REVIEW

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Modifiable Factors and Genetic Predisposition Associated with Gallbladder Cancer. A Concise Review

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ABSTRACT

Gallbladder cancer (GbCa) is the most frequent malignancy of the biliary tract. It is also the 6th most common gastrointestinal tumor. It is associated with very high lethality, mainly due to the lack of symptoms up to a very late and thus incurable state. As many as 80% of patients are diagnosed at very late stages of disease, which allow only palliative therapy. As a result, most of the patients with GbCa will die within 6 months of the diagnosis, hence the average 5-year survival does not exceed 5%. Currently, surgical resection represents the only curative option in GbCa, but this approach is feasible only at an early stage of the disease. Other oncologic therapies are of limited use. The incidence of GbCa is remarkably increased in certain populations such as Native North Americans, South Indian females and, in Europe, in the Polish population. It is not clear to date if these enhanced risk populations are the result of common environmental exposure or of shared genetic risk factors. In this review we provide an overview of the state-of-art in GbCa research with the focus on the current knowledge concerning genetic and environmental triggers of this tumor.

Key words: biliary malignancy – gallstones – genetic predisposition.

Abbreviations: CI: confidence interval; GbCa: gall bladder cancer; GWAS: genome wide association scan; HCC: hepatocellular carcinoma; HR: hazard ratio; OR: odds ratio; PSC: primary sclerosing cholangitis; RR: relative risk; SIR: standardized incidence ratio.

INTRODUCTION

Gallbladder cancer (GbCa) is relatively rare in developed countries with an estimated overall frequency of about 2-3 cases per 100,000 population [1], but the tumour is highly lethal due to its quiescence, i.e. absence of symptoms, until late in its progression. Thus, survival rates are very low, even below 10% at 5 year [2]. Gallbladder carcinoma is the most common among the biliary tract cancers, accounting for over 80-90% of cases. The remaining patients with biliary tract cancers suffer from cholangiocarcinomas and are further sub-classified as intrahepatic (IHC) when they arise from intrahepatic biliary radicles or extrahepatic (EHC) when they arise from the confluence of the main left and right hepatic ducts or distal in the bile ducts. Gallbladder cancer develops from the epithelium lining the gallbladder wall and is adenomatous in 95% of cases. Once symptoms develop (often unspecific, such as fatigue, upper abdominal pain, weight loss, or, at later stages jaundice, pruritus, cholestasis), the disease has usually progressed beyond curative treatment and the average survival is substantially shorter (4.0 months, 95% CI 3.03 - 5.96) than survival in patients where the tumour is found incidentally during cholecystectomy (11.4 months, 95% CI 5.6 - 13.7, P = 0.01) [3].

The frequency of GbCa is highly variable: the highest numbers have been recorded in Latin America (e.g. 27.3/100,000 in female Mapuche Indians) and in Asia (e.g. 8.1/100,000 in male Koreans) [1]. In Europe, for example, Poland ranks among countries with the highest incidence of this tumor (14/100,000). The frequency of GbCa is up to three times higher among women than men in all populations [4]. Mortality from GbCa has been traditionally high in Eastern Europe, and lower in northern countries. The trends in mortality from gallbladder cancer are likely to be influenced by changes in risk factor exposure, such as diet, tobacco or other environmental factors, but essentially reflect more widespread

and earlier adoption of cholecystectomy in the EU. The data also indicate the need for improving the management of gallbladder disease in Eastern Europe [5]. Death rates from GbCa for the period 1965-1989 from 25 European countries revealed a relatively high-mortality area, i.e. with overall death certification rates around or over 2/100,000 men and 4/100,000 women in Germany and the surrounding central European countries [6]. During the two decades analysed, rates increased in Czechoslovakia and Hungary, remained stable in Poland and decreased in Austria and Germany. Likely, well-funded health systems, offering an easy access to cholecystectomy in gallstone patients, have an impact on GbCa mortality. A study on the Chilean population revealed that the most significant risk factors were female gender, Mapuche Indian origin, and a low education level (schooling < 4yrs). Symptomatic GbCa, particularly when presenting with jaundice, was associated with significantly higher mortality, and the overall median survival was only 3.4 months [7]. Overall, although several therapeutic trials have been performed in GbCa patients, neither chemotherapy nor radiation improved survival significantly. Hence, early detection of GbCa is a *conditio sine qua non* for curative therapy. However, we currently lack rapid cost-effective non-invasive methods that allow diagnosis in patients with GbCa at the early stage. Moreover, in stark contrast to other gastrointestinal tumors, no GbCa-specific serum markers are known at the moment.

