CANCER GENETICS

Implications of Incidental Germline Findings Identified In the Context of Clinical Whole Exome Sequencing for Guiding Cancer Therapy

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PURPOSE Identification of incidental germline mutations in the context of next-generation sequencing is an unintended consequence of advancing technologies. These data are critical for family members to understand disease risks and take action.

PATIENTS AND METHODS A retrospective cohort analysis was conducted of 1,028 adult patients with metastatic cancer who were sequenced with tumor and germline whole exome sequencing (WES). Germline variant call files were mined for pathogenic/likely pathogenic (P/LP) variants using the ClinVar database and narrowed to high-quality submitters.

RESULTS Median age was 59 years, with 16% of patients ≤ 45 years old. The most common tumor types were breast cancer (12.5%), colorectal cancer (11.5%), sarcoma (9.3%), prostate cancer (8.4%), and lung cancer (6.6%). We identified 3,427 P/LP variants in 471 genes, and 84% of patients harbored one or more variant. One hundred thirty-two patients (12.8%) carried a P/LP variant in a cancer predisposition gene, with *BRCA2* being the most common (1.6%). Patients with breast cancer were most likely to carry a P/LP variant (19.2%). One hundred ten patients (10.7%) carried a P/LP variant in a gene that would be recommended by the American College of Medical Genetics and Genomics to be reported as a result of clinical actionability, with the most common being *ATP7B* (2.7%), *BRCA2* (1.6%), *MUTYH* (1.4%), and *BRCA1* (1%). Of patients who carried a P/LP variant in a cancer predisposition gene, only 53% would have been offered correct testing based on current clinical practice guidelines. Of 471 mutated genes, 231 genes had a P/LP variant identified in one patient, demonstrating significant genetic heterogeneity.

CONCLUSION The majority of patients undergoing clinical cancer WES harbor a pathogenic germline variation. Identification of clinically actionable germline findings will create additional burden on oncology clinics as broader WES becomes common.

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INTRODUCTION

Next-generation sequencing (NGS) of a patient's tumor has become a common approach to identify targetable genomic aberrations in patients with a variety of advanced malignancies. Currently, the US Food and Drug Administration has approved at least one panel-based test[1] and, more recently, a comprehensive NGS test with coverage of the whole exome, and more than three quarters of oncologists nationwide use some type of NGS-based test in routine practice.[2] As the number of novel drug targets increases and the cost of sequencing decreases,[3] comprehensive testing will become more cost efficient and more commonly used in the community setting.

One of the unintended consequences of conducting tumor NGS is the incidental identification of pathogenic germline variants.[4-9] For testing that does not include paired germline DNA, the detection of a mutation in a loci known to be consistent with a pathogenic germline mutation must prompt, at the very least, consideration of confirmatory germline testing. For most comprehensive, whole exome–based tests, paired germline is necessary to filter out the vast number of variants tested. In any case, the detection of concerning germline findings is complicated by multiple factors, including the complicated and dynamic characterization of variant pathogenicity (which is obviously designed for determining the targetability of a somatic variant) and the clinical actionability of the various pathogenic variants. The technology to identify such variants exceeds our ability to accurately determine pathogenicity of variants. It is not uncommon for laboratories to have discordant classifications of variants depending on their interpretation of the

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

What are some of the commonly identified germline findings when whole exome sequencing (WES) is performed in an oncology clinic with the intent of guiding cancer therapy?

Knowledge Generated

Patients undergoing WES are often found to carry at least one germline pathogenic variant, adding to the complexity of their care. Pathogenic variants were most commonly identified in non–cancer predisposition genes; however, 12.8% of patients carried a pathogenic variant in a cancer predisposition gene.

Relevance

As paired germline and tumor analysis becomes more common in oncology clinics, careful approaches to pretest counseling and result disclosure are critical. A multidisciplinary approach, including clinical genetics, is necessary to provide optimal care for both patients and their family members.

data.[10-12] These classifications may or may not be routinely updated by laboratories as new data emerge. Furthermore, a particular variant's pathogenicity may be dependent on whether it is somatic or germline in origin.

Equally important is the complex clinical context of such findings given that most patients have a life-limiting malignancy, receive the test without optimal pretest counseling, and have agreed to the test for a completely different reason (ie, to identify a drug target). Currently, ASCO supports the communication of medically relevant and incidental germline findings to patients,[13] and the vast majority of patients agree to receive these findings when given the option to consent.[14,15] Multiple studies have found that the occurrence of germline cancer predisposition variants is not rare in this population, ranging from 3.0% to 12.6%.[4-9] However, these data from panel-based testing are not comprehensive and lack risk-specific details such as family history. The largest study to date from The Cancer Genome Atlas (TCGA; n = 10,398) reported a frequency of 8%[16] but only included cancer-specific variants and did not have companion clinical annotation. Here, we present, to our knowledge, the first expansive germline findings for patients with cancer receiving comprehensive whole exome sequencing (WES) of the tumor and matched germline with annotated history with the primary intent to identify drug targets.

PATIENTS AND METHODS

Patients

Our retrospective analysis included 1,028 consecutive adult patients with any histologic type of metastatic cancer, who were referred to the Indiana University Health Precision Genomics clinic between January 12, 2016, and March 31, 2019, and who underwent paired tumor and germline WES. The primary intent for testing was to guide cancer therapy. Each patient had adequate tumor DNA to meet the minimum standards for WES. All patients also submitted a blood sample or buccal swab for germline analysis. This study was approved by the Indiana University Institutional Review Board.

