Q*=4.311, *P*=0.116, I^2=53.6%). Four studies examined the relationship between GSTM1 polymorphisms and risk of GBC in 260 cases and 549 controls [23, 48, 52-53]. Null allele of *GSTM1* did not cause any increase in the risk for GBC (OR=1.08, 95% CI=0.73-1.60,; Table 4). No heterogeneity was found (*Q*=0.369, *P*=0.831, I^2=0%). Four studies evaluated the relationship between *CYP1A1* rs4646903 polymorphism and GBC risk [21, 48, 52-53]. But due to limited data availability, meta-analysis could not be performed. None of the remaining polymorphisms investigated were found to be significantly associated with GBC.

3.2.5 Other genes—The angiogenesis pathway gene *KRAS* Gln25His polymorphism was significantly associated with GBC risk (OR= 2.81; *P*=0.003) in a multivariable study from Eastern India [57]. The polymorphisms in other genes such as angiogenesis pathway (*ACE*), signaling (*TLR*), miRNA, apoptosis (*CASP8*) or methylation (*DNMT3B*) pathway were significantly associated with GBC (additive model) [58-62].

3.3 Gene-Gene Interactions

There were fourteen studies that investigated gene-gene interactions and their association with GBC. The combinations studied were mostly between genes of metabolic, DNA repair, inflammatory and hormone pathways. Srivastava et al. [63] performed Classification and Regression Tree Analysis (CART) and Grade of Membership (GoM) analysis on 16 polymorphisms in 8 genes involved in DNA repair, apoptotic and inflammatory pathways to identify combinations of alleles contributing to GBC risk. The CART analysis revealed *OGG1* Ser326Cys, and *OGG1* IVS4-15C>G polymorphisms as the best polymorphic signature for discriminating between cases and controls. GoM analysis categorized the data into low risk (controls) and high risk groups (patients) on the basis of risk alleles. Baez et al. [64] investigated the combination of “at-risk” genotypes of the *APOB* rs693 and *CETP* rs708272 polymorphisms with GBC risk. They found that compared with all remaining combinations, patients with [C/C (apoB) +T/T (CETP)] genotypes had an elevated risk for GBC (age adjusted OR= 4.75; 95% CI= 1.16 -19.4). Xu et al. [65] found that the carriers of the *ABCG8* (rs4148217 and rs11887534) C-C haplotype had >4 fold greater risk of GBC (95% CI=1.71-10.1). Srivastava et al. [56] found that the C-T and C-C haplotype frequencies of the *CYP7A1* gene were significantly higher in GBC group compared to healthy controls and imposed higher risk for the disease (ORs= 1.84 and 3.10, respectively). They hypothesized that the altered gallbladder bile composition to lithogenic profile due to *CYP7A1* −204 A>C and −469 T>C promoter polymorphisms may impair fatty acid metabolism and decrease hepatic canalicular bile acid transport, causing accumulation of free radicals and other toxic products by lipid peroxidation, leading to GBC susceptibility. Srivastava et al. [17] found that compared with the most common haplotype of the *PTGS2* gene, $G_{-1195}G_{-765}T_{+8473}$, the $A_{-1195}G_{-765}T_{+8473}$ haplotype was associated with a significantly increased risk of GBC (OR= 2.20; 95%CI= 1.1–4.4). Srivastava et al. [49] performed a correlation and regression tree analysis (CART) to identify risk sets of polymorphisms in *OGG1*, *MSH2* and *ERCC2* genes. Compared with the low-risk group combining terminal nodes with a case ratio <45%, the medium-risk (case ratio between 45%and 55%) and high-risk groups (case ratio >55%) were both associated with a significantly increased GBC risk (ORs = 7.6 and 1.7, respectively; P_{trend} <0.001). Hsing et al. [20] found that none of the 5 inferred haplotypes involving three separate SNPs in the

IL8RB gene (Ex3+811C>T, Ex3+1235T>C, Ex3−1010G>A) were associated with GBC risk. None of the haplotypes for *LDLR* IVS9−30C>T-EX10+55G>A-EX15−80G>A-IVS17−42A>G-EX18+88G>A, *APOB* IVS6+360C>T-EX4+56C>T and *APOB* EX26-3573T>C-IVS23-79T>C were found to be significantly associated with GBC risk in a study by Andreotti et al. [66]. Also, no associations for any of six major haplotypes for *ESR1* gene were found relative to the most common haplotype with GBC risk in a study by Park et al [55].

Meta-analysis was also performed for polymorphisms with more than 2 studies which involved the *OGG1* Ser326Cys, *TP53* Pro72Arg, *GSTM1* null and *CYP1A1* Ile462val polymorphisms (Table 4 and Fig. 2). Meta-analysis of *TP53* Pro72Arg polymorphism showed significant association at the allelic level (OR= 1.38). Based on Venice criteria, for "amount of evidence", this association was graded as C (n<100 variant Pro allele in cases and controls combined), grade C for "replication consistency" due to high between-study inconsistency (I^2 >50%) and grade C for "protection from bias". Thus, *TP53* Pro72Arg polymorphism would be graded as having weak credibility according to the Venice criteria. Rest of the studied polymorphisms revealed no association with GBC at genotypic level and allelic level or under recessive model and dominant models (Table 4; Fig. 2).

3.4 Meta-regression

We performed the regression analysis for three predefined potential sources of heterogeneity, the ethnic background, study size, and also genotyping methods adding single covariates at a time in a series of univariate models. Dummy variables were created for sample size (0 for < 200 cases) vs. (1 for >200 cases) and for genotyping methods as TaqMan (1), otherwise (0); and PCR-RFLP (1), otherwise (0). Univariate regression analyses showed study size (P_{Het}< 0.01) as the source of heterogeneity. TaqMan genotyping method emerged as significant source of heterogeneity but not PCR-RFLP (P_{Het} = < 0.001 and 0.569, respectively, for Taqman and PCR-RFLP methods).

3.5 Sensitivity Analysis

A single study involved in the meta-analysis was removed each time to reflect the influence of the individual data set to the pooled ORs, and the corresponding pooled ORs were not significantly altered (Fixed effect OR range= 1.12-1.14; Random effect OR range= 1.13-1.15).

