

ORIGINAL ARTICLE

Comprehensive genetic analysis in biliary tract cancer: a prospective single-center experience

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Available online 29 August 2025

Background: Recent data reported a significant incidence of germline aberrations in biliary tract cancer (BTC) patients, even if conclusive data on that and on its clinical implication are lacking.

Methods: We selected patients for genetic counselling basing on four criteria: personal history of oncologic disease other than BTC; familial history of oncologic disease (considering relatives of first and second grade); patients ≤50 years old; patients presenting a somatic mutation in genes involved in DNA damage repair pathways and mismatch repair.

Results: A total of 22/150 patients met at least one criterion and were directed to genetic counselling. Of these, 17 received the germline test. Four patients carried a pathogenic variant. Some 18/22 patients received the somatic test on tissue samples. Patients who carried a germline pathogenic variant had the same variant also at a somatic level. No statistically significant differences were found in terms of incidence of germline pathogenic variants between patients diagnosed with BTC before the age of 50 years versus after the age of 50 years and in patients with intrahepatic cholangiocarcinoma and extrahepatic cholangiocarcinoma.

Conclusion: The present study is one of the first prospective experiences investigating the role of genetic counselling and germline testing in a BTC setting, thus suggesting several improvements in the selection criteria of patients directed to genetic counselling.

Key words: biliary tract cancer, germline test, genetic predisposition

INTRODUCTION

Biliary tract cancer (BTC) comprises a group of malignant tumors originating from various levels of the biliary tract, including intrahepatic cholangiocarcinoma (iCCA), extrahepatic cholangiocarcinoma (eCCA), perihilar cholangiocarcinoma (pCCA), and gallbladder cancer (GBC).¹⁻⁴ The aggressive biological behavior of BTC results in poor treatment responses and a dismal prognosis. Since 2010, platinum-based

chemotherapy has been the cornerstone of treatment of advanced and metastatic BTC, regardless of the site of origin of the tumor.^{5,6} Recently, substantial progress has been made in this complex patient population. Notably, immunotherapy combined with chemotherapy has shown a survival benefit over chemotherapy alone: durvalumab, an anti-programmed death-ligand 1 (anti-PD-L1) antibody, combined with cisplatin and gemcitabine, is now the new first-line standard of care for advanced and metastatic BTC.⁷ Furthermore, advances in high-throughput genomic analysis techniques have identified new potential molecular targets, including kinases such as FGFR1, FGFR2, FGFR3, PIK3CA, ALK, EGFR, ERBB2, BRAF, and AKT3, as well as tumor suppressor genes involved in DNA damage repair pathways and oncogenes such as *IDH1* and *IDH2*.⁸⁻¹⁰ Some of these targets have shown promise in phase II and phase III clinical trials for targeted therapies. Despite these recent

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advancements, prognosis for patients affected by advanced BTC remains poor and results from the clinical trials are unsatisfactory; moreover, the increasing incidence, mainly of iCCA in the Western world, makes a deeper comprehension of the underlying biological pathways and the identification of new potential targets for innovative treatments an urgent need. If several risk factors are counted for this setting of patients, including primary sclerosing cholangitis, chronic inflammations associated with gallstones, hepatobiliary flukes, and viral hepatitis, only few data, mainly retrospective, are already available concerning the genetic predisposition and its impact in this group of patients. In other cancers, such as breast and pancreatic cancer, investigations into genetic predispositions through germline evaluations have had significant therapeutic implications. Patients with certain pathogenic germline variants (PGVs) have been shown to respond better to specific chemotherapy agents and biological treatments.^{11,12} The prevalence of germline mutations in BTC, however, remains unclear, and their therapeutic implications are still unknown. Moving on from these premises, the aim of the present study was to implement a germline testing protocol within our institution's cohort of patients to investigate the genetic profile of those with advanced BTC who may have a hereditary predisposition. The primary objective was to generate new prospective data on the prevalence of PGVs in BTC patients and explore the potential clinical utility of germline testing.

MATERIALS AND METHODS

Patient selection

Patients were selected from a large cohort affected by BTC, thus including iCCA, eCCA, pCCA, and GBC, who received systemic treatment of locally advanced, metastatic or recurrent disease in our institution from August 2021 to February 2024.