Molecular Analysis of Patient Samples

DNA samples were obtained from each patient and sent to NantOmics (Culver City, CA) for paired germline/somatic testing. Clinical Laboratory Improvement Amendments (CLIA)–based somatic WES was performed on each sample.

In addition, exome sequencing of germline DNA was performed with CLIA reporting of the American College of Medical Genetics and Genomics (ACMG) cancer predisposition genes. DNA sequencing libraries were prepared from normal blood or buccal samples using the KAPA HyperPrep kit (Roche, Indianapolis, IN) and sequenced on an Illumina Sequencing Platform (Illumina, San Diego, CA). DNA sequencing data were aligned to the human genome (hg19) using the Burrows-Wheeler Aligner algorithm. Duplicated reads were marked by samblaster, and indel realignment and base quality recalibration were performed using GATK v2.3. Each variant was sequenced to a minimum depth of 10 reads and had a minimum alternate allele fraction of 0.25 in the normal sequencing data. Variant call format (VCF) files containing germline variants were generated. NantOmics WES CLIA sequencing has demonstrated $>$ 95% sensitivity and $>$ 99% specificity for germline single nucleotide polymorphisms and germline insertions and deletions.

Variant Interpretation for Hereditary Disease

Variants from germline VCF files were then annotated with ClinVar pathogenicity classifications using the Golden Helix SVS 8.8.3 software (Golden Helix, Bozeman, MT). Variants were filtered for those that were classified as either pathogenic or likely pathogenic (P/LP) by any one of the following ClinVar submitters: Ambry Genetics (Aliso Viejo, CA), Invitae (San Francisco, CA), or GeneDx (Gaithersburg, MD). These laboratories were selected because of their robust testing menu, thorough variant interpretation

processes, and frequent submissions and updates to ClinVar. All genes were included in this analysis regardless of disease category. Known artifacts of the assay were removed for quality control.

RESULTS

Patient Characteristics

One thousand twenty-eight patients who underwent paired tumor and germline WES were included in this analysis. The patient characteristics are listed in Table 1. The median age was 59 years, with 16% of patients < 45 years of age and 54% < 60 years of age. Eighty-six percent of the population self-defined their race as White or Caucasian and 8% as Black or African American. The most common tumor types were breast cancer (12.5%), colorectal cancer (11.5%), sarcoma (9.3%), prostate cancer (8.4%), and lung cancer (6.6%).

TABLE 1. Patient Characteristics and Tumor Types

Characteristics	No. of Patients (N = 1,028; %)
Age, years	
≤ 45	164 (16)
46 60	390 (38)
61 75	401 (39)
≥ 76	73 (7)
Sex	
Female	542 (53)
Male	486 (47)
Race	
White or Caucasian	890 (87)
Black or African American	84 (8)
Unknown	34 (3)
Asian	17 (2)
American Indian	3 (0.3)
Tumor type	
Breast	146 (13)
Colorectal	134 (12)
Sarcoma	109 (9)
Prostate	98 (8)
Lung	77 (7)
Head and neck	59 (5)
CNS tumor	52 (5)
Ovarian	58 (5)
Pancreas	47 (4)
Melanoma	44 (4)
Urothelial	42 (4)
Renal	30 (3)
Cholangiocarcinoma	26 (2)
Thymoma	24 (2)
Endometrial	23 (2)
Thyroid	23 (2)
Unknown primary	18 (2)
Other	157 (14)

Genomic Landscape

A total of 3,427 P/LP germline variants were identified. Of these, there were 855 unique P/LP variants in a total of 471 genes. The frequency and breakdown of P/LP variants is summarized in Figure 1. Eight hundred sixty-two (84%) of 1,028 patients carried a P/LP variant of any type (cancer or noncancer) in any gene (ACMG- or non–ACMG-designated variant; Data Supplement). Of 471 mutated genes, a single P/LP variant was identified in 231 genes (49%), demonstrating significant genetic heterogeneity. The most commonly mutated genes (frequency > 1.5%) are listed in Table 2, with *HFE* being the most common (37.4%) followed by *MCR1* (33.7%) and *GALT* (17%). The vast majority of these 29 genes have an autosomal recessive inheritance pattern, and only two (6.9%) of 29 are recommended by the ACMG to have results returned to the patient.

