Gallbladder cancer epidemiology, pathogenesis and molecular genetics: Recent update

Aarti Sharma, Kiran Lata Sharma, Annapurna Gupta, Alka Yadav, Ashok Kumar

Aarti Sharma, Annapurna Gupta, Alka Yadav, Ashok Kumar, Department of Surgical Gastroenterology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow 226014, India

Kiran Lata Sharma, Department of Biochemistry and Medical Genetics, John Buhler Research Centre, University of Manitoba, Winnipeg MB R3E 3P4, Canada

Author contributions: Sharma A, Sharma KL and Kumar A have written the manuscript; Sharma A, Sharma KL and Gupta A has contributed in literature search; Sharma A, Sharma KL, Gupta A and Yadav A have contributed in designing tables as well as referencing; Kumar A is senior Corresponding author; Sharma KL is co-corresponding author; all the authors have approved the final version of the manuscript.

Conflict-of-interest statement: We declare that we have no conflict of interest.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Manuscript source: Invited manuscript

Correspondence to: Dr. Ashok Kumar, Professor, Department of Surgical Gastroenterology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raebareli Road, Lucknow 226014, India. akgupta@sgpgi.ac.in
Telephone: +91-522-2494423
Fax: +91-522-2668017

Received: December 24, 2016
Peer-review started: December 29, 2016
First decision: January 19, 2017
Revised: February 1, 2017

Accepted: June 1, 2017
Article in press: June 1, 2017
Published online: June 14, 2017

Abstract

Gallbladder cancer is a malignancy of biliary tract which is infrequent in developed countries but common in some specific geographical regions of developing countries. Late diagnosis and deprived prognosis are major problems for treatment of gallbladder carcinoma. The dramatic associations of this orphan cancer with various genetic and environmental factors are responsible for its poorly defined pathogenesis. An understanding to the relationship between epidemiology, molecular genetics and pathogenesis of gallbladder cancer can add new insights to its undetermined pathophysiology. Present review article provides a recent update regarding epidemiology, pathogenesis, and molecular genetics of gallbladder cancer. We systematically reviewed published literature on gallbladder cancer from online search engine PubMed (http://www.ncbi.nlm.nih.gov/pubmed). Various keywords used for retrieval of articles were Gallbladder, cancer Epidemiology, molecular genetics and bullion operators like AND, OR, NOT. Cross references were manually searched from various online search engines (http://www.ncbi.nlm.nih.gov/pubmed, https://scholar.google.co.in/, http://www.medline.com/home.jsp). Most of the articles published from 1982 to 2015 in peer reviewed journals have been included in this review.

Key words: Gallbladder cancer; Epidemiology; Molecular genetics; Pathogenesis

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.
Core tip: The Gallbladder cancer is a fatal malignancy which displays considerable differences in certain ethnicities and geographic regions. Indo-Gangetic plains of India, Mapuche Indians in Chile and South America are most affected regions with this cancer. Because of this cancer is largely unstudied as compare to other cancers Present review provides a comprehensive summery of the studies conducted regarding its Epidemiology, Pathogenesis and molecular genetics. This will be helpful for the researchers to understand the current scenario of research work and how much success we have gained till now. Based on which future research work can be planned in appropriate directions.

Sharma A, Sharma KL, Gupta A, Yadav A, Kumar A. Gallbladder cancer epidemiology, pathogenesis and molecular genetics: Recent update. World J Gastroenterol 2017; 23(22): 3978-3998. Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i22/3978.htm  DOI: http://dx.doi.org/10.3748/wjg.v23.i22.3978

INTRODUCTION

Gallbladder cancer (GBC) is a rare biliary tract malignancy in most western countries, but is much widespread in some other regions of the world. Moreover, this carcinoma is infrequent in developed countries but more common in some developing countries, characterized by its lack of symptoms at initial stage leading to difficulties in treatment.

The extensive variation in geography, ethnicity, and cultural differences in the incidence of gallbladder cancer suggests the role of key genetic and environmental factors associated with the development and progression of the disease[1,2]. The lack of a serosal layer of gallbladder adjacent to the liver thus enabling hepatic invasion and metastatic progression is one of the major cause of its miserable prognosis[3]. The present review provides a recent update of studies regarding epidemiology, pathogenesis and molecular genetics of gallbladder cancer as available in literature.

EPIDEMIOLOGY OF GALLBLADDERS CANCER

Gallbladder cancer shows an unusual geographic distribution worldwide with substantial geographic variation. Data from Mapuche Indians from Valdivia, Chile, South America shows the rate of gallbladder cancer as: 12.3/100000 for males and 27.3/100000 for females[3]. The native people are these countries exceed for gallbladder cancer mortality rates from cervical (8.0/100000), breast (8.7/100000), pancreatic (7.4/100000), and ovarian cancers (7.3/100000)[3].

American Indians in New Mexico, USA, have also very high average annual rate of GBC (8.9/100000)[4],[Surveillance, Epidemiology and End Results Program (SEER) The Four Most Common Cancers for Different Ethnic Populations 2013. Bethesda, MD: National Cancer Institute; 2013].

Although the worldwide occurrence of gallbladder cancer is less than 2/100000, but this has been recorded with extensive variance[5]. The residents of Indo-Gangetic belt particularly females of northern India (21.5/100000) and south Karachi Pakistan (13.8/100000) have been reported as one of the highest affected regions[6]. Gallbladder cancer is also found in high frequency in Eastern Europe include Poland (14/100000 in Poland), Czech Republic, and Slovakia and Asia whereas south Americans of Indian descent (3.7 to 9.1 per 100000), Israel (5/100000) and Japan (7/100000) have shown intermediate prevalence of gallbladder cancer[4,6]. The residents of Andean-area, North American Indians and Mexican-Americans are specially predisposed of GBC[6]. The majority of the world has decreasing mortality trends in gallbladder cancer but GBC frequency is constantly rising in Shanghai, China which is substantial cause of mortality[7]. Although Gallbladder cancer is more common in females still in some countries like Korea, Iceland and Costa Rica, higher mortality rate has been reported for males as compare to females[8]. The data from National Cancer Institute; SEER Program (http://seer.cancer.gov/) has revealed only little turn down in incidence over the past few decades.

ETIOLOGICAL FACTORS FOR GBC PATHOGENESIS

The development of gallbladder cancer has been linked to various genetic and environmental factors. Chronic infection of gallbladder or/and environmental exposure to specific chemicals, heavy metals, and even many dietary factors, have been found to be associated with GBC formation. The dramatic association of GBC with female gender and certain geographical regions (mostly developing countries) has been proposed to be influenced by various female hormones, cholesterol cycling and salmonella infections in existing literature[9,10]. Worldwide GBC affects females 2-3 times more commonly than males, but bias varies greatly in different parts of the world mostly in high prevalent regions of GBC[4,6].

To some extent, the female hormone estrogen causes increased cholesterol super saturation in bile and hence involved in gallstone mediated GBC pathogenesis[11]. Although the female gender GBC can be linked with the role of female hormones. However an article published previously has questioned the association of hormone receptor expression to tumor differentiation[12]. So the extent of female hormones contribution in Gallbladder cancer is still not certain and requires more investigation.

Other well-known GBC associated risk factors
Dependent Etiological factors of carcinoma gallbladder has also been reported in two siblings from Brazil as reported by Trajber the clustering of gallbladder cancer within families paternal in familial gallbladder cancers (10.2 million individuals from the year 1961-1998), Cancer Data base from the Swedish Cancer Registry environmental factors. The nationwide Swedish Family to a large extent to this cancer further modulated by The high risk heritable factors are likely to contribute and the disease manifestation in several high risk pedigrees as reported in previous studies gives a strong indication for genetic susceptibility to GBC. The significant risk in 3rd degree relatives and the disease manifestation in several high risk pedigrees as reported in previous studies gives a strong indication for genetic susceptibility to GBC. The significant risk in 3rd degree relatives and the disease manifestation in several high risk pedigrees as reported in previous studies gives a strong indication for genetic susceptibility to GBC. The high risk heritable factors are likely to contribute to a large extent to this cancer further modulated by environmental factors. The nationwide Swedish Family-Cancer Data base from the Swedish Cancer Registry (10.2 million individuals from the year 1961-1998), has reported maternal transmission favoring over paternal in familial gallbladder cancers. Furthermore, the clustering of gallbladder cancer within families is suggestive of a critical role of genetics in its development. Carcinoma gallbladder was detected in two siblings from Brazil as reported by Trajber et al. Role of allele specific mutations in pathogenesis of carcinoma gallbladder has also been reported.

Another report by Pandey et al. has shown higher frequency of carcinoma gallbladder in patients with A+ and AB+ blood groups to which the reason is still unknown.

### GENETIC AND MOLECULAR ALTERATION REPORTED IN GALLBLADDER CARCINOMA

The present existing information regarding genetic and molecular alterations in GBC is still very much limited. Like other neoplasms, GBC is a multifactorial disorder involving multiple genetic alterations. Abnormality in tumor suppressor genes, oncogenes, and DNA repair genes, presence of microsatellite instability (MSI) and epigenetic alterations mainly caused by aberrant promoter methylation of gene areas are some of the various well known factors reported till now. The serious of genetic alteration leading to gallbladder cancer formation is still not established clearly. Some of the molecular alterations reported so far are enumerated in Tables 2-4.

### GENETIC ALTERATIONS IN GBC

#### KRAS

KRAS act as initial key player in numerous signal transduction mechanisms and associated pathways. Many pathogenic mutations have been reported in KRAS oncogene in Gallbladder cancer tissue. KRAS gene mutations identified in GBC mostly affects codons 12, 13 and 61. In north India KRAS codon 13 mutation is more common (about one third) than codon 12 and 61. However many other studies have not detected any mutations in this gene. Any activating point mutations in KRAS oncogene can give rise to abnormal growth signals which is one of the hallmarks of cancer. The previous reports have correlated a condition called anomalous arrangement of the pancreatico-biliary duct with presence of gallbladder cancer as patients harboring this condition have a higher frequency of KRAS gene mutation as compare

| Major Independent Etiological factors | Dependent Etiological factors |
|---------------------------------------|------------------------------|
| Age[6] | Tobacco consumption[14] |
| Sex[8], BMI[26] | Mustard oil[36,37] |
| Family history[7,36] | Argemone oil (AO) and butter yellow (BY)[18] |
| Cholelithiasis[27,28] | Early age at first pregnancy[21] |
| Chronic cholecystitis, porcelain gallbladder[27,28] | Use of Oral contraceptives[15,23-26] |
| Chronic infection by Salmonella species, S. paratyphi or S. typhican[6,7,10,13-50] | Red Chili pepper[10,24] |
| Helicobacter pylori[9,26,27] | Occupational exposure, Benzene[17,23] |
| High parity[13] | Secondary bile acids[13,36-50] |
| Porcelain gallbladder[26] | Xanthogranulomatous cholecystitis[61] |
| Gallbladder polyp[25] | Heavy metals[44,45] |
| Obesity[40] | Genetic factors[40] |
| Free radical oxidation products[44] |
deregulated survival of genetically impaired abnormal cells which can lead to neoplastic conversion of later on\[70\]. TP53 mutations are relatively more common in later stages of the disease\[63,66,71-73\]. Most of the TP53 mutations associated with GBC are missense mutations that produce a non-functional protein with an increased half-life. The existing literature has reported mutations of the TP53 gene in between approximately 27% to 70% of gallbladder carcinomas\[74\]. Many codons of the TP53 codons are affected by pathogenic to normal condition\[65,67,68\]. However mutation of KRAS gene has never been detected in GBC having adenoma carcinoma sequence of development\[69\] (Table 2).