3.6 Heterogeneity and Publication Bias

Begger's funnel plot and Egger's test were performed to assess the publication bias. Review of funnel plot could not completely rule out the potential for publication bias at allelic level but Egger's test result did not reveal publication bias (Y axle intercept = 0.31, (95%CI) = −0.39-1.01; t = 0.878, p = 0.382 for allelic model, Fig. 3). Also, Begg and Mazumdar rank correlation test also indicated absence of publication bias ($P_{2tailed}$=0.162) However, the fail-safe number was large enough to provide credence to our findings ($N_{fs0.05}$=417). Duval and Tweedie's trim and fill method showed that, if the publication bias was the only source of the funnel plot asymmetry, it needed two more studies to be symmetrical. The value of Log OR did not change too much after the adjustment (Fig. 4). A cumulative meta-analysis was also done by sorting the studies in the sequence of largest to smallest, and analysis performed with the addition of each study. The point estimate of the study did not deviate with the addition of smaller studies, ruling out the possibility of publication bias.

4. Discussion

Although there have been several descriptive reviews on this topic, to the best of authors' knowledge, this is the first systematic review analyzing the effect of genetic determinants in GBC. In summary, we reviewed the available literature on genetic studies of GBC and conducted four independent meta-analyses for association between GBC and *OGG1* rs1052133, *TP53* rs1042522, *GSTM1* and *CYP1A1* rs1048943 polymorphisms. There was minimal evidence for a probable overall association between the studied polymorphisms and increased odds of GBC. However, due to small number of studies with an overall mediocre quality and lack of confirmatory studies, it is very difficult to draw any definitive conclusions.

There are two different classes of genes, mutations in which may give rise to cancer; high penetrance genes and low penetrance genes. Most of the cancer susceptibility alleles identified so far are rare and highly penetrant (for example, *APC, BRCA1, BRCA2, MSH2, LMH1, PTEN, CDNK2A*). Although these genes are directly involved in pathogenesis of various cancers, but their effect on all cancers is relatively small. The low penetrance genes show marked locus and allelic heterogeneity with multiple alleles of individual gene influencing cancer.

The pathophysiology of gallbladder carcinogenesis is still obscure and a combination of various factors including gallstones, infection, environmental carcinogens, diet and genetic variations might be involved (Table 5). Various evidences suggest that the causal pathway for GBC pathogenesis involves chronic inflammation of the biliary epithelium, which may be either due to mechanical irritation by gallstones or by bacterial infection [67-68]. A study from Utah Cancer Registry (UCR) estimated that 26% of all gallbladder cancers are familial [69].The Swedish Family-Cancer Database from the Swedish Cancer Registry also reported high risk for familial gallbladder cancer [70]. Genetic susceptibility to developing GBC requires an association between different polymorphic genes and a patient's ability to respond to an environmental insult. Although a number of studies have reported positive associations between genetic polymorphisms and GBC, most of them are inconsistent and have not been replicated. Moreover, gallbladder cancer being multigenic and multifactorial, the prior probability of a particular genetic variation increasing the risk of gallbladder cancer is miniscule even with very significant p-values. Thus, in the present meta-analysis, we incorporated data from 1046 cases and 2310 controls retrieved from forty five studies to evaluate the association of various SNPs in GBC pathogenesis. Overall, the evidence in this review supports a modest involvement of inflammatory and DNA repair pathway genes in GBC susceptibility.

To identify heterogeneity among studies due to genotyping methods used, we did metaregression analysis which revealed significant difference between TaqMan genotyping and PCR-RFLP based genotyping, indicating potential misclassification It should be noted that although TaqMan is a better genotyping method than PCR-RFLP, it is not immune to errors [71]. Meta regression also showed study size to be a potential source of between-study heterogeneity.

One of the important issues in every meta-analysis is publication bias. Since meta-analysis summarizes quantitative evidence from multiple studies, the publication bias effect of the literature included in the analysis can bias the meta-analytic results, potentially generating overstated conclusions. In the present study, the funnel plot for overall results was symmetrical, suggestive of a small probability of publication bias. The Egger's test and Begg and Mazumdar rank correlation test also did not indicate statistically significant potential for publication bias. However, we cannot completely rule out the possibility of

publication bias as the significance of these tests and the typical funnel plot have been questioned [72]. We also performed sensitivity analyses by using HWE-adjusted ORs and corresponding variances. The results did not modify the risks of development of GBC.

One other factor that we examined was the timing of publication. We were interested to know whether the average scores observed in each year improved over time or not. We did observe an improvement in the quality score of published studies with respect to time.

Although we have only analyzed the effect of polymorphisms on GBC risk, the polymorphisms may interact with environmental exposures which might act as modifiers of gallbladder carcinogenesis. However, interactions between environmental factors and genetic factors have not yet been evaluated completely. A prospective study by Yagyu et al [73] evaluated the association of cigarette smoking and alcohol consumption with the risk of GBC death. The study associated smoking with an elevated risk of death from GBC. Drinking posed an elevated risk only among males. Another study by Shukla et al [74] associated tobacco chewing with an increased risk of GBC. A study by Pandey et al [21] looked at a possible association between *CYP1A1* rs4646903 polymorphism and tobacco status. In the case-only analysis, they found an elevated GBC risk with TC genotype (OR 4.1, 95% CI=1.3–11.9). Srivastava et al analyzed the interaction of *CCR5* Δ32 and *OGG1* and *XRCC1* polymorphisms with tobacco usage in 2 separate studies [10, 75] but the authors did not find any associated significantly increased risk with GBC.

About 40%–100% cases of GBC have been found to be associated with gallstones [76-77]. Many studies have evaluated a potential interaction between polymorphisms and gallstones on GBC risk. Srivastava et al [75] looked for a relationship between polymorphisms of DNA repair pathway genes and modulation of GBC risk in presence of gallstones. The frequency distribution of *OGG1* Cys/Cys genotype in GBC patients with gallstone was significantly higher and conferred high risk for GBC (OR=5.50). This result is consistent with an earlier study, in which Jiao et al [51] showed a near-significant increase in risk for gallbladder cancer for gallstone presence with the *OGG1* Ser/Cys and Cys/Cys genotypes (OR=2.2 and OR=6.1, respectively). Patients with the Cys allele at codon 326 have a lower DNA-repair activity, and this decreased activity might be expected to increase GBC risk, especially among those who are exposed to high levels of reactive oxygen species (ROS) generated by gallstones. On the other hand, significant negative association was also observed for *XRCC1* Arg399Gln Gln/Gln genotype in GBC patients without gallstones compared with controls (OR = 0.27). Another study by the same authors observed difference in frequency of *CCR5* Δ32 allele in GBC patients without gallstones when compared with healthy controls conferring high GBC risk (OR = 3.21) [10]. Hou et al [78] reported, for *CYP17* rs743572 polymorphism, that relative to individuals with the A2/A2 genotype, those with the A1 allele and having a history of diabetes along with biliary stones had a 3-fold risk of gallbladder cancer.

