CANCER GENETICS

Multigene Panel Testing in Individuals With Hepatocellular Carcinoma Identifies Pathogenic Germline Variants

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abstract

PURPOSE Hepatocellular carcinoma (HCC) has well-defined environmental risk factors. In addition, epidemiologic studies have suggested hereditary risk factors. The goals of this study were to determine the rate of pathogenic and likely pathogenic (P/LP) germline variants in cancer predisposition genes in patients with HCC, possible enrichment of P/LP variants in particular genes, and potential impact on clinical management.

MATERIALS AND METHODS A prospective study at a tertiary medical center enrolled 217 patients with a personal history of HCC. Multigene panel testing was performed for 134 cancer predisposition genes in all patients. The rate of P/LP variants was compared with population rates. A separate retrospective cohort included 219 patients with HCC who underwent testing at a commercial laboratory.

RESULTS In the prospective cohort, P/LP germline variants were identified in 25 of 217 patients with HCC (11.5%). Four patients (1.8%) had P/LP variants in the highly penetrant cancer genes *BRCA2* (n = 2), *MSH6* (n = 1), and *PMS2* (n = 1). In addition, multiple patients had P/LP variants in *FANCA* (n = 5) and *BRIP1* (n = 4), which were significantly enriched in HCC compared with the general population. Detection of P/LP variants led to changes in clinical management in regard to therapy selection, screening recommendations, and cascade testing of relatives. In a separate retrospective analysis of 219 patients with HCC, 30 (13.7%) were positive for P/LP variants including 13 (5.9%) with highly penetrant genes *APC* (n = 2), *BRCA1* (n = 1), *BRCA2* (n = 6), *MSH2* (n = 2), or *TP53* (n = 2).

CONCLUSION P/LP germline variants in cancer predisposition genes were detected in 11%-14% of patients with HCC. Inherited genetics should not be overlooked in HCC as there are important implications for precision treatment, future risk of cancers, and familial cancer risk.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fourth most common cause of cancer mortality worldwide.[1] The development of HCC is strongly linked to underlying chronic liver disease and cirrhosis, most commonly because of chronic infection with either hepatitis C virus (HCV) or hepatitis B virus (HBV).[2] Other HCC risk factors include nonalcoholic fatty liver disease; excessive alcohol use; exposure to hepatotoxins; and rare genetic disorders such as hereditary hemochromatosis, hereditary tyrosinemia, and acute hepatic porphyrias.[1,3] Analyses of HCC tumor tissues have revealed an array of somatic mutations, the most common being *TERT* promoter–activating mutations, *CTNNB1*-activating mutations, and *TP53*-inactivating mutations.[2] HCC has not been linked with pathogenic and likely pathogenic (P/LP) variants in germline cancer predisposition genes to date.

Familial aggregation studies and case-control series have demonstrated that a hereditary component influences the risk of HCC, suggesting an interaction between genetic and environmental factors.[4,5] A meta-analysis of studies that included 3,600 individuals with liver cancer concluded that a family history of liver cancer increased the risk of HCC by more than 2.5-fold.[6]

As the detection of inherited P/LP variants in cancer predisposition genes would have profound implications for surveillance and management of patients and their family members,[7] it is important to know the yield of germline multigene panel testing in patients with HCC. The goals of this study were to determine the rate of P/LP variants in patients with HCC, the possible associations with particular cancer predisposition genes, and the potential impact on clinical management, including surveillance and precision therapy.

MATERIALS AND METHODS

Prospective Cohort (Penn cohort)

Study design. The prospective cohort is a cross-sectional study of patients seen in the hepatology, oncology, or radiology clinics at Penn Medicine (an

CONTEXT

Key Objective

Hepatocellular carcinoma (HCC) has been linked to a number of environmental risk factors. An additional hereditary component has remained unexplored. The objective of this study was to determine the yield of testing for cancer predisposition genes in the germline of patients with HCC and to explore the potential implications for clinical management.

Knowledge Generated

Pathogenic and likely pathogenic variants in cancer predisposition genes were detected in the germline of 11%-14% of patients with HCC, with 1.8%-5.5% of these occurring in highly penetrant cancer genes. These findings affected the clinical treatment and risk reduction strategies for patients and their family members.

Relevance

Treating physicians should be aware of the possibility of germline variants in cancer predisposition genes in patients with HCC. Multigene panel genetic testing should be considered, at least for the patients who meet testing criteria on the basis of a family history of cancers.

academic hospital system in Philadelphia, PA) with a history of HCC diagnosed in their native liver. The diagnosis was corroborated by review of radiology and/or pathology reports by the board-certified gastroenterologists and hepatologists on the study team according to American Association for the Study of Liver Diseases guidelines (M.A.H. and K.J.W.).[8] Patients were enrolled between April 30, 2019 and June 24, 2020. The study was approved by the University of Pennsylvania Institutional Review Board (IRB) (Protocol No. 832453).

Study population and data collection. Eligible patients were at least 18 years of age. Patients provided informed consent for genetic testing and submitted a saliva or blood sample. Patients were excluded if actively listed for solid organ transplantation at the time of enrollment, to eliminate any potential that genetic testing results could influence eligibility for transplantation. Questionnaires were used to collect data on ethnicity, history of liver disease risk factors, history of other primary cancers, and a multigenerational family history of cancer. Self-reported data were corroborated using the electronic health record.

Sample collection and genetic testing. Patient saliva or blood samples were sent to a commercial laboratory (Invitae, San Francisco, CA) for processing and testing. The DNA extracted from saliva or blood samples was tested using a Clinical Laboratory Improvement Amendments (CLIA)–certified next-generation sequencing–based panel of 134 cancer-associated genes (Appendix Table A1). Full-gene sequencing and deletion and duplication analysis, including coding exons and 10-20 base pairs of adjacent intronic sequence on either side of the coding exons, was performed to detect single-nucleotide variants, insertions and deletions < 15 bp in length, and exon-level deletions and duplications, as previously described.[9] The variant interpretation was performed by Invitae scientific and clinical staff using a refinement of the American College of Medical Genetics (ACMG) criteria.[10]

Reporting of genetic test results. As the testing was CLIA-certified, the prospective study released P/LP results to patients and recorded the findings in the electronic health record. Variants of uncertain significance and normal results were not reported to patients. Patients were counseled on recommended follow-up in medical genetics or cancer genetics clinics for further management, as appropriate.