Among our cohort of BTC patients, we selected those who were candidates to receive genetic counseling, according to our internal protocol:

- Patients with personal history of oncologic disease other than BTC;
- Patients with familial history of oncologic disease (considering relatives of first and second grade);
- Patients ≤ 50 years old;
- Patients presenting a somatic mutation in genes involved in DNA damage repair pathways and mismatch repair.

All patients who met at least one of the above-mentioned criteria were referred to a genetic counselor. After genetic evaluation, patients were eventually indicated for germline testing.

Data collection

For patients directed to the genetic counselor, both medical and family histories of any cancer in all relatives were recorded, with specifics about the age at onset.

From the molecular point of view, data about the germline next-generation sequencing (NGS) testing were recorded and put in correlation with the medical and family history of patients. Genomic DNA was extracted from peripheral blood samples. The gene panel used to carry out the germline testing included the following genes: *BRCA1*, *BRCA2*, *ATM*, *APC*, *BAP1*, *MLH1*, *MSH2*, *MSH6*, *PALB2*, *PMS2*, *RAD51D*, *CDK4*, *BARD1*, *BRIP1*, and *TP53*. The list of genes was defined according to the already available data on the genetic predisposition in BTC.¹³⁻¹⁸

After NGS sequencing on a NextSeq Illumina platform, prioritization and classification of the germinal variants was carried out in agreement with the Standards of the American College of Medical Genetics and Genomics and the Association of Molecular Pathology,¹⁹ as follows: pathogenic variants (PVs) which included pathogenic and likely pathogenic, variant of uncertain significance (VUS), likely benign or benign. NGS data analysis was carried out independently by two trained laboratory medical geneticists.

Moreover, for those patients who had adequate tissue samples, a large somatic NGS analysis was carried out. Particularly, the somatic genomic analysis of the primary tumors was carried out by Foundation Cdx technology (FoundationOne assay) on formalin-fixed paraffin-embedded samples and hematoxylin–eosin staining slides containing at least 20% of tumor cells. Identified alterations included insertions/deletions (indel, 1-40 bp), base substitutions, copy number alterations-amplifications (ploidy <4 , amplification with copy number ≥ 8), copy number alterations-deletions (ploidy <4 , homozygous deletions), and fusion/rearrangements in a total of 324 genes. Molecular pathologists in our institution independently revised the results. The genetic alterations identified using the FoundationOne assay are classified into two categories: known or likely PVs, which are documented in the Foundation Medicine database and include entries from the Catalogue of Somatic Mutations in Cancer (COSMIC) database, and VUS.

The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the ethics committee of each institution involved in the project (number of registry ethics committee: 113/INT/2021). Under the conditions of retrospective archival tissue collection and patients' data anonymization, our study was exempted from the acquisition of informed consent from patients by the institutional review board.

Statistical analysis

Descriptive statistics for medical and family histories of the sample size were examined. A mixed correlation study between early age (<50 years), and medical and family histories was conducted. Moreover, a mixed correlation study between patients with iCCA versus eCCA and medical and family histories was carried out.

Rates of detection of PVs and VUS in the whole samples of patients who carried out the germline test and in different subgroups of patients (those <50 years old; those with iCCA

versus eCCA; those with a medical history of second neoplasia; those with a family history suggestive of genetic background) were calculated. In addition, a comparative analysis including the somatic and germline molecular profile was carried out for patients who had both the somatic and germline test available.

Chi-square and Fisher tests were used to compare the significant difference of alteration in different subtypes or cohorts. A two-sided *P*-value <0.05 was considered statistically significant.

A MedCalc package (MedCalc® version 16.8.4) was used for statistical analysis.

RESULTS

Patient characteristics and medical and family histories of oncologic disease

Overall, 150 patients with a pathologically confirmed diagnosis of BTC were treated with systemic therapy for locally advanced, metastatic or recurrent disease in the oncologic unit of our institution from August 2021 to February 2024. Among them, a total of 22 patients (14.5% of the whole cohort) met at least one of the inclusion criteria of our internal protocol, and thus were directed to genetic counselling. Between them, only 19/22 patients actually went to genetic counselling (the reason why 3/22 patients did not go to genetic counselling as indicated are personal and unknown). Of the 19 patients who underwent genetic counselling, 17 were tested for germline alterations. Two patients did not meet the criteria for testing according to the specialist consult.