Obesity and use of oral contraceptives are important risk factors for gallbladder cancer exerting their effect via increased estrogen levels [79]. Park et al [18] found a statistically significant interaction between BMI and the *CYP1A1* rs2606345 SNP on GBC risk, with non-obese (BMI<23 kg/m^2) carriers of the T allele having a 3.3-fold risk (95% CI=1.8–6.1). The possible reason for this association was not apparent since higher levels of bioavailable estradiol and adipokines which are linked to GBC have been observed in obese subjects [80]. The authors also found female carriers of the *SHBG* rs6259 variant genotype to be at 3.2-fold increased risk of gallbladder cancer (95% CI=1.1–9.1).

There are various limitations in our study the first being the vulnerability to several types of bias, primarily the publication bias. The proportion of published literature with positive

result surpasses the one with negative results resulting in an inherent bias toward the publication with positive results. Another potential limitation is analysis reporting bias caused by researchers who report only a portion of their analyses. There was no access to the unpublished data of many association studies in GBC. Inclusion of data from all the available studies could have improved our power, but attempts for unpublished allele frequency data by directly contacting the authors unfortunately remained futile. All this suggests that in spite of following a systematic methodology, the included samples may not be the true representative of present genetic association studies of GBC. Since meta-analysis is a retrospective research, it cannot avoid the influence of methodological shortcomings.

Also, the present meta-analysis included only case–control studies in GBC which are prone to confounding and selection bias particularly due to the complex aetiology of the disease. Moreover, due to small numbers of studies for *OGG1* rs1052133, *TP53* rs1042522, *GSTM1* and *CYP1A1* rs1048943 polymorphisms, we were unable to construct funnel plots and perform Egger's test distorting the results due to potential publication bias. We also could not perform the ethnic-specific meta-analysis to detect associations in ethnic groups due to limited data. Also, considering the close association of GBC and gallstone, it would have been interesting to examine the candidate gene polymorphisms with gallstone status which could have provided a clue for GBC susceptible individuals at an earlier stage. However, such analysis was not feasible due to limited availability of data on gallstone status.

Our results need to be interpreted with caution as they are based on unadjusted estimates. A more precise analysis stratified by age, sex, and gallstone status could not be performed due to limitation of data which also restricted our ability for detecting possibility sources of heterogeneity.

5. Conclusion

To conclude, although some genes show promise, the existing candidate gene studies in GBC susceptibility have so far been insufficient to confirm any association. The small number of studies decrease the potential generalization of results and also the statistical power [81-82]. Thus, only preliminary conclusions can be drawn at this stage. Owing to its complex aetiology, it is extremely implausible that any single SNP or risk factor contributes significantly to the development of GBC in a large fraction of patients. Therefore, future research should focus on other low penetrance gene polymorphisms with more comprehensive approaches utilizing complex hypotheses for identification of potential gene–gene, gene–environment interactions and high-risk haplotypes. DNA copy number variations (CNVs) are also an important component of genetic variation covering at least 10% of the human genome. It is assumed that CNVs along with SNPs can explain a large portion of the genetic basis of cancer. Whole exome sequencing (WES) and expression microarray might also help to identify new mutations that may contribute in a significant way to gallbladder cancer pathogenesis. Moreover, genome-wide association (GWA) studies using multistage design might be more fruitful to investigate the role of genetic components in complex diseases such as gallbladder cancer. Understanding the intricate mechanisms of genetic pathways involved in GBC etiopathogenesis would be helpful to better identify subjects at high risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported [in part] by the Intramural Research Program of the National Human Genome Research Institute, National Institutes of Health (USA), Indian council of Medical Research (ICMR), DST and CSIR Government of India.

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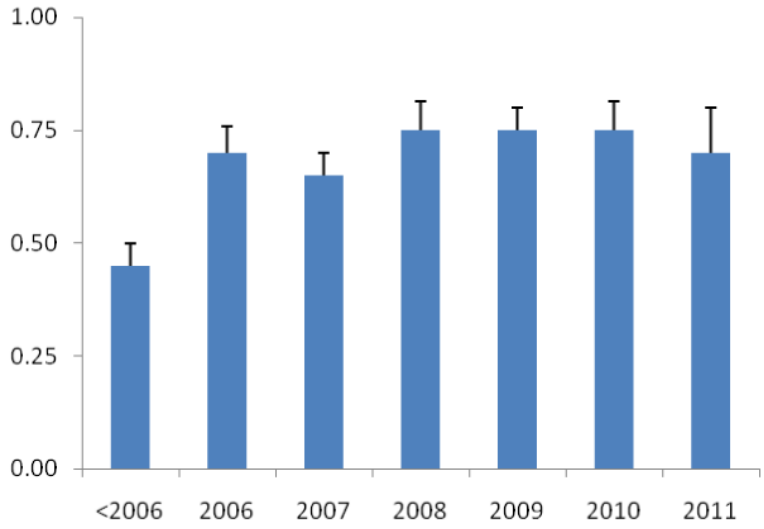

Fig. 1.

The average score of published genetic association studies in GBC, expressed as a percentage of total possible scores from 0 to 1, with standard error of the mean. Studies were divided into year of publication. There was an evidence of a trend towards improvement over time (R^2=0.69; logarithmic trendline).

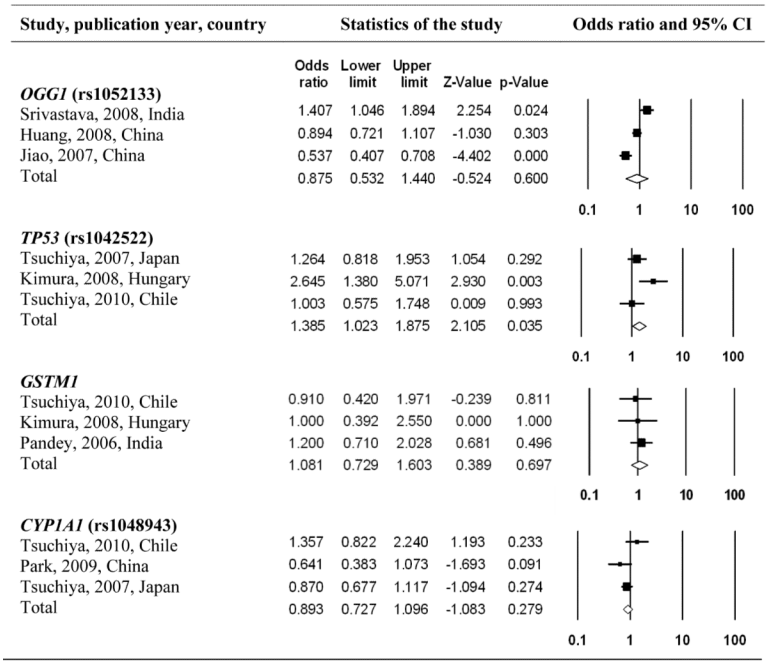

Fig. 2.