ENVIRONMENTAL RISK FACTORS FOR GALLBLADDER CANCER

According to epidemiologic studies, gallstones represent the most important risk factor for GbCa, being present in as many as 96% of patients [8]. Indeed, rates of GbCa increase with the prevalence of gallstone disease, which more commonly affects certain indigenous populations, particularly in North and South America. A case-control study in the Chinese Han population provided strong support for a contribution of identical factors for gallstones and GbCa: family history of gallstones was associated with increased risk of GbCa (OR = 2.1, 95% CI 1.4 - 3.3). Patients with gallstones and a positive GbCa family history displayed a 57-fold increased risk of developing GbCa as compared to the general population [9]. The risk factors for GbCa development in Poland were largely similar to what was seen in the Chilean population, with low education and longstanding gallstone disease (20 yrs or more) being the strongest predictors [10]. Overall, the presence of one or more large gallstones is a major risk factor for GbCa [11]. On the other hand, while gallstone disease is the most significant risk factor for the development of GbCa known to date, prophylactic cholecystectomy is not an effective option, since merely 1% to 2% of gallstone bearers per year develop major complications [12, 13]. Studies suggest that stratification of patients by gallstone size (>3 cm diameter) and persistence (>15 yrs) may provide a cost-effective strategy for GbCa prevention [11, 14]. Indeed, analysis of over 1,600 cholecystectomy reports performed by Lowenfels et al. demonstrated that large stones (e.g. equal or larger than 3.0 cm) were present in 40% of patients with GbCa and in only 12% of patients without this malignancy [15]. Other groups presented comparable findings concerning the size of gallstones and GbCa risk [16]. Gallbladder polyps larger than 1 cm (especially solitary sessile hypoechogenic polyps) are associated with an increased risk of malignancy and in this case a resection is indicated [17]. Of note, some authors suggest prophylactic cholecystectomy for polyps of any size in patients with gallstones (even if silent) [18]. Choledochal cysts and other variations of the biliopancreatic junction are also associated with high risk; cancer may occur at a much younger age and in the absence of gallstones in these patients [19]. Hayes and Muldoon [20] systematically surveyed 2,522 cholecystectomy records from an Irish population and reported similar frequencies of dysplasia (1.15%) and carcinoma (0.48%). These data are in agreement with a Cochrane review on 26 studies including 2,145 patients with a diagnosis of incidental or unsuspected GbCa during or after cholecystectomy, where cancerous lesions were found in 0.7% (95% CI 0.004 - 0.012) [21]. In a small study in an Indian population (310 cases), 27 (8.7%) had acute cholecystitis, 280 (91.2%) chronic cholecystitis, 2 out of 290 cases with cholelithiasis (0.7%) were found to have coexistent histologically proven GbCa [22]. Porcelain gallbladder is a known risk factor for GbCa, particularly when calcifications of the gallbladder mucosa are present [23, 24]. Finally, cancer of both the gallbladder and the bile ducts is more frequent in patients suffering from primary sclerosing cholangitis (PSC).

Apart from gallstones, obesity, inflammation in bile ducts, typhoid, salmonella and, to a lesser extent, *Helicobacter* infections, chronic cholecystitis, gallbladder polyps and female gender are regarded as risk factors for GbCa (Table I) [8, 25-29]. Presence of chronic inflammation is considered as another predisposing factor for gallbladder malignancies. Indeed, inflammation and cancer are tightly linked by various mechanisms such as the production of reactive oxygen and nitrogen species during inflammatory reactions to bacterial invasion or tissue damage [30]. Among chronic inflammatory diseases of the biliary tract, PSC is considered a substantial risk factor for the development of GbCa, possibly due to persistent inflammatory stimuli in the biliary system. Indeed, a survey of malignant liver diseases in patients with PSC and cirrhosis revealed that in contrast to relatively low numbers of hepatocellular carcinomas (HCC), malignancies of the biliary tract, i.e gallbladder cancer (3 in 119 patients) and cholangiocarcinoma (35 patients) were more frequent among PSC patients than in the general population [31]. In line with these results, a Danish study of 4,333 patients with cholangitis revealed a 70-fold increased risk of developing biliary cancer [32]. However, even if the above-cited epidemiological studies provide data concerning risk factors of gallbladder malignancies, most patients with GbCa are not characterised by any of these. Hence, additional modulators seem to play a role in the development of this malignancy.

