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Germline Alterations in Patients with Biliary Tract Cancers: A Spectrum of Significant and Previously Under Appreciated Findings

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Abstract

Background: With limited information on germline mutations in biliary tract cancers, we performed somatic and germline testing in patients at Memorial Sloan Kettering Cancer Center (MSK) with known biliary tract carcinoma with the aim to determine the frequency and range of pathogenic germline alterations (PGA).

Methods: Patients with biliary tract carcinoma were consented for somatic tumor and matched blood testing of up to 468 genes using the MSK – Integrated Mutation Profiling of Actionable Cancer targets (MSK-IMPACT) next-generation sequencing platform. Germline variant analysis was performed on a panel of up to 88 genes associated with increased cancer predisposition. Demographic and diagnostic details were collected.

Results: Germline mutation were tested in 131 patients. Intrahepatic cholangiocarcinoma was the most common cancer (63.4%), followed by gallbladder adenocarcinoma (16.8%), extrahepatic

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cholangiocarcinoma (16%) and otherwise unspecified (3.8%). Known and likely PGAs were present in 21 (16.0%) of patients, with 9.9% of patients harboring a PGA in a high/moderate-penetrance cancer predisposition gene. Among high-penetrance cancer susceptibility genes, PGA were most commonly observed in *BRCA1* and *BRCA2* (33.3%), which makes 5.3% of entire cohort, followed by *PALB2*, *BAP1*, and *PMS2*. Mutations in *ATM*, *MITF* and *NBN*, moderate-penetrance cancer susceptibility genes, were identified in 1 patient each. There was no observed difference in the types of mutations among the subtypes of biliary tract cancer.

Conclusion: The frequency of PGAs found was comparable to existing data on the prevalence of germline mutations in other solid tumor types with matched tumor analysis providing support for the role of *BRCA1/2*, *ATM*, and *BAP1* genes in biliary tract cancer susceptibility.

Precis:

The frequency of pathogenic germline alterations is comparable to existing data on such prevalence in other solid tumor types with matched tumor analysis. This provides support for the role of *BRCA1/2*, *ATM*, and *BAP1* genes in biliary tract cancer susceptibility.

Keywords

BRCA1; BRCA2; ATM; BAP1; biliary tract cancer

Background

Biliary tract carcinoma describes a category of cancer types including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma and gallbladder carcinoma. These are relatively rare tumor types with variable incidences worldwide, although the global incidence of intrahepatic cholangiocarcinoma, the most common biliary tract cancer, has increased in recent decades (1). According to the American Cancer Society, the number of new gallbladder and biliary cancer cases for 2018 was estimated at 12,190 with 3,790 deaths (2). Known risk factors include inflammatory states of the biliary tract attributable to gallstones, hepatobiliary flukes, viral hepatitis and primary sclerosing cholangitis, but these conditions are not required for the development of carcinoma (3). Outcomes for biliary tract carcinomas are poor due to advanced stage disease at detection and modest response to currently available systemic therapies. The 5-year survival is approximately 10% for cholangiocarcinoma and less than 5% for gallbladder carcinoma (4,5). Current treatment recommendations for biliary tract carcinomas include surgery for local disease and combination chemotherapy of gemcitabine plus cisplatin for advanced disease and targeted therapies for selected individuals depending on somatic and or germline findings (3). Median overall survival for patients receiving palliative chemotherapy is less than one year (6).