Frequency of Cancer Predisposition Genes, Concordance, and Predictive Capacity of the Variant

A total of 12.8% of patients carried a cancer predisposition variant (Table 3). The most commonly mutated cancer predisposition genes were *BRCA2* (1.6%), *CHEK2* (1.4%), *MUTYH* (1.4%), *ATM* (1.0%), and *BRCA1* (1.0%). Concordance between genes and tumor types was determined by a group of licensed genetic counselors using well-established, widely accepted cancer risks based on an

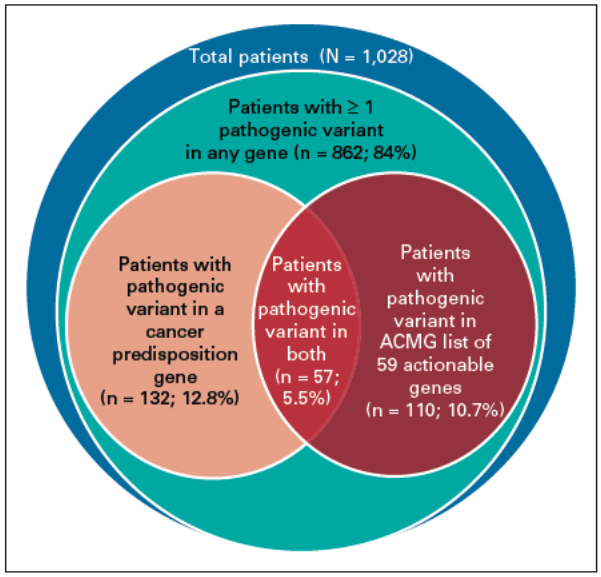

FIG 1. Pathogenic variants in 1,028 patients with advanced cancer who underwent paired tumor and germline whole exome sequencing. ACMG, American College of Medical Genetics and Genomics.

TABLE 2. Genes Mutated in ≥ 1.5% of Our Patient Cohort

Gene	Associated Condition	Inheritance Pattern	Included in ACMG List of 59 Actionable Genes	No. of Patients Carrying P/LP Variant (%)
HFE	Hereditary hemochromatosis	Autosomal recessive	No	357 (34.7)
MC1R	Modifier gene for hair and skin color; possible increased risk for melanoma	Modifier; possible autosomal dominant melanoma risk for some variants	No	346 (33.7)
GALT	Galactosemia	Autosomal recessive	No	175 (17.0)
CBS	Homocystinuria	Autosomal recessive	No	170 (16.5)
FLG	Atopic dermatitis	Modifier: highest risk associated with biallelic mutations	No	116 (11.3)
SERPIN1A	α_1 Antitrypsin	Autosomal codominant	No	115 (11.2)
BTD	Biotinidase deficiency	Autosomal recessive	No	79 (7.7)
GJB2	Nonsyndromic hearing loss	Autosomal recessive	No	63 (6.1)
ACADS	SCAD deficiency	Autosomal recessive	No	58 (5.6)
RBM8A	TAR syndrome	Autosomal recessive	No	54 (5.3)
WNT10A	Hypohidrotic ectodermal dysplasia	Autosomal recessive	No	51 (5.0)
ABCA4	Stargardt macular degeneration	Autosomal recessive	No	48 (4.7)
CFTR	Cystic fibrosis and congenital absence of the vas deferens	Autosomal recessive	No	32 (3.1)
SPG7	Spastic paraplegia 7	Autosomal recessive	No	29 (2.8)
ATP7B	Wilson disease	Autosomal recessive	Yes	28 (2.7)
ABCC6	Pseudoxanthoma elasticum	Autosomal recessive	No	27 (2.6)
MYO6	Deafness, autosomal dominant 22/ deafness, autosomal recessive 37	Autosomal dominant/autosomal recessive	No	24 (2.3)
MPO	Myeloperoxidase deficiency	Autosomal recessive	No	24 (2.3)
ACADM	Medium chain acyl CoA dehydrogenase deficiency	Autosomal recessive	No	23 (2.2)
TNFRSF13B	Common variable immune deficiency	Autosomal dominant/autosomal recessive	No	22 (2.1)
VWF	von Willebrand disease	Autosomal dominant/autosomal recessive	No	18 (1.8)
PAH	Phenylketonuria	Autosomal recessive	No	18 (1.8)
SLC7A9	Cystinuria	Autosomal recessive	No	18 (1.8)
SI	Congenital sucrase isomaltase deficiency	Autosomal recessive	No	17 (1.7)
F2	Prothrombin deficiency	Autosomal recessive	No	17 (1.7)
DHCR7	Smith Lemli Opitz syndrome	Autosomal recessive	No	17 (1.7)
SLC26A4	Pendred syndrome	Autosomal recessive	No	16 (1.6)
BRCA2	Hereditary breast and ovarian cancer syndrome/Fanconi anemia	Autosomal dominant/autosomal recessive	Yes	16 (1.6)
PKHD1	Polycystic kidney disease	Autosomal recessive	No	15 (1.5)

Abbreviations: ACMG, American College of Medical Genetics and Genomics; CoA, coenzyme A; P/LP, pathogenic or likely pathogenic; SCAD, short chain acyl CoA dehydrogenase; TAR, thrombocytopenia with absent radius.

accumulation of current data. After removing heterozygous *MUTYH* and *NTHL1* carriers, only 56% of patients with a cancer predisposition variant had a tumor type concordant with the carriage of that cancer predisposition gene. Specifically, 62% of patients with a *BRCA2* P/LP variant had a malignancy either previously or currently that would have been expected by carriage of a *BRCA2* P/LP variant. Similarly, 64%, 30%, and 90% of the *CHEK2*, *ATM*, and *BRCA1* carriers, respectively, had the tumor type either previously or currently that would have been expected by the P/LP variant. Patients with the following tumor types were most likely to carry a germline P/LP variant: breast cancer (19.2%), prostate cancer (18.4%), ovarian cancer (15.5%), and cholangiocarcinoma (15.4%); Data Supplement. Two of the 26 *BRCA1* or *BRCA2* P/LP variants identified were one of the three common Ashkenazi Jewish