The cohort of patients who met at least one of the criteria for genetic counselling included 10/22 patients with a diagnosis of iCCA and 12/22 patients with a diagnosis of eCCA.

The mean age of the sample was 58.5 years (32-84 years). Some 8/22 patients had onset of BTC at an early age (<50 years old), and 14/22 patients developed BTC after 50 years of age. Concerning personal history, 36% (8/22) had previous other neoplasia(s). More specifically, 6/22 (27%) had a further oncologic diagnosis apart from BTC, including: two cases of breast cancer, one case of colon cancer, one case of prostate cancer, one case of melanoma *in situ*, and one case of granulosa cell tumor. A total of 2/22 patients (9%) had two further oncologic diagnoses: one of them had a double previous history of urothelial cancer, and the second one had a previous history of urothelial cancer and colon cancer. Significantly, one of the breast cancer diagnoses and the melanoma diagnosis were both made at an early age (<50 years).

Concerning the family history, 15/22 (68%) patients had a first-degree relative affected by a neoplasia; 6/22 (27%) of the patients had a first-degree relative with a history of oncologic disease known to be suggestive of a strong genetic predisposition (including positive family history for gynecological neoplasia, breast cancer, prostate cancer, colon cancer at early age, pancreatic cancer). All patients' characteristics are summarized in Table 1.

Table 1. Patients' characteristics

	Analyzed patients in this study (n = 22)
Age, years	
Mean	58.5
Range	32-84
Age BTC onset, n (%)	
<50 years	8 (36)
>50 years	14 (63)
Primary organ, n (%)	
Intrahepatic bile duct	10 (45)
Extrahepatic bile duct	12 (54)
Personal history of other neoplasia	8 (36)
Single neoplasia	6 (27)
Double neoplasia	2 (9)
Family history	
First-degree relative affected by neoplasia	15 (68)
First-degree relative with neoplasia suggestive of strong genetic predisposition	6 (27)

BTC, biliary tract cancer.

Germline and somatic molecular features

In the sample of patients who underwent genetic counselling (*N* = 19), 17/19 received a germline test since in two cases the genetic test was not indicated according to the specialist consult.

In the sample of patients who received the germline test, a total of four patients were detected to have a PGV (*N* = 4/17, 23.5%). In particular, one patient was found to carry a PGV in the *PMS2* gene, while another carried a PGV in the *MSH2* gene. Additionally, two other patients were identified with two PVs in the *MUTYH* gene, which were not included in the main gene panel. Moreover, in the sample of patients who received the germline test, a total of five patients were detected to have a germline VUS (*N* = 4/20, 30%), with one patient being a carrier of a double VUS on the same gene (*MLH1*). In particular, one patient was detected to have a VUS of the gene *BAP1*, one patient was detected to have a VUS of *ATM*, one patient was detected to have a VUS of *BARD1*, another one a VUS of *BRIP1* and finally, one patient presented a double VUS of *MLH1* (Supplementary Figure S1, available at <https://doi.org/10.1016/j.esmogo.2025.100227>, Figures 1 and 2).

Concerning the somatic molecular testing, 18/22 patients presented adequate tissue samples and received the somatic molecular test (FoundationOne assay). Overall, genomic sequencing carried out by FoundationOne assays identified a total of 93 somatic PGVs in the entire sample which involved 54 genes, with a mean of 1.7 alterations per gene (range 0-6). Moreover, genomic sequencing carried out by FoundationOne assays identified a total of 112 somatic VUS in the entire sample which involved 84 genes, with a mean of 1.3 alterations per gene (range 0-4). The most common somatic PVs were found in *CDKN2A* (6/18, 33%), *TP53* (6/18, 33%), *PIK3CA* (5/18, 28%), *ARID1A* (4/18, 22%), *CDKN2B* (4/18, 22%), *PBRM1* (4/18, 22%), *MTAP* (4/18, 22%), *KRAS* (3/18, 16.5%), *MCL1* (3/18, 16.5%) (Supplementary Figure S1, available at <https://doi.org/10.1016/j.esmogo.2025.100227>).