The utility of next-generation sequencing has revealed somatic genetic alterations in biliary cancer that are important prognostic factors and potentially predictive of treatment response and survival (7). Nearly all patients with biliary tract cancer will have at least one oncogenic somatic molecular alteration identified by next generation sequencing analysis (8). The most commonly mutated genes are *CDKN2A*, *TP53*, and *KRAS* with estimated incidence at over

20% of patients with biliary tract tumors. *CDKN2A* mutations have been associated with poor progression-free survival and *TP53* and *KRAS* mutations have been linked to poor overall survival among patients with intrahepatic cholangiocarcinoma (9). Our group and others have shown that within intrahepatic cholangiocarcinoma, the most prevalent biliary tract cancer, *IDH1/2*, *ARID1A* and *BAP1* mutations have been commonly observed (7, 10). *IDH1* and *IDH2* mutations, found almost exclusively in intrahepatic cholangiocarcinomas, represent an important example of a potentially actionable mutation for which there are several ongoing clinical trials (9, 11, 12). In addition, *FGFR2* rearrangements and *HER2* mutations are potentially actionable mutations found in patients with cholangiocarcinoma for which there are with available targeted therapies (13). Overall, the somatic genetic findings in biliary tract cancer have yielded important detail that drives further research for targeted treatments.

Limited research has been published regarding the prevalence of identifying underlying germline alterations in patients with biliary tract carcinoma. In addition, guidelines for genetic counseling and testing are not established in these tumor types. Germline mutations in *BRCA1/2* mutations have been identified in a small subset of cholangiocarcinoma cases, which influence the choice of targeted therapy for these tumors (14). Here, we aimed to define the prevalence of pathogenic germline mutations in an unselected group of patients with biliary tract cancers with and without a personal or family history of cancer to better understand the frequency and spectrum of pathogenic germline mutations in known cancer susceptibility genes.

Methods

We analyzed the paired blood sample of patients with biliary tract carcinomas receiving care at Memorial Sloan Kettering Cancer Center (MSK) and who consented to somatic and germline genetic testing under the Memorial Sloan Kettering - Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) next-generation sequencing part of an institutional review board-approved protocol (NCT01775072). The MSK-IMPACT (IM) platform is a next-generation sequencing assay that includes 341 (IM3), 410 (IM5) and 468 (IM6) gene panel for somatic tumor testing, including known treatment targets and those under investigation (15, 16). Tumor DNA was derived from formalin-fixed and paraffin-embedded tissue. Matched normal DNA was derived from blood samples.

Analysis for germline mutations was performed for a panel of up to 88 genes known to be associated with cancer predisposition (17). The germline testing was performed using the matched peripheral blood sample.

Somatic and germline mutations including likely pathogenic (LP) and pathogenic (P) genetic variants uncovered during testing, were reported to the treating physicians and patients with subsequent genetic counseling provided to each individual with a LP/P variant by the MSK Clinical Genetics Service. Information including demographic information, pathology staging, personal history of other malignancies, and first and second degree family history of malignancy were ascertained through review of the medical records. Results were summarized with descriptive statistics. Similarly, germline mutations were analyzed

distributively according to gene penetrance, and by cancer type. This project was approved by the Institutional Review Board (IRB) at MSK.

Results

Patient Demographics

From January 2016 to June 2017, a total of N=149 patients with a diagnosis of intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma or gallbladder carcinoma, were consented to somatic and germline testing. One hundred and thirty one patients, who completed both somatic and germline testing, were included in this study. Somatic testing was not completed for 18 patients, because they either lacked sufficient tumor tissue, were lost to follow up, or who passed away shortly after consent. The median age of the entire cohort of N= 131 was 59 years of age. Eighty three patients (63.4%) had intrahepatic cholangiocarcinoma with a median age of 59 years. Twenty one patients (16.0%) had extrahepatic cholangiocarcinoma with a median age of 64 years. Twenty two patients (16.8%) had gallbladder adenocarcinoma with a median age of 58 years. Five patients (3.8%) had a cholangiocarcinoma but the origin (intrahepatic or extrahepatic) was undetermined. Table 1 and Table 2 include detailed demographic and clinical data of the study population broken down by specific tumor type. Twenty-one patients (16.0%) were identified to have an underlying germline mutation.