ORs and 95% CIs of individual studies for the association between the *OGG1* (rs1052133) polymorphism Cys allele, *TP53* (rs1042522) polymorphism Arg allele, *GSTM1* null polymorphism and *CYP1A1* (rs1048943) Val allele and GBC. The size of the box is proportional to the weight of the study. CI, confidence interval

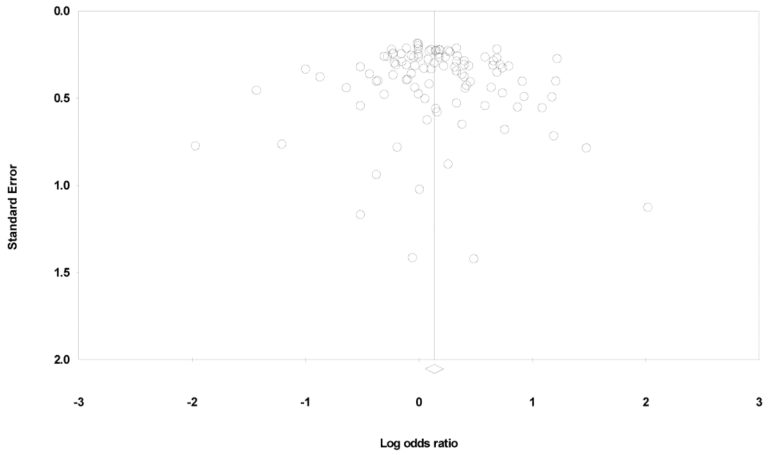

Fig 3.

Funnel plot analysis to detect publication bias for the analyzed polymorphisms in GBC. Each dot represents an individual study for the indicated association. Log [OR], natural logarithm of odds ratio

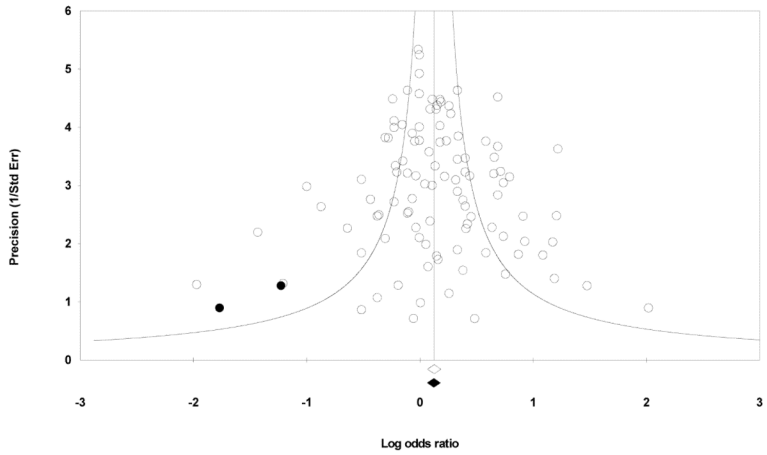

Fig 4.

Funnel plot of Precision by Log odds ratio. The filled circles are missed studies due to publication bias. The bottom diamonds show summary effect estimates before (open) and after (filled) publication bias adjustment.

Table 1

Scoring system for study quality assessment

Components	Quality Criterion	Score
Control group	≥case group, ethnicity matched to cases	Yes = 1 No = 0
Hardy–Weinberg equilibrium	Whether control groups were in HWE	Yes = 1 No = 0
Case group	Inclusion and exclusion criteria defined, adequate definition of GBC included	Yes = 1 No = 0
Primer	Primer sequence or a reference provided	Yes = 1 No = 0
Reproducibility	Described genotyping method to allow replication, validated the accuracy of genotyping	Yes = 1 No = 0
Blinding	Performed the genotyping whilst blind to case/control status	Yes = 1 No = 0
Power calculation	Performed a power calculation	Yes = 1 No = 0
Statistics	Presented major findings with well described tests of significance	Yes = 1 No = 0
Corrected statistics	Correction for the false-positive (type I) error	Yes = 1 No = 0
Independent replication	Performed a second, confirmatory study	Yes = 1 No = 0

Table 2

Characteristics of studies examining genetic risk factors for GBC susceptibility

S. No.	Article (author, year)	Type of study	Gene Polymorphism(s) studied	# of controls	# of cases	Ethnicity	Quality Score	Genotyping method
1	Pramanik, 2011	Retrospective case control	*KRAS* Gln25His	90	60	Asian	8	Sequencing
2	Xu, 2011	Retrospective case control	*ABCG8* (rs4148217, rs11887534), *CETP* (rs708272, rs1800775) and *LRPAP1* rs11267919	422	253	Asian	6	TaqMan
3	Isomura, 2010	Retrospective case control	*CYP2C19* (rs4244285, rs4986893)	566	15	Asian	6	PCR-RFLP
4	Jiao, 2010	Retrospective case control	*XPC* (rs2228000, rs2228001)	329	334	Asian	8	PCR-RFLP
5	Srivastava, 2010	Retrospective case control	*ERCC2* (rs1799793, rs13181); *MSH2* (rs2303426, rs2303425); *OGG1* (rs1052133, rs2072668)	230	230	Asian	8	PCR-RFLP
6	Srivastava, 2010	Retrospective case control	*CASP8* (rs3834129, rs1045485, rs3769818)	230	230	Asian	7	PCR-RFLP
7	Srivastava, 2010	Retrospective case control	*DNMT3B* rs1569686	219	212	Asian	8	PCR-RFLP
8	Tsuchiya, 2010	Retrospective case control	*CYP1A1* (rs4646903, rs1048943); *TP53* rs1042522; *GSTM1* homozygous null deletion polymorphism	70	57	Mixed	6	TaqMan
9	Srivastava, 2010	Retrospective case control	pre-miRNA rs2910164, rs11614913 and rs3746444	230	230	Asian	9	PCR-RFLP
10	Meyer, 2010	Retrospective case control	*AR* CAG repeat length [(CAG)n]	704	215	Asian	5	Automated fragment analysis
11	Srivastava, 2010	Retrospective case control	*TLR2* (Δ22); *TLR4* rs4986791	257	233	Asian	9	PCR-RFLP
12	Srivastava, 2010	Retrospective case control	*ACE* I/D rs4646994	260	233	Asian	9	PCR-RFLP
13	Park, 2010	Retrospective case control	*ESR1* (rs2234693, rs3841686, rs2228480, rs1801132), *ESR2* (rs1256049, rs4986938)	737	237	Asian	7	TaqMan
14	Báez, 2010	Retrospective case control	*ApoB* rs693, *ApoE* (rs7412, rs429358), *CETP* rs708272, *CYP2C9* (rs1057910, rs1799853), *CYP3A4* rs12721627, *CYP2E1* (rs2031920, rs6413432)	70	57	Mixed	6	TaqMan
15	Srivastava, 2010	Retrospective case control	*CYP7A1* (rs3808607, rs3824260)	200	185	Asian	9	PCR-RFLP

