5-2018

CHARACTERISTICS OF INDIVIDUALS UNDERGOING PANEL GENETIC TESTING FOR PRIMARY BRAIN TUMORS

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A

THESIS

Presented to the Faculty of

The University of Texas

MD Anderson Cancer Center UTHealth

Graduate School of Biomedical Sciences

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE

by

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Houston, Texas

May 2018

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Background. Currently, there are no genetic testing guidelines for patients with a primary brain tumor (PBT). This population is largely understudied in terms of the family history, tumor grade, pathology, and their relation to genetic contribution. Our aim was to describe patient-specific characteristics and family histories across mutation-positive, negative, and variant of uncertain significance (VUS) cohorts based on cancer-panel genetic test results among patients with a PBT.

Methods. Subjects were referred for multi-gene panel testing between March 2012 and June 2016. Clinical data were ascertained from test requisition forms. The incidence of pathogenic mutations (including likely pathogenic) and VUS's were calculated for each gene and patient cohort.

Results. Almost all tumors were glial (n=293, 53%) or meningeal pathology (n=222, 40%). Age of diagnosis differed significantly between glial and meningeal tumors ($p<0.001$). Of 654 subjects, panel testing identified 104 (16%) individuals with mutations, with 35 (34%) individuals possessing an isolated PBT. Genes most frequently yielding a positive result were: *CHEK2* (20/104), *BRCA2* (13/104), *PMS2* (10/104), *TP53* (8/104), and *APC* (8/104). Of 165 patients with available family history information, nearly all (n=157, 95%) reported a family history of some cancer.

Conclusions. Our data suggest PBTs can be the primary presenting cancer in hereditary syndromes with a known PBT risk. While pathology is helpful in narrowing down the differential diagnosis, patients' pathology can be atypical in relation to their hereditary cancer syndrome. Family history evaluations are a beneficial risk assessment modality, particularly until testing criteria are developed for PBTs. Further research is necessary for the development of genetic testing criteria in the PBT population and more robust identification of at-risk individuals.

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INTRODUCTION.

The epidemiology of primary brain tumors (PBT), benign or malignant, is poorly understood. Aside from high dose ionizing radiation and a hereditary predisposition, there are no proven risk factors for PBT development as there are for other cancer types such as breast or colon cancers. The 2016 World Health Organization Guidelines classify PBTs according to their histopathology, grade, immunohistochemistry, and molecular data. Histopathology of a brain tumor has historically been critical for classification; assignment of tumor grade is also based off histopathology(1). These characteristics can also be used to develop a differential diagnosis when performing a risk assessment to distinguish PBTs that are more likely to have a hereditary etiology. The major brain tumor predisposition syndromes include Li-Fraumeni syndrome (LFS), neurofibromatosis type 1 or 2 (NF1/NF2), von Hippel-Lindau disease (VHL), tuberous sclerosis complex (TSC), nevoid basal cell carcinoma syndrome (NBCCS), constitutional mismatch repair deficiency (CMMRD), Lynch syndrome, and familial adenomatous polyposis (FAP).

One of the first syndromes which identified PBTs as part of their diagnostic criteria was LFS which results from a germline mutation in the *TP53* gene. PBTs are one of the most common types of neoplasm, constituting around 14% of the malignancies seen(2). Additional cancers include soft tissue sarcoma, breast cancer, osteosarcoma, and adrenocortical carcinoma. Roughly 60% of the PBTs seen in LFS are astrocytomas, with the remaining 40% being medulloblastomas or primitive neuroectodermal tumors (PNETs)(2). All patients with choroid plexus carcinoma should be evaluated for LFS.

NF1 arises from a germline mutation in the *NF1* gene, with around 95% of patients being diagnosed by age 8(3). Common features include axillary and inguinal freckling, Lisch nodules, café au-lait spots, neurofibromas, and optic gliomas. Optic gliomas are low grade pilocytic astrocytomas and are seen in about 15% of NF1 patients. Gliomas arising outside of the optic nerve pathway occur in approximately 5% of patients(4). Additionally, approximately 50% of NF1 patients develop plexiform neurofibromas and 10% develop malignant peripheral nerve sheath tumors(5).

NF2 is characterized by the development of bilateral vestibular schwannomas. The causative gene is *NF2* with the average age of onset spanning from 18-24 years. Almost all individuals present with bilateral vestibular schwannomas by 30(6). Additionally, meningiomas are the second most common tumor type observed in about half of cases with the lifetime risk approaching 80%(7, 8). They are often the primary presenting feature in the pediatric cohort(7) . Two thirds of patients with NF2 will develop spinal tumors, including schwannomas, ependymomas or, rarely, astrocytoma.

VHL carries an increased risk to develop a central nervous system (CNS) malignancy, particularly for hemangioblastoma. Hemangioblastomas of the brain or spinal cord are the most common tumor type seen in 60-80% of patients, with the average age of diagnosis being 33(9-11). This condition is caused by a germline mutation in the *VHL* gene. Features of VHL include tumors in the CNS, retina, kidneys, pancreas, and adrenal glands(10, 12).

TSC is a genetic condition where individuals develop benign tumors in multiple parts of the body such as the skin, lungs, kidneys, and brain. TSC results from a germline mutation in either the *TSC1* or *TSC2* genes. Brain lesions associated with TSC include subependymal nodules (70-80%), subependymal giant cell astrocytoma (5-20%), and cortical tubers (70%)(13).