Distribution of Germline Mutation According to Gene Penetrance

High-Penetrance Genes—The high-penetrance cancer susceptibility genes most commonly mutated were found in patients with *BRCA2* (N=5) and *BRCA1* (N=2). As detailed in tables 3–5, three PGA in *BRCA1/2* were observed in patients with intrahepatic cholangiocarcinoma, one in a patient with extrahepatic cholangiocarcinoma, and three in patients with gallbladder cancer. Three of these mutations were in patients of known Ashkenazi Jewish descent and two of the three were Founder mutations. Notably, three of the PGA in *BRCA1/2* were found in patients of Asian descent, 1 with *BRCA1* and 2 with *BRCA2*. Of 10 patients with PGA in high-penetrance genes, 8 reported family history of cancer, including 5 in at least one first-degree relative. In addition, 4 patients had a personal history of cancer, including a patient with early-onset bilateral breast cancer and a PGA in *BRCA2*, and a patient with ovarian cancer and a PGA in *BRCA2*. Other high-penetrance cancer susceptibility genes that were identified were *PALB2*, *BAP1*, and *PMS2*; all three in patients with intrahepatic cholangiocarcinoma (Table 3). Assessment of the matched tumors revealed loss of heterozygosity in 2 patients with *BRCA1* mutations, 1 with a *BRCA2* mutation, and 1 with a mutation in the *BAP1* gene providing further evidence of the likely role of these germline mutations in cancer development. Notably, the patient with a *PMS2* germline mutation had a microsatellite stable tumor, suggesting that the *PMS2* mutation may not have been causative of the cancer.

Moderate-Penetrance Genes—Among the moderate-penetrance genes, mutations were identified in *ATM*, *MITF*, and *NBN* with one each in 3 patients with intrahepatic cholangiocarcinoma (Table 3). Loss of heterozygosity was noted in the tumor of the patient harboring an *ATM* mutation.

Low-Penetrance Genes—Low-penetrance mutations included *APC* *T1307K* (N=3), a known Ashkenazi Jewish founder mutation associated with an approximate 2-fold increased risk of colorectal cancer, and monoallelic *MUTYH* carriers, was observed in 3 patients.

Recessive carrier genetic changes—Although mutations in *FH* are generally associated with the autosomal dominant Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC), in our cohort, two patients were found to harbor the c.1431_1433dupAAA small duplication which does not cause HLRCC, but rather represents carrier status for the autosomal recessive condition, fumarate hydratase deficiency.

These findings were thought to be incidental germline findings unrelated to the patients biliary cancers (Table 3 and Table 4).

Distribution by Disease

Intrahepatic cholangiocarcinoma—Within the group of 83 patients with intrahepatic cholangiocarcinoma, 12 patients (14.5%) were found to have a pathogenic germline mutation (PGA). These 12 mutations were observed in 10 different genes including *BRCA1*, *BRCA2*, *MUTYH*, *BAP1*, *PMS2*, and *APC*. A detailed look at the spectrum of mutations found in intrahepatic cholangiocarcinoma, the largest cohort patients in this study, can be seen in Table 3. The median age of diagnosis for patients with a germline mutation and intrahepatic cholangiocarcinoma was 51 years of age, as compared to the median age of the patients without a mutation, which was 59 years. Three of the patients with intrahepatic cholangiocarcinoma and a PGA had a personal history of another primary cancer, including both patients with *BRCA2* mutations. Seven of the patients had a family history of cancer in at least one first degree relative. Of the 7 patients with positive 1st degree family history, 2 had high penetrance mutations, 2 had moderate penetrance mutations, 3 had low penetrance mutations. Four patients had no family history of cancer in a first degree relative and all 4 had high penetrance mutations (*BAP1*, *BRCA2*, *PALB2*, *PMS2*). One patient had an unknown family history.