**TP53**

TP53 is a well-known tumor suppressor gene and has various mechanisms of anticancer function and plays significant role in maintenance of genome integrity, apoptosis, genomic stability, and inhibition of angiogenesis etc. Loss of TP53 function allows deregulated survival of genetically impaired abnormal cells which can lead to neoplastic conversion of later on\[70\]. TP53 mutations are relatively more common in later stages of the disease\[63,66,71-73\]. Most of the TP53 mutations associated with GBC are missense mutations that produce a non-functional protein with an increased half-life. The existing literature has reported mutations of the TP53 gene in between approximately 27% to 70% of gallbladder carcinomas\[74\]. Many codons of the TP53 codons are affected by pathogenic

### Table 2 Mutations detected in gallbladder cancer by low throughput methods

| Studied gene | Type of study | Methods used | Studied population | Ref. |
|--------------|--------------|--------------|--------------------|------|
| KRAS         | Mutation at codon-12 (8%) | PCR-RFLP | India | [64] |
|              | Mutation at codon-12 (20%-30%) | PCR-RFLP | Chile | [76,77] |
|              | Mutation at codon-12 (0%-59%) | PCR-RFLP, Direct sequencing | Japan | [60,78,79] |
| INK4A (p16)  | Mutation, deletion | PCR-RFLP, direct sequencing, IHC | Japan, Chile | [54,79,81,82] |
| D310 mtDNA   | Mutation (Displacement loop) | PCR-based assay, direct sequencing | Chile | [83] |
| TP53         | Mutation, overexpression, LOH | PCR-RFLP, direct sequencing, IHC | Greece, Japan, Chile | [84-86] |

### Table 3 Mutations studies in gallbladder cancer by high throughput methods

| Platform                          | Number of samples | Study population | Research planned | Key findings | Ref. |
|-----------------------------------|-------------------|------------------|------------------|--------------|------|
| Sequenom Mass ARRAY technology    | 49 FFPE           | India            | PIK3CA (4%), KRAS (2%), CTNNB1 (4%), TP53 (18%) | [95] |
| Mass spectroscopy-based           | 57 FFPE           | MD Anderson Centre | 14 hotspot mutations in 9 cases including (KRAS, NRAS, PIK3CA, IDH1, ALK, MET) | [94] |
| Next-generation sequencing (NGS)  | 15 FFPE           | NGS of 182 cancer-related genes | Preponderance of mutations involving the PI3 kinase pathway | [94] |
| Whole Exome and transcriptome     | 29 Fresh Frozen   | Japan            | EGFR, ERBB3, PTK6, ARID2, MLH1, MLL3, APOBEC, TERT | [96] |
| Exome sequencing and targeted gene sequencing | 57 Fresh Frozen China | Whole exome sequencing | TP53 (47.1%), KRAS (7.8%) and ERBB (11.8%) | [93] |

**FFPE:** Fresh frozen paraffin embedded.

### Table 4 Summary of global gene expression studies in gallbladder cancer

| Biological sample used | Platform/studies key findings | Ref. |
|------------------------|------------------------------|------|
| 17 gallbladder tissue specimens (6 advanced GBC, 6 early GBC cancers and 5 normal control) | Oligonucleotide Microarray platform | 2270 Unregulated genes: 2270 Downregulated genes: 2412 | [97] |
| 5-Normal biliary epithelial scrapings, 11- surgically resected biliary carcinomas, 9-biliary cancer cell lines | Oligonucleotide Microarray platform | 282 Unregulated genes: 282 Downregulated genes: 513 | [98] |
| 37 biliary tract carcinomas (15 bile duct, 11 gallbladder, 11 of ampulla of Vater) | cDNA array platform | Upregulated: (TP50 II-alpha, cyclin B2, CDC28, ubiquitin-conjugating enzyme E2C), and one metabolism-related: (gamma-glutamyl hydrolase) | [99] |
| 12 advanced gallbladder carcinoma tissue 3 samples of normal control gallbladder epithelium | Oligonucleotide Array platform | 118 genes were identified with a prognostic value | [100] |
| 34 biliary tract cancers including 13 intrahepatic (IHC), 12extrahepatic (EHC), 9 (GBC) | Oligonucleotide Array platform | 1281 genes with deregulated expression pattern | [101] |
Sharma A et al. GBC recent update

mutations of this gene. Functional molecular studies have discovered that mutations in exons 5 and 8 of TP53 gene causes deregulation of this gene[73]. Details are shown in various existing literature is shown in Table 2[54,60,63,64,76-86].

C-ERB-B2
The oncogene c-erb-B2 is a homologue for epidermal growth receptor, encoding a protein with tyrosine kinase activity. The immunohistochemical expression of c-erb-B2 has been found positive between 10%-46% of gallbladder cases. However its expression has been found to be absent in dysplasia or adenomas as shown by previous reports[87,88]. Animal model studies in transgenic mice have shown that erbB2 overexpression in the basal layer of the biliary tract epithelium led to the development of GBC in all (100%) of mice. Moreover, the expression of HER2/neu was positively observed in 28% of GBCs which was directly correlated with advanced stage of cancer[89]. Therefore, it can be hypothesized that some oncogene is associated with in Gallbladder cancer progression. In a study from India, C-erbB2 was frequently expressed in well differentiated and stage I to stage IV in about 9.4% of GBC cases[90]. A recent report showed HER2/neu overexpression occurred in 14% of the advanced gallbladder cancer cases, and this subgroup was expected to be benefited from HER2/neu pathway inhibitors[91]. Therapeutic targeting of EGFR/HER2 pathways boosts the anti-proliferative effect of gemcitabine in biliary tract and gallbladder carcinomas as shown by a previous study[92]. Based on facts it can be concluded that C-ERB-B2 expression can become a marker for a poor prognosis.

HIGH THROUGHPUT MUTATION STUDIES IN GBC
High throughput research has made large scale repetition of experiments feasible as it automates the experiments thus it has now become possible to study how all 21000 genes potentially contribute to cell function or disease. But in case of gallbladder cancer there are very limited high throughput studies. One of the pioneer studies published in nature genetics using high throughput approach by Chinese population has found recurrent mutations in ErbB pathway[93]. Javle et al[94] has found 26 missense mutations with more common TP53 and PIK3CA mutations in GBC tumor using NGS technology. Mutation profiling of gallbladder cancer tissue in Indian population has found PIK3CA and KRAS mutations as most common among this ethnicity[95]. The variability in the results is an indicator of intra-tumoral heterogeneity of cancer, which describes the observation of different tumor cells showing distinct morphological and molecular profiles including variable gene expression but ultimately leading to a common phenotype. The high throughput mutation studies in GBC are presented in Table 3[95-98].

GENE EXPRESSION STUDIES IN GBC
In order to identify potential biomarkers for GBC progression, many studies have been performed to find out the differential gene expression profiles between normal and tumor cells. Existing data varies greatly, despite of same grade and stage of the included study subjects. Table 4[97-101] and Table 5[94,96,75,86,90,102-104] are summarizing global and single gene expression studies reported in GBC respectively.

LOSS OF HETEROZYGOSITY AND MICROSATELLITE INSTABILITY
Loss of heterozygosity (LOH) is a common genetic alteration in cancer genome. The events like heterozygous deletion of one of the two alleles, or duplication of a maternal or paternal chromosome or chromosomal region and concurrent loss of the other allele gives rise to LOH. The studies focused to detect loss of heterozygosity (LOH) in GBCs have shown frequent heterozygous allelic loss which spans in 18 different chromosomal regions[57]. Cytogenetic locations involved in frequent loss of heterozygosity i.e., 3p, 8p, 9p, and 22q regions have also been identified in GBC from different populations; which have also been reported in several other cancers like Retinoblastoma, melanoma, Squamous cell carcinoma of larynx[181-183]. In particular, gallbladder tumor shows numerous site of allelic loss in the short arm of chromosome 3, which harbors several known or putative tumor suppressor genes[105,181]. High degree of microsatellite instability (MSI) in 10% of GBC cases was observed as reported in research article published by Goldin et al[184]. A different pattern of allelic loss has also been detected in Japanese population. In this report the allelotype analysis of gallbladder carcinoma revealed an interesting associated with anomalous junction of pancreatico-biliary duct[185]. Table 6[54,76,68,109,112,185-193] enlists various studies conducted in GBC regarding LOH and MSI.

METHYLATION AND GALLBLADDER CANCER
Understanding of DNA methylation patterns of gallbladder tumors can prove to be important biomarkers to refine the diagnosis and prognostic information which ultimately helps in appropriate therapeutic selection. Hypermethylation in gene promoter regions is a common epigenetic mechanism for the inactivation of tumor suppressor genes. One of the important research article published previously has found an important link between methylation and survival. In this study methylation of genes p73, MGMT, and DCL1 was significantly associated with...
| Studied single genes | Expression pattern | Studied population | Ref. |
|----------------------|--------------------|--------------------|------|
| TP53                | Expression (20%-70%) | India, Slovenia, Greece, Taiwan, Japan, Chile | [75,84-86,102-106] |
| p16                 | Overexpression     | South Korea        | [107] |
| FHT                 | Expression loss (45%-75%) | Japan, Chile       | [108,109] |
| ERBB2               | Overexpression (25%-64%) | India, Japan, China, South Korea | [66,103,110,111] |
|                    | Exp. in 9.4% cases of well differentiated and stage II to stage IV tumors | India | [90] |
| RB                  | 20% cases allelic loss 4%-14% - loss of expression | Japan | [54,112] |
| CDKN1A              | Reduced expression 49% cases | Japan | [113] |
| Cyclin D1, Cyclin E | Overexpression (41%-49%) | Japan | [114,115] |
| COX2                | Over-expressed     | Slovenia, Japan, Chile | [104,116,117] |
| BCL2                | Over-expressed     | Japan               | [118] |
| CKIT                | Expression 45%     | Japan               | [119] |
| SOX-4               | Overexpression     | China               | [120] |
| Chemokine (C-X-C motif) ligand 12 | Increased expression | South Korea | [121] |
| CXCR4, CXCR7        | Increased expression | China | [122] |
| hedgehog pathway components (Shh, Ptc1 and Gli1) | Shh: 81.7% of cases expressed, Ptc1: 75.3% of cases, Gli1: 70.0% of cases | China | [123] |
| CD56, CD99          | Altered expression | South Korea         | [124] |
| CD97, CD55          | CD97: 69.6% of cases expressed, CD55: 62.5% of cases | China | [125] |
| HMGA2 and CD9       | HMGA2 positive expression, CD9 negative expression | China | [126] |
| cholecystokinin type-A | 44.1% of cases expressed | India | [127] |
| vascular endothelial growth factor-A | 53.6% of cases expressed | China | [128] |
| VEGF-C, VEGF-D      | VEGF-C: 64.0% of cases, VEGF-D: 62.0% of cases | China | [129] |
| Tumor endothelial marker 8 protein | Increased expression | India | [130] |
| L1 cell adhesion molecule | Increased expression | South Korea | [131] |
| Tissue factor pathway inhibitor-2 | Down-regulated | China | [132] |
| HIF-1α              | Increased expression | China               | [133] |
| VHL                 | Reduces expression | China               | [134] |
| ERCC1(excision repair cross-complementing 1) | High expression in best differentiated tumors | Chile | [135] |
| NF-E2-related factor 2 (Nf2) | Increased expression | China | [136] |
| CD34, CA13-3        | Highly expressed in stromal and epithelial | Italy | [137] |
| ADAM-17             | Overexpression     | China               | [138] |
| Cdx2                | Aberrant expression | Japan               | [139] |
| TLR4                | Expressed in glandular and luminal epithelium | China | [140] |
| MIRNA               | Loss of Dicer and Drosha expression | China | [141] |
| Inducible Nitric Oxide Synthase iNOS | Expressed | China | [142] |
| Prostate stem cell antigen (PSCA) | Down-regulated | Japan, China | [143] |
| OCT-4               | Down-regulated    | China               | [144] |
| bTEKT/Telemerase    | Expressed in 86.66% cases | India | [145] |
| Aquaglycerins (AQPs) | Positive expression | Japan | [146] |
| Ornithine decarboxylase (ODC) and glutamate decarboxylase 65 (GAD65) | Overexpression | China | [147] |
| Alpha-methylacyl coenzyme A (racecurne) | Overexpression | Taiwan | [148] |
| AMACR               | Overexpression     | Taiwan              | [149] |
| Sonic Hh (Sshh)     | Elevated expression | Japan               | [150] |
| TGF-beta induced miR-182 | Overexpression | China | [151] |
| SLP-2               | Overexpression     | China               | [152] |
| TMPRSS4             | Higher expression  | China               | [153] |
| zinc finger X-chromosomal protein | Suppressed | China | [154] |
| multidrug resistance-associated protein 2 (MRP2) | Overexpression | South Korea | [155] |
| HuR                 | Overexpression     | Taiwan              | [156] |
| miR-1-155           | Overexpression     | Japan               | [157] |
| LAPT4M4B-35         | Overexpressed(76%) | China               | [158] |
| p27, p21            | p21 (75% cases) and p27 (25% cases) | Jordan | [159] |
| Thymidylate synthase (TS) | Low expression | Japan | [160] |
| CD146               | Elevated expression | China               | [161] |
| AEG-1               | Highly expressed (63.4%) | China | [162] |
| CCKAR               | Expression increased (76.6%) | India | [163] |
| Nemo-like kinase (NLK) | Overexpression of NLK | China | [164] |
| C-erbB2             | Overexpression (9.4%) | India | [165] |
survival of gallbladder cancer patients.\[194,195\] The study was conducted in a series of 109 advanced gallbladder cancer cases. However genes like \textit{CDH13} and \textit{FHIT} did not show any significant tendency with respect to gallbladder cancer patient’s survival.\[194,195\]

Multivariate analysis found \textit{MGMT} gene to be an independent prognostic factor for survival found, representing the important role of epigenetic process in gallbladder carcinogenesis.\[195\] The recent report showed that promoter methylation of specific genes like \textit{CDH1}, \textit{CDKN2A-p16}, \textit{REPRIMO} (tumor suppressor gene family) and \textit{UCHL1} (also known as PGP9.5) have important role in gallbladder carcinogenesis.\[196\]

Some other studies conducted on GBC have shown variable methylation pattern of a number of genes Table 7.\[81,82,193-208\]

