Published in final edited form as:

Clin Cancer Res. 2016 August 15; 22(16): 4087–4094. doi:10.1158/1078-0432.CCR-16-0015.

Germline Analysis from Tumor-Germline Sequencing Dyads to identify clinically actionable secondary findings

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Abstract

PURPOSE—To evaluate germline variants in hereditary cancer susceptibility genes among unselected cancer patients undergoing tumor-germline sequencing.

EXPERIMENTAL DESIGN—Germline sequence data from 439 individuals undergoing tumor-germline dyad sequencing through the LCCC1108/UNCseq™ (NCT01457196) study were analyzed for genetic variants in 36 hereditary cancer susceptibility genes. These variants were analyzed as an exploratory research study to determine if pathogenic variants exist within the germline of patients undergoing tumor-germline sequencing. Patients were unselected with respect to indicators of hereditary cancer predisposition.

RESULTS—Variants indicative of hereditary cancer predisposition were identified in 19 (4.3%) patients. For about half (10/19), these findings represent new diagnostic information with potentially important implications for the patient and their family. The others were previously identified through clinical genetic evaluation secondary to suspicion of a hereditary cancer predisposition. Genes with pathogenic variants included *ATM*, *BRCA1*, *BRCA2*, *CDKN2A*, and *CHEK2*. In contrast, a substantial proportion of patients (178, 40.5%) had Variants of Uncertain Significance (VUS), 24 of which had VUS in genes pertinent to the presenting cancer. Another

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Disclaimers: We declare no financial conflict of interest. The views expressed in the submitted article are exclusively of the authors and not an official position of the University of North Carolina at Chapel Hill School of Medicine.

143 had VUS in other hereditary cancer genes, and 11 had VUS in both pertinent and non-pertinent genes.

CONCLUSION—Germline analysis in tumor-germline sequencing dyads will occasionally reveal significant germline findings that were clinically occult, which could be beneficial for patients and their families. However, given the low yield for unexpected germline variation and the large proportion of patients with VUS results, analysis and return of germline results should adhere to guidelines for secondary findings rather than diagnostic hereditary cancer testing.

Keywords

Cancer susceptibility genes; Cytogenetics and clinical molecular genetics; Molecular diagnosis and prognosis; Suppressor genes; Familial and hereditary cancers

Introduction

With the technological advancement and continued reduction in cost associated with massively parallel sequencing, application of this technology to tumor sequencing has enabled clinicians and scientists to elucidate molecular mechanisms in cancer(1) and recognize the potential of personalized oncology(2,3). When tumor-germline dyad sequencing is performed on entire genomes, exomes, or selected genes, germline variants are “subtracted out” from those found in the tumor in order to identify somatic mutations(4). The primary focus is then the analysis of somatic mutations to identify driver mutations with existing targeted therapies(2,3,5). While the process of germline variant subtraction enhances the specificity of detecting somatic mutations(3), in some instances, a pathogenic germline mutation may be overlooked that predisposes a patient to increased cancer susceptibility(6–8). Ignoring the germline data post “subtraction” will likely miss these critical variants and identifying such germline variants via tumor-only sequence analysis would be challenging and imprecise(9).

In the course of tumor/germline sequencing, the incidental detection of germline mutations of potentially diagnostic clinical significance can and does occur as previously described by other groups (10,11). As these concomitant and potentially unexpected findings could have significant implications for the patient and their family members, we conducted an exploratory study within patients undergoing tumor-germline sequencing to explore the frequency of “opportunistically identified” pathogenic germline variants within cancer predisposing genes.

Experimental Design

Patients, germline sequencing, and variant calling

Participants were enrolled in the LCCC1108 study (UNC clinical sequencing study, referred to hereafter as UNCseq™). Informed consent and whole blood DNA (or buccal as appropriate) were obtained from all patients through an institutional review board (IRB)-approved protocol at the Lineberger Comprehensive Cancer Center and the University of North Carolina, Chapel Hill(5) (NCT01457196). The UNCseq™ study aims to associate known molecular alterations with clinical outcomes in oncology and uses this information to

support treatment decisions through reporting of genetic profiling to clinicians. The overarching study consent describes the collection and analysis of both tumor and germline tissue including the explicit possibility for identification of an underlying hereditary cancer predisposition. Participants consent to the reporting of all results deemed clinically significant. Consent was obtained by UNCseq™ study staff for the primary study at enrollment. Patients were referred into the UNCseq™ study team by their clinic physician and enrolled according to their treated cancer (Table 1) and thus the tumor tissue to be analyzed. All patients enrolled between 11/2011 and 06/2014 for the cancer types listed in Table 1 were included in our data capture for exploratory germline analysis.