TABLE 3. Concordance of Cancer Diagnoses for Those Carrying a Cancer Predisposition Gene and Predictive Capacity of Pedigree

Cancer Predisposition Gene	Tumor Types Seen	No. of Tumor Types Concordant With Gene (%)	No. of Patients Who Would Not Have Been Tested for Variant Based on Personal and Family History (%)
BRCA2 (n 16)	Breast[a] (n 4), prostate[a] (n 4), lung (n 1), sarcoma (n 1), salivary gland (n 1), head and neck (n 1), unknown primary (n 1), breast[a], vulvar, small bowel and anal (n 1)[b], breast[a] × 2 (n 1)[b], thymoma and lung (n 1)[b]	11 (52)	3 (19)
CHEK2 (n 14)	Breast[a] (n 5), colon[a] (n 3), prostate[a] (n 1), lung (n 1), nerve sheath (n 1), sarcoma (n 1), cholangiocarcinoma (n 1), granuloma cell tumor (n 1)	9 (64)	5 (36)
MUTYH (n 14)[c]	Pancreas (n 2), sarcoma (n 2), breast[a] (n 1), ACC (n 1), colon[a] (n 1), GIST (n 1), prostate (n 1), adenoid cystic (n 1), oligodendroglioma (n 1), bladder (n 1), SCC (n 1), neuroendocrine small bowel and melanoma (n 1)[b]	2 (13)	12 (86)
ATM (n 10)	Prostate[a] (n 2), colon (n 2), renal (n 1), lung (n 1), uterine (n 1), sarcoma (n 1), melanoma (n 1), hepatocellular, CLL, and prostate[a] (n 1)[b]	3 (25)	5 (50)
BRCA1 (n 10)	Breast[a] (n 2), breast[a] and ovarian[a] (n 2)[b], ovarian[a] (n 1), prostate[a] (n 1), glioblastoma (n 1), GIST and prostate[a] (n 1)[b], breast × 2, uterine, and colon (n 1)[b], ovarian[a] × 2 (n 1)[b]	13 (77)	0 (0)
MITF (n 8)	Breast (n 3), cholangiocarcinoma (n 2), pancreas (n 1), ACC (n 1), sarcoma and GIST (n 1)[b]	0 (0)	7 (88)
NTHL1 (n 8)[c]	Colon (n 2), sarcoma (n 1), uterine (n 1), glioblastoma (n 1), parotid (n 1), lung × 2 (n 1)[b], renal, bladder, melanoma, and prostate (n 1)[b]	0 (0)	7 (88)
TP53 (n 5)	Prostate[a] (n 1), breast[a] × 2 and sarcoma[a] (n 1)[b], ovarian[a] (n 1), PNET[a] (n 1), myeloma[a] and prostate[a] (n 1)[b]	8 (100)	0 (0)
CDKN2A (n 4)	Breast (n 1), bladder (n 1), pancreas[a] (n 1), ovarian (n 1)	1 (25)	3 (75)
HOXB13 (n 4)	Prostate[a] (n 2), breast (n 1), SCC (n 1)	2 (50)	2 (50)
FH (n 3)	GIST (n 1), breast and glioblastoma (n 1)[b], Leydig cell tumor and lung (n 1)[b]	0 (0)	3 (100)
NF1 (n 3)	Sarcoma[a] (n 2), sarcoma[a] × 2 (n 1)[b]	4 (100)	0 (0)
FANCC (n 3)	Sarcoma (n 1), ovarian (n 1), sarcoma × 2 (n 1)[b]	0 (0)	3 (100)
NBN (n 3)	Breast[a] (n 2), bladder (n 1)	2 (67)	1 (33)
PALB2 (n 3)	Ureter (n 1), ovarian[a] (n 1), melanoma (n 1)	1 (33)	1 (33)
MSH2 (n 2)	Colon[a] (n 2)	2 (100)	0 (0)
BRIP1 (n 2)	Cholangiocarcinoma (n 1), breast[a] and ovarian[a] (n 1)[b]	2 (66)	1 (50)
MLH1 (n 2)	Colon[a] (n 1), gastric[a] (n 1)	2 (100)	0 (0)
MEN1 (n 2)	Pancreas[a] and thymic[a] (n 1)[b], thyroid[a] and pancreas[a] (n 1)[b]	4 (100)	0 (0)
RECQL4 (n 2)	Gastroesophageal junction (n 2)	0 (0)	2 (100)
SDHB (n 1)	Pheochromocytoma[a] (n 1)	1 (100)	0 (0)

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TABLE 3. Concordance of Cancer Diagnoses for Those Carrying a Cancer Predisposition Gene and Predictive Capacity of Pedigree (Continued)