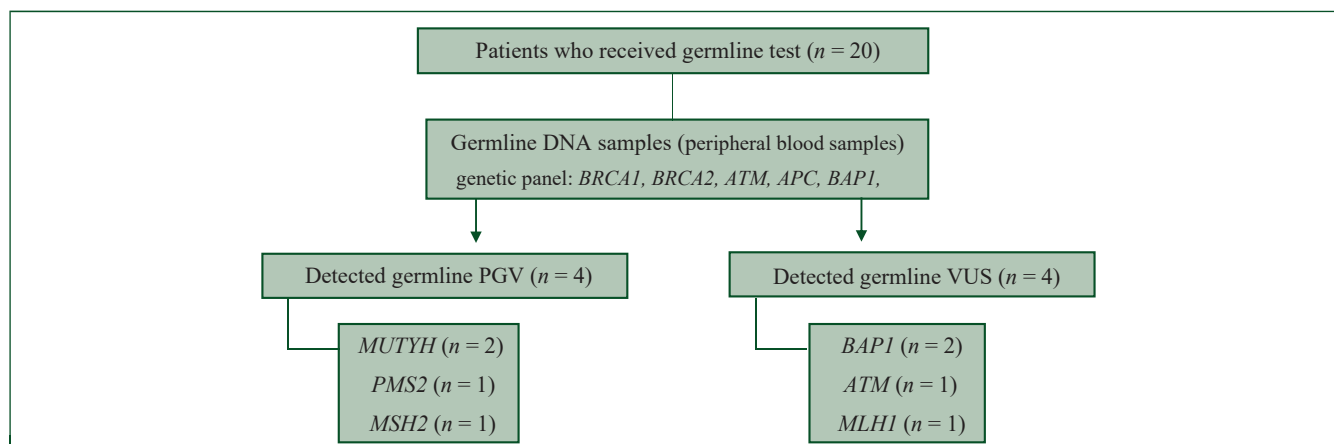


Figure 1. Molecular study design.

PGV, pathogenic germline variant; VUS, variant of uncertain significance.

Somatic PVs of all remaining genes were detected in <15% of the entire sample, including *BAP1*, *FGFR2*, *IDH1*, *IDH2*, *MUTYH*, *NF1*, and *SMAD4*.

Correlation study: early age (<50 years) and personal and family history of oncologic disease

A correlation study between early age versus not early age and medical and family histories was carried out. In the group of patients who were diagnosed with BTC at an early age ($N = 8/22$), only two had a previous diagnosis of a second neoplasm (one was previously affected by a

granulosa cell tumor, and the second one received previous local surgery for a melanoma *in situ*). In the same group of patients with diagnosis of BTC at an early age, however, only two had a positive family history for oncologic disease suggestive of a strong genetic background.

In the group of patients who were diagnosed with BTC not at an early age ($N = 14/22$), six had a previous diagnosis of a second neoplasm (two breast cancers, one prostate cancer, two intestinal cancers, and two urothelial cancers) and three of them were diagnosed with three oncologic diseases in their lifetime overall. In the same group of patients with a diagnosis of BTC not at early age,