NBCCS results from a germline mutation in the *PTCH1*, *SUFU*, and possibly *PTCH2* genes. Predominant clinical characteristics include cutaneous findings of basal cell carcinoma, palmoplantar pits and epidermoid cysts(14-16). Approximately 5-10% of patients with *PTCH1* mutations develop medulloblastoma; however, patients with SUFU mutations have an up to 20 times higher risk to develop these tumors(17). Patients also have a 5% risk to develop meningiomas(18). They generally present within the first two years of life as compared to the general population where age of onset can be expected between ages 7-8(14). Often, this malignancy can be the presenting feature of NBCCS.

CMMRD results from biallelic germline mutations in the mismatch repair, (MMR) genes: *MLH1, MSH2, MSH6, PMS2*. Affected individuals are at increased risk for CNS, hematologic, and gastrointestinal malignancies. Bakry et al.(19), found PBTs to be the most common type of malignancy, specifically high-grade gliomas, followed by medulloblastoma, and then low-grade glioma. The average age of diagnosis for PBTs in CMMRD ranges from 2-11 years of age(19). Currently, the risk to develop

a PBT with CMMRD is unknown due to the rarity and the still developing identification protocol of this condition, but estimated to be very high(20).

Similar to CMMRD, recently the predisposition of developing a PBT with Lynch syndrome has been further investigated. Lynch syndrome is the most common hereditary, adult-onset form of colorectal cancer and caused by a monoallelic germline mutation in one of the MMR genes or *EPCAM*. A study by Therkildsen et al.(21) investigating brain tumors in those with Lynch syndrome found glioblastoma was the most common histological subtype followed by astrocytoma and oligodendroglioma, with high grade gliomas being more frequent than low grade. Overall, the risk to develop a PBT with Lynch syndrome is estimated to be 1-6%, with the higher end of that range being attributed to a *MSH2* mutation. The average age of diagnosis for a glioma in the general population is 50 years of age, while the average case of Lynch syndrome is diagnosed in the fourth decade of life.

FAP results from a germline mutation in the *APC* gene. Classically, affected individuals present with hundreds to thousands of adenomatous colorectal polyps. On average, polyps present around age 16(22) . Risks for extra colonic cancers include CNS malignancies. Medulloblastoma accounts for 80% of PBTs with the remainder including high grade astrocytomas and ependymomas(5). It typically presents within the first decade of life with 70% of cases occurring prior to age 16. The lifetime risk of developing any PBT in families affected with FAP is seven times higher than the general population risk(9).

As exhibited above, syndromic PBTs are frequently diagnosed at a younger age, with some syndromes predisposing to pediatric PBTs. In contrast, the average age of diagnosis in the general population is 59 years(23). Early identification of at risk or affected individuals can be valuable in prognosis and management recommendations. In patients with CMMRD, it has been shown to be crucial in predicting prognosis and allows for earlier tumor resection(20, 24). It also results in screening for other associated cancers or prophylactic surgery options. Other benefits include targeted treatment options such as avoiding radiation therapy when possible in individuals with LFS(25). Testing at risk individuals can result in starting appropriate screening and management as with FAP and LFS. Identification of individuals in need of genetic testing or referral to genetic counseling is challenging due

to our limited understanding about this population's landscape. Perhaps most importantly, there are currently no guidelines for genetic testing in patients who present with a PBT.

Features of genetic testing guidelines for other hereditary cancers include characteristic family history, tumor pathology, and age of diagnosis. For example, up to 20% of cases of triple negative (estrogen negative, progesterone negative, and HER-2 negative) breast cancer are *BRCA* positive, particularly *BRCA1(26)*. Lynch syndrome colorectal cancers have distinct pathologic features including: signet ring cell, mucinous or medullary growth pattern, poor differentiation, and Crohn's like lymphocytic reaction(27, 28). Additionally, with these syndromes, it is understood that a younger age of diagnosis and positive family history correlate with an increased risk of a genetic etiology(29-31). Such characteristics have not been evaluated in the PBT population.

One of the first and only studies to examine the contribution of germline mutations toward the development of a PBT was by Bergner and Jackson(32). Their analysis focused on individuals with positive genetic testing results, revealing that over half the germline mutations identified were in the *BRCA2, CHEK2, PMS2,* and *TP53* genes. This suggests the potential for a spectrum of germline mutations to be an underlying etiology for the development of a PBT.

To our knowledge no study has comprehensively examined patient-specific characteristics, such as tumor grade, pathology, age at diagnosis, and the presence of other primary cancers, or family history information among all individuals undergoing panel testing for a PBT. It is unknown how these characteristics may differ across individuals testing positive, negative, or with a VUS. Additionally, minimal data exists on the germline genetic testing outcomes for patients with a PBT. It is unclear if multi-gene panel genetic testing provides answers for these patients and if so, at what frequency. We aimed to help clinicians better identify individuals who should undergo genetic testing. Our aim was to investigate how patient-specific characteristics and family history differ across mutation positive, negative, and VUS cohorts based on cancer-panel genetic test results among patients diagnosed with a PBT.

METHODS.