Extrahepatic cholangiocarcinoma—Four of 21 (19.0%) patients with extrahepatic cholangiocarcinoma were found to have a PGA. These alterations were identified in *APC*, *BRCA1*, *FH*, and monoallelic *MUTYH*. Detailed information regarding these patients is found in Table 4. The median age of diagnosis for patients with a mutation and extrahepatic cholangiocarcinoma was 65 years of age, as compared to the median age for germline wildtype patients with extrahepatic cholangiocarcinoma, which was 62. None of the patients with underlying mutations had a personal history of another primary cancer, however all four patients had at least one first degree relative with a history of cancer. Of these 4 patients, one had a high penetrance mutation, two had a low penetrance mutations and one had a recessive mutation.

Gallbladder adenocarcinoma—Five of the total of 22 (22.7%) patients with gallbladder adenocarcinoma were identified to have a PGA. Three patients had a germline *BRCA2* mutation. The other two mutations were found in the *APC* and *NBN* genes. Table 5 has information on the five patients with pathogenic germline alterations and gallbladder

adenocarcinoma. The median age of diagnosis was 53 in patients with a PGA, and 61 in patients with gallbladder adenocarcinoma and no underlying germline mutation. Two of the five patients with a PGA and gallbladder adenocarcinoma had a personal history of cancer. Of these two patients, one had a high penetrance mutation while the other had a low penetrance mutation. Four out of the five patients had no family history of cancer in first degree relatives. Of these four patients with no family hx of cancer in 1st degree relatives, two had high, one had moderate, and one had low penetrance mutations.

Somatic Mutations

Of the 131 patients in the study, 7 had non-oncogenic, and 15 oncogenic somatic mutations in BRCA1, BRCA2, BAP1, ATM, and PALB2. All BRCA1 and BRCA2 somatic mutations were non-oncogenic. The 15 patients oncogenic somatic mutation were in BAP1 or ATM. The BAP1 somatic mutations were 12 all in intrahepatic cholangiocarcinoma cases, plus one in a case of mixed intrahepatic cholangiocarcinoma plus hepatocellular carcinoma. ATM somatic mutations were more limited and disease varied: 3 intrahepatic cholangiocarcinoma, and 1 each extrahepatic cholangiocarcinoma, gallbladder cancer, and intrahepatic cholangiocarcinoma plus hepatocellular carcinoma.

Discussion

Mandelker et al. described a cohort of over 1000 patients with advanced solid tumors who underwent germline analysis of 76 known cancer predisposition genes via the MSK-IMPACT platform, unselected for personal or family cancer history. The detection rate of pathogenic germline alterations among the study cohort was higher than would have been expected given current screening guidelines for germline mutations. In fact, 19.7% of patients were found to have a pathogenic germline alteration associated with a cancer predisposition. Among these patients, 55.5% would not have been identified using current clinical guidelines for genetic testing (18).

The practice of germline testing in patients with cancer has implications for potential therapeutic targeting in addition to prevention and screening in family members. Though the utility of genetic screening in biliary cancers is not yet established, there is clear clinical benefit to patients with other cancer types including colon, breast, and ovary. Very little has been reported on the topic of germline mutations in biliary tract cancers. *BRCA2* mutations and Lynch Syndrome have previously been associated with biliary tract carcinoma (19, 20). Our data is comparable with these few studies as pathogenic *BRCA1* or *BRCA2* mutations were identified in seven patients, with 43% of these patients demonstrating evidence of biallelic inactivation with loss of heterozygosity in the tumor corresponding to the *BRCA1* or *BRCA2* regions supporting a role of the *BRCA* germline mutation in tumor etiology. Let alone the potential therapeutic value, considering that the current standard of care regimen for biliary tract cancers includes cisplatin (6); and the recently reported on maintenance olaparib for germline *BRCA*-mutated metastatic pancreatic cancer (21), that may suggest the need for similar trials for biliary tract cancers.