P/LP rate in genome aggregation database. A method adapted from Hu et al was used to estimate the P/LP carrier rate in the general population for *BRIP1* and *FANCA* using the genome aggregation database (gnomAD v2.1.1), which includes more than 134,000 unrelated individuals from various international genome projects.[11,12] P/LP variants were selected from whole-exome sequences with > 50× coverage, including all frameshift, stop gained, splice donor, and splice acceptor variants (*BRIP1*, n = 232; *FANCA*, n = 456), less those reported as benign, or likely benign or of uncertain significance by a majority of laboratories on ClinVar (*BRIP1*, n = 97; *FANCA*, n = 16), plus all missense variants reported as P/LP in ClinVar (*BRIP1*, n = 92; *FANCA*, n = 169). P/LP variants were then weighted on the basis of self-reported ancestry for a total of 202 P/LP variants of 115,244 individuals for *BRIP1* and 535 P/LP variants of 111,718 individuals for *FANCA*.

Statistical analysis. Descriptive statistics were used to summarize the clinical and demographic characteristics. The rates of P/LP variants of *BRIP1* and *FANCA* were compared with the population rates using the Fisher's exact test with *P* value reported.

Retrospective Comparison Cohort (Invitae cohort)

Under IRB approval, de-identified clinical information was retrospectively reviewed on a separate cohort of 219 consecutive participants who underwent genetic testing for HCC between December 2015 and September 2020 at a commercial laboratory (herein referred to as the Invitae cohort). HCC cases were determined from phenotypic data

and International Classification of Diseases–10 codes reported by clinicians on the test requisition forms. Sample types for this cohort included germline DNA extracted from blood or saliva. Once extracted, DNA was processed and tested as described above.[9] There was no overlap in cases between the prospective and retrospective cohorts.

RESULTS

Patient Characteristics: Prospective Cohort

Table 1 summarizes the demographic characteristics of study participants from the prospective cohort. A total of 232 patients enrolled in the study, and 217 (93.5%) patients completed testing.

The distribution of sex, history of underlying liver disease and transplantation status, Barcelona Clinic Liver Cancer stage,[8] and ethnicity was similar between patients found to have P/LP variants compared with those who did not (Table 1). Of 18 patients who had small HCCs found incidentally on explant and completed genetic testing, all were negative for P/LP variants. Of the 25 patients with P/LP gene variants, one (4.0%) had a first- or second-degree relative with HCC versus 12 of 207 (6.3%) patients without any such variant. Considering family history of other cancers, 88.0% of patients with P/LP variants had a relative with cancer as compared with 77.1% of those without.

Spectrum of Pathogenic and Likely Pathogenic Variants in the Prospective Cohort

The cancer predisposition multigene panel used for the study comprises 134 genes linked to risks for a large variety of cancers (Appendix Table A1). This includes all 24 cancer predisposition genes recommended for reporting by the ACMG in patients undergoing whole-exome sequencing as they are considered highly penetrant and clinically actionable.[13]

Among the 217 consecutive patients undergoing testing in the prospective cohort, there were 28 heterozygous P/LP germline variants in cancer-associated genes identified in the germline of 25 patients (11.5%) (Table 2 and Fig 1A). Three patients had P/LP variants in two different genes.

There were an additional 20 heterozygous P/LP variants in *CFTR*, which is associated with autosomal recessive cystic fibrosis. This gene is included on the panel because of an association with chronic pancreatitis, which in turn is linked to pancreatic cancer. Since heterozygous *CFTR* variants occurred at population rates,[14,15] and are implausibly involved in the pathogenesis of HCC, they were excluded from the analyses.

ACMG-reportable genes. Of the 217 patients completing testing, four (1.8%) had P/LP variants in highly penetrant genes recommended to be reported by the ACMG (Table 2).[13] Two patients had *BRCA2* pathogenic variants, which are causative of hereditary breast and ovarian cancer syndrome, and two patients had pathogenic variants in mismatch repair genes causative of Lynch syndrome,

namely *MSH6* and *PMS2*. Both patients with *BRCA2* pathogenic variants had compensated cirrhosis and multifocal HCC at presentation (Barcelona Clinic Liver Cancer stage B and C), and one had a second primary cancer, renal cell carcinoma. A patient with *PMS2*-associated Lynch syndrome had an adrenal adenoma. All four patients with pathogenic variants in ACMG-reportable genes had first- or second-degree relatives with cancers and were slightly younger at HCC diagnosis with average age 56 (range 47-66) versus 61 years.

Variants in FANCA. Five patients (2.3% of total) had heterozygous P/LP variants in the gene *FANCA* (Table 2). Preliminary data support an association between variants in *FANCA* and prostate cancer.[16-18] Interestingly, one patient with a *FANCA* pathogenic variant had prostate cancer in addition to HCC, and another patient had a first-degree relative with prostate cancer.

There was a significant enrichment of P/LP variants in *FANCA* among patients with HCC as compared with the rates in the general noncancer population on gnomAD[11] (Fisher's exact *P* value = .0043), demonstrating a possible association in HCC for P/LP variants in *FANCA*.

Variants in BRIP1. Four P/LP variants (1.8% of total) were detected in *BRIP1* (Table 2), a gene linked to ovarian cancer susceptibility.[19,20] The rate of P/LP variants in *BRIP1* reached significant enrichment in HCC as compared with individuals without cancer in gnomAD (Fisher's exact *P* value = .0007).

