Prevalence of Germline Mutations and Homologous Recombination Deficiency in a Real-World Biliary Tract Cancer Cohort

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INTRODUCTION

Biliary tract cancers (BTC) are a heterogeneous family of cancers that include intrahepatic and extrahepatic cholangiocarcinomas (ICC, ECC) and gallbladder carcinomas (GBC). Treatment options remain limited, and prognosis is poor for advanced BTC, underscoring the need for early detection and new therapeutic targets. Next-generation sequencing (NGS) studies have identified mutations associated with homologous recombination deficiency (HRD) in up to 25% of BTC patients, but the proportion with germline (GMut) versus somatic (SMut) mutations has not been established.

This retrospective study aimed to determine the prevalence of HRD-associated GMut and SMut in a large BTC cohort and the association of these mutations with key clinical parameters.

METHODS

We identified 804 BTC patients who received tumor/normal (T/N) matched NGS between 2017-2021 via the Tempus xT assay (DNA-seq of 648 genes, including all exons and select intronic regions for specific genes, at 500x coverage; full transcriptome RNA-seq). This targeted panel detects single nucleotide variants (SNVs), indels, copy number variants (CNVs), and a selection of rearrangements. Large insertions and deletions can be missed by NGS.

Pathogenic/likely pathogenic (P/LP) SMut and CNVs and incidental GMut (limited to SNVs and small indels) were identified in 50 pre-selected hereditary cancer genes based on ACMG (American College of Medical Genetics) recommendations, NCCN Genetic/Familial High-Risk Assessment guidelines, and other published literature.

HRD-associated GMut and SMut were analyzed across 9 and 18 HRD pathway genes, respectively. GMut and SMut were analyzed to determine any relationships to clinical covariates including anatomic subsite of BTC and demographics.

SIGNIFICANCE

- A significant proportion of BTC patients harbors **potentially clinically-relevant GMut and Smut** (at least 13% in this cohort) in HRD associated genes.
- Future studies are warranted to define the role of germline testing and to examine the activity of HRD-targeted therapies in BTC patients.

RESULTS

Table 1. Patient Demographics

Characteristic	Overall, N = 804[1]	Extrahepatic bile duct, N = 83[1]	Gallbladder, N = 222[1]	Intrahepatic bile duct, N = 499[1]	p-value[2]
Age at Diagnosis	66 (58, 73)	68 (60, 73)	68 (59, 74)	65 (57, 72)	0.011
Unknown	1	5	23	53	
Gender					<0.001
Male	362 (45%)	49 (59%)	58 (26%)	255 (51%)	
Female	441 (55%)	34 (41%)	164 (74%)	243 (49%)	
Unknown	1	0	0	1	
Race					
White	337 (78%)	35 (83%)	83 (66%)	219 (83%)	
Black or African American	51 (12%)	2 (4.8%)	25 (20%)	24 (9.1%)	
Asian	26 (6.0%)	4 (9.5%)	11 (8.8%)	11 (4.2%)	
Native Hawaiian or Other Pacific Islander	1 (0.2%)	0 (0%)	0 (0%)	1 (0.4%)	
American Indian or Alaska Native	2 (0.5%)	0 (0%)	1 (0.8%)	1 (0.4%)	
Other Race	13 (3.0%)	1 (2.4%)	5 (4.0%)	7 (2.7%)	
Unknown	374	41	97	236	
Ethnicity					0.3
Not Hispanic or Latino	179 (80%)	13 (81%)	48 (74%)	118 (83%)	
Hispanic or Latino	45 (20%)	3 (19%)	17 (26%)	25 (17%)	
Unknown	580	67	157	356	

[1]Median (IQR); n (%)
[2]Kruskai-Wallis rank sum test; Pearson's Chi-squared test; Fisher's exact test

Table 2. Prevalence of Germline Mutations Across BTC Types

Characteristic	Overall, N = 804[1]	Extrahepatic bile duct, N = 83[1]	Gallbladder, N = 222[1]	Intrahepatic bile duct, N = 499[1]	p-value[2]
Has any P/LP germline mutation in any of the 50 genes of interest	37 (4.6%)	4 (4.8%)	8 (3.6%)	25 (5.0%)	0.7
Has any P/LP germline mutation in HRD	26 (3.2%)	3 (3.6%)	5 (2.3%)	18 (3.6%)	0.7
Has any P/LP germline mutation in BRCA1/2	11 (1.4%)	1 (1.2%)	1 (0.5%)	9 (1.8%)	0.4