Participants:

Consecutive subjects were referred for multi-gene panel testing for a primary brain tumor between March 2012 and December 2016 at Ambry Genetics. Panel types ordered included: CancerNext, CancerNext Expanded, PancNext, ColoNext, BRCAPlus, PGLNext, GYNPlus, BreastNext, OvaNext, CustomNext-Cancer, RenalNext, BRCAPlus Expanded, and BrainTumorNext. The number of genes ranged from 6-67. For this retrospective cohort study, all information was de-identified in a secure database. This study was conducted in accordance with all regulations set for by the Institutional Review Board (IRB) of the University of Texas at Houston Health Sciences Center, Houston, TX and Western IRB. Inclusion criteria were a personal history of at least one primary brain tumor. Exclusion criteria were a brain tumor as a site of metastasis. Demographic, clinical history, and family history of cancer information were collected from test requisition forms, clinic notes, and pedigrees provided by ordering clinicians at the time of testing. Information was collected on current age, personal history, and age at diagnosis of other primary cancers, tumor pathology and grade, family history of cancer with cancer type, and age at diagnosis among relatives.

Panel Type	Genes (as of December 2016)
CancerNext	APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, CDH1, CDK4, CDKN2A, CHEK2, DICER1, EPCAM, GREM1 HOXB13, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PMS2, POLD1, POLE, PTEN, RAD50, RAD51C, RAD51D, SMAD4, SMARCA4, STK11, TP53
CancerNext Expanded*	AIP, ALK, APC, ATM, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, CDH1, CDK4, CDKN1B, CDKN2A, CHEK2, DICER1, EPCAM, FANCC, FH, FLCN, GREM1, HOXB13, MAX, MEN1, MITF, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, NF2, PALB2, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RB1, RET, SDHA, SDHB, SDHC, SHDD, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TMEM127, TP53, TSC1, TSC2, VHL, XRCC2
PancNext*	APC, ATM, BRCA1, BRCA2, CDKN2A, EPCAM, MLH1, MSH6, PALB2, PMS2, STK11, TP53
ColoNext	APC, BMPR1A, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2, POLD1, POLE, PTEN, SMAD4, STK11, TP53
PGLNext*	FH, MAX, MEN1, NF1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, VHL
BRCAPlus*	BRCA1, BRCA2, CDH1, CHEK2, PALB2, PTEN, TP53
BRCAPlus-Expanded*	ATM, BRCA1, BRCA2, CDH1, PALB2, PTEN, TP53
GYNPlus*	BRCA1, BRCA2, BRIP1, EPCAM, MLH1, MSH2, MSH6, PALB2, PMS2, PTEN, RAD51C, RAD51D, TP53
BreastNext	ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, NF1, PALB2, PTEN, RAD50, RAD51C, RAD51D, TP53
OvaNext	ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, DICER1, EPCAM, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, SMARCA4, STK11, TP53
CustomNext-Cancer*	AIP, ALK, APC, ATM, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDKN1B, CDKN2A, CHEK2, DICER1, EPCAM, FANCC, FH, FLCN, GALNT12,GREM1, HOXB13, MAX, MEN1, MET, MITF, MEN1, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, NF2, PALB2, PHOX2B, PMS2, POT1, POLD1, POLE, PRKAR1A, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RB1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TMEM127, TP53, TSC1, TSC2, VHL, XRCC2
RenalNet*	BAP1, EPCAM, FH, FLCN, MET, MITF, MLH1, MSH2, MSH6, PMS2, PTEN, SDHA, SDHB, SDHC, SDHD, TP53, TSC1, TSC2, VHL
BrainTumorNext*	AIP, ALK, APC, CDKN1B, CDKN2A, DICER1, MEN1, MLH1, MSH2, MSH6, NBN, NF1, NF2, PHOX2B, PMS2, POT1, PRKAR1A, PTCH1, PTEN, SMARCA4, SMARCB1, SMARCE1, SUFU, TP53, TSC1, TSC2, VHL

Table 1. Genetic testing panel types ordered *denotes panels which were not available in March 2012

Mutation identification and analysis:

Genetic testing was completed in Ambry's laboratory using the following protocol. Genomic deoxyribonucleic acid was isolated from subject's whole blood or saliva samples using the QIAsymphony instrument (Qiagen, Valencia, CA) according to manufacturer's protocols. Deoxyribonucleic acid was quantified using a spectrophotometer (Nanodrop, Thermoscientific, Pittsburgh, PA or Infinite F200, TECAN, San Jose, CA). Genomic deoxyribonucleic acid was then combined with primer pairs in micro-droplets designed to the specified targets for each gene to complete sequence enrichment (Raindance Thunderstorm Technologies, Billerica, MA). Using Illumina HiSeq technology (Illumina, San Diego, CA) enriched libraries were applied to the solid surface flow cell for clonal amplification and sequencing. Sanger sequencing was performed for any region with insufficient depth of coverage, defined as 50×. Additionally, bi-directional Sanger sequencing was performed to confirm all variant calls, other than known previously defined benign and likely benign alterations. To detect large deletions and duplications, a targeted chromosomal microarray with increased probe density in regions of interest was completed (Aglient, Santa Clara, CA).

Initial data processing and base calling, including extraction of cluster intensities, was done using RTA 1.12.4 (HiSeq Control Software 1.4.5). Sequence quality filtering was executed with the Illumina CASAVA software (ver 1.8.2 Illumina, Hayward, CA). Sequence fragments were aligned to the reference human genome (GRCh37) and variant calls were generated using CASAVA. A minimum quality threshold of Q30 was applied which translates to an accuracy of >99.9% for called bases and mean coverage was >300×.

Annotated variants were then analyzed to determine the likelihood of pathogenicity and classified into five tiers based on the recommendations of the American College of Medical Genetics and Genomics(33). Alterations were classified in the following categories: pathogenic mutation, variant likely pathogenic, variant of unknown significance (VUS), variant likely benign, and benign based on a previously described multifactorial algorithm(34).