Other genes. A number of additional genes associated with cancer predisposition were identified in patients with HCC in the Penn cohort (Fig 1A and Table 2). There were three P/LP variants in *CHEK2*, which is associated with an autosomal dominant risk of breast, prostate, colon, and thyroid cancers.[21] There were three heterozygous P/LP variants in *MUTYH*, a gene associated with autosomal recessive polyposis.[13] Heterozygosity for *MUTYH* occurs in up to 2% of European populations[22,23] and has been linked to a slightly increased risk of colon cancer.[24]

Additional heterozygous variants were identified in genes associated with autosomal recessive Fanconi anemia including *FANCD2* (n = 2), *FANCM*, and *ERCC4*. There were single cases of P/LP variants in *RAD50*, *NF1*, *TMEM127*, and *MITF*, genes associated with autosomal dominant susceptibility to a variety of cancers.[25-30] There was a heterozygous pathogenic variant in *MSH3*, which is linked to autosomal recessive polyposis.[31]

Impact on Clinical Management: Prospective Cohort

P/LP results and post-test genetic counseling were provided to patients. In several instances, the genetic testing results had an immediate impact on the clinical management of patients, particularly for those cured of HCC by liver transplantation. In one patient who had progression of HCC on standard therapy, the discovery of a germline

TABLE 1. Demographic and Clinical Features of 232 Enrolled Participants (Prospective Cohort)

Characteristic	All Patients (N = 232), No. (%)	Testing Not Completed (n = 15), No. (%)	P/LP Cancer-Associated Gene, No. (%)	
			Yes (n = 25)	No (n = 192)
Age at diagnosis, years				
< 40	4 (1.7)	0	0	4 (2.1)
40-49	18 (7.8)	0	2 (8.0)	16 (8.3)
50-59	73 (31.5)	6 (40.0)	7 (28.0)	60 (31.25)
60-69	117 (50.4)	7 (46.7)	13 (52.0)	97 (50.5)
70-79	18 (7.8)	2 (13.3)	3 (12.0)	13 (6.8)
> 80	2 (0.9)	0	0	2 (1.0)
Sex				
Male	193 (83.2)	14 (93.3)	22 (88.0)	157 (81.8)
Female	39 (16.8)	1 (6.7)	3 (12.0)	35 (18.2)
Underlying etiology of liver disease				
Cirrhosis	214 (92.2)	14 (93.3)	24 (96.0)	176 (91.7)
HCV	136 (58.6)	11 (73.3)	14 (56.0)	111 (57.8)
Alcohol	49 (21.1)	5 (33.3)	3 (12.0)	41 (21.4)
Nonalcoholic fatty liver disease	41 (17.7)	3 (20.0)	7 (28.0)	31 (16.2)
HBV	20 (8.6)	1 (6.7)	2 (8.0)	17 (8.9)
Other diagnosis	18 (7.8)	0	3 (12.0)[a]	15 (7.2)[b]
Underwent liver transplantation	166 (71.6)	11 (73.3)	16 (64.0)	139 (72.4)
BCLC stage at presentation				
0	27 (11.6)	0	3 (12.0)	24 (12.5)
A	139 (59.9)	10 (66.7)	17 (68.0)	112 (58.3)
B	31 (13.4)	1 (6.7)	4 (16.0)	26 (13.5)
C	15 (6.5)	2 (13.3)	1 (4.0)	12 (6.3)
Incidental HCC[c]	20 (8.6)	2 (13.3)	0	18 (9.4)
Family history (first- or second-degree relative)				
HCC	15 (6.4)	2 (13.3)	1 (4.0)	12 (6.3)
Other cancer	180 (77.6)	10 (66.7)	22 (88.0)	148 (77.1)
Personal medical history				
Colon polyps	133 (57.3)	10 (66.7)	16 (64.0)	107 (55.7)
Regular EtOH	138 (59.5)	9 (60.0)	11 (44.0)	118 (61.5)
Smoking	148 (63.8)	11 (73.3)	18 (72.0)	119 (62.0)
Other primary cancers	89 (38.4)	4 (26.7)	9 (36.0)	76 (40.0)
Ethnicity				
White	187 (80.6)	10 (6.7)	21 (84.0)	156 (81.3)
Black or African American	32 (13.8)	4 (26.7)	3 (12.0)	25 (13.0)
Asian	10 (4.3)	0	1 (4.0)	9 (4.7)
More than one	3 (1.3)	1	0	2 (1.0)
Hispanic or Latino	15 (6.5)	1 (6.7)	1 (4.0)	13 (6.8)
Ashkenazi Jewish	10 (4.3)	0	3 (12.0)	7 (3.7)

NOTE. *CFTR* is excluded from this analysis as described in the text.

Abbreviations: BCLC, Barcelona Clinic Liver Cancer; EtOH, ethyl alcohol; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; P/LP, pathogenic and likely pathogenic.

[a]Polycystic liver disease and autoimmune hepatitis with cirrhosis, toxin exposure, and idiopathic noncirrhotic HCC.

[b]Cryptogenic cirrhosis (three), cardiogenic liver disease (three), primary sclerosing cholangitis, primary biliary cholangitis (two), acquired hemochromatosis with cirrhosis, alpha-1-antitrypsin disease (two), and unspecified (three).

[c]Incidental HCC indicates that a focus of HCC was found in the explant in patients undergoing liver transplantation for end-stage liver disease.