Cancer Predisposition Gene	Tumor Types Seen	No. of Tumor Types Concordant With Gene (%)	No. of Patients Who Would Not Have Been Tested for Variant Based on Personal and Family History (%)
KIT (n 1)	GIST[a] (n 1)	1 (100)	0 (0)
FLCN (n 1)	Unknown primary (n 1)	0 (0)	1 (100)
AXIN2 (n 1)	Colon[a] (n 1)	1 (100)	0 (0)
RB1 (n 1)	Sarcoma[a] and retinoblastoma[a] (n 1)[b]	2 (100)	0 (0)
RAD51D (n 1)	Esophageal (n 1)	0 (0)	1 (100)
APC (n 1)	Thymic (n 1)	0 (0)	1 (100)
RAD50 (n 1)	Prostate and tongue (n 1)[b]	0 (0)	1 (100)
VHL (n 1)	Breast and pheochromocytoma[a] (n 1)[b]	1 (50)	0 (0)
SDHA (n 1)	GIST[a] (n 1)	1 (100)	0 (0)
RET (n 1)	Medullary thyroid[a] (n 1)	1 (100)	0 (0)
BMPR1A (n 1)	Colon[a] (n 1)	1 (100)	0 (0)
RECQL (n 1)	Melanoma (n 1)	0 (0)	1 (100)
Overall (%)			47

Abbreviations: ACC, adrenocortical carcinoma; GIST, GI stromal tumor; PNET, pancreatic neuroendocrine tumor; SCC, squamous cell carcinoma.
[a]Tumor determined to be concordant with gene.
[b]Indicates patient had more than one primary malignancy.
[c]Autosomal recessive condition; all identified patients in this category carried a single heterozygous mutation.

founder mutations known to be more common in that population.

Frequency of ACMG-Recommended Actionable P/LP Variants

The ACMG has assembled a list of 59 genes deemed to be clinically actionable and for which the results should be offered to patients undergoing clinical exome and genome analysis.[17] Importantly, not all cancer predisposition variants are considered actionable, and not all actionable genes are cancer genes; the latter includes genes that can increase the risk of cardiac conditions, metabolic conditions, and others. The most commonly mutated ACMG genes with proposed management guidelines by the National Comprehensive Cancer Network (NCCN) and GeneReviews identified in our analysis were *ATP7B* (2.7%), *BRCA2* (1.6%), *MUTYH* (1.4%), and *BRCA1* (1%; Table 4). After removing heterozygous *ATP7B* and *MUTYH* carriers, 69 patients (6.7%) carried a mutation that would be recommended by the ACMG to be returned to the patient. In addition, 5.5% of patients (4.2% of patients after removing *MUTYH* heterozygotes) carried an ACMG-designated, clinically actionable cancer P/LP gene variant.

Relevance and Concordance of Pedigree With Cancer Risk Allele Carriage

The electronic medical record of each patient carrying a P/LP variant in a cancer predisposition gene was reviewed by a licensed genetic counselor. Of 132 patients carrying a cancer predisposition variant, 51.9% had a three-generation pedigree with a focus on cancer history taken by a licensed genetic counselor. The family history for the remaining 48.1% of patients was taken from the clinic notes of the medical oncologist in the Precision Genomics clinic. As a result of the patients undergoing germline analysis, a thorough family history was obtained for each patient. On the basis of the patients' pertinent demographics and family history, we used current clinical practice guidelines[18-20] to determine whether a patient would have been recommended to have genetic evaluation. If a patient's personal and family history would result in recommendation for genetic evaluation, a licensed genetic counselor determined which genetic test would likely have been offered to that patient using a multigene panel encompassing the tumor types identified in that patient's personal and family history. For patients who carried a cancer predisposing P/LP variant, 34% would not have been recommended to undergo testing, 13% would have been recommended to have a genetic test that would not have identified the uncovered P/LP variant, and 53% would have had the P/LP variant identified. For patients who carried an ACMG-recommended clinically actionable cancer predisposition P/LP variant, 23% would not have been recommended to undergo testing, 7% would have been recommended to have a genetic test that would not have identified the uncovered P/LP variant, and 70% would have had the P/LP variant identified. When considering the entire population, 6% and 1.7% of patients would not have had a cancer predisposition variant and an ACMG-recommended clinically actionable cancer predisposition variant, respectively, identified.

TABLE 4. Patients With P/LP Variant in ACMG Recommended Gene and Associated Condition

ACMG-Recommended Gene	Associated Condition	Inheritance Pattern	Proposed Management Guidelines	No. of Patients Carrying P/LP Variant (n = 1,028; %)
ATP7B	Wilson disease[a,b]	AR	Initiate treatment with copper chelating agents or zinc as soon as possible	28 (2.7)
			At least twice annually: serum copper and ceruloplasmin, liver biochemistries, international normalized ratio, CBC, urinalysis, and physical examination including neurologic assessment	
			At least once annually: 24 hour urinary excretion of copper	
BRCA2	Hereditary breast and ovarian cancer	AD	Women: clinical breast exam every 6 12 months starting at 25 years; annual MRI at 25 75 years; annual mammogram at 30 75 years; consider bilateral mastectomy; RRSO between age 35 and 40 years for *BRCA1* carriers but may be delayed until 40 45 years for *BRCA2*; consider TVUS/CA 125 starting at 30 35 years until RRSO	16 (1.6)
			Men: clinical breast exam every 12 months starting at 35 years; prostate screening at 40 years	
			Women and men: no specific guidelines for melanoma screening; pancreatic cancer screening using MRI/MRCP and/or EUS may be considered based on family history	
MUTYH	*MUTYH* associated polyposis[b,c]	AR	Heterozygotes: if first degree relative with CRC, colonoscopy every 5 years starting at 40 years; otherwise, no changes to management	14 (1.4)
			Homozygotes: colonoscopy starting at 25 30 years, every 2 3 years if negative; if positive, every 1 2 years; consider upper endoscopy starting at 30 35 years	
BRCA1	Hereditary breast and ovarian cancer	AD	Refer to *BRCA2* management	10 (1.0)
MYBPC3	Dilated cardiomyopathy 1A	AD	< 12 years: individualized based on personal/family history	6 (0.6)
			Between 12 and 18 years: physical exam, echocardiography, and ECG every 12 18 months	
			≥ 18 years: physical exam, echocardiography, and ECG at least every 5 years but may be individualized based on personal/family history	
TP53	Li Fraumeni syndrome	AD	Clinical breast exam every 6 12 months starting at age 20 years	5 (0.5)
			Annual breast MRI at 20 75 years	
			Annual mammogram at 30 75 years	
			Consider bilateral mastectomy	
			Colonoscopy and upper endoscopy every 2 5 years starting at 25 years	
			Physical exam including neurologic exam every 6 12 months	
			Annual dermatology exam starting at 18 years	
			Annual whole body MRI	
			Annual brain MRI	
			Pancreatic cancer screening using MRI/MRCP and/or EUS may be considered based on family history	