Data Analysis:

Data were described using frequencies (with percentages) and medians (with interquartile ranges, IQR) for categorical and continuous variables, respectively. Contingency tests (Chi-square or Fisher exact) were used to compare categorical variables across groups. Distribution of continuous variables across groups were performed using a Kruskal-Wallis test with a post-hoc Dunn's test. All analyses were performed using STATA (v.13, College Station, TX). Statistical significance was assumed at $p<0.05$.

Patients were categorized according to pathology and tumor grade for some analyses, as reported by the ordering provider. Patients diagnosed with an astrocytoma, oligodendroglioma, oligoastrocytoma, ependymoma, optic glioma, glioma, or glioblastoma multiforme (GBMs) were categorized as glial tumors. Those with a meningioma were categorized as meningeal. Those with medulloblastoma, primitive neuroectodermal tumors (PNETs), or choroid plexus carcinoma were classified as embryonal. Those with a hemangioblastoma were classified as mesenchymal. Regarding tumor grade, grade I and II glial tumors were categorized as low grade. Juvenile pilocytic astrocytomas were considered to be low grade. Grade III and IV glial tumors were categorized as high grade. Glial tumors described as "anaplastic" were considered to be grade 3 and GBMs were considered to be grade IV. All meningeal tumors were reported to be "benign" and were assumed to be grade I, categorized as low grade.

RESULTS.

Demographics

A total of 658 patients were selected for this study based upon an initial query of test requisition forms of patients identified as having at least one PBT. Four patients were excluded because they did not have a diagnosis of a PBT, but an osteoma, epidermoid tumor, cerebellar venous angioma, and dermoid tumor. Therefore, a total of 654 patients were included in the final analyses. Seventy-five percent of the cohort was female (488/654) and 25% (166/654) male. About two-thirds of the patients were non-Hispanic white (n=424, 65%). The remaining third of the patients were either Ashkenazi Jewish, (n=54, 8%), mixed ethnicity (n=41, 6%), African American (n=31, 5%), Hispanic (n=29, 4%), Asian (n=21, 3%), and Middle Eastern (n=6, 1%). Ethnicity was unknown for 48 patients (8%) (Table 1).

Demographics	Frequency (n, %)
Ethnicity	
African american/black	31 (5)
Ashkenazi jewish	54 (8)
Asian	21 (3)
Caucasian	424 (65)
Hispanic	29 4)
Middle eastern	6 (1)
Mixed ethnicity	41 (6)
Unknown	48 (7)
Gender	
Male	166 (25)
Female	488 (75)
Total	654

Table 2. Demographics

Pathology

Tumor pathology was available for 558 individuals. Slightly more than half of all tumors were glial (n=293, 53%), with nearly all of the remaining tumors classified as meningeal, (n=222, 40%). Less than 10% of the tumors were classified as mesenchymal (n=22, 4%) and embryonal (n=18, 3%). Germ cell, nerve sheath tumors, and choroid plexus carcinoma were present in only a single individual for each. The distribution of tumor pathology was not significantly different across the positive, negative, and VUS genetic testing cohorts. Details about tumor pathology and other patient characteristics can be found in Table 2.

		Glial	Meningeal	Embryonal	Mesenchymal
		(n= 293)	(n= 222)	(n= 18)	(n= 22)
Age of Diagnosis, years, median (IQR)		39 (29 - 51)	50 (43 - 58)	7.5 (6-19)	47.5 (32-56)
Tumor Grade, n (%)					
	1	11 (8)	213 (100)	NA	3 (100)
	2	6 (4)	0	NA	0
	3	18 (14)	0	NA	0
	4	98 (74)	NA	NA	NA
Number of primaries, n (%)					
	1	162 (55)	44 (20)	6 (33)	10 (45)
	2	90 (31)	98 (44)	8 (44)	6 (27)
	3	30 (10)	57 (26)	3 (17)	5 (23)
	4 or more	11 (4)	23 (10)	1 (6)	1 (5)

Table 3. Patient characteristics by pathology type

Age at diagnosis

Age at diagnosis was available for 506 patients. Fifty-seven (11%) were diagnosed under the age of 18, 282 (56%) were diagnosed between the ages of 18-50, and 167 (33%) were diagnosed over the age of 50. The median age of diagnosis for this cohort was 44 years (STD=17), which is significantly younger than the general population, at 59 years ($p<0.001$).

Glial (median: 39, IQR: 29-51), meningeal (median: 50, IQR: 43-58), and mesenchymal (median: 47.5, IQR: 32-56) tumors were diagnosed in an exclusively adult population. Embryonal tumors were diagnosed in a predominantly pediatric population (median: 7.5, IQR: 6-19). This was significantly earlier than glial tumors ($p<0.001$) and meningeal tumors ($p<0.001$). Glial tumors were diagnosed at significantly younger ages than meningeal tumors at ($p<0.001$). Evaluating age within the subtypes of glial tumors, oligodendrogliomas, astrocytomas, and oligoastrocytomas were all diagnosed at significantly younger ages compared to glioblastoma multiformes (GBM), ($p=0.043$, $p<0.001$, $p<0.001$, respectively). Of note, test result positive astrocytomas were diagnosed at a significantly younger age compared to test result negative and VUS astrocytomas ($P=0.021$). No other significant differences were noted between the ages of diagnosis in the positive, negative, and VUS cohorts when compared across tumor types.