TABLE 2. Germline P/LP Variants in Cancer Predisposition Genes in Patients With HCC (prospective cohort)

Age at Diagnosis (years), Sex	Gene	Heterozygous Variant	Pathogenicity	gnomAD v2.1.1 (Noncancer) Allele Frequency[a]	Self-Reported Ancestry	Underlying Liver Disease	Personal History of Other Primary Neoplasm, Age (years)	Family History of Cancer
ACMG-reportable genes								
47, male	BRCA2	c.8702del (p.Gly2901fs)	Pathogenic	—	Asian, non-Hispanic	HBV cirrhosis	—	SDR: leukemia
66, male	BRCA2	c.3967A>T (p.Lys1323*)	Pathogenic	—	Black, non-Hispanic	HCV cirrhosis	Renal cell carcinoma, 68	FDR: lung
51, male	MSH6	c.1242G>A (p.Trp414*)	Pathogenic	—	White, non-Hispanic	HCV cirrhosis	—	FDR: breast
61, male	PMS2	Deletion (exons 6-7)	Pathogenic	—	White, non-Hispanic	HCV cirrhosis	Adrenal adenoma, 61	FDR: uterine, unknown cancer type
FANCA								
67, male	FANCA	c.3915_3916del (p.Phe1306Serfs*6)	Pathogenic	1.12×10^{-5}	White, non-Hispanic	HCV cirrhosis	—	FDR: colon, ovarian, multiple myeloma
73, male	FANCA	c.65G>A (p.Trp22*)	Pathogenic	1.23×10^{-4}	White, non-Hispanic, Ashkenazi	NAFLD cirrhosis	Prostate, 68	FDR: pancreatic SDR: pancreatic, H&N, mesothelioma, lung, esophageal, stomach
58, female	FANCA	c.65G>A (p.Trp22*)	Pathogenic	1.23×10^{-4}	White, non-Hispanic, Ashkenazi	Autoimmune hepatitis, polycystic liver disease, cirrhosis	—	FDR: lung SDR: breast
68, male	FANCA	c.2852G>A (p.Arg951Gln)	Pathogenic	6.34×10^{-5}	White, non-Hispanic	NAFLD cirrhosis	Basal cell carcinoma, 63	FDR: lung × 2, breast
71, male	FANCA	c.3791_3793del (p.Ser1264del)	Likely pathogenic	—	Black, non-Hispanic	HCV and alcoholic cirrhosis	—	FDR: breast, prostate
BRIP1								
69, male	BRIP1	Deletion exon 8	Likely pathogenic	—	White, non-Hispanic	NAFLD cirrhosis	Transitional cell carcinoma, 61 and 69, papillary urothelial carcinoma, 67	FDR: ovarian, lung × 2 SDR: prostate, HCC
54, male	BRIP1	c.2392C>T (p.Arg798*)	Pathogenic	1.40×10^{-4}	White, non-Hispanic, Ashkenazi	HCV cirrhosis	—	—
69, male	BRIP1	c.290_293del (p.Asn97Metfs*3)	Pathogenic	4.22×10^{-6}	White, non-Hispanic, Ashkenazi	Environmental toxin exposure	—	FDR: leukemia, lung, cervical or ovarian, skin × 2
46, male	BRIP1	c.206-1G>T (splice acceptor)	Likely pathogenic	None	White, non-Hispanic	HCV cirrhosis	—	SDR: breast, lung, brain
	CHEK2	c.58C>T (p.Gln20*) (possibly mosaic)	Pathogenic	1.5×10^{-4}				
Other cancer predisposition genes								
65, male	CHEK2	c.470T>C (p.Ile157Thr)	Pathogenic (low penetrance)	4.88×10^{-3}	White, non-Hispanic	HCV cirrhosis	—	FDR: colon, tonsil SDR: unknown cancer type
52, female	CHEK2	c.1100del (p.Thr367Metfs*15)	Pathogenic	2.12×10^{-3}	Black, non-Hispanic	HCV cirrhosis	Anal cancer, 60	FDR: breast, lung SDR: breast, bone, skin
61, female	MUTYH	c.1187G>A (p.Gly396Asp)	Pathogenic	2.99×10^{-3}	White, non-Hispanic	HCV cirrhosis	—	FDR: prostate × 6 SDR: breast
61, male	MUTYH	c.1187G>A (p.Gly396Asp)	Pathogenic	2.99×10^{-3}	White, non-Hispanic	NAFLD cirrhosis	—	FDR: prostate SDR: breast
58, male	MUTYH	c.1187G>A (p.Gly396Asp)	Pathogenic	2.99×10^{-3}	White, non-Hispanic, Ashkenazi	NAFLD cirrhosis	—	—
60, male	FANCD2	c.3922C>T (p.Gln1308*)	Pathogenic	—	White, non-Hispanic	NAFLD cirrhosis	Testicular cancer 30, basal cell carcinoma 63	FDR: breast SDR: leukemia × 2
63, male	FANCD2	c.2444G>A (p.Arg815Gln)	Pathogenic	1.35×10^{-4}	White, Hispanic	HCV cirrhosis	—	—
59, male	TMEM127	Deletion (entire coding sequence)	Pathogenic	—	White, non-Hispanic	Alcoholic and NAFLD cirrhosis	—	FDR: cervical, breast SDR: colon
	FANCM	c.5647C>T (p.Gln1883*)	Pathogenic	—				

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TABLE 2. Germline P/LP Variants in Cancer Predisposition Genes in Patients With HCC (prospective cohort) (Continued)

Age at Diagnosis (years), Sex	Gene	Heterozygous Variant	Pathogenicity	gnomAD v2.1.1 (Noncancer) Allele Frequency[a]	Self-Reported Ancestry	Underlying Liver Disease	Personal History of Other Primary Neoplasm, Age (years)	Family History of Cancer
72, male	RAD50	c.2934_2935del (p.Glu979Thrfs*2)	Pathogenic	8.51×10^{-6}	White, non-Hispanic	Noncirrhotic, HCC found incidentally	—	FDR: colon SDR: breast
72, male	NF1	c.1466A>G (p.Tyr489Cys)	Pathogenic	8.47×10^{-6}	White, non-Hispanic	HBV and HCV cirrhosis	Gallbladder adenocarcinoma, 72	FDR: breast, pancreatic
56, male	ERCC4	c.2395C>T (p.Arg799Trp)	Pathogenic	4.03×10^{-4}	White, non-Hispanic	HCV cirrhosis	Gastric marginal zone lymphoma, 59	FDR: breast
62, male	MITF	c.952G>A (p.Glu318Lys)	Pathogenic	1.29×10^{-3}	White, non-Hispanic	HCV cirrhosis	—	FDR: unknown cancer type
	MSH3	c.513del (p.Asp171Glufs*62)	Pathogenic	—				

Abbreviations: ACMG, American College of Medical Genetics; FDR, first-degree relative; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; H&N, head and neck; NAFLD, nonalcoholic fatty liver disease; P/LP, pathogenic and likely pathogenic; SDR, second-degree relative.