The overall prevalence of high/moderate penetrance mutation in our cohort was 9.9%. Our results are similar to a study that identified deleterious germline mutations in 11% of biliary tract cancer patients of Japanese descent, based on an analysis of selected predisposing genes, including *BRCA1*, *BRCA2*, *RAD51D*, *MLH1* and *MSH2* (22). These results are comparable to the findings reported herein in terms of the genes identified with alterations, but there were clear differences in demographics among to two study populations. Notably, germline mutations in two additional genes of interest, namely germline mutations in *ATM* and *BAP1*, were identified in our cohort. Although *ATM* has long been recognized as a moderate-penetrance breast cancer susceptibility gene (23), more recently, germline mutations in *ATM* have also been found in individuals with pancreatic cancer (24). Other cancer associations linked to heterozygote *ATM* mutation carriers are still under investigation. For example, a role for *ATM* in ovarian cancer susceptibility is now emerging (25). Notably, in our study, an *ATM* mutation was identified in a 36 year-old woman with intrahepatic cholangiocarcinoma with the tumor demonstrating loss of heterozygosity at the *ATM* region. The patient's family cancer history was notable for presence of breast cancer and absence of pancreatic cancer. *ATM*-deficient cancers targeted therapy continue to be in development (26). Similarly, the spectrum of cancers associated with *BAP1* germline mutations is still under investigation but susceptibility to mesothelioma, uveal and cutaneous melanoma, as well as renal cell cancer have been reported (27–29). In our cohort, we identified a 51 year-old patient with intrahepatic cholangiocarcinoma, with a prior diagnosis of oral cancer, who harbored a likely pathogenic *BAP1* mutation with interrogation of the tumor revealing loss of heterozygosity involving the *BAP1* gene locus. Notably, a prior case report also noted cholangiocarcinoma in a germline *BAP1* mutation carrier (30), suggesting that cholangiocarcinoma may be among the expanding spectrum of cancers associated with mutations in this tumor suppressor gene. Further large germline studies of biliary tract cancers are warranted to better assess the association of *ATM* and *BAP1* with this rare cancer type.

Additionally, we also identified one patient with a mutations in the *PMS2* gene, consistent with a diagnosis of Lynch syndrome. However, somatic analysis indicated be a microsatellite stable tumor, without evidence of *PMS2* LOH or a second somatic mutation, suggesting that the PGA in *PMS2* was likely not a driver of this patients biliary tract cancer. We also identified three patients with underlying monoallelic *MUTYH* mutations. Monoallelic *MUTYH* mutations have not been linked to cancer predisposition, aside from a modest increased risk for colorectal cancer (31). On the contrary, biallelic mutations in *MUTYH* are diagnostic of *MUTYH*-associated polyposis (MAP), a syndrome characterized by a large burden of colonic polyps and predisposition to colorectal cancer. One prior study specifically argued against a role of monoallelic *MUTYH* alterations in increased risk of cholangiocarcinoma (32). The prevalence of *MUTYH* monoallelic mutations in the general population is 1–2%, suggesting that in our study, the identification of *MUTYH* carrier status was likely incidental with a prevalence of 2.2%, in line with population prevalence.

Germline mutations appeared to be equally distributed among the intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma and gallbladder subtypes, and, albeit limited in sample size, there did not appear to be any association with specific mutations with disease type. While many patients did have a second primary cancer or a first-degree

family member with a history of cancer, this information alone was not sufficient to identify those likely to harbor a germline mutation in a cancer susceptibility gene. The majority of patients with PGA, 16 of 21 patients (76.2%), had no personal history of another primary cancer, and eight of the 21 with PGA (38.1%) patients with germline mutations had no family history of cancer in a first degree relative. These results indicate that many patients who carry PGA alterations may not meet current clinical criteria for germline testing. Specifically, among the 13 patients with high or moderate-penetrance germline mutations, only four met criteria for germline genetic testing for the identified mutation (all for the identified *BRCA 1/2* mutations) (33, 34). Regardless, results were transmitted in the context of genetic counseling to 19 of 21 patients (90.5%) with germline PGA. As per MSK institutional protocol, notification letters offering follow-up genetic counseling for patients and/or designated family members were sent to two patients who did not return for genetic counseling.