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TABLE 4. Patients With P/LP Variant in ACMG Recommended Gene and Associated Condition (Continued)

ACMG-Recommended Gene	Associated Condition	Inheritance Pattern	Proposed Management Guidelines	No. of Patients Carrying P/LP Variant (n = 1,028; %)
KCNQ1	Long QT syndrome type 1	AD	β Blockers	3 (0.3)
			ICD and/or LCSD may be considered based on personal/family history	
			Sodium channel blockers may be considered based on personal history	
DSG2	Arrhythmogenic right ventricular cardiomyopathy type 10	AD	Antiarrhythmic medication, ICD, and/or heart transplantation based on personal history	2 (0.2)
KCNH2	Long QT syndrome type 2	AD	Refer to *KCNQ1* management	2 (0.2)
LDLR	Familial hypercholesterolemia	SD	Lifestyle modifications to reduce CAD risk factors	2 (0.2)
			Consider low dose aspirin	
			Pharmacotherapy (statins with or without the use of other medication) to reduce lipid levels	
MEN1	Multiple endocrine neoplasia type 1	AD	Beginning at 5 years: serum concentration of prolactin, IGF 1, fasting glucose, and insulin; head MRI every 3 5 years	2 (0.2)
			Beginning at 8 years: fasting total serum calcium concentration (corrected for albumin) and/or ionized serum calcium concentration, chromogranin A, pancreatic polypeptide, glucagon, vasoactive intestinal peptide for other pancreatic NET	
			Beginning at 20 years: fasting serum gastrin concentration; abdominal CT or MRI every 3 5 years	
			Consider fasting serum concentration of intact (full length) PTH and yearly chest CT, somatostatin receptor scintigraphy octreotide scan	
MLH1	Lynch syndrome	AD	Consider BSO	2 (0.2)
			Colonoscopy every 1 2 years starting at 20 25 years	
			Consider hysterectomy	
			Endometrial biopsy every 1 2 years may be considered	
			TVUS may be considered at clinician's discretion	
			Consider upper endoscopy every 3 5 years starting at 40 years (stronger evidence for those of Asian descent)	
			Consider urinalysis starting at 30 35 years based on personal/family history	
			Consider annual physical/neurologic exam starting at 25 30 years	
			Pancreatic cancer screening using MRI/MRCP and/or EUS may be considered based on family history	
MSH2	Lynch syndrome	AD	Refer to *MLH1* management	2 (0.2)
MYH7	Familial hypertrophic cardiomyopathy type 1	AD	Refer to *MYBPC3* management	2 (0.2)
RYR1	Malignant hyperthermia	AD	Avoid exposure to potent volatile agents and succinylcholine	2 (0.2)
			Monitor temperature for individuals undergoing general anesthetics exceeding 30 minutes	

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TABLE 4. Patients With P/LP Variant in ACMG Recommended Gene and Associated Condition (Continued)