Dietary patterns were studied in the Chilean population in 2002 [33] to focus on risk factors for GbCa and explain its sharp and constant increase in incidence in Chile since the 1970s. Gallbladder cancer patients differed from controls and had lower socioeconomic status, a much longer history of gallstone disease and a dietary pattern characterized by high red chilli pepper consumption and low fresh fruit intake. These factors may have been confounding variables in an earlier study on

Table I. Selected environmental risk factors in gallbladder cancer (GbCa)

Factor	Study size	Ethnicity	Year and Reference
Typhoid infection	37 GbCa 80 controls	India	2000 [25]
Helicobacter infection	meta-analysis	mixed	2013 [89] 2011 [90] 2015 [27]
Red chili peppers	114 GbCa 114 gallstone patients	Chile	2002 [33]
Aflatoxin	36 GbCa 29 gallstone patients 47 community controls	Chile	2015 [35]
Obesity and insulin resistance	627 biliary tract cancers 1037 biliary stones 959 controls	China	2011 [36]
Environmental toxins	Inhabitants of 148 towns within 5 km of cement and lime factories or waste incinerators	Spain	2015 [37] 2013 [91]
Environmental toxins	10,701	USA	2015 [38]
Alcohol	meta-analysis	mixed	2015 [39]
Smoking	414 GbCa 230 controls	India	2014 [40]
Female sex hormones, number of pregnancies	257 GbCa deaths from a total of 1,292,462 women	Taiwan	2015 [42]

Aymara and Chechua Indios in Bolivia [34] and in Chile [7]. A recent study reported a potential association of aflatoxin contamination in *ají rojo* (red chili peppers) with GbCa in Chile [35]. Aflatoxin is a liver carcinogen associated with bile duct epithelium proliferation in humans and animals. The level of aflatoxin-albumin adducts was used as a substitute measurement for exposure, and was compared between GbCa patients and two sets of controls either with or without gallstones. The presence of aflatoxin adducts was significantly more frequent in patients with gallbladder disease, and levels of adducts were higher in the patients with GbCa (median value 7.6 pg/mg of albumin) compared to aflatoxin-positive gallbladder controls (3.5 pg/mg of albumin) and community controls without gallstones (2.4 pg/mg of albumin) [35]. These data suggest a role for carcinogenic contaminants in the diet and warrants closer examination of food contaminants in other populations with a higher incidence of GbCa.

Studies among indigenous populations in Mexico and Bolivia showed that race was a very strong risk factor for GbCa in Bolivia [34]. Language was used as a surrogate marker for being of South American Indio ethnic origin. Relative to mestizos who spoke neither indigenous language, the odds ratio (OR) for cases versus control subjects without stones for those who spoke Aymara well was 15.9 (95% CI 1.9 - 179), whereas it was 1.4 (95% CI 0.2 - 8.2) for those who spoke Quechua well [34]. This means that gallstone-free individuals who spoke the language of the indigenous Aymara population had a 16-fold risk of developing GbCa compared to gallstone-free individuals from a mixed or European ethnic background, whereas people from the Quechua ethnic group had a relative risk of only 1.4 fold, i.e. rather identical compared to people of European or mixed background. An increased risk was noted for elevated maximum body mass index (P = 0.03), family history of gallstones (OR = 3.6, 95% CI 1.3 - 11.4), and physician-diagnosed typhoid (OR = 12.7, 95% CI 1.5 - 598). A number of associations with certain dietary and cooking habits were also noted [34].

Obesity and insulin resistance were confirmed as risk factors for cholelithiasis and for gallbladder malignancies in an Asian population with metabolic syndrome (OR = 2.75, 95% CI = 1.82-4.15) and biliary stones (OR = 1.64, 95% CI 1.24 - 2.16) [36]. Increasing the number of metabolic syndrome components exerted a significant dose effect (P < 0.0001). The observed association persisted among subjects without a history of diabetes, while association between insulin resistance and GbCa was borderline (P trend = 0.06) [36]. Considering the worldwide increase in the rates of obesity, we expect a concomitant rise in gallstones and, at least in countries where obesity is not matched by the widespread availability of cholecystectomy and yet-to-come targeted intervention in high-risk cases, a rise in GbCa rates.