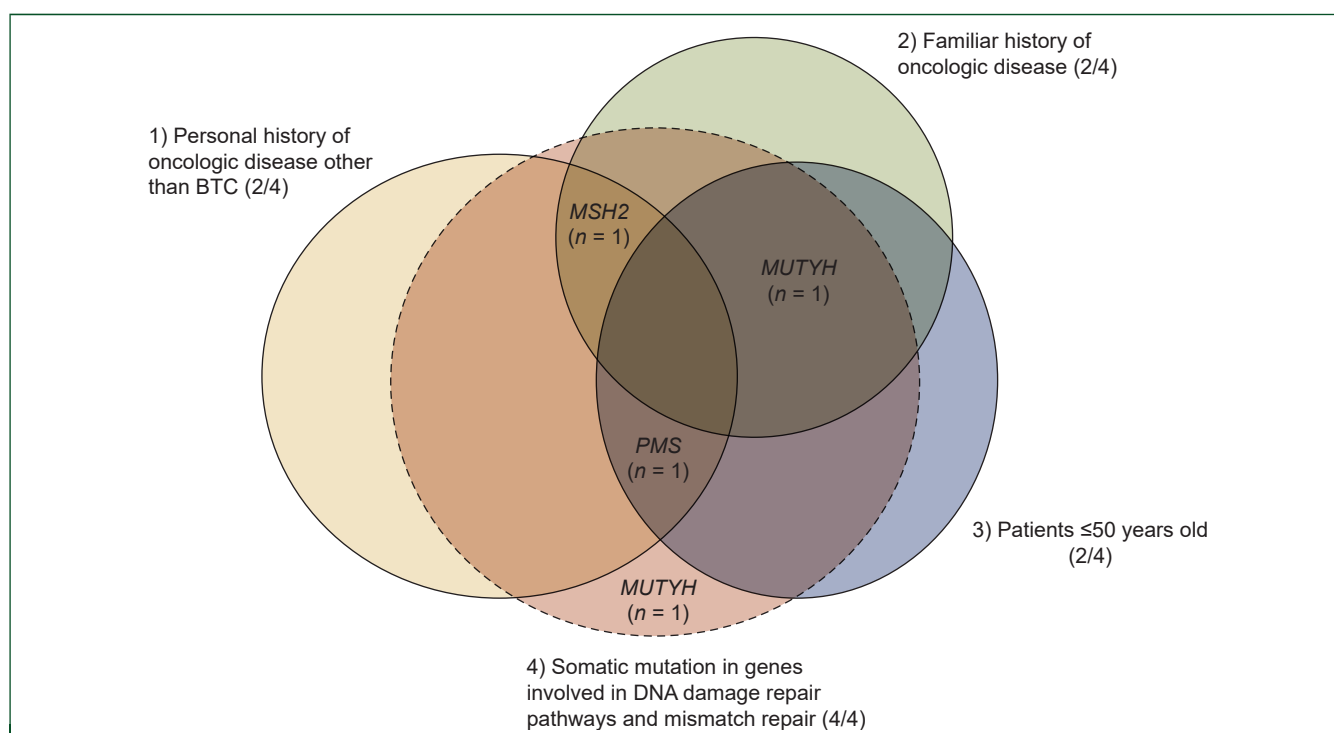


Figure 2. Patients with PGVs grouped accordingly to the selection criteria.

BTC, biliary tract cancer; PGV, pathogenic germline variant.

Table 2. Correlation study between clinical–pathological characteristics and germline profile

	Overall (n = 22)	Germline tested, n (%)	Detected PGV, n (%)	PGV detected	Detected germline VUS, n (%)
Age, n (%)					
<50 years	8 (36.4)	7 (87.5)	2 (28.5)	<i>PMS2, MUTYH</i>	2 (28.5)
>50 years	14 (63.6)	13 (92.8)	2 (15.4)	<i>MSH2, MUTYH</i>	1 (7.7)
Primary organ, n (%)					
iCCA	10 (45.5)	9 (90.0)	2 (22.2)	<i>PMS2, MUTYH</i>	0
eCCA	12 (54.5)	11 (91.6)	2 (18.2)	<i>MSH2, MUTYH</i>	3 (27.3)

eCCA, extrahepatic cholangiocarcinoma; iCCA, intrahepatic cholangiocarcinoma; PGV, pathogenic germline variant; VUS, variant of uncertain significance.

however, only three patients had a positive family history for oncologic disease suggestive of a strong genetic background.

Correlation study: primitive tumor site and personal and family history of oncologic disease

A correlation study between the primitive tumor site (iCCA versus eCCA) and medical and family histories was carried out.

In the group of patients who were diagnosed with iCCA ($N = 10/22$), four patients had a previous diagnosis of a second neoplasm (two breast cancers, one prostate cancer, and a melanoma *in situ*). In the same group of patients with diagnosis of iCCA, however, four patients had a positive family history for oncologic disease suggestive of a strong genetic background.

In the group of patients who were diagnosed with eCCA ($N = 12/22$), two had a previous diagnosis of a second neoplasm (one colon cancer and one granulosa cell tumor), whereas two other patients received three oncologic diagnoses throughout their life. In the same group of patients with a diagnosis of eCCA, however, only two patients had a positive family history for oncologic disease suggestive of a strong genetic background.