Tumor grade

Of the 354 individuals for whom tumor grade information was available, almost all tumors had either glial (137/354, 39%) or meningeal pathology (213/350, 61%). Eighty-six percent of glial tumors were high grade (116/138) with 14% being low grade (22/138). Conversely, all meningeal tumors were low grade. When comparing high grade and low grade glial tumors, high grade tumors were significantly more likely to be diagnosed at an older age ($p<0.001$). The grade of the glial tumor was not predictive of a positive genetic test result in our cohort. Among glial tumors, there was a trend of increasing age of diagnosis with increasing tumor grade. Grade IV tumors (GBMs) were observed in a significantly older population (median: 49, IQR: 37-61) compared to grade I (median: 15.5, IQR: 12-35, $p <0.001$), grade II

(median: 18.5, p=0.006) and grade III (median= 32, p<0.001) (Table 3). There were no statistically significant differences in age of diagnosis between grades I, II and III glial tumors.

		Positive	VUS	Negative
Tumor Type, n (%)				
	Glial	48 (17)	57 (20)	182 (63)
	Meningeal	27 (12)	51 (23)	144 (65)
	Embryonal	3 (17)	7 (39)	8 (44)
	Mesenchymal	4 (18)	5 (23)	13 (59)
Tumor Grade, n (%) *				
	Low grade	5 (23)	3 (14)	14 (64)
	High Grade	21 (18)	25 (22)	70 (60)

Table 4. Tumor pathology and grade by genetic testing outcome
***all meningeal tumors were grade I**

Number of primaries

Of the 654 patients, 239 (36%) had one primary cancer (brain tumor), 254 (39%) patients had 2 primaries, 117 (18%) patients had 3, and 44 (7%) patients had 4 or more. The most common additional primary cancers were breast (226/654, 35%), colon (55/654, 8%), and thyroid (36/654, 6%). Individuals with multiple primary cancers were significantly more likely to be diagnosed with a PBT at a later age compared to those who only had a PBT (p<0.001). Compared to those with a glial tumor, individuals with a meningeal tumor were significantly more likely to develop additional primaries (p<0.001). The number of additional primary cancers was not predictive of a positive genetic test result.

Test results

One hundred and four of 654 (16%) patients had a positive genetic test result, 141 (21%) with a variant of uncertain significance (VUS), and 410 (84%) with negative results. There were 24/141 patients who had multiple VUS reported. In the positive testing cohort, five patients were *MUTYH* carriers and five patients had multiple mutations in: *APC* and *ATM*, biallelic *PMS2* mutations (2), *EPCAM* and *MSH2*, biallelic *TSC1* mutations. The five genes most frequently yielding a positive result were *CHEK2* (20/104, 19%), *BRCA2* (13/104, 13%), *PMS2* (10/104, 10%), *TP53* (8/104, 8%), and *APC* (8/104, 8%). The five genes most frequently yielding a VUS were *ATM* (17/141, 12%), *APC* (12/141, 9%), *MSH6* (8/141, 6%), *BRCA2* (7/141, 5%), and *MLH1* (7/141, 5%).

Eighty-four of 104 (81%) patients who tested positive had pathology information available. In the positive cohort, glial tumors were the most common (50/84, 60%) followed by meningeal tumors (27/84, 32%). Astrocytomas made up 42% (21/50) of glial tumors followed by GBM at 34% (17/50). The genes most frequently yielding a positive result in glial tumors were *CHEK2* (9/50, 18%), *PMS2* (6/50, 12%), *TP53* (5/50, 10%). Within glial tumors, astrocytomas returned positive results in *APC* (2/21, 10%), *CHEK2* (5/21, 24%), *PMS2* (4/21, 19%), and *TP53* (2/21, 10%). GBM yielded positive results most frequently in *BRCA1* (2/17, 12%), *CHEK2* (2/17, 12%), *MUTYH* (2/17, 12%), and *TP53* (2/17, 12%). In meningeal tumors, *BRCA2* (5/27, 19%) and *CHEK2* (6/27, 22%) most frequently yielded a positive result. Additional details on pathology and results can be found in Table 4.

		Number of Positive test results	APC	ATM	BARD1	BRCA1	BRCA2	BRIP1	CDKN2A	CHEK2	EPCAM	MLH1	MSH6	MUTYH
Tumor Type (n, %)														
	Glial	50	3 (6)	2 (4)	---	3 (6)	4 (8)	1 (2)	---	9 (18)	1 (2)	1 (2)	---	3 (6)
	Meningeal	27	1 (4)	2 (7)	1 (4)	---	1 (4)	1 (4)	1 (4)	6 (22)	---	---	2 (7)	---
	Embryonal	2	1 (50)	---	---	---	---	---	---	---	---	---	---	---
	Mesenchymal	4	---	---	---	---	---	---	---	2 (50)	---	---	---	---
Glial Subsets (n, %)														
	GBM	17	---	1 (6)	---	1 (6)	1 (6)	1 (6)	---	2 (12)	1 (6)	1 (6)	---	2 (12)
	Astrocytoma	21	2 (10)	1 (5)	---	1 (5)	1 (5)	---	---	5 (24)	---	---	---	---
	Oligodendroglioma	3	---	---	---	---	---	---	---	1 (33)	---	---	---	---