In addition, with the help of advanced technologies like high resolution allele stratification (allelotyping analysis) investigated very high frequencies of 3p (100%), 8p (100%), 9q (88%), 22q (92%) sites in gallbladder cancer that lead to positional identification of tumor suppressor genes associated with GBC malignancies and pathogenesis.\[57,58,109,209\] Moreover, some well-known tumor suppressor genes that

---

**Table 6**: Loss of heterozygosity and microsatellite instability studies reported in gallbladder cancer

| Studied reported in respective population | LOH/MSI | Ref. |
|------------------------------------------|---------|------|
| Chilean                                  | LOH reported in : 3p, 6q, 7q, 8p, 9p, 9q, 11q, 12q, 17p, 18q, 19p, 22q, and Xq | [57] |
| Japanese                                 | LOH reported in : 2p, 4p, 4q, 8q, 9q, 10p,14q,14q16q, 19p, 21p and Xp [Maximum deletion-2p24, 14q22 and 21q22] | [68] |
| Chilean, Japanese                        | p53, 9p,8p, DCC, KRAS, p16, 16q24, 3p, 9q, 22q and p161NK4 | [54,66,109,112,185] |
| Greek                                    | BAT-26 | [186] |
| Chile, Japan                             | MSI reported (20%-33%) | [187,188] |
| India                                    | E-cadherin (CDH1) 2p, 2q, 6q, 7q,17p | [189] |
| India                                    | Fragile histidine triad (FHIT ) MSI-H 17.5% LOH :27.5% | [190] |
| Japan                                    | High incidences of LOH at 1p56 (19/36:53%), 9p21 (12/32:38%), 13q14 (20/36: 56%), 16q24 (31/34: 61%), and 17p13 (15/36: 42%) | [191] |
| Chile                                    | FHIT gene locus (3p14.2) | [109] |
| India                                    | LOH at 8 loci, that is 3p12, 3p14.2, 5q21, 9p21, 9q, 13q, 17p13, and 18q for tumor suppressor genes (DUTT1, FHIT, APC, p16, FCMD, RBL1, p53, and DCC genes) | [192] |
| India                                    | genomic instability at 2p, 2q, 6q, 7q, and 17p loci | [189] |
| Chile                                    | DUTT1 (3p12), FHIT (3p14.2), BLU, RASSF1A, SEMA3B and hMLH1 (3p21.3) | [193] |

LOH: Loss of heterozygosity; MSI: Microsatellite instability.
| Gene     | Full name                                      | Function                                      | Meth Freq | Population               | Ref.                   |
|----------|-----------------------------------------------|-----------------------------------------------|-----------|--------------------------|------------------------|
| CDH1     | Cadherin 1, type 1, E-cadherin (epithelial)   | Tissue invasion (cell-cell adhesion)          | 11%-65%   | Japan, Chile             | [194-200]              |
| FHT      | Fragile histidine triad gene                  | Regulation of DNA Replication, and apoptosis  | 30%-57%   | Chile                    | [81,193-195,199]       |
| APC      | Adenomatous polyposis coli                    | Tumor suppressor gene (Cell migration, adhesion and apoptosis) | 26%-35%   | Chile, United States     | [81,194,195,198,199]   |
| hMLH1    | Human homologs of Mutl, gene of bacteria      | Mismatch repair                               | 0%-14%    | Chile, United States     | [81,193-195,199]       |
| p16      | Cyclin-dependent kinase inhibitor 2A          | Cell cycle regulation                         | 15%-60%   | Chile, United States, [81,82,195,197-199,201,202] | Germany [81,198]       |
| p15      | Cyclin-dependent kinase inhibitor 2B          | Cell cycle regulation                         | 22%-44%   | Chile                    | [81,198]               |
| DAPK1    | Death-associated protein kinase 1             | Serine-threonine kinase                       | 8%-61%    | Japan, Chile             | [81,197,198]           |
| DLC1     | Deleted in liver cancer 1                     | GTPase-activating protein                     | 39%-55%   | Chile                    | [81]                   |
| MGMT     | O-6-methylguanine-DNA methyltransferase       | Methyltransferase                             | 13%-30%   | Chile, United States     | [81,195]               |
| RARβ2    | Retinoic acid receptor, beta-isoform 2        | Encodes retinoic acid receptor beta           | 4%-44%    | Chile, United States     | [81,198]               |
| REPRIMO  | TP53 dependent G2 arrest mediator candidate   | Cell cycle regulation (p53 mediator)         | 62%       | Chile                    | [204]                  |
| SHP1     | Protein tyrosine phosphatase, non-receptor 6  | Regulate cell growth, differentiation, mitotic cycle | 80%       | Chile                    | [198]                  |
| 3-OST-2  | Heparan sulfate (glucosamine) 3-O-sulfotransferase 2 | O-sulfotransferase | 72%       | Chile                    | [198]                  |
| RUNX3    | Runx-related transcription factor 3           | TGF-beta signal pathway                       | 22%-32%   | Chile                    | [197,198]              |
| RIZ1     | PR domain containing 2, with ZNF domain       | Histone/protein methyltransferase            | 26%       | Chile                    | [198]                  |
| HPP1     | Transmembrane protein with EGF-like and two follistatin-like domains 2 | TGF-beta signal pathway                       | 20%       | [198]                    |
| P73      | Tumor protein p73                             | Induction of apoptosis and cell cycle regulation | 14%-28%   | Chile, United States     | [81,198]               |
| SOCS-1   | Suppressor of cytokine signaling 1            | JAK-STAT pathway                             | 12%       | Chile                    | [198]                  |
| DCR2     | Tumor necrosis factor receptor superfamily, member 10d | TNF-receptor superfamily                  | 6%        | Chile                    | [198]                  |
| SEMA3B   | Sema domain, immunoglobulin domain (lg), short basic domain, secreted,(semaphorin) 3B | Induction of apoptosis | 92%       | Chile                    | [193]                  |
| DLITT1   | Human homolog of Drosophila Roundabout (ROBO1) | Cell migration and metastasis               | 22%       | Chile                    | [193]                  |
| BLU      | Zinc finger, MYND-type containing 10          | Cell cycle regulation                         | 26%       | Chile                    | [193]                  |
| p14      | Ribonuclease P/MRP 14 kDa subunit             | Cell cycle regulation                         | 40%       | Germany                  | [201]                  |
| MASP1    | Mammary serine protease inhibitor              | Tumor suppressor gene                         | 70%       | India                    | [205]                  |
| THBS1    | Thrombospondin 1                              | Platelet aggregation, angiogenesis, and tumorigenesis | 52%       | [205]                    |
| HLT      | Helicase-like transcription factor            | Regulate transcription                        | 16%       | Brazil                   | [206]                  |
| MYC      | V-Myc Avian Myelocytomatosis Viral Oncogene Homolog transcription factor | Cell cycle progression, apoptosis and cellular transformation | 80%       | Brazil                   | [206]                  |
| APC      | Adenomatous polyposis coli                    | Tumor suppressor gene                         | 71%-95%   | Chile                    | [207]                  |
| CDKN2A   | Cyclin-dependent kinase inhibitor 2A          | Cell cycle                                    | 71%-95%   | Chile                    | [207]                  |
| ESR1     | Estrogen receptor 1                           | Transcription factor                           |            |                         |                       |
| PGP9.5   | Protein gene product 9.5                      | Neural and/or nerve sheath differentiation     |            |                         |                       |
| SSBP2    | Single-stranded DNA-binding protein 2         | Microsatellite instability                    |            |                         |                       |
are present in chromosomes like 3p, 5q, 8p, 13q and 18q can also influence the gallbladder cancer formation.\textsuperscript{[57,58,109,209]}

**Candidate genes for gallbladder cancer susceptibility**

The merely successful mechanism for identifying low or moderate penetrance cancer genes, is the analysis of genes involved in candidate loci. Therefore, these genes are also termed as candidate genes. The candidate gene analysis is done via case-control study, in which allele frequencies in cancer patients and healthy controls are compared and obtained results are analyzed statistically. Candidate modifier genes are selected on the basis of biological plausibility. Most studies are based on genes that encode proteins, thought to be involved in carcinogenesis, such as those involved in apoptosis, cell-cycle control, DNA repair, xenobiotic metabolism, hormonal and inflammatory pathway or other risk factors. Moreover, known genes account for a small proportion of the heritability of gallbladder cancer, and it is likely that many genes with modest effects are yet to be found.

A study by Wang et al.\textsuperscript{[210]} from China suggested about CCK-induced impaired gallbladder emptying in patients having gallstones. Most of the candidate genes identified so far are related to the classical rate limiting enzymes and proteins of lipid metabolism, steroidogenesis, lipid transport, bile acid synthesis, bile canalicular transport, gallbladder contractility, cell cycle, DNA repair and Inflammatory pathway\textsuperscript{[211-233]}. Till now there are very limited studies in GBC which are independently replicated which includes \textit{OGG1}, \textit{TP53}, \textit{GSTM1} null polymorphism and \textit{CYP1A1} polymorphism\textsuperscript{[48]}. No definitive conclusions can be drawn due to limited number of studies. Hence there is a great need to explore genes related to GBC susceptibility. Table 8\textsuperscript{[208,214-273]} shows an overview of candidate gene studies reported in GBC.

The only one genome-wide association study conducted in gallbladder cancer identified a SNP (rs7504990) in \textit{DCC} gene which was associated with six times gallbladder cancer risk in the Japanese population. It has also been reported that reduced expression of \textit{DCC} gene (deleted in colorectal cancer, 18q21.3) was designated to be associated with the greater aggressiveness of the disease which include increased proliferation, poorly differentiated histology, and metastasis through loss of adhesiveness\textsuperscript{[234]}. However genome wide association study (GWAS) identified SNPs was replicated in Indian population and the study found no individual association of \textit{DCC}\textsuperscript{rs7504990} but haplotype analysis of \textit{DCC} gene found the cumulative effect of \textit{G}=\textit{A}\textsuperscript{rs4078288} + \textit{G}\textsuperscript{rs7504990} + \textit{A}\textsuperscript{rs1714} haplotypes in Gallbladder Cancer predisposition\textsuperscript{[235]}.

**Molecular pathogenesis of GBC**

Gallbladder carcinoma develops through a serious of events before converting in to invasive malignancy. Any exposure to carcinogens may convert normal gallbladder epithelium to condition called metaplasia which subsequently forms dysplasia to carcinoma \textit{in situ} (CIS), and finally proceeding to invasive carcinoma in about 15 years\textsuperscript{[274,275]}. The multistage pathogenesis of gallbladder carcinoma begins with gallstones giving rise to a condition called chronic cholecystitis, which increases to risk to gallbladder cancer formation. More than 90% of patients with gallbladder carcinoma show dysplasia and CIS\textsuperscript{[274,275]}. There is an unusual asymmetric thickening of the gallbladder wall with infiltration to surrounding structures in gallbladder cancer. Maximum cases reported in carcinomas of gallbladder are adenocarcinomas (80%-95%). Adenocarcinomas can further be of papillary, tubular, mucinous, or signet cell type. Some other types which are present in very low frequency include: squamous cell carcinoma (16%), undifferentiated or anaplastic carcinoma (2%-7%), and adenosquamous carcinoma (1%-4%)\textsuperscript{[276]}. Most of GBCs (60%) are found in the fundus, near about 30% in the body, and 10% in the neck region.