[a]Karczewski et al.[11]

BRCA2 variant prompted the use of the PARP inhibitor olaparib, as there is evidence that this class of drug can be used as a targeted therapy in BRCA1 and BRCA2 carriers with breast, ovarian, prostate, and pancreatic cancer.[16,21,32] Unfortunately, this patient has progressive disease on olaparib. One patient with a pathogenic deletion of TMEM127, which is associated with autosomal dominant hereditary paraganglioma and pheochromocytoma syndromes, was recommended to undergo surveillance imaging with MRI.[33] Two patients were recommended to undergo regular screening according to clinical guidelines for the management of Lynch syndrome. Multiple patients' families were recommended to undergo cascade genetic testing.

Variants Detected in the Retrospective Cohort

The spectrum of variants among 219 patients with HCC referred for testing at a large-volume commercial laboratory (Invitae) was examined retrospectively. These patients underwent testing with a variety of cancer predisposition gene panels ranging in size between 1 and 154 genes. In contrast to the prospective cohort, the retrospective cohort had a majority of females (Table 3).

Among the 219 patients with HCC who underwent genetic testing, there were 31 P/LP variants in 30 patients (13.7%) (Fig 1B and Table 4). There were 13 (5.9%) patients with P/LP variants in ACMG-reportable cancer predisposition genes including BRCA2 (n = 6) and BRCA1 (n = 1) for an overall rate of BRCA1/2 of 2.7%, MSH2 (n = 2, associated with Lynch syndrome), TP53 (n = 2, associated with Li Fraumeni syndrome), and APC (n = 2, associated with familial adenomatous polyposis syndrome). The patients with APC P/LP variants likely had hepatoblastoma based on their early age of diagnosis of liver cancer (4 and 13 years) and the known association with APC.[34] In addition to the
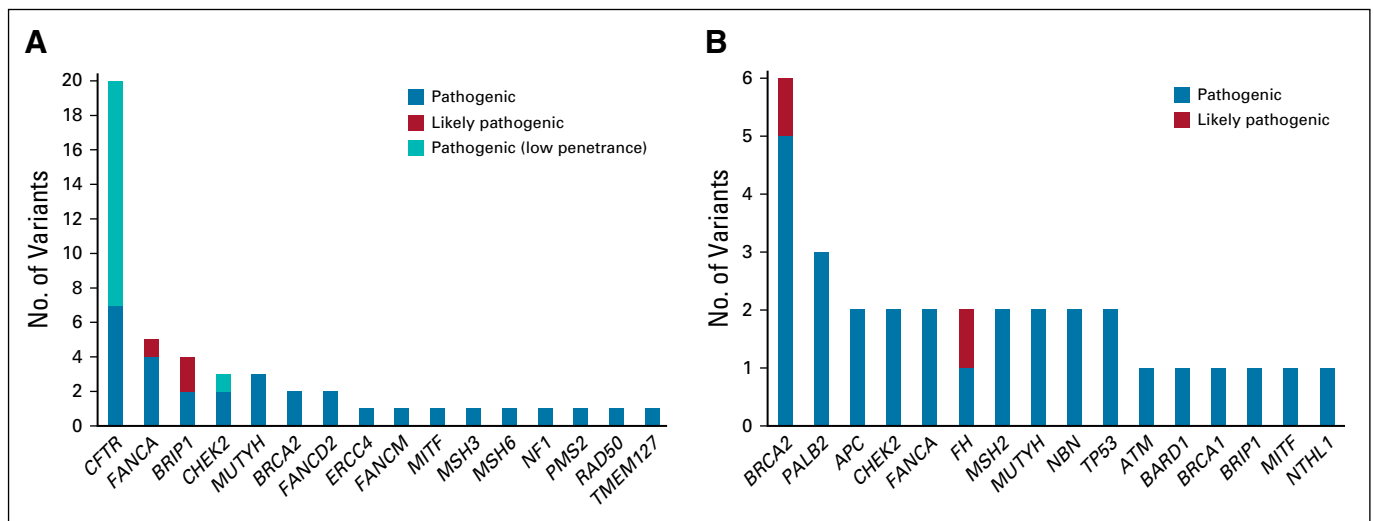
FIG 1. Distribution of P/LP counts for each gene with at least one P/LP variant identified in patients with HCC. (A) Prospective cohort: a consecutive series of patients with HCC undergoing multigene panel testing at a tertiary medical center. Pathogenic (low penetrance) indicates variants with sufficient evidence of pathogenicity but with an attenuated phenotype as compared with other P/LP variants of the gene. (B) Retrospective cohort: a consecutive series of patients with HCC completing testing at a commercial laboratory. HCC, hepatocellular carcinoma; P/LP, pathogenic and likely pathogenic.

ACMG-reportable genes, of 12 who underwent testing for *FANCA*, one patient was found to have two pathogenic variants (c.709+5G>A splice site and c.1304G>A, phase unknown). Of 190 patients tested for *BRIP1*, there was one pathogenic variant.

In addition to the ACMG-reportable genes, there were a number of other variants in genes with potential for precision therapy, clinical treatment trial, and/or management implications including cascade family variant testing (Fig 1B).

DISCUSSION

In this study, which is the largest analysis of multigene panel testing in HCC, the overall rate of P/LP germline variants was 11%-14%, or more than one of nine patients. The rate was 1.8%-5.9% for highly penetrant cancer genes. Positive test results led to changes in clinical management for some patients and recommendations for cascade genetic testing in at-risk relatives.

Hereditary predisposition may have been overlooked in HCC because of clear environmental risk factors. Indeed, there were no distinguishing clinical features in patients harboring P/LP variants, and they had a variety of underlying acquired risk factors such as chronic HCV, HBV, and nonalcoholic fatty liver disease. This may help to explain the absence of established, clinical criteria–based recommendations for germline testing in patients with HCC.