Correlation study: clinical–pathological characteristics and germline molecular profile

A correlation study between clinical–pathological characteristics and germline molecular profile was carried out. By considering the classification of patients based on early age onset (<50 versus >50 years old), in the group of patients who were diagnosed with BTC before 50 years old ($N = 8$), six received the germline test. Only two patients (29% of

patients with early age diagnosis of BTC) were detected to have a PGV (*PMS2* and *MUTYH*), whereas two patients presented germline VUS. Curiously, both of the early age patients who were detected to have a PGV had a diagnosis of iCCA. In the group of patients who were diagnosed with BTC after 50 years old ($N = 14$), 11/14 received the germline test. Only two patients (15.4% of patients with a diagnosis of BTC after 50 years old) were detected to have a PGV (*MSH2* and *MUTYH*), whereas 3/13 patients were detected to have a germline VUS. No statistically significant differences were found in terms of incidence of PGV between patients diagnosed with BTC before 50 years of age versus after 50 years of age ($P = 0.58$) (Table 2).

By considering the classification of patients based on the primitive tumor site, in the group of patients who were diagnosed with iCCA ($N = 10/22$), seven received the germline test. Only two patients (22% of patients with diagnosis of iCCA) were detected to have a PGV (*PMS2* and *MUTYH*), whereas one patient presented germline VUS of *BRIP1*. In the group of patients who were diagnosed with eCCA ($N = 12/22$), 10/12 received the germline test. Only two patients (18% of patients with a diagnosis of eCCA) were detected to have a PGV (*MSH2* and *MUTYH*), whereas four patients were detected to have a germline VUS. No statistically significant differences were found in terms of incidence of PGV between patients affected by iCCA and eCCA ($P = 0.62$) (Table 2).

Correlation study: medical and family histories and germline molecular profile

A correlation study between medical and family histories and germline molecular profile was carried out. When considering personal medical history, in the group of

Table 3. Correlation study between anamnestic data and germline profile

	Overall (n = 22)	Germline tested, n (%)	Detected PGV, n (%)	Germline PGV detected	Detected germline VUS, n (%)
Personal history					
Previous oncological disease	8 (36.4)	7 (87.5)	2 (28.5)	<i>PMS2, MSH2</i>	1 (14.3)
No previous oncological disease	14 (63.6)	13 (92.8)	2 (15.4)	<i>MUTYH, MUTYH</i>	2 (15.4)
Family history					
suggestive of genetic background	6 (27.3)	6 (100)	1 (16.7)	<i>MSH2</i>	0
Not suggestive of genetic background	16 (72.7)	14 (87.5)	3 (21.4)	<i>PMS2, MUTYH, MUTYH</i>	3 (21.4)

PGV, pathogenic germline variant; VUS, variant of uncertain significance.

patients with a positive medical history of previous oncologic disease(s) ($N = 8$), seven received the germline test. Only two patients [28.5% of patients with positive medical history of previous oncologic disease(s)] were detected to have a PGV (*PMS2* and *MSH2*), whereas two patients presented germline VUS.

In the group of patients without a positive medical history of previous oncologic disease(s) ($N = 14$), 10 received the germline test. Only two patients [15.4% of patients without a positive medical history of previous oncologic disease(s)] were detected to have a PGV (both in *MUTYH*), whereas three patients were detected to have a germline VUS. No statistically significant differences were found in terms of incidence of PGV between patients with a positive medical history of previous oncologic disease(s) and those without ($P = 0.62$) (Table 3).

By considering the family history, patients with a family history suggestive of a strong genetic background ($N = 6$), five received the germline test. Only one patient (16.7% of patients with a family history suggestive of a strong genetic background) was detected to have a PGV (*MSH2*), whereas one patient presented germline VUS. In the group of patients without a family history suggestive of a strong genetic background ($N = 16$), 10 received the germline test. Three patients (21.4% of patients without a family history suggestive of a strong genetic background) were detected to have a PGV (one in *PMS2* two in *MUTYH*), whereas three patients presented germline VUS.

No statistically significant differences were found in terms of incidence of PGV between patients with a family history suggestive for a strong genetic background and those without ($P = 1.00$) (Table 3).