A recent investigation in Spain [37] studied cancer incidence and mortality due to 33 types of cancer (period 1997-2006) in towns in the vicinity of installations for the production of cement, lime, plaster, and magnesium oxide. Population exposure to pollution was estimated on the basis of distance from town to industrial facility. The third highest associated risk ratio (RR = 1.21, 95% CI 1.02 - 1.42) was found for GbCa in men, indicating a small but significant contribution of environmental factors apart from food and beverages. In a study of cancer mortality in the meat and delicatessen departments of supermarkets (1950-2006), increased standardized mortality ratios (SMR) from gallbladder and biliary tract cancer were detected in white male meatcutters [38] with a relative risk of 2.8 (95% CI 1.3 - 5.3) compared to the general US population. Alcohol consumption and abuse is a risk factor for many cancers, including GbCa: a meta-analysis of site-specific cancer risk in heavy drinkers including 486,538 subjects from 572 studies, revealed a significantly higher risk of, among various other cancer types, GbCa (RR = 2.64). The only cancer types with a higher risk ratio conferred by alcohol were oral and pharyngeal cancer (RR = 5.13), oesophageal squamous cell carcinoma (RR = 4.95) and laryngeal cancer (RR = 2.65). The relative risk of liver cancer was lower at RR = 2.07 [39]. These data need to be considered in view of the fact that smoking is a significant co-morbidity in alcohol drinkers. The hypothesis that the increased risk ratio for GbCa in drinkers compared to liver cancer might be due to an additional impact of smoking via xenobiotics contained in cigarette smoke and exported via bile is supported by a small study in an Indian population: Rai et al. describe the association of a polymorphism (rs743572) in the CYP17 xenobiotic metabolism pathway with GbCa in smokers [40]. Of note, the CYP17 is also an important enzyme in estrogen and testosterone hormone biosynthesis, which have been implied in the aetiology of GbCa by a strong female bias [41].

The role of female sexual hormones was confirmed by a study into GbCa risk and parity in a Taiwanese population,

confirming an increasing number of pregnancies as a risk factor. The authors followed 1,292,462 women who were pregnant with their first child between 1978 and 1987. Altogether, there were 257 GbCa deaths during 34,980,246 person-years of follow-up, a mortality rate of 0.73 cases per 100,000 person-years. Compared with monoparous women, the adjusted hazard ratio (HR) was 1.20 (95% CI 0.79 - 1.83) for women who had two children, 1.47 (95% CI 0.95 - 2.29) for women who had three children, and 1.68 (95% CI 0.99 - 2.85) for women with four or more births [42].

HERITABILITY OF GALLBLADDER CANCER

As we outlined in a previous publication [43], hepatic malignancies develop as a result of interaction between environmental and genetic factors (Fig. 1). Evidence for this combinatorial interaction in the aetiology of GbCa comes for example from Korean males, who show a relatively high incidence of this malignancy when resident in Korea (8.1/100000), and maintain a slightly lower but still relatively high frequency of GbCa after emigration to the United States (5.1/100,000) [44].

More evidence of genetic contribution comes from a study of Chinese Han in Shanghai, which showed that an individual with gallstones and a family history of GbCa had more than double the risk of developing GbCa compared to an individual with gallstones but without a family history. The risk in the former was increased 21 fold compared to the general population, whereas it was increased 57 fold in the latter [9]. The authors confirmed that the association with a family history of gallstones was seen in all first-degree relatives, including parents, siblings and offspring, but not in spouses, who in this case represent a valuable control population exposed to a shared environment [9]. To our knowledge, no twin studies have been published to date, which is not overly surprising in view of the relative rarity of GbCa and the late age of onset.

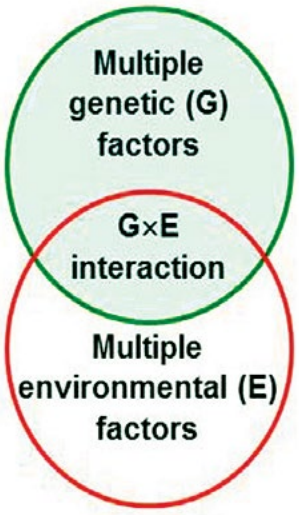

Fig. 1. Hepatic malignancies result from interaction between genetic predisposition and environmental triggers. Single polymorphisms might have only minor effect on the cumulative disease risk. However, their presence coupled with environmental determinants can substantially enhance the overall risk of developing neoplasia. Adapted from Krawczyk/Lammert 2010 [43].