S. No.	Article (author, year)	Type of study	Gene Polymorphism(s) studied	# of controls	# of cases	Ethnicity	Quality Score	Genotyping method
16	Srivastava, 2010	Retrospective case control	*CR1* (rs2274567, rs12144461)	200	185	Asian	9	PCR-RFLP
17	Srivastava, 2009	Retrospective case control	*PTGS2* (rs689466, rs20417, rs5275)	184	167	Asian	7	PCR-RFLP
18	Srivastava, 2009	Retrospective case control	*OGG1* rs1052133, *XRCC1* (rs25487, rs1799782)	204	173	Asian	8	PCR-RFLP
19	Park, 2009	Retrospective case control	*COMT* (rs4633, rs4818), *CYP1A1* (rs2606345, rs1048943), *CYP1B1*(rs10012, rs1056836), *CYP19A1* (rs1065778, rs700518, rs2304463, rs700519, rs1065779, rs4646), *HSD3B2* (rs1819698, rs1361530), *HSD17B3* (rs2066479), *HSD17B1* (rs2830), *SHBG* (rs6259), *SRD5A2* (rs523349)	737	237	Asian	8	TaqMan
20	Srivastava, 2008	Retrospective case control	*ABCG8* rs11887534	221	171	Asian	9	PCR-RFLP
21	Kimura, 2008	Retrospective case control	*CYP1A1* (rs4646903, rs1048943), *GSTM1* del polymorphism, *TP53* rs1042522	100	43	European	6	PCR-RFLP
22	Vishnoi, 2008	Retrospective case control	*IL1RN* 86-bp VNTR and *IL1B* rs16944	166	124	Asian	7	PCR-RFLP
23	Zhang, 2008	Retrospective case control	*MGMT* rs12917, *RAD23B* rs1805335, rs1805329, *CCNH* rs2266690, *XRCC3* rs861539	737	236	Asian	7	TaqMan
24	Hsing, 2008	Retrospective case control	*IL1A* (rs17561, rs2856841, rs2071374, rs1800587), *IL1B* (rs16944, rs1143634, rs1143627), *IL4* (rs2243250, rs2243248, rs2070874, rs2243267, rs2243268, rs2243290), *IL5* (rs2069812, rs2069807, rs2069818), *IL6* (rs1800795, rs1800796, rs1800797), *IL8* (rs4073, rs2227307, rs2227306), *IL10* (rs3024496, rs3024491, rs1800871, rs1800872, rs1800896), *IL13* (rs1800925, rs1295686, rs20541), *IL16* (rs859, rs11325), *IL8RA* (rs2234671), *IL8RB* (rs1126579, rs1126580, rs2230054), *PPARD* (rs2016520), *PPARG* (rs2938392, rs3856806), RNASEL (rs486907, rs11072), SOD2 rs4880, *MPO* (rs2333227, rs2243828), *NOS2* (rs944722, rs2297518), *NOS3* (rs1799983, rs1007311), *TGFB1* (rs1800469, rs1982073), *TNF* (rs1799964, rs1800630, rs1799724, rs1800750, rs1800629, rs361525, rs1800610), *VCAM1* (rs1041163, rs3176878, rs3176879), *VEGF* rs3025039	737	237	Asian	8	TaqMan
25	Vishnoi, 2008	Retrospective case control	*EGF* rs4444903, *TGFB1* rs1800469	190	126	Asian	6	PCR-RFLP

S. No.	Article (author, year)	Type of study	Gene Polymorphism(s) studied	# of controls	# of cases	Ethnicity	Quality Score	Genotyping method
26	Srivastava, 2008	Retrospective case control	*CCR5* rs333	210	144	Asian	6	PCR-RFLP
27	Chang, 2008	Retrospective case control	*PPARG* rs3856806, *PPARD* rs2016520, *RXRA* (rs1536475, rs1805343), *RXRB* (rs2744537, rs2076310) and *INS* rs689	737	237	Asian	8	TaqMan
28	Andreotti, 2008	Retrospective case control	*ApoB* (rs676210, rs673548, rs520354, rs1367117), *ApoE* rs440446, *LDLR* (rs1003723, rs5930, rs5927, rs6413504, rs14158), *LPL* rs263, *ALOX5* rs2029253	730	235	Asian	7	TaqMan
29	Pandey, 2008	Retrospective case control	*CYP1A1* rs4646903	171	142	Asian	7	PCR-RFLP
30	Srivastava, 2008	Retrospective case control	*CYP7A1* rs3808607	200	141	Asian	7	PCR-RFLP
31	Huang, 2008	Retrospective case control	*XRCC1* (rs1799782, rs25489, rs25487), *APEX1* rs3136820, *OGG1* rs1052133	737	237	Asian	8	TaqMan
32	Srivastava, 2008	Retrospective case control	*MTHFR* rs1801133	210	146	Asian	7	PCR-RFLP
33	Srivastava, 2008	Retrospective case control	*CCKAR* rs1800857	190	139	Asian	7	PCR-RFLP
34	Vishnoi, 2007	Retrospective case control	*TNFA* rs1800629, *IL6* rs1800795	200	124	Asian	7	PCR-RFLP
35	Pandey, 2007	Retrospective case control	*ApoB* rs693 and rs17240441	232	123	Asian	7	PCR-RFLP
36	Tsuchiya, 2007	Retrospective case control	*CYP1A1* (rs4646903, rs1048943), *GSTM1* homozygous null deletion polymorphism, *TP53* rs1042522	178	54	Asian	7	PCR-RFLP
37	Jiao, 2007	Retrospective case control	*OGG1* rs1052133	209	204	Asian	7	PCR-RFLP
38	Pandey, 2007	Retrospective case control	*NAT2* (rs1799929, rs1799930, rs1799931)	147	124	Asian	6	PCR-RFLP
39	Pandey, 2006	Retrospective case control	*LRPAP1* rs11267919	208	129	Asian	6	PCR-RFLP
40	Pandey, 2006	Retrospective case control	*GSTM1* and *GSTT1* homozygous null deletion polymorphism, *GSTP1* rs1695, *GSTM3* rs1799735	201	106	Asian	6	PCR-RFLP
41	Hou, 2006	Retrospective case control	*CYP17* rs743572	818	254	Asian	7	PCR-RFLP
42	Sakoda, 2006	Retrospective case control	*PTGS2* (rs20420, rs5277, rs20432, rs4648276, rs5273, rs4648291, rs5275, rs689470)	737	237	Asian	8	TaqMan