ACMG-Recommended Gene	Associated Condition	Inheritance Pattern	Proposed Management Guidelines	No. of Patients Carrying P/LP Variant (n = 1,028; %)
APC	Familial adenomatous polyposis[d]	AD	Annual colonoscopy starting 10 15 years	1 (0.1)
			If AFAP, may have scopes every 1 2 years	
			Upper endoscopy starting at 20 25 years; consider baseline upper endoscopy earlier if colectomy before age 20 years	
			Annual thyroid exam, consider ultrasound	
			Annual physical exam	
			Annual abdominal palpation, consider abdominal MRI with and without contrast or CT with contrast within 1 3 years after colectomy and then every 5 10 years if family history of symptomatic desmoids	
			Consider liver palpation, abdominal ultrasound, and AFP level every 3 6 months for first 5 years of life	
APOB	Familial hypercholesterolemia	SD	Refer to *LDLR* management	1 (0.1)
BMPR1A	Juvenile polyposis syndrome	AD	Beginning at 15 years: annual colonoscopy and upper endoscopy; surveillance may be completed every 2 3 years if no polyps are found	1 (0.1)
DSC2	Arrhythmogenic right ventricular cardiomyopathy type 11	AD	Refer to *DSG2* management	1 (0.1)
FBN1	Marfan syndrome	AD	β Blockers or angiotensin receptor blockers	1 (0.1)
			Annual ophthalmology exam	
			Annual echocardiography; frequency may be increased based on personal history	
			Intermittent CT or MRA scans of the entire aorta	
LMNA	Dilated cardiomyopathy type 1A	AD	Refer to *MYBPC3* management	1 (0.1)
PCSK9	Familial hypercholesterolemia	AD	Refer to *LDLR* management	1 (0.1)
RB1	Retinoblastoma	AD	Eye exam under anesthesia every 3 4 weeks until 6 months and then less frequently until 3 years	1 (0.1)
			Clinical exams every 3 6 months until 7 years and then annually or biennially for life	
RET	Multiple endocrine neoplasia types 2a and 2b	AD	Annual serum calcitonin; serum calcium, PTH, plasma catecholamines, and metanephrines	1 (0.1)
			Prophylactic thyroidectomy	
			Prophylactic parathyroidectomy and autotransplantation	
			The age to initiate screening and/or undergo prophylactic surgery is dependent on genotype and family history	
SCN5A	Long QT type 3	AD	Refer to *KCNQ1* management	1 (0.1)
SDHB	Pheochromocytoma paraganglioma syndrome	AD	Annual plasma free fractionated metanephrines or 24 hour urine fractionated metanephrines	1 (0.1)
			Cross sectional imaging (preference of nonradiating imaging) of skull base to pelvis every 2 years	

(Continued on following page)

TABLE 4. Patients With P/LP Variant in ACMG Recommended Gene and Associated Condition (Continued)

ACMG-Recommended Gene	Associated Condition	Inheritance Pattern	Proposed Management Guidelines	No. of Patients Carrying P/LP Variant (n = 1,028; %)
VHL	Von Hippel Lindau syndrome	AD	Beginning at 1 year: ophthalmology exam, evaluation for neurologic symptoms and hearing loss, blood pressure monitoring (all completed annually)	1 (0.1)
			Beginning at 5 years: annual plasma or 24 hour urine for fractionated metanephrines; audiology assessment every 2 3 years	
			Beginning at 16 years: annual abdominal ultrasound; MRI of the brain, spine, and abdomen every 2 years	

Abbreviations: ACMG, American College of Medical Genetics and Genomics; AD, autosomal dominant; AFAP, attenuated familial adenomatous polyposis; AFP, α fetoprotein; AR, autosomal recessive; BSO, bilateral salpingo oophorectomy; CAD, coronary artery disease; CRC, colorectal cancer; CT, computed tomography; EUS, endoscopic ultrasound; ICD, implantable cardioverter defibrillator; IGF 1, insulin like growth factor 1; LCSD, left cardiac sympathetic denervation; MRA, magnetic resonance angiography; MRCP, magnetic resonance cholangiopancreatography; MRI, magnetic resonance imaging; NET, neuroendocrine tumor; P/LP, pathogenic or likely pathogenic; PTH, parathyroid hormone; RRSO, risk reducing salpingo oophorectomy; SD, semidominant; TAH, total abdominal hysterectomy; TVUS, transvaginal ultrasound.

[a]AR condition; one patient in this category carried biallelic mutations.

[b]ACMG recommends disclosure of biallelic mutations only. Whole exome sequencing does not routinely include deletion and duplication analysis. Additional genetic testing, additional screening for the condition, and/or thorough family history review may be considered to assess for a second germline mutation or moderately affected heterozygote carriers.

[c]AR condition; all identified patients in this category carried a single heterozygous mutation.

[d]Patient has I1307K variant, which is associated with a modest risk for colon cancer, not considered familial adenomatous polyposis.

DISCUSSION

Tumor NGS has become an efficient approach for identifying actionable drug targets, and several studies have demonstrated benefit in outcomes for patients with advanced disease who receive this testing.[21,22] Although the more comprehensive assessment of multiple targets increases efficiency of testing and potentially uncovers more drug options, it has also created the dilemma of triaging unexpected germline findings, which occur in 3.0% to 12.6%[4-9] of patients tested. This is further complicated by the fact that most patients do not have appropriate pretest counseling and that the purpose of the test is to identify a targeted agent, usually in the metastatic setting.[23] These series all represent an assessment of germline variants initially identified on noncomprehensive panel-based testing, which does not account for all possible pathogenic variants and does not include paired germline testing.[7] The former limitation likely underestimates the actual volume of germline carriers. The latter makes it impossible to know whether the finding is a reflection of a germline change.[4] TCGA performed comprehensive WES on 10,389 patients and identified P/LP variants in 8%.[16] That study, however, focused entirely on cancer-relevant variants, and there were no clinical data to determine whether those patients should have been tested based on pedigree and other risk-specific factors. WES of tumor with matched normal sample in a small population of pediatric patients (n = 150) revealed that 10% had a germline P/LP mutation related to their disease and an additional 5% carried incidental germline mutations.[8]