**Tumor markers in GBC**

Till date there is no reliable tumor marker developed which can be employed in diagnosis of gallbladder cancer. The only two markers \textit{i.e.}, carcino-embryonic antigen (CEA) and carbohydrate antigen 19-9 are most often elevated in advanced stages with a low specificity. So most often they are not used in stand-alone diagnosis of GBC\textsuperscript{[277]}. However, there are other tumor markers like CA125, CA199, CEA (carcino-embryonic antigen), cancer antigens (CA) and CA242, which are for diagnosis of different other types of cancer (\textit{e.g.}, gastric, liver, pancreatic), have also been researched in diagnosis of gallbladder cancer but the obtained results are highly inconsistent\textsuperscript{[278-280]}. In addition some previous reports have shown CA 242, RCAS1 (receptor binding cancer antigen expressed on SiSo cells) CA15-3, Mac-2BP (macrophage...
Table 8  Candidate gene studies (low susceptibility genes) in gallbladder cancer

| Pathway involved          | Gene     | Polymorphism                   | Population                  | Ref.       |
|---------------------------|----------|--------------------------------|----------------------------|------------|
| DNA repair pathway genes  | XPC      | (rs2280303) Ala499Val          | China                       | [236]      |
|                           |          | (rs2280001) Lys593Gln          | China                       |            |
|                           | ERCC2    | (rs1799793) Asp312Asn          | North Indian                | [232]      |
|                           |          | (rs13181) Lys751Gln           | North Indian                |            |
|                           | MSH2     | (rs2303426) IVS1+9G>C          |                             |            |
|                           |          | (rs2303425) -1187T>C          |                             |            |
|                           | OGG1     | (rs2012668) 748-15C>G         |                             |            |
|                           | TP53     | (rs1042522) Pro72Arg          | North Indian, Hungary, Japan| [237-239] |
|                           | XRCC1    | (rs1799792) Arg194Trp         | North Indian, Shanghai, China| [222,231]|
|                           | APEX1    | (rs25487) Arg599Gln           | Shanghai, China             | [222]      |
|                           | RAD23B   | (rs1805335) IV5-15A>G         |                             |            |
|                           |          | (rs1805329) Ex7+65C>T         |                             |            |
| Hormonal pathway genes    |          | (rs1308577) IV5-15C>G         | Shanghai, China             | [241]      |
|                           |          | (rs2071011G>C, rs915889C/T,   | Shanghai, China             | [241]      |
|                           |          | rs3822222C/T, rs180085317/A   |                             |            |
|                           | ESR1     | (rs2334545) IVS1-397T>C       | Shanghai, China, North India| [241-243]|
|                           |          | (rs3841686) IV55-34>G>T       |                             |            |
|                           |          | (rs2228480) Ex8+229G>A        |                             |            |
|                           |          | (rs1801132) Ex4-122G>C        |                             |            |
|                           |          | (rs9340799) IVS5-351A>G       |                             |            |
|                           | ESR2     | (rs1256049) Val328Val         | Shanghai, China             | [224]      |
|                           | PGR      | Ins/Del                       | North India                 | [244]      |
|                           | AR       | (CAG)n                        | Shanghai, China             | [224]      |
|                           |          | (rs4633) His62His             | Shanghai, China             | [224]      |
|                           | COMT     | (rs4818) Leu136Leu            | Shanghai, China             | [224]      |
|                           | CYP1A1   | (rs2600345) IVS1+606G>T       | Shanghai, China             | [224]      |
|                           | CYP1B1   | (rs100122) Arg485Gy           | Shanghai, China             | [224]      |
|                           | CYP19A1  | (rs1065778) IV54-76A>G        | Shanghai, China             | [224]      |
|                           |          | (rs700518) Val800A            | Shanghai, China             | [224]      |
|                           | HSD3B2   | (rs2034463) IVS5-106T>G       | Shanghai, China             | [224]      |
|                           |          | (rs700519) Arg264Cys          | Shanghai, China             | [224]      |
|                           | HSD17B3  | (rs1065779) IV59-53C>G        | Shanghai, China             | [224]      |
|                           |          | (rs4646) Ex11+410G>C         | Shanghai, China             | [224]      |
|                           | HSD17B1  | (rs1819608) Ex4-133C>G        | Shanghai, China             | [224]      |
|                           | SHBG     | (rs2064479) Gly829Arg         | Shanghai, China             | [224]      |
|                           | SREDS2   | (rs6259) Ex8+66G>A           | Shanghai, China             | [224]      |
|                           | RXR-a    | (rs1356475) IVS6+70A>G        | Shanghai, China             | [224]      |
|                           | RXR-b    | (rs1805343) IVS1-27A>G        | Shanghai, China             | [224]      |
|                           |          | (rs2744337) G292T             | Shanghai, China             | [224]      |
|                           | INS      | (rs669) A-6T                  | Shanghai, China             | [224]      |
|                           | PPARC    | (rs2016520) Ex4+15C>G         | Shanghai, China             | [224]      |
|                           | PPARG    | (rs3856086) His477His         | Shanghai, China             | [224]      |
|                           | CR1      | (rs2274567) His1208Arg        | Shanghai, China             | [224]      |
| Inflammatory pathway genes|          | (rs12144461) Intron 27, HindIII|                             |            |
|                           | IL1RN    | 86 bp VNTR                    | North Indian                | [223]      |
|                           |          | -1195G>A                     | North Indian                | [233]      |
|                           | PTGS2    | (rs20417) -765G>C            | North Indian, Shanghai, China| [233,246]|
|                           | ILS1     | (rs20575) +8437T>C           | Shanghai, China             | [220,247] |
|                           | ILS10    | (rs16944) -1067T>C           | Shanghai, China             | [247]      |
|                           |          | (rs1800871) -7334T>C         | Shanghai, China             | [247]      |
|                           | ILS-8    | (rs18008566) 1L.8-1308C>G     | Shanghai, China             | [247]      |
|                           | EGFR     | (rs444903) +61A>G            | Shanghai, North Indian      | [222]      |
|                           | TGFBI    | (rs1800469) -509C>T          | Shanghai, North Indian      | [219,221,247]|
|                           | TNF-a    | (rs18000629) -308C>A         | Shanghai, North Indian      | [219,221,247]|
|                           | IL6      | (rs1800795) 236C>G           | Shanghai, North Indian      | [219,221,247]|
|                           | IL8      | (rs10805066) -13985C>G       | Shanghai, North Indian      | [219,221,247]|

Sharma A et al. GBC recent update
### Metabolic pathway genes

| Gene  | SNP Reference | Population |
|-------|---------------|------------|
| MMP-2 | (rs2285653) -735 C>T | North Indian |
| MMP-7 | (rs11568318) -181 A>G | North Indian |
| TIMP2 | (rs1797090) -418 G>C | North Indian |
| MTHFR | (rs1801133) Ala222Val | Indian, Chilean, Hungarian, Japanese |
| APOB | (rs17240441) 35_43del9 | Indian, Chilean, Hungarian, Japanese |
| NAT2 | (rs1799929) NAT2*5A (rs1799930) NAT2*6B | Indian, Chilean, Hungarian, Japanese |
| GSTT1 | Null polymorphism (rs1695) Ile105Val | Indian, Chilean, Hungarian, Japanese |
| GSTP1 | (rs743572) Ex1+27T>C | Shanghai, India |
| CYP17 | (rs11568818) -181 A>G | North Indian |
| CYP1A1 | (rs1048943) Ile105Val | Shanghai, India |

### Apoptosis pathway

| Gene  | SNP Reference | Population |
|-------|---------------|------------|
| CASP8 | (rs3834129, -652 6N ins/del) | North Indian |
| APOB | (rs676219) Pro2739Leu | Shanghai, India |

### Nuclear Receptors

| Gene  | SNP Reference | Population |
|-------|---------------|------------|
| LXR-α, LXR-β | | North Indian |

### Cancer Stem cell gene

| Gene  | SNP Reference | Population |
|-------|---------------|------------|
| CD44 | (rs13347) C>T, CD44 (rs356369) A>C, CD44 (rs187116) G>A, CD44 (rs187115) T>C | North Indian |

### Prostate stem cell antigen mRNA

| Gene  | SNP Reference | Population |
|-------|---------------|------------|
| PSA  | (rs2910164) C>G | India, Japan |
| hsa-mir-146a | (rs2994087) T/C and rs2978974 | India, Japan |

### GWAS-associated genes

| Gene  | SNP Reference | Population |
|-------|---------------|------------|
| DCC  | (rs7054990) C>T, (rs7054989) A>G | Japan, North Indian |

---

**References:**

1. Sharma A et al. GBC recent update
2. [https://www.wjgnet.com](https://www.wjgnet.com)
galactose-specific lectin-2 binding protein). Fragments of cytokeratin-19 (CYFRA 21-1) are frequently present in blood of cancer patients and shown to be associated with GBC with variable sensitivity and specificity[277,281,282].

CONCLUSION

Various lines of evidence suggest role for various environmental risk factors in Gallbladder carcinoma. Despite of many articles regarding genetic predisposition of gallbladder cancer there is no established genetic marker. Also, very limited Genome wide association studies (GWAS) have been conducted in gallbladder cancer till now.

The evidence-based model of gallbladder carcinogenesis and its dissemination by Barreto et al[283] serves as a basic platform for elucidation of molecular mechanisms involved in cancer development which based on recent data can be improved by discovery of other signature mutations using high throughput studies. Technological advancement can be helpful more understanding of pathogenic mechanisms underlying neoplastic conversion of gallbladder cancer mucosa. The tumor markers available for diagnosis GBC has also not of very high specificity and not discovered until advanced stage of the disease leading to complexity of the treatment. Exome sequencing of gallbladder cancer tissue has found ERBB pathway as most dysregulated pathway in this disease. Although study has not of very high specificity and not discovered until advanced stage of the disease leading to complexity of the treatment. Exome sequencing of gallbladder cancer tissue has found ERBB pathway as most dysregulated pathway in this disease. Although present review provides a comprehensive summary of the studies conducted regarding its Epidemiology, Pathogenesis and molecular genetics under a single umbrella. This will be helpful for the researchers to understand the current scenario of research work and how much success we have gained till now. Based on that future research work can be planned in appropriate directions.

REFERENCES

1 Misra S, Chaturvedi A, Misra NC, Sharma ID. Carcinoma of the gallbladder. Lancet Oncol 2003; 4: 167-176 [PMID: 12623362]

2 Andia ME, Hsing AW, Andreotti G, Ferreccio C. Geographic variation of gallbladder cancer mortality and risk factors in Chile: a population-based ecologic study. Int J Cancer 2008; 123: 1411-1416 [PMID: 18566990 DOI: 10.1002/ijc.23662]

3 Hundal R, Shaffer EA. Gallbladder cancer: epidemiology and outcome. Clin Epidemiol 2014; 6: 99-109 [PMID: 24634588 DOI: 10.2147/CLEP.S373574.e6-099]

4 Randi G, Franceschi S, LaVecchia C. Gallbladder cancer worldwide: geographical distribution and risk factors. Int J Cancer 2006; 118: 1591-1602 [PMID: 16397865 DOI: 10.1002/ijc.21683]

5 Shaffer EA. Gallbladder cancer: the basics. Gastroenterol Hepatol (N Y) 2008; 4: 737-741 [PMID: 21960896]

6 Lazcano-Ponce EC, Miquel JF, Muñoz N, Herrera R, Ferreccio C, Wistuba II, Alonso de Ruiz P, Arísti Urista G, Nervi F. Epidemiology and molecular pathology of gallbladder cancer. CA Cancer J Clin 2001; 51: 349-364 [PMID: 11760569]

7 Hsing AW, Bai Y, Andreotti G, Rashid A, Deng J, Chen J, Goldstein AM, Han TQ, Shen MC, Fraumeni JF, Gao YT. Family history of gallstones and the risk of biliary tract cancer and gallstones: a population-based study in Shanghai, China. Int J Cancer 2007; 121: 832-838 [PMID: 17450525 DOI: 10.1002/ijc.22756]

8 Hariharan D, Saied A, Kocher HM. Analysis of mortality rates for gallbladder cancer across the world. HPB (Oxford) 2008; 10: 327-331 [PMID: 18992147 DOI: 10.1080/13651820802007464]

9 Pilgrim CH, Groeschl RT, Christians KK, Gamblin TC. Modern perspectives on factors predisposing to the development of gallbladder cancer. HPB (Oxford) 2013; 15: 839-844 [PMID: 23458506 DOI: 10.1111/hpb.12046]
in Japan. World J Surg 1991; 15: 337-343 [PMID: 1853612]
47 Wiles R, Varadpande M, Muly S, Webb J. Growth rate and malignant potential of small gallbladder polyps—systematic review of evidence. Surgeon 2014; 12: 221-226 [PMID: 24502936 DOI: 10.1016/j.surge.2014.01.003]
48 Srivastava K, Srivastava A, Sharma KL, Mittal B. Candidate gene studies in gallbladder cancer: a systematic review and meta-analysis. Mutat Res 2011; 728: 67-79 [PMID: 21708280 DOI: 10.1016/j.mrrev.2011.06.002]
49 Larsson SC, Wolk A. Obesity and the risk of gallbladder cancer: a meta-analysis. Br J Cancer 2007; 96: 1457-1461 [PMID: 17375043]
50 Shukla VK, Shukla PK, Pandey M, Rao BR, Roy SK. Lipid peroxidation product in bile from patients with carcinoma of the gallbladder: a preliminary study. J Surg Oncol 1994; 56: 258-262 [PMID: 8057656]
51 Jackson HI, Glasgow RE, Mulvihill SJ, Cannon-Albright LA. Cannon-Albright. Familial risk in gallbladder cancer. J Am Coll Surg 2007; [205]: S38-S138
52 Hemminki K, Li X. Familial liver and gall bladder cancer: a nationwide epidemiological study from Sweden. Gut 2003; 52: 592-596 [PMID: 12631675]
53 Trajber HJ, Szego T, de Camargo HS, Mester M, Marujo WC, Roll S. Adenocarcinoma of the gallbladder in two siblings. Cancer 1982; 50: 1200-1203 [PMID: 7104965]
54 Wistuba II, Sugio K, Hsu K, Kimishima Y, Virmani AK, Ito A, Albores-Saavedra J, Gazdar AF. Allele-specific mutations involved in the pathogenesis of endemic gallbladder carcinoma in Chile. Cancer Res 1995; 55: 2511-2515 [PMID: 7790959]
55 Pandey M, Khatri AK, Dubey SS, Gautam A, Shukla VK. Erythrocyte membrane fatty acid profile in patients with primary carcinoma of the gallbladder. J Surg Oncol 1995; 59: 31-34 [PMID: 7745974]
56 Sasatomi E, Tokunaga O, Miyazaki K. Precancerous conditions of gallbladder carcinoma: overview of histopathologic characteristics and molecular genetic findings. J Hepatobiliary Pancreat Surg 2000; 7: 556-567 [PMID: 11180887 DOI: 10.1007/s005400602341]
57 Wistuba II, Tang M, Maitra A, Alvarez H, Troncoso P, Pimentel F, Gazdar AF. Genome-wide allelotyping analysis reveals multiple sites of allele loss in gallbladder carcinoma. Cancer Res 2001; 61: 3795-3800 [PMID: 11325854]
58 Rashid A. Cellular and molecular biology of biliary tract cancers. Surg Oncol Clin N Am 2002; 11: 995-1009 [PMID: 12607385]
59 Imai M, Hoshi T, Ogawa K. K-ras codon 12 mutations in biliary tract tumors detected by polymerase chain reaction denaturing gradient gel electrophoresis. Cancer 1994; 73: 2727-2733 [PMID: 8194013]
60 Ajiki T, Fujimori T, Onoyama H, Yamamoto M, Kitazawa S, Maeda S, Saitoh Y. K-ras gene mutation in gall bladder carcinomas and dysplasia. Gut 1996; 38: 426-429 [PMID: 8675098]
61 Ito T, Watanabe H, Ajieko Y, Oohashi Y, Takel K, Nishikura K, Nakamura Y, Horai A, Saito T. APC, K-ras codon 12 mutations and p53 gene expression in carcinoma and adenoma of the gall-bladder suggest two genetic pathways in gall-bladder carcinogenesis. Pathol Int 1996; 46: 333-340 [PMID: 8809879]
62 Ito T, Watanabe H, Ajieko Y, Nishikura K, Saito T. Correlation of p53 protein expression with gene mutation in gall-bladder carcinomas. Pathol Int 1997; 47: 525-530 [PMID: 9293532]
63 Masuhara S, Kasuya K, Aoki T, Yoshimatsu A, Tsuaida A, Koyanagi Y. Relation between K-ras codon 12 mutation and p53 protein overexpression in gallbladder cancer and biliary ductal epithelia in patients with pancreatobiliary malignancy. J Hepatobiliary Pancreat Surg 2000; 7: 198-205 [PMID: 10982614 DOI: 10.1007/s0054000007019.534]
64 Singh MK, Chetri K, Pandey UB, Kapoor VK, Mittal B, Choudhuri G. Mutational spectrum of K-ras oncogene among Indian patients with gallbladder cancer. J Gastroenterol Hepatol 2004; 19: 916-921 [PMID: 15242496 DOI: 10.1111/j.1440-1746.2004.03555.x]
Expression of p16 protein may explain the poor prognosis.