		Number of Positive test results	NF1	PALB2	PMS2	POLE	RAD51C	RET	SDHB	SDHD	SMARCB1	TP53	TSC2	VHL
Tumor Type (n, %)														
	Glial	50	4 (8)	---	6 (12)	2 (4)	---	1 (2)	1 (2)	---	---	7 (14)	1 (2)	
	Meningeal	27	---	1 (4)	1 (4)	---	---	---	1 (4)	1 (4)	1 (4)	---	---	
	Embryonal	2	---	---	1 (50)	---	---	---	---	---	---	---	---	---
	Mesenchymal	4	---	---	---	---	1 (25)	---	---	---	---	---	---	1 (25)
Glial Subsets (n, %)														
	GBM	17	2 (12)	---	1 (6)	1 (6)	---	---	---	---	---	2 (12)	---	
	Astrocytoma	21	1 (5)	---	4 (20)	1 (5)	---	1 (5)	1 (5)	---	---	2 (10)	1 (5)	
	Oligodendroglioma	3	---	---	1 (33)	---	---	1 (33)	---	---	---	---	---	

Table 5. Tumor pathology by genetic testing result

Of the 104 patients with a positive test result, 35 (34%) had an isolated PBT with no additional primary cancers. These individuals yielded mutations in the genes: *APC, ATM, BRCA1, BRCA2, CHEK2, MUTYH, NF1, PMS2, POLE, SMARCB1, TP53* and *TSC2*. Three most common additional primaries were breast (26/104, 25%), colon (14/104, 13%), and thyroid (4/104, 4%). Individuals with breast cancer as an additional primary possessed mutations in the genes: *APC, ATM, BARD1, BRCA1, BRCA2, CHEK2, CDKN2A, MSH6, NF1, PALB2, PMS2,* and *PTEN*. Those with colorectal cancer possessed mutations in the genes: *APC, BRCA1, BRIP1, CHEK2, MLH1, MSH6, MUTYH, PMS2*, and *VHL*. Those with thyroid cancer possessed mutations in the genes: *APC, ATM,* and *CHEK2*. Additional primaries included: melanoma, pancreatic, gastric, prostate, renal, ovarian, sarcoma, leukemia, uterine, and other CNS malignancies (Table 5).

		Number of Positive test results	APC	ATM	BAP1	BARD1	BRCA1	BRCA2	BRIP1	CDKN2A	CHEK2	EPCAM	MLH1	MSH6
Additional primary cancer														
	Breast	26	2	2	---	---	1	5	1	1	7	---	---	1
	Colon	14	1	---	---	---	1	---	1	---	2	---	2	1
	Renal	5	1	---	1	---	---	---	---	---	1	---	---	---
	Sarcoma	8	---	---	---	---	1	2	---	---	---	---	---	---
	Thyroid	4	1	1	---	---	---	---	---	---	2	---	---	---
	Melanoma	4	---	---	---	---	---	---	---	1	1	---	1	---
	Ovarian	4	---	---	---	---	---	---	---	1	---	---	---	---
	Pancreatic	3	1	---	---	---	---	---	---	---	1	1	---	---
	Uterine	3	---	---	---	---	---	---	---	---	---	---	---	1
	Prostate	2	---	1	1	---	---	---	---	---	---	---	---	---
	CNS	1	---	---	---	---	1	---	---	---	---	---	---	---
	Gastric	1	---	---	---	---	---	---	---	---	---	---	1	---
Isolated PBT		35	3	1	---	---	1	7	---	---	8	---	---	---

		Number of Positive test results	MUTYH	NF1	PALB2	PMS2	POLE	PTEN	RAD51C	SMARCB1	TP53	TSC2	VHL
Additional primary cancer													
	Breast	26	---	1	1	2	---	1	---	---	---	---	---
	Colon	14	1	---	---	4	---	---	---	---	---	---	1
	Renal	5	---	---	---	---	---	---	---	---	---	---	1
	Sarcoma	8	---	1	---	---	---	---	---	---	4	---	---
	Thyroid	4	---	---	---	---	---	---	---	---	---	---	---
	Melanoma	4	---	---	---	1	---	---	---	---	1	---	---
	Ovarian	4	---	---	---	1	---	1	1	---	---	---	---
	Pancreatic	3	---	---	---	---	---	---	---	---	---	---	---
	Uterine	3	---	---	---	2	1	---	---	---	---	---	---
	Prostate	2	---	---	---	---	---	---	---	---	---	---	---
	CNS	1	---	---	---	---	---	---	---	---	---	---	---
	Gastric	1	---	---	---	---	---	---	---	---	---	---	---
Isolated PBT		35	3	4	---	2	1	---	---	1	3	1	---

Table 6. Additional primary cancers and genetic testing results

Family history

Family history information was available for 821 relatives of 165 patients. Among these patients, nearly all (n=157, 97%) reported a family history of some cancer and 55 (35%) reported a first-degree relative with cancer. The most common cancers in the family history were breast (220/821, 27%), colorectal (73/821, 9%), and prostate (62/821, 8%). A family history of brain tumors was noted in 30/165 (18%) patients, with 13 patients reporting a first-degree relative with a brain tumor. There was no difference in frequency of positive family history between patients that tested positive compared to those that tested negative or had a VUS. Overall, patients with multiple primary cancers tended to have a stronger family history of cancer. Among patients with family history information, 100% of the patients with 3 or more additional primaries reported a positive family history.