Correlation study: germline molecular profile and somatic molecular profile

When comparing the germline and somatic profile of each patient, a specific correlation was observed. In particular, the patient who was detected to have a somatic PV of *MSH2* was observed to present also the same PV at germline level. Both patients who were detected to have a somatic PV in the gene *MUTYH*, were also observed to have the same genomic alteration at the germline level. Finally, the patient who was detected to have a somatic PV in the gene *PMS2*, was also observed to present the same alteration at the germline molecular test.

DISCUSSION

Despite the recent new molecular highlights in the BTC field, the role of genetic predisposition in this setting of patients remains unclear, due to a lack of studies on the prevalence of germline mutations in cancer predisposition genes, with a majority constituting retrospective analyses. With the undoubtable limit of the small sample size, the present work reports one of the first exploratory analysis from the real-world setting investigating the germline profile of BTC patients, starting from selection criteria assumed by our institution based on the previous literature. Indeed, no conclusive data

about the criteria useful to direct patients to a genetic counselor and eventually to germline testing are available for BTC patients. The first important point worthy of discussion is the protocol we applied in terms of both selection criteria of patients and gene panels used for the germline test. We applied four selection criteria: the first three concerned the clinical and anamnestic areas (the onset at early age, the presence of personal and/or familial oncologic history), and the last one concerned the somatic mutation profile. The first three criteria are well known 'red flags' that increase the likelihood of developing a hereditary cancer syndrome.²⁰⁻²² Nevertheless, in a large prospective study conducted in 205 liver cancer patients, the authors highlighted that only 15% of PVs were detected in patients that met at least one clinical and anamnestic criterion, thus the 75% of patients with positive results would not have been detected using international guidelines for genetic evaluation.¹⁸ So, the molecular criterion was added moving from both evidence of a certain concordance between germline and somatic molecular alterations in several neoplasias,²³ and the extreme heterogeneity of the somatic molecular landscape in the BTC setting. Of note, the proportion of patients who met at least one criterion and thus were directed to genetic counseling was 14.5%. This uptake is notably low but comparable to the eligibility and referral rates in other oncologic settings. In breast, ovarian, and prostate cancer fields, awareness about the genetic predisposition is such that not only have numerous studies expanded treatment options for patients with germline mutations, but evidence-based screening programs have also been implemented in the general population. Nevertheless, real-world evidence reported low rates of referrals to genetic counselors also in those oncologic settings: it was reported that only 25%-30% of eligible breast cancer patients undergo genetic testing, <30% of ovarian cancer patients, and only 35% of eligible prostate cancer patients are referred for testing.^{24,25} These scarce results are probably due to a still scarce awareness about the importance and clinical implications, to the unavailability of germline testing or, in some cases, to already unclear guidelines.²⁶

Another important topic of discussion is the gene panel which might be used in this setting of patients. The following genes were included in the gene panel proposed for the germline test: *BRCA1*, *BRCA2*, *ATM*, *APC*, *BAP1*, *MLH1*, *MSH2*, *MSH6*, *PALB2*, *PMS2*, *RAD51D*, *CDK4*, *BARD1*, *BRIP1*, and *TP53*. The gene panel has been defined by an internal consensus within our institution between physicians and genetic counselors on the basis of the previous evidence in the BTC setting and in pancreatic cancer, due to its biological similarity.²⁶ Of interest, a certain flexibility has been maintained, thus patients who revealed somatic gene alterations of notable genetic interest were tested for the gene involved, also at the germline level, regardless of whether it was included in the germline panel or not. Thanks to this kind of approach, two cases of germline *MUTYH* mutations have been highlighted in our sample of patients (and directed to a specific screening program also for first-degree relatives carrying the same mutation), which would not have been discovered if a strict adherence

to the gene panel had been followed. The mutY homolog (*MUTYH*) gene encodes a protein involved in oxidative DNA damage repair. Biallelic mutations in *MUTYH* are pathognomonic of the recessive syndrome *MUTYH*-associated polyposis, which is related to a high incidence of colorectal cancer.^{27,28} To date, no conclusive data about the role of germinal mutation in the *MUTYH* gene in BTC patients are available; only a few cases are described in literature,^{15,29,30} and one prior study specifically argued against a role of monoallelic *MUTYH* mutations in the BTC setting.³¹ Of note, the 50% of the PVs we reported in the present cohort involved the *MUTYH* gene. Nevertheless, the prevalence of *MUTYH* monoallelic mutations in the general population is ~1%-2%, and a random effect derived by the small sample size has to be considered.