Lowe AW. Gene expression profiles in gallbladder cancer: the close association of p16 with survival and near absence of amplification of cMYC defines a novel subclass of gallbladder carcinoma. *Nat Genet* **7**, 283-288 [PMID: 10817595].

Kim JH, Kim HN, Lee KT, Lee JK, Choi SH, Paik SW, Rhee JC, Park KH, Shin WJ, Shin J, Chung SS, Kim Y, Lee JH, Lee J, Jang K, Hong J, Lim J, et al. Expression of p53 and c-erbB-2 protein in adenocarcinoma of the gallbladder. *J Cancer Res Clin Oncol* **134**, 1055-1059 [PMID: 18497548 DOI: 10.1159/00013257000132570].

Hansel DE, Rahman A, Hidalgo M, Thuluvath PJ, Lillemoe KD, Schulick R, Ku JL, Park JG, Miyazaki K, Ashfaq R, Wistuba II, Varma R, Hawthorne L, Geradiz J, Argani P, Maitra A. Identification of novel cellular targets in biliary tract cancers using global gene expression technology. *Am J Pathol* **2003; 163**: 217-229 [PMID: 12819026].

Murakawa K, Tada M, Takada M, Tamoto E, Shindoh G, Hama N, Hosoda F, Urushidate T, Ohashi S, Hiraoka N, Ojima N, Kamada H, Katoh H, Yoshiki T, Moriiuchi T. Prediction of lymph node metastasis and perineural invasion of biliary tract cancer by selected features from cDNA array data. *J Surg Oncol* **2004; 122**: 184-194 [PMID: 15555617].

Washiro M, Ohtsuka M, Kimura F, Shimizu H, Yoshidome H, Sugimoto T, Seki N, Miyazaki M. Upregulation of topoisomerase I alpha expression in advanced gallbladder carcinoma: a potential chemotherapeutic target. *J Cancer Res Clin Oncol* **2003; 134**: 793-801 [PMID: 18204862 DOI: 10.1007/s00432-007-0348-0].

Miller G, Socci ND, Dhali D, D'Angelica M, DeMatteo RP, Allen PJ, Singh B, Fang Y, Blumgart LH, Klimstra DS, Jarnagin WR. Genome-wide analysis and clinical correlation of chromosomal and transcriptional mutations in cancers of the biliary tract. *J Exp Clin Cancer Res* **2009; 28**: 62 [PMID: 19435499 DOI: 10.1186/1756-9966-28-62].

Misra S, Chaturvedi A, Goel MM, Mehrotra R, Sharma ID, Srivastava AN, Misra NC. Overexpression of p53 protein in gallbladder carcinoma in North India. *Eur J Surg Oncol* **2000; 26**: 164-167 [PMID: 10744937 DOI: 10.1016/s0300-9802(00)00141-9].

Chaubé A, Tewari M, Garbály RS, Singh U, Shukla HS. Preliminary study of p53 and c-erbB-2 expression in gallbladder cancer in Indian patients manuscript id: 896201628764582. *BMC Cancer* **2006; 6**: 126 [PMID: 16686942].

Legan M, Luzzar B, Ferlan-Marotol V, Cór A. Cyclooxygenase-2 expression determines neo-angiogenesis in gallbladder carcinomas. *Biochimica et Biophysica Acta* **2006; 1717**: 56-63 [PMID: 17177652].

Wang SN, Chung SC, Tsai KB, Chai CY, Chang WT, Kao KK, Chen JS, Lee KT. Abrupton p53 expression and the development of gallbladder carcinoma and adenoma. *Kaohsiung J Med Sci* **2006; 22**: 53-59 [PMID: 16568721].

Ghosh M, Sakhija P, Singh S, Agarwal AK. p53 and beta-catenin expression in gallbladder tissues and correlation with tumor progression in gallbladder cancer. *Saudi J Gastroenterol* **2013; 19**: 34-39 [PMID: 23319036 DOI: 10.4103/1319-3767.105922].

Choi HJ, Yun SS, Kim HJ, Choi JH. Expression of p16 protein in gallbladder adenocarcinoma and its precancerous conditions. *Hepatogastroenterology* **2010; 57**: 18-21 [PMID: 20422865].

Koda M, Yashima K, Kawaguchi K, Andachi H, Hosoda A, Shiota G, Ito H, Murawaki Y. Expression of Fhit, Mlh1, and P53 protein in human gallbladder carcinoma. *Cancer Let* **2003; 199**: 131-138 [PMID: 12967975].

Wistuba II, Ashfaq R, Maitra A, Alvarez H, Riquelme E, Gazzard A. Fragile histidine triad gene abnormalities in the pathogenesis of gallbladder carcinoma. *Am J Pathol* **2002; 160**: 2073-2079 [PMID: 12057912].

Suzuki T, Takano Y, Kakita A, Okudaira M. An immunohistochemical and molecular biological study of c-erbB-2 amplification and prognostic relevance in gallbladder cancer. *Pathol Res Pract* **1993; 189**: 283-292 [PMID: 8101375].

Chow NH, Huang SM, Chan SH, Mo LR, Hwang MH, Su WC. Significance of c-erbB-2 expression in normal and neoplastic epithelium of biliary tract. *Anticancer Res* **1995; 15**: 1055-1059 [PMID: 7645925].

Shi YZ, Hui AM, Li X, Takayama T, Makuchii M. Overexpression of retinoblastoma protein predicts decreased survival and correlates with loss of p16INK4 protein in gallbladder carcinomas. *Clin Cancer Res* **2000; 6**: 4096-4100 [PMID: 11051262].

Li X, Hui AM, Shi YZ, Takayama T, Makuchii M. Reduced p21(WAF1/CIP1) expression is an early event in gallbladder carcinogenesis and is of prognostic significance for patients with
carcinomas of the gallbladder. *Hum Pathol* 2001; 32: 771-777 [PMID: 11522188]

114 *Eguchi* N, Fujii K, Tsuchida A, Yamamoto S, Sasaki T, Kajiyama G. Cyclin D overexpression in human gallbladder carcinomas. *Onco Rep* 1999; 6: 93-96 [PMID: 9864402]

115 *Hui AM*, Li X, Shi YZ, Takayama T, Torzilli G, Makuuchi M. Cyclin D1 overexpression is a critical event in gallbladder carcinogenesis and independently predicts decreased survival for patients with gallbladder carcinoma. *Clin Cancer Res* 2000; 6: 4272-4277 [PMID: 11106243]

116 *Asano* T, Shoda J, Ueda T, Kawamoto T, Todoroki T, Shimonishi M, Tanabe T, Sugimoto Y, Ichikawa A, Mutoh M, Tanaka N, Miwa M. Expressions of cyclooxygenase-2 and prostaglandin E-receptors in carcinoma of the gallbladder: crucial role of arachidonate metabolism in tumor growth and progression. *Clin Cancer Res* 2002; 8: 1157-1167 [PMID: 11948128]

117 *Kawamoto* T, Shoda J, Asano T, Ueda T, Funakawa M, Koike K, Tanaka N, Todoroki T, Miwa M. Expression of cycloxygenase-2 in the subserosal layer correlates with postsurgical prognostic value of pathological tumor stage 2 carcinoma of the gallbladder. *Int J Cancer* 2002; 98: 427-434 [PMID: 11920955 DOI: 10.1002/ijc.10222]

118 *Sasamoto* E, Tokunaga O, Miyazaki K. Spontaneous apoptosis in gallbladder carcinoma. Relationships with clinicopathologic factors, expression of E-cadherin, bcl-2 protooncogene, and p53 oncopressor gene. *Cancer* 1996; 78: 2101-2110 [PMID: 8918403]

119 *Tanaka* S, Tanaka H, Yamamoto T, Shuto T, Takemura S, Hsi H, Sakabe K, Uenishi T, Hirohashi K, Kubo S. Immunohistochemical demonstration of e-Kit protooncogene product in gallbladder cancer. *J Hepatobiliary Pancreat Dis Int* 2011; 18: 220-224 [PMID: 16708300 DOI: 10.1007/s00534-005-0774-0]

120 *Wang* C, Zhao H, Lu Y, Jin Y, Zang L, Song N, Dong R, Wu T. *Clin. Pathol*: Clinical significance of SOX4 expression in primary gallbladder carcinoma. *Diagn Pathol* 2012; 7: 41 [PMID: 22510499 DOI: 10.1186/1746-1596-7-41]

121 *Lee JJ*, Lee K, Lee DG, Bae KH, Kim JS, Jiang ZL, Huang SM, Suk Oh Y, Kim HY, Jo DY, Min JK, Kim JM, Lee HJ. Chenomine (C-X-C motif) ligand 12 is associated with gallbladder carcinoma progression and is a novel independent poor prognostic factor. *Clin Cancer Res* 2012; 18: 3270-3280 [PMID: 22553346 DOI: 10.1158/1078-0432.CCR-11-2417]

122 *Yao* X, Zhou L, Han S, Chen Y. High expression of CXCR4 and CXCR7 predicts poor survival in gallbladder cancer. *J Int Med Res* 2011; 39: 1253-1264 [PMID: 21996127]

123 *Li J*, Wu T, Li J, Cao Y, Song N, Yang T, Dong R, Yang Y, Zang L, Du X, Wang S. Immunohistochemical evidence of the prognostic value of hedgehog pathway components in primary gallbladder carcinoma. *Surg Today* 2012; 42: 770-775 [PMID: 22407314 DOI: 10.1007/s00595-012-1051-7]

124 *Choi YL*, Xuan YH, Shin YK, Chae SW, Kook MC, Sung RH, Youn SJ, Choi JW, Kim SH. An immunohistochemical study of expression pattern of tumor endothelial marker 8 protein in gallbladder carcinomas. *Ann Anat* 2010; 22: 190-195 [PMID: 20450586 DOI: 10.1016/j.annanat.2010.01.003]

125 *Choi SY*, Jo YS, Huang SM, Liang ZL, Min JK, Hong HJ, Kim JM. L1 cell adhesion molecule as a novel independent poor prognostic factor in gallbladder carcinoma. *Hum Pathol* 2011; 42: 1476-1483 [PMID: 21496863 DOI: 10.1016/j.humpath.2011.01.003]

126 Qin YG, Gong W, Weng MZ, Li JY, Quan ZW. [The role of tissue factor pathway inhibitor-2 gene in gallbladder cancer]. *Zhonghua Wai Ke Za Zhi* 2012; 50: 1099-1103 [PMID: 23336488]