Of patients for whom family history information was available, 22 of 165 (13%) received positive genetic test results. Sixteen of these 22 (73%) patients did not have a family history of brain tumors. Their test results revealed mutations in the genes: *APC, BRCA2, CDKN2A, CHEK2, MLH1, MSH6, MUTYH, NF1, PMS2, SDHB, TP53,* and *VHL*. The 6 individuals who did have a family history of brain tumors possessed mutations in the genes: *APC, BRCA2, CHEK2, MUTYH, TP53,* and *VHL*. Thus, having a family history of a brain tumor was not predictive of a positive genetic test result. Sixteen relatives were noted to previously have genetic testing with 7 yielding positive results in *BRCA1* (5), *PMS2,* and *BRCA2.* The other 9 relatives were either negative or the outcome was not otherwise specified.

DISCUSSION.

This is the first study, to our knowledge, to describe patient specific characteristics in a population of individuals undergoing genetic testing for a PBT. We investigated how these characteristics and family histories differ across mutation positive, negative, and VUS cohorts based on cancer panel genetic test results. We are laying the groundwork for development of future genetic testing criteria for a PBT, based off patient-specific characteristics, as well as aiding clinicians in better recognition of individuals requiring genetic testing. Typical indicators for other hereditary cancer syndromes can include, but are not limited to early age of diagnosis, family history, rare/specific pathology, and presence of additional primary cancers(25, 35-38).

According to the American Brain Tumor Association, the median age of diagnosis for a PBT of any pathology is 59 years(23). In our population, however, the median age of diagnosis was significantly younger at 44 years ($p<0.001$). This indicates the population of patients undergoing genetic testing for a PBT are younger than typically observed. This is consistent with the National Society of Genetic Counselors (NSGC) and American College of Medical Genetics and Genomics (ACMG) joint practice guidelines(38) which suggest earlier age of brain tumor diagnosis can be utilized with other patient characteristics as a marker for referral to genetic counseling. In our cohort, there was no significant difference in age at diagnosis between the positive and negative cohorts, likely since 70% of our study population was under the age of 50 when diagnosed.

Test result positive astrocytomas were found to be diagnosed at a significantly younger age than test result negative and VUS astrocytomas ($p=0.021$). Other studies have shown utility in pathology and younger age at diagnosis for PBTs. Pathmaban, et al reported an isolated meningioma or schwannoma diagnosed <25 yielded a positive test result 38% and 20% of the time, respectively(39). None of the patients in our cohort with an isolated meningioma or schwannoma were diagnosed at this early age. While no other pathology types noted age of diagnosis differences across test results, the astrocytoma findings suggest more research in this area is necessary. With a larger sample size, the potential role of pathology in association with earlier ages of diagnosis could be explored further.

Another indicator for referral to genetic counseling is tumor pathology, which can be used to create a differential diagnosis of various genetic syndromes. Typically, it is used in conjunction with another patient characteristic. We evaluated mutation positive individuals to see how their tumor pathology corresponded with associated syndromes. Out of the 84 patients with positive genetic testing results and available pathology, 17 (20%) had tumor pathology which was associated with their respective syndrome. The other 70 had pathology types which were not previously reported in individuals with that gene or syndrome. For example, there were 4 individuals with meningioma and MMR gene mutations. While the MMR genes can predispose a risk for PBTs, meningiomas are not the typical pathology noted in patients with Lynch syndrome or CMMRD. There have been case reports of patients with atypical PBT pathology and Lynch syndrome, including pituitary tumors(40) and choroid plexus carcinoma(41). Additionally, 3 individuals harbored mono-allelic *MUTYH* mutations, 2 of which had GBMs and 1 an oligodendroglioma. *MUTYH* carriers are not expected to be at increased risk to develop cancer and PBTs have not yet been observed as part of the MAP cancer spectrum. Case reports outline patients with astrocytoma(42) and high-grade glioma(43) in mono-allelic *MUTYH* mutation carriers. These findings suggest the spectrum of PBT pathology seen with these syndromes may be broader than we currently appreciate. It is also possible that some affected patients develop a PBT based on the general population risks unrelated to their genetic mutation.

While tumor grade is not currently an indication for a genetic counseling referral, it may be important in risk assessment for a PBT. As expected based on the natural history of gliomas, our results indicated lower grade glial tumors were diagnosed at earlier ages than higher grade glial tumors. However, our results did not show an association between tumor grade and genetic testing results. Clinically, high grade PBTs are associated with some genetic conditions, such as CMMRD and LFS(5, 20, 44). Our results suggest that while tumor grade is currently being used as an indicator for genetic counseling referral, more research is necessary to better understand the relationship with testing outcomes and the weight which should be placed on tumor grade in making genetic testing recommendations.

The NSCG/ACMG guidelines also suggest family history as an indicator for referral to genetic counseling. Family history information was available for 165 patients, 95% (157/165) of whom had a family history of cancer. Thus, positive family history appeared to be a referral and/or testing indication for this subset of patients. Our data echo the NSGC/ACMG recommendations and reflect the utility in a thorough pedigree evaluation. A family history of PBTs was noted in 30 (18%) patients and 127 (80%) patients reported a family history of cancer aside from PBTs. There were 111 (67%) patients with a family history of breast cancer and 57 (35%) with colorectal cancer. Twenty-two patients (13%) had mutations in 12 genes: *APC, BRCA2, CDKN2A, CHEK2, MLH1, MSH6, MUTYH, NF1, PMS2, SDHB, TP53,* and *VHL*. Nine of 12 genes were related to breast or colorectal cancer. Of 8 individuals with family histories of only breast cancer, 5 (63%) possessed mutations in related genes (*BRCA2* (4) and *CHEK2*). For 7 individuals with a family history of both breast and colorectal cancer, 5 (71%) had mutations in colorectal cancer genes (*APC* (2), *MLH1, MSH6, PMS2*) and 1/7 (14%) in a breast cancer gene (*TP53*). Although, these results represent a small subset of individuals, it suggests family history is a valuable risk assessment modality until specific PBT testing criteria are established. Family history could provide clues for the presence of a genetic etiology, as PBTs are associated with numerous hereditary cancer predisposition syndromes. Thus, non-PBT family history is as critical to evaluate as family history of brain tumors.