Hemminki and Li studied the Swedish Family-Cancer Database covering 10.2 million individuals for the years 1961–1998 from the Swedish Cancer Registry [45]. Altogether, liver and biliary tract cancers were identified in 1,121 offspring between 0 and 66 years of age, and 17,131 parents. The standardised incidence ratio (SIR) for familial GbCa was 5.21 (95% CI 2.07 - 10.80). The risk to offspring from parental gallbladder cancer ranks second only to thyroid cancer among all main cancers in the Family-Cancer Database [46]. Considering the relatively late age of onset for GbCa, a longer observational period for the offspring may increase the incidence, but diminish the familial incidence ratio due to the increased contribution from „random" cancer of old age as outlined by Tomasetti and Vogelstein [47]. To assess the contribution of shared environmental factors towards increased family risk, the researchers compared GbCa rates between spouses, who share a common environment but no genetic factors. No spouse effect was observed for GbCa, indicating a prominent role for inheritable factors, particularly for relatively early onset' GbCa [45]. Although the authors speculated that „*the demonstration of candidate genes would help to further characterise the familial risks*", no major studies into the genetic aetiology of GbCa have materialised to date.

Interesting epidemiological studies were performed by Lisa Cannon-Albright and colleagues in Salt Lake City/Utah, who attempted to quantify the genetic component of GbCa in a study of the Utah Population Database (UPDB) that links Utah genealogy to the Utah Cancer Registry (UCR) [48]. In this database, approximately 100,000 UCR patients are linked to a genealogy of nearly 2.5 million Utahns. The group estimated a relative risk in 1st, 2nd and 3rd degree relatives, and identified high-risk GbCa pedigrees. Altogether, 364 GbCa patients were identified, with 26% (94/364) having at least 1 affected relative and a significant excess of cases within their pedigree. Notably, 1st and 2nd degree relative risks were not significantly elevated, whereas significantly elevated relative risk (RR 2.28, 95% CI 1.35-3.60) was observed among 33,351 3rd degree relatives. Based on the incidence of pedigrees with increased GbCa incidence, the group estimates that about 26% of cases are caused by genetic factors [48]. The observation of familial clustering had previously been hindered by the late onset of disease and the observed lack of effect in first-degree relatives. The same group studied the incidence of non-breast cancer in 173 breast-ovarian cancer families with BRCA2 mutations at 20 centers in Europe and North America. The risk of gallbladder and bile duct cancer was increased five-fold (RR 4.97, 95% CI 1.50-16.52), suggesting common genetic factors underlying both cancer types [49].

POTENTIAL SUSCEPTIBILITY GENES FOR GALLBLADDER CANCER

Nevertheless, to date no major and frequent genetic triggers of GbCa have been detected. A substantial number of case-control association studies in patients from India and China are providing interesting leads as to candidate gene SNPs for GbCa susceptibility. Results from most of these studies have been comprehensively reviewed previously [50], thus we are

not presenting a full list of candidate association studies in this review but rather an exemplary selection. Variants in genes for, among others, estrogen receptors [51], cholecystokinin type A receptor [52], adrenergic receptor [53] or the cholesterol hemitransporter ABCG8 [54, 55] increase the risk for GbCa by about 2-3 fold (Table II). Such associations, however, have only been found in single populations and need to be confirmed in larger populations of different ethnicity. A systematic review and, where sufficient data available, meta-analysis of 80 candidate gene variants and 173 polymorphisms among 1046 cases and 2310 controls revealed that most studies were underpowered or, as observed for 8-oxoguanine DNA glycosylase (*OGG1*, rs1052133), *TP53* (rs1042522), *CYP1A1* (rs1048943) and glutathione S-transferase mu 1 (*GSTM1*) Null polymorphisms, could not confirm previous association results upon meta-analysis [50].