127 *Yang Z*, Yang Z, Xiong L, Huang S, Liu J, Yang L, Miao X. Expression of VHL and HIF-1a and Their Clinicopathologic Significance in Benign and Malignant Lesions of the Gallbladder. *Appl Immunohistochem Mol Morphol* 2011; 19: 534-539 [PMID: 21415706 DOI: 10.1097/TA.0b013e3182128001]

128 *Roa* I, de Arctab卡拉, X, Lantaddilla S, Munoz S. ERCC1 (excision repair cross-complementing 1) expression in pT2 gallbladder cancer is a prognostic factor. *Histol Histopathol* 2011; 26: 37-43 [PMID: 21117025]

129 Wang J, Zhang M, Wang L, Cao Y, Zhou S, Zhang J, Wang Y. Correlation of Nrg2, HO-1, and MRPS in gallbladder cancer and their relationships to clinicopathologic features and survival. *J Surg Res* 2010; 164: e99-105 [PMID: 20828733 DOI: 10.1016/j.jss.2010.05.058]

130 Artico M, Bronzetti E, Alicino V, Ionta B, Bosco S, Grande C, Bruno M, Tranquilli Leali FM, Ionta G, Fumagalli L. Human gallbladder carcinoma: Role of neurotrophins, MB-1, CD34 and CAIX-3. *Eur J Histoch Histopathol* 2010; 54: e10 [PMID: 20359905]

131 Wu K, Liao M, Liu B, Deng Z. ADAM-17 over-expression in gallbladder carcinoma correlates with poor prognosis of patients. *Med Oncol* 2011; 28: 475-480 [PMID: 20300669 DOI: 10.1007/s12032-010-9481-8]

132 *Wani* Y, Notohara K, Fujisawa M. Absent expression of an “intestinal marker” Cdx2 in pyloric gland adenoma of the gallbladder. *Virchows Arch* 2008; 453: 521-527 [PMID: 18843504 DOI: 10.1007/s00428-008-0680-z]

133 *Huang* P, Maosheng T, Zhiqian H, Long C, Xiaojun Y. TLR4 expression in normal gallbladder, chronic cholecystitis and gallbladder carcinoma. *Hepatogastroenterology* 2012; 59: 42-46 [PMID: 22251522 DOI: 10.5754/htg.10258]

134 *Shu* GS, Yang ZL, Liu DC. Immunohistochemical study of Dicer and Drosha expression in the benign and malignant lesions of gallbladder and their clinicopathologic significances. *Pathol Res Pract* 2012; 208: 392-397 [PMID: 22658478 DOI: 10.1016/j.prp.2012.05.001]

135 *Zhang* M, Pan JW, Ren TR, Zhu YF, Han YJ, Kühnel W. Correlated expression of inducible nitric oxide synthase and P53, Bax in benign and malignant diseased gallbladder. *Ann Anat* 2003; 185: 549-554 [PMID: 14704000]

136 *Ono* H, Hiroaka N, Lee YS, Woo SM, Lee WJ, Choi JH, Saia T, Yanagihara K, Kanai Y, Ohnami S, Chikaiwaki F, Sasaki H, Sakamoto H, Yoshida T, Saeki N. Prostate stem cell antigen, a presumable organ-dependent tumor suppressor gene, is down-regulated in gallbladder carcinogenesis. *Genes Chromosomes Cancer* 2012; 51: 30-41 [PMID: 21936014 DOI: 10.1002/gcc.20928]

137 *Zou* Q, Yang L, Yang Z, Huang J, Fu X. PSCA and Oct-4 expression in the benign and malignant lesions of gallbladder: implication for carcinogenesis, progression, and prognosis of gallbladder adenocarcinoma. *Biomed Res Int* 2013; 2013: 648420 [PMID: 23984394 DOI: 10.1155/2013/648420]

138 Shukla VK, Chauhan VS, Kumar M. Telomerase activation—one step on the road to carcinoma of the gallbladder. *Anticancer Res* 2013; 33: 4207-4213 [PMID: 24070689 DOI: 10.21873/anticanres.512]
Overexpression of LAPTM4B-35 closely correlated with carcinomas. 

Sun W, Fan YZ, Xi H, Lu X, Ye C, Zhang JT. Astrocyte elevated gene-1 overexpression in human primary gallbladder carcinomas: an unfavorable and independent prognostic factor. Oncol Rep 2011; 26: 1133-1142 [DOI: 10.3892/oor.2011.1387]

Li M, Zhang S, Wang Z, Zhang B, Wu X, Weng H, Ding Q, Tan Z, Zhang N, Mu J, Yang J, Shi Y, Bao R, Ding Q, Wu W, Cao Y, Liu Y. Prognostic significance of n-myc-like kinase (NLK) expression in patients with gallbladder cancer. Tumour Biol 2013; 34: 3995-4000 [DOI: 23857283 DOI: 10.1007/s13277-013-0988-4]

Leal P, García P, Sandoval A, Letelier P, Brebi P, Ilic I, Álvarez H, Tapia O, Roa JC. Immunohistochemical expression of phospho-mTOR is associated with poor prognosis in patients with gallbladder adenocarcinoma. Arch Pathol Lab Med 2013; 137: 552-557 [DOI: 23544944 DOI: 10.5858/arpa.2012-0032-OA]

Debkashmi RK, Deka M, Saikia AK, Sharma DK, Singh N, Das NN, Bose S. Prognostic relevance of human telomerase reverse transcriptase (hTERT) expression in patients with gall bladder disease and carcinoma. Asian Pac J Cancer Prev 2015; 16: 2923-2928 [DOI: 25853484]

Lu W, Gao J, Yang J, Cao Y, Jiang L, Li M, Zhang Y, Zhou J, Liu Y. Down-Regulated Phosphoglycerate Kinase 1 Expression Is Associated With Poor Prognosis in Patients With Gallbladder Cancer. Medicine (Baltimore) 2015; 94: e2244 [DOI: 26656369 DOI: 10.1097/MD.0000000000002244]

Liu L, Yang ZL, Wang C, Xiao M, Liu Z, Li D, Zou Q, Li J, Liang L, Zeng G, Chen S. The Expression of Notch 1 and Notch 3 in Gallbladder Cancer and Their Clinicopathological Significance. Pathol Oncol Res 2016; 22: 483-492 [DOI: 26634853 DOI: 10.1007/s12253-015-0019-4]

Faridi MS, Jaiswal MS, Goel SK. Expression of CKK Receptors in Carcinoma Gallbladder and Cholelithiasis: A Pilot Study. J Clin Diagn Res 2015; 9: PC04-PC07 [DOI: 26391162 DOI: 10.1080/16623680.2015.1266798]

Lian S, Shao Y, Liu H, He J, Lu W, Zhang Y, Jiang Y, Zha J. PDK1 induces JunB, EMT, cell migration and invasion in human gallbladder cancer. Oncotarget 2015; 6: 29076-29086 [DOI: 26318166 DOI: 10.18632/oncotarget.49314]

Ma F, Zhang M, Gong W, Weng M, Quan Z. MiR-138 Suppresses Cell Proliferation by Targeting Bag-1 in Gallbladder Carcinoma. PLoS One 2015; 10: e0126499 [DOI: 25962180 DOI: 10.1371/journal.pone.0126499]

Chen Y, Chen C, Ma C, Sun S, Zhang J, Sun Y. Expression of heat-shock protein gp96 in gallbladder cancer and its prognostic clinical significance. Int J Clin Exp Pathol 2015; 8: 1946-1953 [DOI: 25973087]

Ma MZ, Kong X, Weng MZ, Zhang MD, Qin YY, Gong W, Zhang WJ, Quan ZW. Long non-coding RNA-LET is a positive prognostic factor and exhibits tumor-suppressive activity in gallbladder cancer. Mol Carcinog 2015; 54: 1397-1406 [DOI: 25213660 DOI: 10.1002/mc.22215]

Nigam J, Chandra A, Kazmi HR, Parmar D, Singh D, Gupta V. Prognostic significance of survivin in resected gallbladder cancer. J Surg Res 2015; 194: 57-62 [DOI: 25472573 DOI: 10.1016/j.jss.2014.07.054]

Ma MZ, Chiu BF, Zhang Y, Weng MZ, Qin YY, Gong W, Quan ZW. Long non-coding RNA CCA1 promotes gallbladder cancer development via negative modulation of miRNA-218-5p. Cell Death Dis 2015; 6: e1583 [DOI: 25569100 DOI: 10.1038/cddis.2014.541]

Song SP, Zhang SB, Liu R, Yao L, Hao YQ, Liao MM, Zhang YD, Li ZH. NDRG2 down-regulation and CD24 up-regulation promote tumor aggravation and poor survival in patients with gallbladder carcinoma. Med Oncol 2012; 29: 1879-1885 [DOI: 22135002 DOI: 10.1007/s12052-011-0110-y]

Zhang M, Gong W, Zhang Y, Yang Y, Zhou D, Weng M, Qin Y,
Jiang A, Ma F, Quan Z. Expression of interleukin-6 is associated with epithelial-mesenchymal transition and survival rates in gallbladder cancer. *Med Mol Rep* 2015; 11: 3539-3546 [PMID: 25572091 DOI: 10.3892/mmr.2014.1314]

175 Liang PL, Li CF, Chen LT, Sun DP, Chen TJ, Hsing CH, Hsu HP, Lin CY. BCL6 overexpression is associated with decreased p19 ARF expression and confers an independent prognosticator in gallbladder carcinoma. *Tumour Biol* 2014; 35: 1417-1426 [PMID: 24114011 DOI: 10.1007/s12277-013-1195-z]

176 Letelier P, Garcia P, Leal P, Ili C, Buchegger K, Riquelme I, Kapoor VK, Krishnani N, Agrawal V, Agarwal S. Fragile histidine triad (FHT) gene and its association with p53 protein expression in the progression of gall bladder cancer. *Cancer Invest* 2009; 27: 764-773 [PMID: 19452299 DOI: 10.1080/073579009030211304]

177 Matsuo K, Kuroki T, Kitaoka F, Tajima Y, Kanematsu T. Loss of heterozygosity of chromosome 16q in gallbladder carcinoma. *J Surg Res* 2002; 102: 133-136 [PMID: 11796009 DOI: 10.1006/jscr.2001.6297]

178 Jain K, Mohapatra T, Das P, Misra MC, Gupta SD, Ghosh M, Kabra M, Bansal VK, Kumar S, Sreenivas V, Garg PK. Sequential occurrence of preneoplastic lesions and accumulation of loss of heterozygosity in patients with gallbladder stones suggest causal association with gallbladder cancer. *Ann Surg* 2014; 260: 1073-1080 [PMID: 24827397 DOI: 10.1097/SLA.0b013e3182a1898]

179 Riquelme E, Tang M, Baez S, Diaz A, Pruyas M, Wistuba II, Corvalan A. Frequent epigenetic inactivation of chromosome 3p candidate tumor suppressor genes in gallbladder carcinoma. *Cancer Lett* 2007; 250: 100-106 [PMID: 17084965]

180 Roa JC, Anabalón L, Roa I, Melo A, Araya JC, Tapia O, de Andrade JD, Gazdar AF. High resolution chromosome 3p, 8p, 9q and 22q allelotyping analysis in the pathogenesis of gallbladder carcinoma. *Carcinog* 2002; 23: 25-30 [PMID: 11858568]

181 Liu DC, Yang ZL, Jiang S. Identification of PEG10 and TSG101 as carcinogenesis, progression, and poor-prognosis related biomarkers for gallbladder adenocarcinoma. *Pathol Oncol Res* 2011; 17: 859-866 [PMID: 21455631 DOI: 10.1007/s12253-011-9394-7]

182 Kalekos A, Miliaras D. Cytokeratin 7 and 20 expression in neoplastic lesions of the gallbladder. *Ann Surg* 2003; 238: 218-229 [PMID: 12738733 DOI: 10.1097/01.sjl.0000049000.04500.f6]

183 Goldin RD, Roa JC. Gallbladder cancer: a morphological and molecular update. *Histopathology* 2009; 55: 218-229 [PMID: 19490172 DOI: 10.1111/j.1365-2559.2008.03192.x]

184 Yanagisawa N, Miki T, Takeda H, Nakamura T, Arai K, Miwa Y, Yabe Y, Ito Y, Shinoda K, Kikumoto T, Maruyama Y, Tanaka S, Hasegawa Y, Takakura M, Okada Y, Kato Y. Identification of PEG10 and TSG101 as frequent epigenetic inactivation of chromosome 3p in gallbladder carcinomas. *Carcinog* 2009; 30: 133-136 [PMID: 18679877 DOI: 10.1002/ijc.20910]

185 Tozawa T, Tamura G, Honda T, Nawata S, Kimura W, Makino N, Kawata S, Sugai T, Suto T, Motoyama T. Promoter hypermethylation of DAP-kinase is associated with poor survival in primary biliary tract carcinoma patients. *Cancer Sci* 2004; 95: 736-740 [PMID: 15471559]