Moving on from the highlights of the present work, further investigations focusing on the role of *MUTYH* in this setting of patients are warranted. Another two patients in our sample were highlighted to carry a PGV: one in the *PMS2* gene, and one in the *MSH2* gene, configuring a microsatellite instability and thus, a Lynch syndrome. Significantly, no patients were highlighted to carry germline mutations in genes involved with the hereditary breast and ovarian syndrome, like *BRCA1/2*, *PALB2* or *ATM*. Both *PMS2* and *MSH2* mutations, however, have been revealed to be germline mutated also in previous retrospective cohorts of BTC patients,^{14,15} thus reinforcing the role of these mutated genes in the development of BTC.

The correlations analysis we carried out in the present work showed us scarce correlation between clinical–pathological characteristics and prevalence of PGVs. In particular, no differences were found in terms of PVs between patients with iCCA and patients with eCCA, consistent with previous retrospective reports.^{15,16} In the same way, no statistically significant differences were found in terms of incidence of PVs between patients with early onset BTC (<50 years old) and patients with not early onset BTC (>50 years old), thus meaning that age at BTC alone may not be a useful predictor of PGVs. This latter result is noteworthy. Even if the genetic testing indication has often been heavily weighted by the early age at onset, in several oncologic settings the conviction is growing that the age must be contextualized and scrutinized with the other risk modifiers,^{32,33} which seems to be a similar conclusion to the present work.

A strong correlation was found between the somatic molecular profile and the germline molecular profile in the present analysis. This result is not surprising, if comparing to other oncologic settings. Indeed, several oncology practice guidelines have included recommendations for germline testing when a PV with clinical implications is highlighted in the tumor somatic profiling,³⁴⁻³⁸ since evidence demonstrated that germline analysis following the somatic test may identify PVs in patients who would not otherwise have met the criteria for genetic testing.³⁸ Tumor profiling is not a sufficient criterion for genetic testing alone, however, since it could present several limitations and leads to false negatives. One of the main reasons is that somatic gene panels often do not encompass all genes associated with hereditary cancer

predisposition syndromes, and their analysis methods may lack complete gene sequencing or thorough deletion/duplication analysis.³⁹⁻⁴²

The results from the present analysis must be interpreted after considering a number of limitations. Firstly, the small sample size makes the present results useful as hypothesis-generating data, but no conclusions could be reached, since a validation on larger cohorts of patients must be carried out. Secondly, an important limitation has to be considered: the inclusion criteria we adopted are based on previous results from retrospective cohorts. Indeed, the germline test is not used routinely in clinical practice and is not reimbursed for advanced BTC patients, thus a selection must be done, even if the available evidence is scarce and enriched by a number of biases. Moreover, the variant allele fraction (VAF) was not evaluated at a somatic level in the present analysis. It could constitute a limitation, since VAF is well known to be related to the likelihood to present a germline mutation in the same gene.

Despite the above-mentioned limits, the present study is one of the first prospective experiences investigating the role of genetic counselling and germline testing in the BTC setting. The incidence of PGVs from previous evidence has been reported to be ~15%, which is comparable with the proportion observed in other oncologic settings, including pancreatic cancer, where guidelines recommend universal genetic testing.⁴³ The issue of health costs could not be ignored, however, thus a better definition of patients who could present a higher genetic predisposition to develop BTC is crucial in order to improve clinical outcomes. The results we reported could suggest several improvements in the selection criteria which could be implemented: if age and primitive tumor location could not be considered exclusive criteria of selection, the somatic test is of crucial interest for the identification of patients who are likely to present a higher incidence of PGVs. In line with that, a personalization of the gene panel used for germline testing after genetic counselling is warranted. A deeper definition of clinical and pathological risk factors must be carried out, in order to better define patients to direct to genetic counseling and germline testing.

FUNDING

None declared.

DISCLOSURE

The authors have declared no conflicts of interest.

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