Since gallstones represent a significant risk factor for GbCa, the *Lith* genes predisposing to gallstones represent creedal genetic risk factors [56]. Several genes have been associated with the development of human gallstone disease [57]. Candidate genes tested in human cohorts include for example the apolipoproteins E (*APOE*) [58] and B (*APOB*) [59, 60], cholesterol 7 α-hydroxylase [61, 62], cholecystokinin receptor A (*CCKAR*) [63], the low density lipoprotein receptor related protein-associated protein (*LRPAP1*) [64] and the cholesterol ester transporting protein (*CETP*) [65]. Finally, genome-wide association analyses and functional studies have identified the p. D19H variant of the hepatic cholesterol hemitransporter (*ABCG8*) associated with susceptibility for human gallstones [55, 66, 67]. To our knowledge the only genome-wide association study (GWAS) of patients with gallbladder cancer published to date (https://www.ebi.ac.uk/gwas/) was conducted in a relatively small Japanese discovery cohort of 41 GC patients and 866 controls, and a subsequent validation cohort of 30 cases and 898 controls [68]. A strong positive signal (combined $P = 7.46 \times 10^{-8}$ OR=6.95; 95% CI=3.43-14.08) was detected for rs7504990 within the *DCC* gene (deleted in colorectal cancer) on chromosome 18q21.3. A subsequent SNP study using 4 variants from the same region in 406 Indian GbCa cases and 260 controls confirmed the role of this gene in the pathogenesis of GbCa and identified a risk haplotype that might be useful for patient stratification [69]. A recent study [70] reports two new genes associated with the development of gallstones due to obesity. Analysis of a cohort of 15,241 women of European ethnicity using a focused 50,000 SNP array inquiring selected genes involved in cardiovascular disease [CVD bead chip containing up to ~ 53,000 single-nucleotide polymorphisms (SNPs) from over 2000 selected genes] detected strong associations of 13 SNPs in and around the ABCG5/G8 locus. Two SNPs from the tetratricopeptide repeat domain 39B (*TTC39B*) gene and one SNP from the glucokinase (hexokinase 4) regulator (*GCKR*) also showed significant associations. When lowering the threshold for significance, a further 13 gallstone-associated genes were identified [70], some of which may deserve closer scrutiny as candidates for the development of GbCa due to their involvement in either inflammation (chemokine (C-C motif) ligand 22, *CCL22*) and/or metabolism (*CYP4F2*). To add biological plausibility to the candidacy for gallbladder disease, the authors confirmed that *CCL20* (highest expression in gallbladder), ABCG5, *ABCG8, CYP4F2, GCKR*, runt-related transcription factor 1 *RUNX1* and *TTC39B* all showed predominant expression in hepato-biliary and gastrointestinal tissues [70]. Among all genes that predispose to the development of gallstones or bile transport abnormalities, there are various molecules involved in metabolism and transport of xenobiotics or toxic drug compounds, such as the UGT transferases [71]. Besides their potential role in nucleating cholesterol stone formation [71, 72], they may also contribute to a more toxic bile constitution and possibly trigger the development of biliary malignancies but this hypothesis still needs to be confirmed in large studies. Various association studies in different populations confirmed a potential role for *CYP1A1*, which metabolises among others estrogen and xenobiotics, in Indian, Japanese and Hungarian cases [73-76]. A meta-analysis of 268 studies examining the role of *CYP1A1* polymorphisms in cancer susceptibility indicated that further investigation of the role of this polymorphism in gallbladder cancer are warranted [77]. There is also a question in the matter of the selection of controls for genetic studies of GbCa. Considering that the aetiology of gallstones is about 25% genetics/predisposition plus 75% environment [78], any association study should use a GbCa-free population of gallstone-sufferers as a control group to address the genetic factors that predispose to cancer rather than gallstone development. With an age of onset around 60 years of age, a suitable control population has to be most likely drawn from octagenarians with gallstones, which is a tedious but feasible option. The actual paucity of GWAS studies for GbCa might in part be caused by the difficulties of recruiting a sufficiently large, well-matched gallstone-bearing control group. The usefulness of stratification by presence or absence of gallstones was shown by Sharma et al. [79] when 410 gallbladder cancer cases and 230 healthy controls from North India were analysed for the association of genetic variants in matrix metallproteinases (*MMP-2*, *7* and *9*) and tissue inhibitor of metallproteinase (*TIMP-2*) with gallbladder disease. Different associations were found in patients with or without accompanying gallstones, indicating differences in the genetic aetiology between both entities. Unfortunately, no gallstone-bearing but cancer-free controls were available.

Another classic way of identifying candidate genes and regions for cancer is the cytogenetical analysis of non-tumor chromosomes. A cytogenetical analysis of blood cells from 30 patients with GbCa revealed deletions and or translocation involving chromosome 1 in 4 samples, while a t(4;6) was observed in a single case [80]. Considering the paucity of cytogenetic studies in GbCa, further in depth analyses of translocations and/or deletions in patients seem justified, particularly because technical progress in array-CGH or Sky-FISH enables a better characterisation of samples from cultured blood or primary tumour cells [81, 82]. Cytogenetic analysis of five primary GbCa and two metastases has shown that the most frequently affected areas during carcinogenesis involved chromosomes 7, 1, 3, 11, 6, 5 and 8 [83]. While some of these areas harbour the usual suspects such as P53, the co-localisation of pre-cancerous aberrations and tumour-specific translocation on the long arm of chromosome 1 might be suggestive of an „early locus" in the aetiology of GbCa.