186 Takahashi T, Shivapurkar N, Riquelme E, Shigematsu H, Reddy J, Suzuki M, Miyajima K, Zhou X, Bekele BN, Gazdar AF, Wistuba II. Aberrant promoter hypermethylation of multiple genes in gallbladder adenocarcinoma and chronic cholecystitis. *Cancer Res* 2004; 64: 6126-6133 [PMID: 15447999 DOI: 10.1158/1078-0432.CCR-04-0579]

187 Roa S JC, Garcia M P, Melo A A, Tapia O, de Andrade JD, Gazdar AF. Gene methylation patterns in digestive tumors. *Rev Med Chil* 2008; 136: 451-458 [PMID: 18769877]

188 Koga Y, Kitajima Y, Miyoshi A, Sato K, Kitahara H, Soejima H, Miyazaki K. Tumor progression through epigenetic gene silencing of O(6)-methylguanine-DNA methyltransferase in human biliary tract cancers. *Ann Surg Oncol* 2005; 12: 354-363 [PMID: 15915369 DOI: 10.1245/ASO.2005.07.020]

189 Klump B, Hsieh CJ, Dette S, Holzmann K, Kiebetalich R, Jung M, Sinn U, Orntr M, Porschken R, Gregor M. Promoter methylation of INK4a/ARF as detected in bile-significance for the differential diagnosis in biliary disease. *Clin Cancer Res* 2003; 9: 1773-1778 [PMID: 12738733]

190 Takahashi T, Suzuki M, Shigematsu H, Shivapurkar N, Echebiri C, Nomura T, Stasny V, Augustus M, Wu CW, Wistuba II, Meltzer SJ, Gazdar AF. Aberrant methylation of Reprimo in human lung cancer. *Carcinog* 2005; 26: 6396-6403 [PMID: 16287758 DOI: 10.1038/sj.can.3301519]

191 Singh TD, Gupta S, Shrivastav BR, Tiwari PK. Epigenetic characterization of...
of role in tumour associated genes. *Gene* 2016; 576: 743-752 [PMID: 26456195 DOI: 10.1016/j.gene.2015.10.004]

206 Ishak G, Leal MF, Dos Santos NP, Demachihi S, Nunes CA, do Nascimento Borges B, Calcagno DQ, Smith MC, Assunção PP, Burbano RR. Deregulation of MYC and TP53 through genetic and epigenetic alterations in gallbladder carcinomas. *Clin Exp Med* 2015; 15: 421-426 [PMID: 25200035 DOI: 10.1007/s10238-014-0311-8]

207 Kagohara LT, Schussel JL, Subbannayya T, Sahasrabuddhe N, Lebron C, Brait M, Maldonado LA, Valle BL, Pirini F, Jahuira M, Lopez J, Letelier P, Brebi-Mieville P, Li C, Pandey A, Chatterjee A, Siddransky D, Guerrero-Preston R, Global and gene-specific DNA methylation pattern discriminates cholecytitis from gallbladder cancer patients in Chile. *Future Oncol* 2015; 11: 233-249 [PMID: 25066711 DOI: 10.2217/fon.14.165]

208 Lee YM, Lee JY, Kim MJ, Bae HI, Park JY, Kim SG, Kim DS. Hypomethylation of the protein gene product 9.5 promoter region in gallbladder cancer and its relationship with clinicopathological features. *Cancer Sci* 2006; 97: 1205-1210 [PMID: 16965602]

209 Kuroki T, Tajima Y, Matsuo K, Kanematsu T. Genetic alterations in gallbladder carcinoma. *Surg Today* 2005; 35: 101-105 [PMID: 15674488 DOI: 10.1007/s00595-004-2906-2]

210 Wang Z, Wu J, Miao X. Study on CCK-induced gallbladder emptying with real-time ultrasonography. *Zhonghua Nei Ke Za Zhi* 1995; 34: 385-387 [PMID: 8582185]

211 Lammert F, Carey MC, Paigen B. Chromosomal organization of candidate genes involved in cholesterol gallstone formation: a murine gallstone map. *Gastroenterology* 2001; 120: 221-238 [PMID: 11208732]

212 Mittal B, Mittal RD. Genetics of gallstone disease. *J Postgrad Med* 2002; 48: 149-152 [PMID: 12215703]

213 Wittenburg H, Lyons MA, Paigen B, Carey MC. Mapping cholesterol gallstone susceptibility (Lith) genes in inbred mice. *Dig Liver Dis* 2003; 35 Suppl 3: S2-S7 [PMID: 12974501]

214 Pandey SN, Dixit M, Choudhuri G, Mittal B. Lipoprotein receptor associated protein (LRPAP1) insertion/deletion polymorphism: association with gallbladder cancer susceptibility. *Int J Gastrointest Cancer* 2006; 37: 124-128 [PMID: 17987404 DOI: 10.1007/s10209-007-9002-y]

215 Pandey SN, Jain M, Nigam P, Choudhuri G, Mittal B. Genetic polymorphisms in GSTM1, GSTT1, GSTP1, GSTM3 and the susceptibility to gallbladder cancer in North India. *Biomarkers* 2006; 11: 250-261 [PMID: 16760134]

216 Pandey SN, Modi DR, Choudhuri G, Mittal B. Slow acetylator genotype (N-acetyltransferase2 (NAT2) is associated with increased susceptibility to gallbladder cancer: the cancer risk not modulated by gallstone disease. *Cancer Biol Ther* 2007; 6: 91-96 [PMID: 17224641]

217 Pandey SN, Srivastava A, Dixit M, Choudhuri G, Mittal B. haplotype analysis of signal peptide (insertion/deletion) and XbaI polymorphisms of the APOB gene in gallbladder cancer. *Liver Int* 2007; 27: 1008-1015 [PMID: 17694941 DOI: 10.1111/j.1478-3231.2007.01516.x]

218 Pandey SN, Choudhuri G, Mittal B. Association of CYP1A1 Msp1 polymorphism with tobacco-related risk of gallbladder cancer in a population-based case-control study in China. *Carcinogenesis* 2009; 30: 606-614 [PMID: 19168589 DOI: 10.1093/carcin/bgp024]

219 Srivastava A, Pandey SN, Choudhuri G, Mittal B. CCR5 Delta32 polymorphism: associated with gallbladder cancer susceptibility. *Scand J Immunol* 2008; 67: 516-522 [PMID: 18405329 DOI: 10.1111/j.1365-3083.2008.02097.x]

220 Srivastava A, Pandey SN, Choudhuri G, Mittal B. Role of genetic variant A-204C of cholesterol 7alpha-hydroxylase (CYP7A1) in susceptibility to gallbladder cancer. *Mol Genet Metab* 2008; 94: 83-89 [PMID: 18178499 DOI: 10.1016/j.ymgep.2007.11.014]

221 Srivastava A, Pandey SN, Dixit M, Choudhuri G, Mittal B. Cholecystokinin receptor A gene polymorphism in gallstone disease and gallbladder cancer. *J Gastroenterol Hepatol* 2008; 23: 970-975 [PMID: 17944886 DOI: 10.1111/j.1365-3083.2007.05170.x]

222 Srivastava A, Pandey SN, Pandey P, Choudhuri G, Mittal B. No association of Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism in susceptibility to gallbladder cancer. *DNA Cell Biol* 2008; 27: 127-132 [PMID: 17979520 DOI: 10.1089/dna.2007.0679]

223 Srivastava A, Tulsyan S, Pandey SN, Choudhuri G, Mittal B. Single nucleotide polymorphism in the ABCG8 transporter gene is associated with gallbladder cancer susceptibility. *Liver Int* 2009; 29: 831-837 [PMID: 19189757 DOI: 10.1111/j.1478-3231.2008.01907.x]

224 Srivastava A, Mittal B. Complement receptor 1 (A3650G Rsal and intron 27 HindIII) polymorphisms and risk of gallbladder cancer in north Indian population. *Scand J Immunol* 2009; 70: 614-620 [PMID: 19066204 DOI: 10.1111/j.1365-3083.2009.02329.x]

225 Srivastava A, Srivastava K, Pandey SN, Choudhuri G, Mittal B. Single-nucleotide polymorphisms of DNA repair genes OGG1 and XRCC1: association with gallbladder cancer in North Indian population. *Ann Surg Oncol* 2009; 16: 1695-1703 [PMID: 19266243 DOI: 10.1245/s10434-009-0354-3]

226 Srivastava K, Srivastava A, Mittal B. Polymorphisms in ERCC2, MSH2, and OGG1 DNA repair genes and gallbladder cancer risk in a population of Northern India. *Cancer* 2010; 116: 3160-3169 [PMID: 20564624 DOI: 10.1002/cncr.25063]

227 Srivastava K, Srivastava A, Pandey SN, Kumar A, Mittal B. Functional polymorphisms of the cytochromeoxygenase (PTGS2) gene and risk for gallbladder cancer in a North Indian population. *J Gastroenterol* 2009; 44: 774-780 [PMID: 19455278 DOI: 10.1007/s00535-009-0071-5]

228 Cha PC, Zembutsu H, Takahashi A, Kubo M, Kamatani N, Nakamura Y. A genome-wide association study identifies SNP in DCC as associated with gallbladder cancer in the Japanese population. *J Hum Genet* 2012; 57: 235-237 [PMID: 22318345 DOI: 10.1038/jhg.2012.9]

229 Rai R, Sharma KL, Tiwari S, Misra S, Kumar A, Mittal B. DCC (deleted in colorectal carcinoma) gene variants confer increased susceptibility to gallbladder cancer (Ref. No.: Gene-D-12-01446). *Gene* 2013; 518: 303-309 [PMID: 23353777 DOI: 10.1016/j.gene.2013.01.019]

230 Jiao X, Ren J, Chen H, Ma J, Rao S, Huang K, Wu S, Fu J, Su X,
Luo C, Shi J, Broelsch CE. Ala499Val (C&gt;G) and the risk of primary gallbladder adenocarcinoma: a case-control study in China. Carcinogenesis 2011; 32: 496-501 [PMID: 21113018 DOI: 10.1093/carcin/bgr250]

243 Tsuichy Y, Baez S, Calvo A, Praytas M, Nakamura K, Kiyohara Y, Oyama M, Ikegami K, Yamamoto M. Evidence that genetic variants of metabolic detoxication and cell cycle control are not related to gallbladder cancer risk in Chinese women. Int J Biol Markers 2010; 25: 75-78 [PMID: 20544687]

244 Kimura A, Tsuichy Y, Lang J, Zolitan S, Nakadaira H, Ajiko Y, Kiyohara Y, Oyama M, Nakamura K. Effect of genetic predisposition on the risk of gallbladder cancer in Hungary. Asian Pac J Cancer Prev 2008; 9: 391-396 [PMID: 18990008]

245 Tsuichy Y, Kiyohara C, Sato T, Nakamura K, Kimura A, Yamaji Y, Ohta M, Seto M, Asaoka Y, Tanaka Y. Polymorphisms and haplotypes in Flap endonuclease 1 and risk of gallbladder cancer and gallstones: a population-based study in Shanghai, China. Sci Rep 2015; 5: 18160 [PMID: 26668074 DOI: 10.1038/srep18160]

246 Xu HL, Hising AW, Vogtmann E, Chu LW, Cheng JR, Gao J, Tan YT, Wang BS, Shen MC, Gao YT, Rashid A, Wang BS, Deng J, Han TQ, Zhang BH, Meyers DA, Fraumeni JF, Hsing AW. Androgen receptor CAG repeat length and risk of developing gallbladder cancer in Japanese. Carcinogenesis 2016; 37: 1342-1348 [PMID: 26838322 DOI: 10.1046/j.1365-2125.1995.01031.x]

247 Meyer TE, O'Brien TG, Andretti G, Yu K, Li Q, Gao YT, Rashid A, Shen MC, Wang BS, Han TQ, Zhang BH, Niwa S, Fraumeni JF, Hsing AW. Androgen receptor CAG repeat length and risk of biliary tract cancer and gallstones. Cancer Epidemiol Biomarkers Prev 2010; 19: 787-793 [PMID: 20200439 DOI: 10.1158/1055-9965. EPI-09-0973]

248 Chang SC, Rashid A, Gao YT, Andreotti G, Shen MC, Wang BS, Han TQ, Zhang BH, Sakoda LC, Leitzmann MF, Chen BE, Rosenberg PS, Chen J, Canchon SJ, Hsing AW. Polymorphism of genes related to insulin sensitivity and the risk of biliary tract cancer and biliary stone: a population-based case-control study in Shanghai, China. Carcinogenesis 2008; 29: 944-948 [PMID: 18375961 DOI: 10.1093/carcin/bgn025]

249 Sakoda LC, Gao YT, Chen BE, Chen J, Rosenberg PS, Rashid A, Deng J, Shen MC, Wang BS, Han TQ, Zhang BH, Cohen-Webb H, Yeager M, Welch R, Canchon S, Fraumeni JF, Hsing AW. Prostaglandin-endoperoxide synthase 2 (PTGS2) gene polymorphisms and risk of biliary tract cancer and gallstones: a population-based study in Shanghai, China. Carcinogenesis 2006; 27: 1251-1256 [PMID: 16361272 DOI: 10.1093/carcin/bgl114]