There were several limitations of our study, which was meant to be an exploratory evaluation of germline mutations present in patients with biliary tract cancers. The sample size was limited. In addition, although germline mutations were identified in 11 different genes among patients with biliary tract cancers, it is unclear whether these are causative of the malignancy, although our integrated tumor analysis did identify biallelic inactivation in 42% of patients with high/moderate-penetrance germline mutations. Our study was further limited by the demographics included in this study, e.g. it consisted primarily of Caucasian patients accounting for over 75% of the entire cohort. In addition and despite the lack of any validation, the incidence of 16% of germline aberration may be artificially high. Younger patients are the ones more likely to seek care at a tertiary/quaternary referral center such as MSK, yet this may be speculative in our analyzed database, considering the median age per demographics was 59 years old.

In summary, our results show that 16% of patients with biliary tract cancers to have a pathogenic or likely pathogenic germline mutations. This is in line with the emerging literature in other solid tumor malignancies (18, 35, 36). The potential association between *ATM* and *BAP1* with biliary tract cancer susceptibility, two tumor suppressor genes where cancer spectrum and risk estimates are still being defined, with biliary tract cancer susceptibility warrants further investigation. In the interim, germline testing for all patients with biliary tract carcinoma patients, is warranted, particularly, for patients up to their 50's at diagnosis, with tumor mutations known to be potentially germline, and with family history.

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Figure 1:
Germline Alterations in Biliary Tract Cancer, among the 21 patients with PGA.

Table 1:

Demographics and Clinical Characteristics

Clinical Characteristics	N= 131	Germline Alteration	
Sex		Yes (N= 21)	No (N= 110)
Male	65 (49.6%)	9 (42.9%)	56 (50.9%)
Female	66 (50.4%)	12 (57.1%)	54 (49.1%)
Stage of Disease			
Resected	46 (35.1%)	5 (23.8%)	41 (37.3%)
Locally advanced/Metastatic	85 (64.9%)	16 (76.2%)	69 (62.7%)
Age at Diagnosis (years)			
< 40	11 (8.4%)	3 (14.3%)	8 (7.3%)
40–49	23 (17.5%)	3 (14.3%)	20 (18.2%)
50–59	37 (28.2%)	9 (42.9%)	28 (25.5%)
60–69	36 (27.5%)	4 (19%)	32 (29.1%)
70–79	23 (17.6%)	2 (9.5%)	21 (19.1%)
80+	1 (0.8%)	-	1 (0.9%)
Primary Tumor Location			
Intrahepatic	83 (63.4%)	12 (57.1%)	71 (64.5%)
Extrahepatic	21 (16%)	4 (19%)	17 (15.5%)
Gallbladder	22 (16.8%)	5 (23.8%)	17 (15.5%)
Unspecified biliary tract	5 (3.8%)	-	5 (4.5%)
Race			
Caucasian	100 (76.3%)	16 (76.2%)	84 (76.4%)
Ashkenazi Jewish (AJ)	25 (25%)	6 (37.5%)	19 (22.6%)
Known Non-AJ	17 (17%)	3 (18.8%)	14 (16.7%)
Unknown	58 (58%)	7 (43.8%)	51 (60.7%)
Asian	14 (10.7%)	4 (19%)	10 (9.1%)
African American	7 (5.3%)	1 (4.8%)	6 (5.5%)
Hispanic	6 (4.6%)	-	6 (5.5%)
Unknown	4 (3.1%)	-	4 (3.6%)

Table 2:

Age at Diagnosis By Cancer Type

Clinical Characteristics	N=131	Germline Alteration	
		Yes (N=21)	No (N=110)
Median Age, Total	59	53	59
Intrahepatic	59	51	59
Extrahepatic	64	65	62
Gallbladder	58	53	61
Age Range	21 – 84	36 – 72	21 – 84

Table 3:

Germline Mutation Detail, Intrahepatic Cholangiocarcinoma

Mutation	Transcript	Alteration	Penetrance	Age	Sex	Ethnicity	Personal History of Other Cancer	1st Degree Family Hx	2nd Degree Family Hx
<i>APC</i>	NM_000038	c.3920T>A (p.Ile1307Lys)	Low	72	F	AJ		Y	Y
<i>ATM</i>	NM_000051	c.3669_3670insTAG (p. Leu1224*)	Moderate	36	F	Caucasian		Y	Y
<i>BAP1</i>	NM_004656	c.784-1G>A	High	51	F	African American	Y		
<i>BRCA1</i>	NM_007294	c.68_69delAG (p.Glu23Valfs*17)	High	72	M	AJ		Y	Y
<i>BRCA2</i>	NM_000059	c.4131_4132insTGAGG A (p.Thr1378*)	High	56	F	AJ	Y	Y	Y
<i>BRCA2</i>	NM_000059	c.3215T>G (p.Leu1072*)	High	50	M	Asian	Y		Y
<i>FH*</i>	NM_000143	c.1431_1433dupAAA (p.Lys477dup)	Recessive	60	M	Caucasian		Unknown	Unknown
<i>MITF</i>	NM_000248	c.952G>A (p.Glu318Lys)	Moderate	52	F	Caucasian		Y	Y
<i>MUTYH</i>	NM_001128425	c.1187G>A (p.Gly396Asp)	Low	38	M	Caucasian		Y	Y
<i>MUTYH</i>	NM_001128425	c.1437_1439delGGA (p.Glu480del)	Low	41	M	Caucasian		Y	Y
<i>PALB2</i>	NM_024675	c.3549C>G (p.Tyr1183*)	High	43	F	Caucasian			
<i>PMS2</i>	NM_000535	c.943C>T (p.Arg315*)	High	36	F	Caucasian			Y

Table 4:

Germline Mutation Detail, Extrahepatic Cholangiocarcinoma

Mutation	Transcript	Alteration	Penetrance	Age	Sex	Ethnicity	Personal History of Other Cancer	1st Degree Family Hx	2nd Degree Family Hx
<i>APC</i>	NM_000038	c.3920T>A p.Ile1307Lys	Low	57	F	AJ		Y	Y
<i>BRCA1</i>	NM_007294	c.2411_2412del (p.Gln804Leufs*5)	High	68	M	Asian		Y	
<i>FH*</i>	NM_000143	c.1431_1433dupAAA (p.Lys477dup)	Recessive	64	M	Caucasian		Y	Y
<i>MUTYH</i>	NM_001128425	c.312C>A (p.Tyr104*)	Low	66	F	Caucasian		Y	Y

Table 5:

Germline Mutation Detail, Gallbladder Adenocarcinoma

Mutation	Transcript	Alteration	Penetrance	Age	Sex	Ethnicity	Personal History of Other Cancer	1st Degree Family Hx	2nd Degree Family Hx
<i>APC</i>	NM_000038	c.3920T>A (p.Ile1307Lys)	Low	53	M	AJ	Y		Y
<i>BRCA2</i>	NM_000059	c.7542_7549dup (p.Thr2517Lysfs*10)	High	56	F	Caucasian		Y	Y
<i>BRCA2</i>	NM_000059	c.5946delT (p.Ser1982Argfs*22)	High	59	F	AJ	Y		Y
<i>BRCA2</i>	NM_000059	c.5604_5605delCA (p.Asp1868Glu fs*4)	High	50	M	Asian			Y
<i>NBN</i>	NM_002485	c.383T>G (p.Leu128*)	Moderate	48	F	Asian			