Table II. Genetic analyses in gallbladder cancer (GbCa)

Genes tested	Method	Patient numbers	Ethnicity	Odds ratio (OR) and P-value	Year and reference
ABCG8 D19H (rs11887534)	PCR-RFLP	171 GbCa 221 controls	North Indian	OR = 1.79 p = 0.011	2009 [54]
CYP1A1 (2 SNPs) *GSTM1* deletion, TP53 Arg72Pro	n.a.	37 GbCa 100 controls	Hungarian	*CYP1A1* Ile/Val OR = 4.4	2008 [74]
Death receptor DR4 (3 SNPs), FAS & FASL (1 SNP each)	Taqman	400 GbCa 246 controls	North Indian	„twofold" for DR4 haplotype, negative for FAS and FASL	2014 [92]
CYP1A1 (2 SNPs) and *CYP1B1* SNP	Taqman	410 GbCa 230 controls	North Indian	*CYP1A1* Val P = 0.006 *CYP1B1* negative	2014 [73]
CYP1A1 MspI	PCR-RFLP	142 GbCa 171 controls	North Indian	OR = 2.3 p = 0.026 OR = 4.2 p = 0.001 in males	2008 [76]
CETP, *ABCG8*, and *LRPAP1*	Taqman	439 biliary tract cancer (253 GbCa) 429 biliary stones 447 controls	Chinese	*ABCG8* rs11887534 OR = 4.3 for GbCa OR = 2.3 for gallstones	2011 [55]
CYP17 (rs2486758 and rs743572)	Taqman	414 GbCa 230 controls	North Indian	risk in tobacco users	2014 [40]
ADRA2A C-1291G, *ADRβ3* T190C or *Trp64Arg*, and *ADRβ1* C1165G or Arg389Gly	PCR-RFLP	400 GbCa 230 controls	North Indian	*ADRβ3* T190C OR = 2.7 p = 0.014	2014 [53]
PSCA (rs2294008 and rs2978974)	Taqman	405 GbCa 247 controls	North Indian	OR = 1.6 P = 0.021 (in females)	2013 [93]
CD44 (4 SNPs)	PCR-RFLP Taqman	405 GbCa 200 controls	North Indian	OR = 0.47 P = 0.04	2014 [94]
PLCE1 (rs2274223 and rs7922612)		416 GbCa 225 controls	North Indian	OR = 1.9 P = 0.002; OR = 2.0 P = 0.04	2013 [95]
VEGF (4 SNPs)	ARMS and PCR-RFLP	195 GbCa 140 cholecystitis 47 XGC 300 controls	North Indian	g.43737830A>G (OR = 1.48) c.*237C>T (OR = 1.63)	2013 [96]
LXR-α (rs7120118) and *LXR-β* (rs35463555 and rs2695121)	Taqman	400 GbCa 200 controls	North Indian	*LXR-β* (rs35463555) (OR = 1.46 P = 0.03) *LXR-β* (rs2695121) (OR = 1.52 P = 0.01)	2013 [97]
ApoB-100	PCR-RFLP (Xba digest)	Meta analysis	Chinese, Indian, Caucasian	OR = 2.37	2013 [98]
CCK (cholecystikinin) and *CCKAR* (9 SNPs)	Taqman	439 biliary tract cancer (253 GbCa) 429 biliary stones 447 controls	Chinese	*CCKAR* rs1800855 (OR = 2.37 P = 0.0056)	2013 [52]
50000 SNPs (GWAS)	Array hybridisation	71 GbCa 1764 controls	Japan	*DCC* rs7504990 (OR = 6.95, p = 7.46×10^{-8})	2012 [68]
DCC (4 SNPs)	PCR-RFLP Taqman	406 GbCa 60 controls	North Indian	*DCC* rs714 (OR = 1.37 p = 0.01)	2013 [69]
Estrogen (*ESR1*, *ESR2*) and progesterone (*PGR*) receptors	PCR-RFLP	410 GbCa 230 gallstones 220 controls	North Indian	*ESR1*-397TT (rs2234693) OR = 1.8 worst combination *ESR1-ESR2*: OR = 3.9	2012 [51]
MMP2 (2 SNPs), *MMP7* (rs 11568818) *MMP9* (3 SNPs) and *TIMP2* (rs8179090)	PCR-RFLP	410 GbCa 230 controls	North Indian	*MMP-2* c.735C>T [CT] OR = 1.85, *MMP-2* c.1306C>T [CT] OR=1.8, *MMP-9* p.R668Q [RQ] OR = 1.9, *TIMP2* c.418G>C [GC] OR = 1.7	2012 [79]
Motilin, Somatostatin and their receptors	Taqman	439 biliary tract cancer (253 GbCa) 429 biliary stones 447 controls	Chinese	SSTR5 rs169068 (OR = 2.40) for extrahepatic bile duct cancer	2014 [99]
MMP-2 (2 SNPs), MMP-9 (3 SNPs), TIMP-2 (rs8179090), CYP1A1 (2 SNPs), CYP1B1 (rs1056836), LXR-α (rs7120118), LXR-β (2 SNPs)	Taqman and PCR-RFLP	400 GbCA 200 controls	North Indian	Various SNPs associated, with higher order interactions included	2014 [100]