S. No.	Article (author, year)	Type of study	Gene Polymorphism(s) studied	# of controls	# of cases	Ethnicity	Quality Score	Genotyping method
43	Singh, 2004	Retrospective case control	*ApoB* rs693	137	153	Asian	4	PCR-RFLP
44	Tsuchiya, 2002	Retrospective case control	*CYP1A1* (rs4646903, rs1048943)	104	52	Asian	5	PCR-RFLP
45	Unpublished report	Retrospective case control	*ESR1* (rs2234693, rs9340799, rs1801132), *PROGINS* 306 bp insertion (rs10428388)	220	243	Asian	-	PCR-RFLP

Table 3

Association studies of candidate gene polymorphisms and GBC risk

Article (author, year)	Gene	Variant	Risk allele	Gene Polymorphism(s) studied	Total cases	Total controls	OR (95% CI)[a]	No of studies	Reference
DNA repair pathway genes									
Jiao, 2010	*XPC*	rs2228000	T	Ala499Val	334	329	1.93 (1.04-3.55)	1	[54]
Jiao, 2010	*XPC*	rs2228001	C	Lys939Gln	334	329	1.41 (0.85-2.36)	1	[54]
Srivastava, 2010	*ERCC2*	rs1799793	A	Asp312Asn	230	230	2.10 (1.10-4.01)	1	[49]
Srivastava, 2010	*ERCC2*	rs13181	T	Lys751Gln	230	230	1.50 (0.85-2.64)	1	[49]
Srivastava, 2010	*MSH2*	rs2303426	C	IVS1+9G>C	230	230	1.80 (1.07-3.04)	1	[49]
Srivastava, 2010	*MSH2*	rs2303425	C	−118T>C	230	230	1.40 (0.49-3.96)	1	[49]
Srivastava, 2010	*OGG1*	rs2072668	G	748−15C>G	230	230	2.00 (1.17-3.48)	1	[49]
Srivastava, 2010, Huang, 2008, Jiao 2007	*OGG1*	rs1052133	G	Ser326Cys	671	1176	1.76 (0.63-4.91)	3	[49-51]
Tsuchiya, 2010, Kimura, 2008, Tsuchiya, 2007	*TP53*	rs1042522	G	Pro72Arg	154	348	1.28 (0.68-2.42)	3	[48, 52-53]
Srivastava, 2009, Huang, 2008	*XRCC1*	rs1799782	T	Arg194Trp	410	941	0.94 (0.58-1.54)	2	[14, 50]
Srivastava, 2009, Huang, 2008	*XRCC1*	rs25487	A	Arg399Gln	410	941	0.59 (0.38-0.93)	2	[14, 50]
Huang, 2008	*APEX1*	rs3136820	T	Asp148Glu	237	737	0.79 (0.51-1.22)	1	[50]
Zhang, 2008	*RAD23B*	rs1805335	G	IVS5−15A>G	236	737	1.40 (0.71-2.76)	1	[83]

Article (author, year)	Gene	Variant	Risk allele	Gene Polymorphism(s) studied	Total cases	Total controls	OR (95% CI)[a]	No of studies	Reference
Zhang, 2008	*RAD23B*	rs1805329	T	EX7+65C>T	236	737	0.97 (0.41-2.30)	1	[83]
Hormone pathway genes									
Srivastava, 2008	*CCKAR*	rs1800857	C	IVS1−5T>C	139	190	1.05 (0.55-2.01)	1	[12]
Park, 2010, unpublished report	*ESR1*	rs2234693	T	IVS1−397T>C	480	957	1.46 (1.00-2.12)	2	[55]
Park, 2010	*ESR1*	rs3841686	T	IVS5−34−>T	237	737	0.80 (0.49-1.29)	1	[55]
Park, 2010	*ESR1*	rs2228480	G	Ex8+229G>A	237	737	0.70 (0.32-1.54)	1	[55]
Park, 2010, unpublished report	*ESR1*	rs1801132	G	Ex4−122G>C	480	957	0.88 (0.60-1.29)	2	[55]
unpublished report	*ESR1*	rs9340799	G	IVS1−351A>G	243	220	2.50 (1.29-4.85)	1	-
Park, 2010	*ESR2*	rs1256049	A	Val328Val	237	737	1.00 (0.61-1.63)	1	[55]
Meyer, 2010	*AR*	-	-	(CAG)n	215	704	0.99 (0.68-1.43)	1	[84]
Park, 2009	*COMT*	rs4633	T	His62His	237	737	0.90 (0.49-1.66)	1	[18]
Park, 2009	*COMT*	rs4818	C	Leu136Leu	237	737	1.20 (0.74-1.95)	1	[18]
Park, 2009	*CYP1A1*	rs2606345	T	IVS1+606G>T	237	737	2.00 (1.29-3.09)	1	[18]
Park, 2009	*CYP1B1*	rs10012	C	Arg48Gly	237	737	0.90 (0.41-1.96)	1	[18]
Park, 2009	*CYP19A1*	rs1065778	G	IVS4−76A>G	237	737	1.00 (0.65-1.54)	1	[18]
Park, 2009	*CYP19A1*	rs700518	A	Val80Val	237	737	1.00 (0.67-1.49)	1	[18]
Park, 2009	*CYP19A1*	rs2304463	T	IVS7−106T>G	237	737	0.90 (0.59-1.37)	1	[18]
Park, 2009	*CYP19A1*	rs700519	T	Arg264Cys	237	737	1.10 (0.48-2.51)	1	[18]