P/LP variants in *FANCA* and *BRIP1* were found in more than 4% of patients with HCC in the prospective cohort, representing a new potential association. Both *FANCA* and *BRIP1* are involved in the homologous recombination DNA damage repair (HR-DDR) pathway, and biallelic inactivating mutations in these genes cause Fanconi anemia.[35] Growing evidence also implicates monoallelic variants in predisposition to prostate cancer[16-18] and ovarian cancer,[19,20] respectively. Intriguingly, Oussalah et al[22] identified a haplotype of three single-nucleotide polymorphisms in *BRIP1* that was significantly associated with the risk of HCC in patients of European and African ancestry with cirrhosis because of viral hepatitis, supporting links for this gene and HCC. Our retrospective cohort had too small of a sample size to replicate the findings for *BRIP1* and *FANCA*, and a larger study will be needed to evaluate the generalizability of these results.

A majority of P/LP variants identified in our study were in genes involved in HR-DDR pathways. Recent studies support a role for this pathway in HCC pathogenesis. In one molecular profiling study of solid tumor samples, somatic homologous variants in HR-DDR genes were found in 20.9% of 115 tested HCC samples.[23] An analysis of data from the cancer genome atlas identified 15 cases of HCC with germline or somatic variants in genes related to the HR-DDR pathway.[24] Among a sample of 214 patients with HCC, somatic and germline variants in HR-DDR genes were found in 42 and seven patients, respectively, the latter encompassing variants in *BRCA1* (n = 2), *ATM* (n = 1), *PMS2* (n = 2), *BLM* (n = 1), and *RAD50* (n = 1).[25] Together, this suggests that HR-DDR genes may be involved in the

TABLE 3. Demographic Features of 219 Patients With HCC Undergoing Genetic Testing at a Commercial Laboratory (Retrospective Cohort)

Characteristic	All Patients (N = 219), No. (%)	P/LP Cancer-Associated Gene, No. (%)	
		Yes (n = 30)	No (n = 189)
Age at time of testing, years			
< 40	37 (16.9)	4 (13.3)	33 (17.5)
40-49	20 (9.1)	1 (3.3)	19 (10.1)
50-59	30 (13.7)	4 (13.3)	26 (13.8)
60-69	68 (31.1)	14 (46.7)	54 (28.6)
70-79	47 (21.5)	3 (10.0)	44 (23.3)
> 80	17 (7.8)	4 (13.3)	13 (6.8)
Sex			
Male	96 (43.8)	12 (40.0)	84 (44.4)
Female	123 (56.2)	18 (60.0)	105 (55.6)
Race			
White	157 (71.7)	21 (70.0)	136 (66.7)
Black or African American	19 (8.7)	1 (3.3)	18 (9.5)
Asian or Pacific Islander	10 (4.6)	2 (6.7)	8 (4.2)
Mixed, others, unknown	26 (11.9)	6 (20.0)	20 (10.6)
Hispanic or Latino	6 (2.7)	0	6 (3.2)
Native American	1 (0.5)	0	1 (0.5)

Abbreviations: HCC, hepatocellular carcinoma; P/LP, pathogenic and likely pathogenic.

TABLE 4. P/LP Variants in Cancer-Associated Genes in Patients With HCC (retrospective cohort)

Age (years)	Sex	Ethnicity	No. of Genes Tested	Gene	Variant	Zygosity	Interpretation	Family History of Cancer
20-29	Female	White	80+	APC	c.4216C>T (p.Gln1406*)	Heterozygous	Pathogenic	FDR: breast (39) SDR: brain, eye (40s), colon (50s)
30-39	Female	White	1	APC	c.3183_3187delACAAA (p.Gln1062*)	Heterozygous	Pathogenic	FDR: colon SDR: breast (45)
50-59	Male	White	41-50	ATM	c.3214G>T (p.Glu1072*)	Heterozygous	Pathogenic	FDR: breast, thyroid, RCC SDR: RCC, pancreatic (63), stomach
60-69	Female	White	41-50	BARD1	c.448C>T (p.Arg150*)	Heterozygous	Pathogenic	FDR: stomach (77) SDR: endometrial (50), pancreatic (60), throat
60-69	Male	White	80+	BRCA1	c.3882del (p.Leu1295Cysfs*12)	Heterozygous	Pathogenic	FDR: prostate (58 and 66), kidney (90) SDR: breast (75)
60-69	Female	White	80+	BRCA2	c.8167G>C (p.Asp2723His)	Heterozygous	Pathogenic	FDR: myeloma (53) SDR: gastric (84)
60-69	Male	White	80+	BRCA2	c.68-2A>G (splice acceptor)	Heterozygous	Likely pathogenic	SDR: ovarian, prostate, melanoma
80+	Female	Unknown	80+	BRCA2	c.3680_3681del (p.Leu1227Glnfs*5)	Heterozygous	Pathogenic	—
60-69	Female	White	11-20	BRCA2	c.3975_3978dup (p.Ala1327Cysfs*4)	Heterozygous	Pathogenic	FDR: throat, lymphoma, skin, prostate, breast SDR: bone, others
60-69	Male	White	41-50	BRCA2	c.1189_1190insTTAG (p.Gln397Leufs*25)	Heterozygous	Pathogenic	FDR: ovarian
60-69	Male	Black or African American	2-10	BRCA2	c.4552del (p.Glu1518Asnfs*25)	Heterozygous	Pathogenic	FDR: breast (31)
70-79	Female	White	80+	BRIP1	c.2392C>T (p.Arg798*)	Heterozygous	Pathogenic	FDR: leukemia (60s), prostate (49), breast (44) SDR: stomach (40)
50-59	Female	White	41-50	CHEK2	c.1100del (p.Thr367Metfs*15)	Heterozygous	Pathogenic	SDR: optic nerve tumor
80+	Female	White	51-80	CHEK2	c.1100delC (p.Thr367Metfs*15)	Heterozygous	Pathogenic	—
50-59	Male	Asian	21-30	FANCA	c.709+5G>A (intronic)	Heterozygous	Pathogenic	FDR: breast (40), ovarian (48), leukemia (10) SDR: breast (40), ovarian (55)
				FANCA	c.1304G>A (p.Arg435His)	Heterozygous	Pathogenic	
70-79	Female	White	80+	FH	c.700A>G (p.Thr234Ala)	Heterozygous	Pathogenic	SDR: cholangiocarcinoma (59), breast (35), breast (60)
60-69	Male	White	80+	FH	c.1431_1433dup (p.Lys477dup)	Heterozygous	Likely pathogenic	—
60-69	Female	White	80+	MITF	c.952G>A (p.Glu318Lys)	Heterozygous	Pathogenic	FDR: sarcoma (< 13), acute leukemia (82)
60-69	Male	White	2-10	MSH2	c.942+3A>T (intronic)	Heterozygous	Pathogenic	FDR: uterine (30s) SDR: brain (17), breast × 2 unknown (30s), unknown × 2
60-69	Female	Unknown	80+	MSH2	c.2113delG (p.Val705Trpfs*5)	Heterozygous	Pathogenic	FDR: liver (62) SDR: ovarian, breast, uterine lymphoma (65), unknown (30s), unknown (60s), unknown (60s), unknown (40s)
0-20	Male	Others	1	MUTYH	c.312C>A (p.Tyr104*)	Heterozygous	Pathogenic	—