To date, no prospective, comprehensive evaluation of cancer and noncancer germline variants with married clinical annotation has been reported for adult patients with cancer who are undergoing tumor NGS testing. We now report, to our knowledge, the first study of comprehensive germline WES with matched clinical annotation for adult patients with cancer undergoing testing with the intent of identifying a drug target. This comprehensive evaluation of the genetic landscape of this population highlights the pervasive nature of P/LP variants. The majority of the mutated genes in our data set with a frequency > 1.5% were of autosomal recessive inheritance. In addition, only two (6.9%) of 29 genes were recommended by the ACMG to be reported back to the patient. These findings highlight the pervasiveness of identifying mutations that are not clinically relevant to the patient and require expertise by the interpreting clinician. Nonetheless, 10.7% of patients did carry a P/LP variant in a cancer or a noncancer gene, which was frequently unexpected and would have been recommended by expert guidelines to be returned to the patient and acted on accordingly. Despite the relatively small numbers of ACMG reportable variants, pretest counseling should be performed to mitigate the downstream risks and harm of disclosing or not properly disclosing a potentially critical finding for both the patient and his or her family members.

Although some of these mutation carriers may have been identified from germline screening prompted by risk, recent data from a subgroup of the IMPACT cohort highlighted concerns regarding the inability to adequately screen cancer mutation carriers based on pedigree.[24] The IMPACT (ClinicalTrials.gov identifier: NCT01775022) cohort was a subcohort of 1,040 patients who were referred for additional germline testing, and 19.7% of patients were found to harbor a germline pathogenic variant. Astonishingly, approximately 50% of these patients would not have undergone germline testing based on their demographics, tumor characteristics, and pedigree and thus would have accounted for almost 10% of the population with a pathogenic variant who had not been recommended for testing. In this subcohort, patients were referred specifically for germline testing, and thus, although the pedigree did not always support formal testing, it does infer that the instinct of the treating physician is important.[24] Furthermore, there was a high incidence of Ashkenazi Jewish founder pathogenic variants (27 of 59 *BRCA* variants and 24 of 24 *APC* variants) identified, suggesting a biased patient population that is already known to have a higher frequency of pathogenic germline variants in these genes. In our study, only 12.8% of patients harbored a germline cancer predisposition variant, and 5.5% had an ACMG cancer predisposition variant. Our study is a typical mix of all patients with cancer referred with no consideration of need for germline testing. The lower incidence of P/LP variants in our study is likely in part a result of referral bias, tumor type, and patient population.

Despite the lower baseline rate of germline mutation carriage, we also found that between 30% (ACMG cancer predisposition) and 47% (all cancer predisposition) of variants would not have been identified based on current clinical guidelines. We recognize the current clinical guidelines are designed to identify pathogenic variants in highly penetrant genes. Therefore, with some moderate penetrance genes, such as *CHEK2* and *ATM*, it is expected that a higher number of pathogenic variants would be missed. Regardless, 1.7% of ACMG cancer predisposing variants, which are all in high-penetrance genes, would have been missed in a nonbiased referral population of patients with metastatic cancer intending to find a drug target. Although this represents a relatively small segment of the population, it is sobering that approximately 30%-50% of the patients with metastatic disease in this study harbored a risk allele that would not have been recommended for testing but may have led to risk-reducing screening or procedures had they been known earlier in the patient's life.

The clinical implications of these findings are complex and, based on these data, common. First, the setting itself imposes a unique challenge. Many patients in this setting have an incurable disease, and these findings were neither anticipated nor beneficial to the patient. Instead, the patient is now forced to consider the implications and burden of contacting other healthy family members who may be at risk. This may add an emotionally taxing burden that may also illicit unnecessary guilt and grief.[25] Second, the findings themselves are mired in multiple considerations that are highly challenging for the medical oncologist to navigate.

Specifically, if a possible germline mutation is found on a traditional tumor-only panel-based test, there are no paired germline data and the oncologist is left to decide whether to pursue confirmatory testing. Currently, the NCCN guidelines[18] formally recommend genetic evaluation for patients found to have a pathogenic variant in a cancer susceptibility gene while acknowledging the high frequency of somatic mutations clouding the picture for genes such as *TP53*.[7,26] In addition, the impact of finding a cardiovascular or metabolic risk allele in a patient with end-stage cancer may seem unimportant and out of scope for the treating oncologist. However, there are data to suggest that many patients with metastatic cancer would prefer to receive these secondary findings.[15,27]

Other confounders exist for more comprehensive testing even though most have paired germline results. First, there are massive numbers of variants, and their pathogenicity designation is dynamic and best annotated by teams with extensive expertise in clinical and molecular genetics.[28] Second, not all genes are clinically actionable, and thus, testing may only result in concern without offering an intervention to prevent or delay the onset. Third, even for clinically actionable genes, the penetrance is often variable, making the proposed benefit for family members to undergo testing less clear.[29,30]

The complexity of these findings underscores the concern regarding routine implementation of comprehensive NGS in clinical practice without ready access to expertise in variant interpretation and genetic counseling. This unintended need to consider germline findings places an increasing strain on time and resources for routine oncology practices as well as on the already limited number of genetic counselors.[31] With the field quickly evolving, we foresee that all patients with advanced cancer who plan to undergo NGS for target drug identification should ideally have comprehensive pretest counseling so that the optimal results can be returned and expectations grounded. However, this optimal approach will strain the time and resources available in a routine oncology practice.

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