Our data suggest PBTs can be the presenting feature in hereditary syndromes with a known risk for PBTs. Thirty-five of 104 (34%) individuals who tested positive had an isolated PBT and no additional primaries. These individuals possessed mutations in the genes: *APC, ATM, BRCA1, BRCA2, CHEK2, MUTYH, NF1, PMS2, POLE, SMARCB1, TP53* and *TSC2*. Half of these genes, including—*APC, NF1, PMS2, SMARCB1, TP53,* and *TSC2*—are associated with PBTs. For associated syndromes such as FAP, Lynch syndrome, or NF1, individuals would typically be expected to develop other cancers or have other recognizable, clinical features before a PBT. However, our data indicates individuals can present atypically. One case report details two brothers eventually found to have LFS; one presenting

with a glioblastoma and the other with multiple PBTs including a glioma and astrocytoma(45). Another case report reveals medulloblastoma as the presenting feature of FAP in an 11-year-old(42).

For those genes with no known association with PBTs, we investigated if additional primaries could be an explanatory factor. The presence of multiple primaries in an individual could indicate an increased risk for a genetic syndrome. We evaluated the relationship between mutation positive test results and additional primaries. Half of patients harbored a mutation in a gene with a known association with PBTs. The remaining half returned results in genes with no known association to PBTs, including *BRCA1/2, CHEK2,* and *POLE.* A majority of these individuals (45/52, 87%) had mutations in genes predisposing a risk for breast and/or ovarian cancer, which was thought to be consistent with breast cancer being the most common additional primary cancer in this cohort, (226/654, 35%). However, individuals with breast cancer as an additional primary were largely not the ones testing positive for those mutations. Twenty-six of 45 (58%) individuals who possessed mutations in breast and/or ovarian cancer related genes did not have either cancer. For example, 8 of 14 (57%) individuals with a *BRCA2* mutation and 11 of 15 (73%) females with a *CHEK2* mutation did not have breast or ovarian cancer.

For genes with no known association with PBTs, it is challenging to delineate the relationship between the mutation and the PBT at this time. It is possible a subset poses a risk for PBTs and the spectrum of cancer types seen are broader than we currently understand. Case reports have described PBTs seen with *MUTYH*(43) and *BRCA*(46) mutation carriers, for example. However, since there was limited clinical and family history information available in our study, we are unable to comment on whether or not these results represent an incidental finding or if some of these genes do predispose a risk for PBTs. Ultimately, further research, particularly in an unbiased population, is necessary to for further evaluation. In the future, establishment of genetic testing criteria for PBTs will allow clinicians to appropriately identify patients for genetic testing. Harboring a mutation can alter management and screening of these individuals as they may be at risk for additional cancers.

Limitations of this study include a biased sample population as these data were obtained from individuals in the PBT population who were selected to undergo genetic testing at a commercial

laboratory for a variety of reasons. Thus, our findings cannot be extrapolated to the general PBT population. Since patient information was obtained from the test requisition form, all clinical information is per provider report and not all fields were available for all patients. Thus, our cohorts differed for various comparisons, impacting the power of the associations we studied. Additionally, since clinical information was not available for all patients, variables such as whether or not an individual met various testing criteria or how the genetic test result fit with the patient's clinical phenotype could not be evaluated.

Further studies are needed to continue to understand the landscape of this population, especially on a larger scale. Primary brain tumors encompass a highly heterogeneous group of benign and malignant neoplasms; thus, large numbers are needed to adequately power these studies. Conducting research prospectively in a non-biased population will allow for further extrapolation. For example, since *CHEK2* mutations were identified in 20% of our positive cohort, including patients with a variety of PBT pathologies and additional primary cancers, we feel additional studies are particularly needed in this population. Finally, while the NSGC/ACMG referral guidelines are beneficial resources, developing genetic testing criteria for PBTs is critical. Identification of a hereditary predisposition will allow for tailored treatment and risk-reducing measures for both the patient and at-risk family members. Until such criteria are established, future research could evaluate current testing practices and methods to make genetic counseling referral guidelines more robust.

CONCLUSION.

This study evaluated individuals undergoing genetic testing for a PBT. Our data indicate that currently, despite the lack of genetic testing criteria for PBTs, testing is largely being ordered on individuals diagnosed under the age of 50 as well as those who have a positive cancer family history. Mutations were observed in 26 genes across 104 (13%) individuals. Half of the identified mutations were associated with a known PBT risk, while the remaining mutations were identified in genes with no known PBT risk. Approximately one-third of mutation positive individuals possessed an isolated PBT, suggesting PBTs can be the primary presenting cancer in hereditary syndromes with a known PBT risk. Evaluating the family history of all cancers was found to be a beneficial risk assessment modality, particularly until testing criteria are developed for PBTs. Further research is necessary to better understand the potential risks, benefits, and limitations of germline genetic testing in the PBT patient population.

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