(Continued on following page)

TABLE 4. P/LP Variants in Cancer-Associated Genes in Patients With HCC (retrospective cohort) (Continued)

Age (years)	Sex	Ethnicity	No. of Genes Tested	Gene	Variant	Zygosity	Interpretation	Family History of Cancer
50-59	Female	Others	80+	MUTYH	c.1187G>A (p.Gly396Asp)	Heterozygous	Pathogenic	FDR: unknown type (50s)
30-39	Female	Unknown	2-10	NBN	c.657_661del (p.Lys219Asnfs*16)	Heterozygous	Pathogenic	—
60-69	Male	White	41-50	NBN	c.127C>T (p.Arg43*)	Heterozygous	Pathogenic	—
70-79	Female	White	41-50	NTHL1	c.268C>T (p.Gln90*)	Heterozygous	Pathogenic	FDR: pancreatic (50s) SDR: breast, brain, colon, rectal (50s), gastric (50s)
60-69	Male	White	80+	PALB2	c.2386G>T (p.Gly796*)	Heterozygous	Pathogenic	FDR: ovarian (57), liver (50s), liver (50s) SDR: liver (35), liver (60s)
60-69	Male	White	80+	PALB2	c.1240C>T (p.Arg414*)	Heterozygous	Pathogenic	FDR: other cancer (50s) SDR: pancreatic (50s)
80+	Female	Asian	80+	PALB2	c.2470delT (p.Cys824Valfs*27)	Heterozygous	Pathogenic	FDR: lung
80+	Female	White	41-50	TP53	c.713G>A (p.Cys238Tyr)	Possibly mosaic	Pathogenic	FDR: colon, rectal (50s) SDR: stomach (40s), breast (50s)
40-49	Female	Unknown	51-80	TP53	c.524G>A (p.Arg175His)	Heterozygous	Pathogenic	FDR: rhabdomyosarcoma (15)

Abbreviations: FDR, first-degree relative; HCC, hepatocellular carcinoma; P/LP, pathogenic and likely pathogenic; RCC, renal cell carcinoma; SDR, second-degree relative.

mechanism of HCC carcinogenesis. Examining tumor tissues for loss of heterozygosity in association with germline variants with HR-DDR or Lynch syndrome genes would further suggest that germline variants may be drivers of HCC development. Future studies can evaluate for loss of heterozygosity with paired germline-tumor analysis.

The links between HCC and HR-DDR genes are intriguing as the identification of alterations in these genes may provide therapeutic options. PARP inhibitors have shown efficacy in patients with breast, ovarian, prostate, and pancreatic cancers who have germline or somatic variants in BRCA1, BRCA2, CHEK2, and FANCA.[16,21,32] A clinical trial is underway to study whether PARP inhibitors are effective in patients with germline defects in HR-DDR genes as part of a cancer-agnostic treatment approach for solid tumors (ClinicalTrials.gov identifier: NCT04171700). Other precision medicine opportunities include the use of PD-1 inhibitors in patients found to have Lynch syndrome.[26,27]

Future studies should examine the potential for an interaction between environmental factors and hereditary predisposition to HCC. It is plausible that individuals with germline defects in DNA repair genes may be more prone to the development of HCC in the setting of liver disease, given that chronic inflammation induces double-strand DNA breaks in liver cells.[28] Interestingly, among a cohort of patients with Lynch syndrome in the United Kingdom, a region with relatively low prevalence of chronic viral hepatitis infection, the lifetime incidence of HCC was found to be 0.5%,[29] whereas in a Chinese cohort, where there is a high proportion of chronic HBV infection, the rate of HCC among Lynch syndrome kindreds was 8%.[30]

This study has several strengths, including the prospective design, the enrollment of diverse consecutive patients with HCC seen at a medical center, and the use of CLIA-certified genetic testing, which enabled us to recontact patients, implement germline-based precision treatment changes, and recommend cascade testing for family members when clinically indicated. To our knowledge, this is the first study to systematically perform germline genetic testing in patients with HCC.

This study had several limitations. Recruitment for the prospective cohort was from a single tertiary care medical center and may not be generalizable to other centers. The sample set was biased toward patients who had undergone liver transplantation, which favors earlier-stage HCC. The gnomAD data set was used as the comparison group, whereas the ideal comparison would be with a matched control population from our institution. Nominal P values are shown for the sake of hypothesis generation, but a larger, dedicated study will be needed to replicate the results for FANCA and BRIP1. Finally, the retrospective cohort comprises patients referred for testing, resulting in selection bias.