250 Hising AW, Sakoda LC, Rashid A, Andreotti G, Chen J, Wang BS, Shen MC, Chen BE, Rosenberg PS, Zhang M, Niwa S, Chu L, Welch R, Yeager M, Fraumeni JF, Gao YT, Canchon SJ. Variants in inflammation genes and the risk of biliary tract cancers and stones: a population-based study in China. Cancer Res 2008; 68: 6442-6452 [PMID: 18676870 DOI: 10.1158/0008-5472.CAN-08-0444]

251 Castro FA, Koshiol J, Hising AW, Gao YT, Rashid A, Chu LW, Shen MC, Wang BS, Han TQ, Zhang BH, Niwa S, Yu K, Zhang H, Canchon S, Andreotti G. Inflammatory gene variants and the risk of biliary tract cancers and stones: a population-based study in China. BMC Cancer 2012; 12: 468 [PMID: 23057767 DOI: 10.1186/1471-2407-12-468]

252 Sharma KL, Misra S, Kumar A, Mittal B. Higher risk of matrix metalloproteinase (MMP-2, -9, 7) and tissue inhibitor of metalloproteinase (TIMP-2) genetic variants to gallbladder cancer. Liver Int 2012; 32: 1278-1286 [PMID: 22621573 DOI: 10.1111/j.1478-3231.2012.02822.x]

253 Hou L, Xu J, Gao YT, Rashid A, Zheng SL, Sakoda LC, Shen MC, Wang BS, Deng J, Han TQ, Zhang BH, Meyers DA, Fraumeni JF, Hsing AW. CYP17 MspA1 polymorphism and risk of biliary tract cancers and gallstones: a population-based study in Shanghai, China. Int J Cancer 2006; 118: 2847-2853 [PMID: 16381022 DOI: 10.1002/ijc.21708]

254 Rai R, Sharma KL, Misra S, Kumar A, Mittal B. CYP17 polymorphism (rs743572) is associated with increased risk of gallbladder cancer in tobacco users. Tumour Biol 2014; 35: 6531-6537 [PMID: 24687554 DOI: 10.1007/s13277-014-1876-2]

255 Sharma KL, Agarwal A, Misra S, Kumar A, Kumar V, Mittal B. Association of genetic variants of xenobiotic and estrogen metabolism pathway (CYP1A1 and CYP1B1) with gallbladder cancer susceptibility. Tumour Biol 2014; 35: 5431-5439 [PMID: 24535777 DOI: 10.1007/s13277-014-1708-4]

256 Andreotti G, Chen J, Gao YT, Rashid A, Chen BE, Rosenberg P, Sakoda LC, Deng J, Shen MC, Wang BS, Han TQ, Zhang BH, Yeager M, Welch R, Canchon S, Fraumeni JF, Hsing AW. Polymorphisms of genes in the lipid metabolism pathway and risk of biliary tract cancers and stones: a population-based case-control study in Shanghai, China. Cancer Epidemiol Biomarkers Prev 2008; 17: 525-534 [PMID: 18296645 DOI: 10.1158/1055-9965.EPI-07-2704]

257 Xu HL, Cheng JR, Andreotti G, Gao YT, Rashid A, Wang BS, Shen MC, Chu LW, Yu K, Hising AW. Cholesterol metabolism gene polymorphisms and the risk of biliary tract cancers and stones: a population-based case-control study in Shanghai, China. Carcinogenesis 2011; 32: 58-62 [PMID: 21062971 DOI: 10.1093/carcin/bgq194]

258 Srivastava A, Choudhuri G, Mittal B. CYP1A1 (-204 A&gt;G) and the risk of biliary tract cancers and stones: a population-based case-control study in Shanghai, China. Carcinogenesis 2010; 31: 842-846 [PMID: 20172949 DOI: 10.1093/carcin/bgp038]

259 Meyer TE, O'Brien TG, Andretti G, Yu K, Li Q, Gao YT, Rashid A, Shen MC, Wang BS, Han TQ, Zhang BH, Niwa S, Fraumeni JF, Hsing AW. Androgen receptor CAG repeat length and risk of biliary tract cancer and stones. Cancer Epidemiol Biomarkers Prev 2010; 19: 787-793 [PMID: 20200439 DOI: 10.1158/1055-9965. EPI-09-0973]

260 Chang SC, Rashid A, Gao YT, Andreotti G, Shen MC, Wang BS, Han TQ, Zhang BH, Sakoda LC, Leitzmann MF, Chen BE, Rosenberg PS, Chen J, Canchon SJ, Hsing AW. Polymorphism of genes related to insulin sensitivity and the risk of bile duct cancer and biliary stone: a population-based case-control study in Shanghai, China. Carcinogenesis 2008; 29: 944-948 [PMID: 18375961 DOI: 10.1093/carcin/bgn025]

261 Sakoda LC, Gao YT, Chen BE, Chen J, Rosenberg PS, Rashid A, Deng J, Shen MC, Wang BS, Han TQ, Zhang BH, Cohen-Webb H, Yeager M, Welch R, Canchon S, Fraumeni JF, Hsing AW. Prostaglandin-endoperoxide synthase 2 (PTGS2) gene polymorphisms and risk of biliary tract cancer and gallstones: a population-based study in Shanghai, China. Carcinogenesis 2006; 27: 1251-1256 [PMID: 16361272 DOI: 10.1093/carcin/bgl114]

262 Hising AW, Sakoda LC, Rashid A, Andreotti G, Chen J, Wang BS, Shen MC, Chen BE, Rosenberg PS, Zhang M, Niwa S, Chu L, Welch R, Yeager M, Fraumeni JF, Gao YT, Canchon SJ. Variants in inflammation genes and the risk of biliary tract cancers and stones: a population-based study in China. Cancer Res 2008; 68: 6442-6452 [PMID: 18676870 DOI: 10.1158/0008-5472.CAN-08-0444]
Mittal B. Association of cancer stem cell markers genetic variants with gallbladder cancer susceptibility, prognostic, and survival. *Tumour Biol* 2016; 37: 1835-1844 [PMID: 26318430 DOI: 10.1007/s13277-015-3929-6]

262 Ono H, Chihara D, Chiwaki F, Yanagihara K, Sasaki H, Sakamoto H, Tanaka H, Yoshida T, Saeki N, Matsu K. Missense allele of a single nucleotide polymorphism rs2294008 attenuated antitumor effects of prostate stem cell antigen in gallbladder cancer cells. *J Carcinog* 2013; 12: 4 [PMID: 23599686 DOI: 10.4103/1477-3163.109930]

263 Rai R, Sharma KL, Misra S, Kumar A, Mittal B. PSCA gene variants (rs2294008 and rs2798794) confer increased susceptibility of gallbladder carcinoma in females. *Gene* 2013; 530: 172-177 [PMID: 23988503 DOI: 10.1016/j.gene.2013.08.058]

264 Srivastava K, Srivastava A, Mittal B. Common genetic variants in pre-microRNAs and risk of gallbladder cancer in North Indian population. *J Hum Genet* 2010; 55: 495-499 [PMID: 20520619 DOI: 10.1038/jhg.2010.54]

265 Gupta A, Sharma A, Yadav A, Rastogi N, Agrawal S, Kumar A, Kumar V, Misra S, Mittal B. Evaluation of miR-27a, miR-181a, and miR-570 genetic variants with gallbladder cancer susceptibility and treatment outcome in a North Indian population. *Mod Diagn Ther* 2015; 19: 317-327 [PMID: 26288960 DOI: 10.1007/s40291-015-0159-y]

266 Yadav A, Gupta A, Yadav S, Rastogi N, Agrawal S, Kumar A, Kumar V, Misra S, Mittal B. Association of Wnt signaling pathway genetic variants in codon 25 of the KRAS gene associated with gallbladder carcinoma patients of the eastern part of India. *Genet Test Mol Biomarkers* 2011; 15: 431-434 [PMID: 21375404 DOI: 10.1089/gtmb.2010.0194]

267 Srivastava K, Srivastava A, Mittal B. Angiotensin I-converting enzyme insertion/deletion polymorphism and increased risk of gall bladder cancer in women. *DNA Cell Biol* 2010; 29: 417-422 [PMID: 20438364 DOI: 10.1086/dna.2010.1033]

268 Srivastava K, Srivastava A, Mittal B. DNMT3B -579 G&gt; T promoter polymorphism and risk of gallbladder carcinoma in North Indian population. *J Gastrointest Cancer* 2010; 41: 248-253 [PMID: 20480259 DOI: 10.1007/s12029-010-9156-x]

269 Srivastava K, Srivastava A, Kumar A, Mittal B. Significant association between toll-like receptor gene polymorphisms and gallbladder cancer. *Liver Int* 2010; 30: 1067-1072 [PMID: 20492496 DOI: 10.1111/j.1478-3231.2010.02268.x]

270 Rai R, Sharma KL, Misra S, Kumar A, Mittal B. Association of adrenergic receptor gene polymorphisms in gallbladder cancer susceptibility in a North Indian population. *J Cancer Res Clin Oncol* 2014; 140: 725-735 [PMID: 24556804 DOI: 10.1007/s00432-014-1621-7]

271 Sharma KL., Umar M, Pandey M, Misra S, Kumar A, Kumar V, Mittal B. Association of potentially functional genetic variants of PLCE1 with gallbladder cancer susceptibility in north Indian population. *J Gastrointest Cancer* 2013; 44: 436-443 [PMID: 23975622 DOI: 10.1007/s12029-013-9537-z]

272 Li Z, Yuan WT, Ning SJ, Zhang SJ. Vitamin D receptor genetic variants are associated with susceptibility of gallbladder adenocarcinoma in a Chinese cohort. *Genet Mol Res* 2014; 13: 5387-5394 [PMID: 25078595 DOI: 10.4238/2014.July.24.18]

273 Albores-Saavedra J, Alcaíntra-Vazquez A, Cruz-Ortiz H, Herrera-Goeppert R. The precursor lesions of invasive gallbladder carcinoma. Hyperplasia, atypical hyperplasia and carcinoma in situ. *Cancer* 1980; 45: 919-927 [PMID: 7260842]

274 Roa I, Araya JC, Villaseca M, De Arexabalb X, Riedemann P, Endoh K, Roa J. Preneoplastic lesions and gallbladder cancer: an estimate of the period required for progression. *Gastroenterology* 1996; 111: 232-236 [PMID: 8698204]

275 Vaittinen E. Carcinoma of the gall-bladder. A study of 390 cases diagnosed in Finland 1953-1967. *Ann Chir Gynaecol Fenn Suppl* 1970; 160: 1-81 [PMID: 5268194]

276 Srivastava K, Srivastava A, Mittal B. Potential biomarkers in gallbladder cancer: present status and future directions. *Biomarkers* 2013; 18: 1-9 [PMID: 22913835 DOI: 10.3109/13547560.2012.717105]

277 He CZ, Zhang KH, Li Q, Liu XH, Hong Y, Lv NH. Combined use of AFP, CEA, CA125 and CA19-9 improves the sensitivity for the diagnosis of gastric cancer. *BMC Gastroenterol* 2013; 13: 87 [PMID: 23672279 DOI: 10.1186/1471-230X-13-87]

278 Zur B, Holdeneried S, Walgenbach-Brünagel G, Albers E, Stoffel-Wagner B. Method comparison for determination of the tumor markers AFP, CEA, PSA and free PSA between Immulite 2000 XIIF and Dimension Vista 1500. *Clin Lab* 2012; 58: 97-105 [PMID: 22372351]

279 Zhang D, Yu M, Xu T, Xiong B. Predictive value of serum CEA, CA19-9 and CA125 in diagnosis of colorectal liver metastasis in Chinese population. *Hepatogastroenterology* 2013; 60: 1297-1301 [PMID: 23933921 DOI: 10.5754/hs121125]

280 Koopmann J, Thuluthav P, Zahurak ML, Kristiansen TZ, Pandey A, Schullik R, Argani P, Hidalgo M, Iacobelli S, Goggins M, Maitra A. Mac-2-binding protein is a diagnostic marker for biliary tract carcinoma. *Cancer* 2004; 101: 1609-1615 [PMID: 15378479 DOI: 10.1002/cncr.20469]

281 Huang L, Chen W, Liang P, Hu W, Zhang K, Shen S, Chen J, Zhang Z, Chen B, Han Y, Meng F, DeMorrow S, Yin X, Lai J, Liang L. Serum CYFRA 21-1 in Biliary Tract Cancers: A Reliable Biomarker for Gallbladder Carcinoma and Intrahepatic Cholangiocarcinoma. *Dig Dis Sci* 2015; 60: 1273-1283 [PMID: 25487191 DOI: 10.1007/s00467-014-3472-0]

282 Barreto SG, Dutta A, Chaudhary A. A genetic model for gallbladder carcinogenesis and its dissemination. *Ann Oncol* 2014; 25: 1086-1097 [PMID: 24705974 DOI: 10.1093/annonc/mdu006]

---

P- Reviewer: Barreto S, Lee KT, Suzuki H  S- Editor: Gong ZM  L- Editor: A  E- Editor: Wang CH