Although the last cancer cytogenetic study was published 16 years ago [83], no concerted candidate gene search has been reported in this area to date. Confirmation of 1q as a potential early event in 2012 [80] may provide the necessary impetus for a focussed study in the respective region. A comparative analysis of gene transcription and loss of chromosomal material by comparative genome hybridisation (CGH) in various types of biliary cancers revealed various common regions of genetic loss and amplification as well as specific and common transcriptional alterations. Transcriptome analysis revealed 165 common genes with significantly altered expression in all three biliary tract cancer subtypes, while 790 genes were shown to be altered in GbCa only [84]. The high level of transcriptome and structural alterations observed in this study and others might reflect the late stage at which GbCa is generally detected.

CONCLUSIONS AND FUTURE DIRECTIONS

Several lines of evidence suggest a role for environmental risk factors in GbCa, while no common genetic risk factors have been identified. In a recent investigation, GbCa was considered one of the cancers „due to bad luck", i.e. where the frequency of cancer roughly correlates to the number of doublings of the respective stem cell niche [47]: according to this assessment, roughly two-thirds of the variation in adult cancer risk can be explained primarily by "bad luck," when random mutations occur in genes that can drive cancer growth. The remaining cancer cases are attributed to environmental factors and inherited genetic predisposition. These assumptions need to be verified and considered in future assessments of variable population incidence. Cao et al. [85] replied recently that a combination of tissue metabolic rate and the level of oxidative stress, estimated from expression levels of oxidative-stress response genes, provide a better correlation with cancer rates, with a correlation strength of R2 = 0.96 for 13 leading cancer types in 21 countries compared to R2 = 0.65 for the „stem cell doubling" model. In line, the exome-sequencing-based study by Jiao et al. showed that the mutation profile of GbCa is low with *TP53* and *PI3K* found among the most frequent mutations [86]. This observation might underscore the role of the gallstone-related inflammation and the involvement of epigenetic (environmental) factors in carcinogenesis of GbCa.

Given the decreasing costs of genotyping, one can predict more in-depth genetic analysis, which will provide additional information on the inheritable component of GbCa. As mentioned above, according to our knowledge, only a single, small GWAS has been performed in patients with this tumor yet [68]. Although GWAS analyses can identify only common variants (i.e. present in more than 1% of general population), which usually convey relatively low disease risk, this approach proved successful in identifying several genetic contributors towards liver diseases [43]. It is our belief that only the scarcity of GbCa in the general population has prevented a genome-wide approach to date. When looking for differences in allele frequencies, the usual selection criteria for „healthy controls" may be misleading, since when compared to GbCa sufferers, they most likely will yield positive signals in genes that predispose for gallstone development. Thus, any GWAS trying to address factors underlying GbCa predisposition might need to compare allele frequencies between tumour bearers and age-matched gallstone patients without tumour. Apart from the GWAS, genetic studies using next-generation sequencing techniques of selected genes or even of the whole genome [87] may further help to pin-point the heritability of GbCa. Such studies might also improve our knowledge regarding the pathogenesis of this highly lethal malignancy and finally result in improved prevention and treatment strategies.

Clinical experience shows that groups of patients with longstanding gallstone disease are at increased risk of developing gallbladder cancer [88]. Since excessive preventative cholecystectomy is not a viable option, as it would put an enormous financial strain on the healthcare system, the combination of risk assessment by genetic screening and analysis of biomarkers seems a feasible strategy to tackle this unmet need in the future. Particularly, in view of the ever-decreasing cost of DNA sequence analysis, genetic screening is a stratification option with a high promise. Multiplex cytokine and chemokine assays are being tested and evaluated for risk assessment and prediction of gallstone patients. Once we have linked specific genotypes and serum biomarkers with an increased risk of GbCa, we will be able to focus predictive screening efforts to a subset of individuals bearing higher GbCa-risk and eventually conceive a risk matrix that combines gallstone predisposition and GbCa predisposition with lifestyle and environmental factors. Such an approach could lead to novel cost-effective stratification and surveillance strategy with the aim to screen and survey patients with a suspicion of gallbladder malignancy based on a personal risk profile according to known genetic and environmental risk factors.

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