Article (author, year)	Gene	Variant	Risk allele	Gene Polymorphism(s) studied	Total cases	Total controls	OR (95% CI)[a]	No of studies	Reference
Park, 2009	*CYP19A1*	rs1065779	T	IVS9−53G>T	237	737	1.00 (0.65-1.54)	1	[18]
Park, 2009	*CYP19A1*	rs4646	T	Ex11+410G>T	237	737	1.00 (0.59-1.68)	1	[18]
Park, 2009	*HSD3B2*	rs1819698	T	Ex4−133C>T	237	737	1.40 (0.79-2.47)	1	[18]
Park, 2009	*HSD3B2*	rs1361530	G	Ex4−88C>G	237	737	1.50 (0.82-2.76)	1	[18]
Park, 2009	*HSD17B3*	rs2066479	A	Gly289Arg	237	737	0.60 (0.32-1.13)	1	[18]
Park, 2009	*HSD17B1*	rs2830	A	Ex1−486G>A	237	737	1.00 (0.69-1.58)	1	[18]
Park, 2009	*SHBG*	rs6259	A	Ex8+6G>A	237	737	1.900 (0.80-4.51)	1	[18]
Park, 2009	*SRD5A2*	rs523349	G	Ex1−17G>C	237	737	1.00 (0.69-1.45)	1	[18]
Chang, 2008	*RXR*-α	rs1536475	A	IVS6+70A>G	237	737	0.65 (0.32-1.32)	1	[85]
Chang, 2008	*RXR*-α	rs1805343	A	IVS1−27A>G	237	737	0.80 (0.49-1.31)	1	[85]
Chang, 2008	*RXR*-β	rs2744537	T	G392T	237	737	0.82 (0.45-1.51)	1	[85]
Chang, 2008	*RXR*-β	rs2076310	C	C51T	237	737	1.30 (0.83-2.04)	1	[85]
Chang, 2008	*INS*	rs689	A	A-6T	237	737	0.96 (0.57-1.62)	1	[85]
Chang, 2008[b]	*PPARD*	rs2016520	A	Ex4+15C>T	237	737	1.09 (0.63-1.89)	1	[85]
Chang, 2008[b]	*PPARG*	rs3856806	T	His477His	237	737	1.25 (0.67-2.33)	1	[85]
Inflammatory pathway genes									
Srivastava, 2010	*CR1*	rs2274567	G	His1208Arg	185	200	1.94 (1.10-3.41)	1	[9]
Srivastava, 2010	*CR1*	rs12144461	T	Intron 27, HindIII	185	200	1.27 (0.75-2.14)	1	[9]

Article (author, year)	Gene	Variant	Risk allele	Gene Polymorphism(s) studied	Total cases	Total controls	OR (95% CI)[a]	No of studies	Reference
Vishnoi, 2008	*IL1RN*	-	-	86-bp VNTR	124	166	3.25 (1.23-8.58)	1	[26]
Srivastava, 2009	*PTGS2*	rs689466	A	−1195G>A	167	184	2.98 (1.00-8.89)	1	[17]
Srivastava, 2009	*PTGS2*	rs20417	C	−765G>C	167	184	1.06 (0.39-2.85)	1	[17]
Srivastava, 2009, Sakoda, 2006	*PTGS2*	rs5275	C	+8473T>C	404	921	1.28 (0.78-2.14)	2	[17, 86]
Hsing, 2008, Vishnoi, 2008	*IL1B*	rs16944	C	−1060T>C	361	903	3.36 (1.52-7.43)	2	[20, 26]
Hsing, 2008	*IL10*	rs1800871	T	−7334T>C	237	737	0.74 (0.44-1.24)	1	[20]
Hsing, 2008	*IL10*	rs1800872	A	−6653A>C	237	737	0.76 (0.45-1.27)	1	[20]
Vishnoi, 2008	*EGF*	rs4444903	A	+61A>G	126	190	2.22 (1.19-4.15)	1	[28]
Vishnoi, 2008, Hsing, 2008	*TGFβ1*	rs1800469	T	−509C>T	361	937	1.47 (0.72-3.01)	2	[20, 28]
Vishnoi, 2007, Hsing, 2008	*TNFα*	rs1800629	A	−308G>A	361	937	1.47 (0.41-5.28)	2	[20, 27]
Vishnoi, 2007, Hsing, 2008	*IL6*	rs1800795	C	−236C>G	361	937	0.60 (0.06-5.95)	2	[20, 27]
Metabolic pathway genes									
Srivastava, 2008	*MTHFR*	rs1801133	T	Ala222Val	146	210	1.08 (0.32-3.69)	1	[13]
Pandey, 2007	*APOB*	rs17240441	-	35_43del9	123	232	3.30 (0.81-13.51)	1	[25]
Pandey, 2007	*NAT2*	rs1799929, rs1799930, rs1799931	T, A, A	NAT2*5A, NAT2*6B, NAT2*7A	124	147	3.40 (1.98-5.84)	1	[24]
Pandey, 2006	*GSTT1*	-	-	null polymorphism	106	201	0.24 (0.1-0.59)	1	[23]

Article (author, year)	Gene	Variant	Risk allele	Gene Polymorphism(s) studied	Total cases	Total controls	OR (95% CI)[a]	No of studies	Reference
Pandey, 2006	*GSTP1*	rs1695	A	Ile105Val	106	201	2.10 (0.83-5.30)	1	[23]
Hou, 2006	*CYP17*	rs743572	A	Ex1+27T>C	254	818	1.40 (0.92-2.14)	1	[78]
Tsuchiya, 2010, Kimura, 2008, Pandey, 2006, Tsuchiya, 2007	*GSTM1*	-	-	null polymorphism	260	549	1.08 (0.73-1.60)	4	[23, 48, 52-53]
Tsuchiya, 2010, Kimura, 2008, Pandey, 2008, Tsuchiya, 2007	*CYP1A1*	rs4646903	C	CYP1A1*2A	296	519	1.28 (0.49-3.38)	4	[21, 48, 52-53]
Tsuchiya, 2010, Park, 2009, Kimura, 2008, Tsuchiya, 2007	*CYP1A1*	rs1048943	G	Ile462Val (*2C)	391	1085	0.72 (0.42-1.22)	4	[18, 48, 52-53]
Andreotti, 2008	*LDLR*	rs5930	A	EX10+55G>A	235	730	1.10 (0.70-1.73)	1	[66]
Andreotti, 2008	*LDLR*	rs6413504	G	IVS17−42A>G	235	730	0.81 (0.45-1.46)	1	[66]
Andreotti, 2008	*LDLR*	rs14158	A	EX18+88G>A	235	730	1.12 (0.72-1.74)	1	[66]
Andreotti, 2008	*LPL*	rs263	T	IVS5−540C>T	235	730	0.69 (0.31-1.53)	1	[66]
Andreotti, 2008	*ALOX5*	rs2029253	A	IVS3+100G>A	235	730	1.16 (0.74-1.83)	1	[66]
Pandey, 2007, Báez, 2010	*ApoB*	rs693	T	Thr2515Thr	180	302	0.37 (0.15-0.89)	2	[25, 64]
Xu, 2011, Srivastava, 2008	*ABCG8*	rs11887534	C	Asp19His	424	643	1.63 (0.10-26.53)	2	[15, 65]