In conclusion, universal testing of patients with HCC to identify germline variants should be considered, consistent with the INTERCEPT study supporting universal testing of all patients with cancer.[9] At a minimum, a thorough family history should be obtained for all patients and they should

be referred for testing if they meet established criteria for hereditary breast or ovarian cancer or Lynch syndrome. Future investigations may better define the subgroups of patients who would maximally benefit from genetic testing.

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APPENDIX

TABLE A1. Cancer Predisposition Multigene Panel Used in the Prospective Cohort

Gene Name	Transcript Accession Number
ALK	NM_004304.4
APC	NM_000038.5
ATM	NM_000051.3
AXIN2	NM_004655.3
BAP1	NM_004656.3
BARD1	NM_000465.3
BLM	NM_000057.3
BMPR1A	NM_004329.2
BRCA1	NM_007294.3
BRCA2	NM_000059.3
BRIP1	NM_032043.2
BUB1B	NM_001211.5
CASR	NM_000388.3
CDC73	NM_024529.4
CDH1	NM_004360.3
CDK4	NM_000075.3
CDKN1B	NM_004064.4
CDKN1C	NM_000076.2
CDKN2A	NM_058195.3
CDKN2A	NM_000077.4
CEBPA	NM_004364.4
CEP57	NM_014679.4
CFTR	NM_000492.3
CHEK2	NM_007194.3
CTC1	NM_025099.5
CTNNA1	NM_001903.3
CTRC	NM_007272.2
DICER1	NM_177438.2
DIS3L2	NM_152383.4
DKC1	NM_001363.4
EGFR	NM_005228.3
EGLN1	NM_022051.2
ENG	NM_000118.3
EPCAM[a]	NM_002354.2
ERCC4	NM_005236.2
EZH2	NM_004456.4
FANCA	NM_000135.2
FANCB	NM_001018113.1
FANCC	NM_000136.2
FANCD2[a]	NM_033084.3

(Continued in next column)

TABLE A1. Cancer Predisposition Multigene Panel Used in the Prospective Cohort (Continued)

Gene Name	Transcript Accession Number
FANCE	NM_021922.2
FANCF	NM_022725.3
FANCG	NM_004629.1
FANCI	NM_001113378.1
FANCL	NM_018062.3
FANCM	NM_020937.2
FH	NM_000143.3
FLCN	NM_144997.5
GALNT12	NM_024642.4
GATA1	NM_002049.3
GATA2	NM_032638.4
GPC3	NM_004484.3
GREM1 [a]	NM_013372.6
HOXB13	NM_006361.5
HRAS	NM_005343.2
KIF1B	NM_015074.3
KIT	NM_000222.2
MAX	NM_002382.4
MC1R	NM_002386.3
MDM2	NM_002392.5
MEN1	NM_130799.2
MET	NM_001127500.1
MITF[a]	NM_000248.3
MLH1	NM_000249.3
MLH3	NM_001040108.1
MRE11	NM_005591.3
MSH2	NM_000251.2
MSH3	NM_002439.4
MSH6	NM_000179.2
MUTYH	NM_001128425.1
NBN	NM_002485.4
NF1	NM_000267.3
NF2	NM_000268.3
NHP2	NM_017838.3
NOP10	NM_018648.3
NTHL1	NM_002528.6
PALB2	NM_024675.3
PALLD	NM_001166110.1
PDGFRA	NM_006206.4
PHOX2B[a]	NM_003924.3
PIK3CA	NM_006218.2
PMS2	NM_000535.5
POLD1	NM_002691.3

(Continued on following page)

TABLE A1. Cancer Predisposition Multigene Panel Used in the Prospective Cohort (Continued)

Gene Name	Transcript Accession Number
POLE	NM_006231.3
POT1	NM_015450.2
PRKAR1A	NM_002734.4
PRSS1	NM_002769.4
PTCH1	NM_000264.3
PTCH2	NM_003738.4
PTEN	NM_000314.4
RAD50	NM_005732.3
RAD51C	NM_058216.2
RAD51D	NM_002878.3
RB1	NM_000321.2
RECQL4	NM_004260.3
RET	NM_020975.4
RINT1	NM_021930.4
RPL11	NM_000975.3
RPL26	NM_000987.3
RPL35A	NM_000996.2
RPL5	NM_000969.3
RPS10	NM_001014.4
RPS19	NM_001022.3
RPS20	NM_001023.3
RPS24	NM_033022.3
RPS26	NM_001029.3
RPS7	NM_001011.3
RUNX1	NM_001754.4
SDHA[a]	NM_004168.3
SDHAF2	NM_017841.2
SDHB	NM_003000.2
SDHC	NM_003001.3
SDHD	NM_003002.3
SLX4	NM_032444.2
SMAD4	NM_005359.5
SMARCA4	NM_001128849.1
SMARCB1	NM_003073.3
SMARCE1	NM_003079.4
SPINK1	NM_003122.4
SPRED1	NM_152594.2
STK11	NM_000455.4
SUFU	NM_016169.3
TERC	NR_001566.1
TERT	NM_198253.2
TINF2	NM_001099274.1
TMEM127	NM_017849.3

TABLE A1. Cancer Predisposition Multigene Panel Used in the Prospective Cohort (Continued)

Gene Name	Transcript Accession Number
TP53	NM_000546.5
TSC1	NM_000368.4
TSC2	NM_000548.3
VHL	NM_000551.3
WRN[a]	NM_000553.4
WT1	NM_024426.4
XRCC2	NM_005431.1

[a]FANCD2: deletion and duplication analysis is not performed for exons 14-17, 22. WRN: deletion and duplication analysis is not performed for exons 10-11. EPCAM: sequencing analysis is not performed for this gene. GREM1: promoter region deletion and duplication testing only. MITF: c.952G>A p.Glu318Lys variant only. PHOX2B: alanine repeat numbers for the commonly expanded region in exon 3 are not determined. SDHA: deletion and duplication analysis is not performed for this gene.