[1]Median (IQR); n (%)
[2]Fisher's exact test

Genes selected for germline assessment: *APC, ATM, AXIN2, BMPR1A, BRCA1, BRCA2, BRIP1, CDC73, CDH1, CDKN2A, CEBPA, CHEK2, EGFR, EPCAM, ETV6, FH, FLCN, GATA2, MEN1, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PMS2, POLD1, POLE, PTCH1, PTEN, RAD51C, RAD51D, RB1, RET, RUNX1, SDHAF2, SDHB, SDHC, SDHD, SMAD4, STK11, TP53, TSC1, TSC2, VHL, WT1*. HRD genes for germline assessment included: *ATM, BRCA1, BRCA2, BRIP1, CHEK1, CHEK2, NBN, PALB2, RAD51C, RAD51D,*

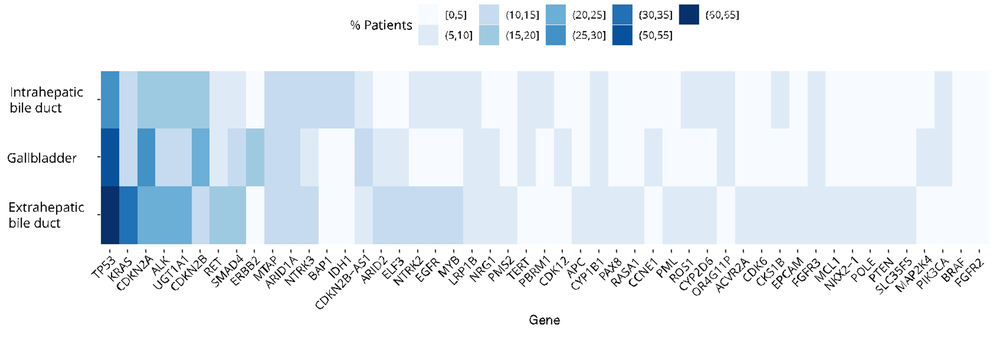
Table 3. Prevalence of Somatic Mutations Across BTC Types

Characteristic	Overall, N = 804[1]	Extrahepatic bile duct, N = 83[1]	Gallbladder, N = 222[1]	Intrahepatic bile duct, N = 499[1]	p-value[2]
Has any P/LP somatic mutation or copy number loss/amplification	731 (91%)	80 (96%)	195 (88%)	456 (91%)	0.058
Has any P/LP somatic mutation or copy number loss/amplification in HRD	105 (13%)	12 (14%)	43 (19%)	50 (10%)	0.002
Has any P/LP somatic mutation or copy number loss/amplification in BRCA1/2	48 (6.0%)	4 (4.8%)	13 (5.6%)	31 (6.2%)	0.8
MSI Status					0.5
Stable	781 (99%)	81 (98%)	218 (99%)	482 (99%)	
High	11 (1.4%)	2 (2.4%)	2 (0.9%)	7 (1.4%)	
Unknown	12	0	2	10	
TMB	1.81 (1.05, 3.16)	2.63 (1.13, 3.68)	2.50 (1.54, 3.68)	1.58 (0.83, 2.63)	<0.001
Unknown	12	0	3	9	
Tumor Mutational Burden					0.9
<10	769 (97%)	80 (96%)	213 (97%)	476 (97%)	
>=10	23 (2.9%)	3 (3.6%)	6 (2.7%)	14 (2.9%)	
Unknown	12	0	3	9	
Any FGFR 1/2/3 Fusion	38 (4.7%)	0 (0%)	2 (0.9%)	36 (7.2%)	<0.001
Any NTRK 1/2/3 Fusion	2 (0.2%)	0 (0%)	1 (0.5%)	1 (0.2%)	0.6

[1]Median (IQR); n (%)
[2]Kruskai-Wallis rank sum test; Pearson's Chi-squared test; Fisher's exact test

Somatic assessment for 648 genes included in the Tempus xT assay. HRD genes for somatic assessment included: *ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCA, FANCL, HDAC2, MRE11, NBN, PALB2, RAD51B, RAD51C, RAD51D, RAD54L*

Figure 1. Somatic Mutational Landscape Across BTC Types



Percentages reflect the proportion of patients with any pathogenic/likely pathogenic variant and/or copy number loss/gain in each gene. The figure is restricted to mutations in coding regions of genes detected in 5% or more of patients within any of the 3 BTC groups: ICC, GBC, ECC.

The prevalence of GMut was 4.6% overall. Subgroup analyses showed GMut in 5% of ICC, 4.8% of ECC, and 3.6% of GBC. For patients <50 years of age at diagnosis, pooled prevalence was 6.1% (n=82) vs. 4.5% (n=641) for age ≥50 years (p=0.6). HRD-associated GMut were present in 3.2% of samples. HRD-associated SMut or CNVs were present in 13% of samples and showed significant variation across subsite (p=0.002) with the highest prevalence in GBC (19%, Table 3). HRD-associated GMut were identified in *ATM* (n=8), *BRCA2* (n=6), and *BRCA1* (n=5). Microsatellite instability was present in 1.4% of samples.

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