Article (author, year)	Gene	Variant	Risk allele	Gene Polymorphism(s) studied	Total cases	Total controls	OR (95% CI)[a]	No of studies	Reference
Xu, 2011, Báez, 2010	*CETP*	rs708272	T	TaqIB	310	492	1.06 (0.55-2.04)	2	[64-65]
Xu, 2011	*CETP*	rs1800775	C	−629C>A	253	422	1.17 (0.75-1.83)	1	[65]
Xu, 2011, Pandey, 2006	*LRPAP1*	rs11267919	-	752-177_752-176ins37	382	630	1.08 (0.71-1.66)	2	[22, 65]
Srivastava, 2010	*CYP7A1*	rs3808607	A	−204 A>C	185	200	2.05 (1.12-3.76)	1	[56]
Srivastava, 2010	*CYP7A1*	rs3824260	C	−469 T>C	185	200	0.86 (0.49-1.54)	1	[56]
Andreotti, 2008	*ApoB*	rs676210	C	Pro2739Leu	235	730	1.15 (0.64-2.07)	1	[66]
Andreotti, 2008	*ApoB*	rs673548	C	IVS23−79T>C	235	730	0.97 (0.52-1.81)	1	[66]
Andreotti, 2008	*ApoB*	rs520354	T	IVS6+360C>T	235	730	1.51 (0.63-3.61)	1	[66]
Andreotti, 2008	*ApoB*	rs1367117	T	Thr98Ile	235	730	2.54 (0.97-6.68)	1	[66]
Andreotti, 2008	*ApoE*	rs440446	G	IVS1+69C>G	235	730	1.32 (0.83-2.10)	1	[66]
Isomura, 2010	*CYP2C19*	rs4244285, rs4986893	A, A	CYP2C19*2, CYP2C19*3	15	566	1.16 (0.39-3.51)	1	[30]
Other genes									
Pramanik, 2011	*KRAS*	-	T	Gln25His	90	60	2.81 (1.42-5.56)	1	[57]
Srivastava, 2010	*hsa-miR-146a*	rs2910164	C	G>C	230	230	2.40 (0.81-7.10)	1	[60]
Srivastava, 2010	*hsa-mir-196a2*	rs11614913	T	C>T	230	230	0.94 (0.46-1.91)	1	[60]
Srivastava, 2010	*hsa-mir-499*	rs3746444	C	T>C	230	230	1.50 (0.71-3.16)	1	[60]
Srivastava, 2010	*ACE I/D*	rs4646994	-	289 bp del polymorphism	233	260	1.38 (0.73-2.60)	1	[62]
Srivastava, 2010	*DNMT3B*	rs1569686	G	−579 G>T	212	219	1.56 (0.84-2.90)	1	[61]

Article (author, year)	Gene	Variant	Risk allele	Gene Polymorphism(s) studied	Total cases	Total controls	OR (95% CI)[a]	No of studies	Reference
Srivastava, 2010	*TLR2*	-	-	−196_174del	233	257	2.14 (0.56-8.14)	1	[58]
Srivastava, 2010	*TLR4*	rs4986791	T	Thr399Ile	233	257	7.57 (0.83-69.16)	1	[58]
Srivastava, 2010	*CASP8*	rs3834129	N/A	−652 6N ins/del	230	230	0.42 (0.20-0.89)	1	[59]
Srivastava, 2010	*CASP8*	rs1045485	C	Asp302His	230	230	0.95 (0.06-15.27)	1	[59]
Srivastava, 2010	*CASP8*	rs3769818	A	IVS12−19 G > A	230	230	0.83 (0.18-3.86)	1	[59]

[a] homozygous variant genotype vs. homozygous wild genotype.

[b] reported by Hsing et al [20] also in same set of samples.

Table 4

Meta-analysis of candidate gene polymorphisms in different genotype models and GBC association

Gene & rs number	Polymorphism	No. of studies	Test of association			Test of heterogeneity			
			OR	95% CI	*P* value	Model	*Q*	P_{het}	I^2
OGG1 (rs1052133)	C vs. S	3	0.87	0.53-1.44	0.600	Random	21.791	<0.001	90.822
	CC+CS vs. SS	3	0.97	0.68-1.39	0.891	Random	6.413	0.046	67.442
	CC vs. CS+SS	3	1.62	0.80-3.30	0.180	Random	9.185	0.010	78.226
	CC v. SS	3	1.76	0.63-4.91	0.277	Random	8.748	0.013	77.139
TP53 (rs1042522)	R vs. P	3	1.38	1.02-1.87	0.035	Fixed	5.268	0.072	62.037
	RR+RP vs. PP	3	1.65	0.74-3.72	0.223	Random	7.822	0.020	74.432
	RR vs. RP+PP	3	1.27	0.71-2.72	0.423	Fixed	0.921	0.64	0
	RR vs. PP	3	1.28	0.68-2.42	0.441	Fixed	0.623	0.732	0
GSTM1 [a]	Null vs. non null	3	1.08	0.73-1.60	0.697	Fixed	0.369	0.831	0
CYP1A1 (rs1048943)[a]	V vs. I	3	0.89	0.73-1.10	0.279	Fixed	4.311	0.116	53.605
	VV+VI vs. II	3	0.89	0.69-1.15	0.366	Fixed	5.237	0.073	61.814
	VV vs. VI+II	3	0.78	0.46-1.32	0.359	Fixed	0.267	0.875	0
	VV vs. II	3	0.72	0.42-1.22	0.221	Fixed	1.009	0.604	0

In the case of significant heterogeneity (P_{het}<0.05), ORs were calculated using random effect model, otherwise fixed-effects model was used.

[a]data from Kimura et al [52] not available.

Table 5

Factors implicated in the etiopathogenesis of gallbladder cancer

Independent / Established/Major	Dependent / Emerging/Novel
NON MODIFIABLE Age [87] Gender [87] Family history [69, 88]	Tobacco [89-90]
MODIFIABLE Gallstone disease [87, 91-94] Chronic cholecystitis [77, 95] Chronic infection [87, 96-97] Obesity [98] High parity [93, 99-101] Anomalous pancreatobiliary duct junction [102] Porcelain gallbladder [103]	Chemical carcinogenesis [104-105]
	Mustard oil [106]
	Early age at first pregnancy [93, 99-101]
	Oral contraceptive use [89, 99, 107]
	Red chili pepper [64, 108]
	Occupational exposure [77]
	Secondary bile acids [109]
	Xanthogranulomatous cholecystitis [110-111]
	Heavy metals [5, 112]
	Genetic factors
	Increased oxidative stress
	Ethnic and geographic variation [87]