Library preparation and gene capture methods have been described previously(5). Briefly, DNA was extracted from blood using a Puregene DNA Purification kit (Gentra Systems), DNeasy Blood and Tissue Kit (Qiagen), or a Maxwell MDx16™ (Promega, Inc.). In each methodology, DNA was extracted according to the manufacturer's protocol. DNA was fragmented to approximately 180-225 base pairs using a Covaris E220 focused ultrasonicator instrument (Covaris, Inc.). Post-fragmentation, the sample was enriched for appropriately-sized fragments using an automated separation step employing AMPure beads (Beckman Coulter). Fragment size enrichment and subsequent library preparation steps involving precise liquid handling steps were performed using the Agilent basic Bravo A and/or the Bravo B robot(s) (Agilent Technologies). Gene capture was performed using a SureSelect^{XT} custom capture kit according to the manufacturer's protocal (Agilent Technologies). All exons of the 247 genes on the UNCseq™ panel were sufficiently captured with average coverage depth of 750X (see Supplementary Table 1-Capture V6 within Jeck et al.(5) listing all 247 genes).

Library quality was assessed with a Bioanalyzer or Tapestation 2200 (Agilent Technologies) using either D1K Screentapes or High Sensitivity D1K Screentapes (Agilent Technologies). Completed libraries were normalized and pooled using Bravo robots guided by vWorks automation control software (Agilent Technologies), and sequenced at the UNC High Throughput Sequencing Facility (HTSF) using a HiSeq2500™ (Illumina). Alignment and variant calling of the sequencing reads have been described previously, with the addition of Isaac and FreeBayes for variant calling as well as ABRA for read realignment(5,14–17). In brief, germline sequencing reads were mapped to the hg18 reference genome using the Burrows Wheeler Aligner(10) and ABRA(13). ABRA is a bioinformatics platform designed to improve indel detection and accuracy for estimation of variant allele frequency(13). The germline variants were then called using Varscan(19), FreeBayes haplotype-based variant detector(15), and Isaac to improve calling near indels by local realignment(12). Lastly, variants were annotated using ANNOVAR(20). Generally, mean target coverage for all patients ranged from 100-2000X, with the average being approximately 750X. Germline variants and variant annotations were stored in a local PostgreSQL database(16).

While the NGS methods used here may detect copy number variation (CNV), we did not use it for this purpose. If we had, any CNV would have been verified through a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory at UNC-Chapel Hill. Validation of the assay including assessment of sensitivity and specificity to detect germline variants was not performed because this is an exploratory research study. Any variants

deemed clinically significant, and thus warranting return to the patient, are confirmed on a new sample through an orthogonal method within the CLIA certified Molecular Genetics Laboratory at the University of North Carolina at Chapel Hill.

Variant classification

Variants were first filtered through a gene list of 36 known hereditary cancer genes and then prioritized for analysis based on minor allele frequencies, protein effect, and existence in databases of previously reported pathogenic variants (see Table 1 for analyzed genes). Allele frequency data were obtained from The 1000 Genomes Browser (http://browser.1000genomes.org/index.html), National Heart, Lung, and Blood Institute Exome Variant Server ESP6500 Data Set (http://evs.gs.washington.edu/EVS/), and/or The Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/).

Online resources for variant classification included The National Center for Biotechnology Information ClinVar database http://www.ncbi.nlm.nih.gov/clinvar/), the Leiden Open-Source Variation Database (LOVD, http://www.lovd.nl/2.0/index_list.php), and the Catalogue of Somatic Mutations in Cancer (COSMIC, http://cancer.sanger.ac.uk/cosmic). COSMIC was used to determine if a variant existed in tumors from similar tissues of origin. After a preliminary computational classification, variant counts were generated using an in-house python script and validated manually.

Variants underwent tiered review by trained molecular analysts in conjunction with discussion in a multidisciplinary group. Evidence curation and variant classification was performed in a manner similar to the more recently published guidelines from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology(17). As the patients were not selected for clinical or family histories suggestive of a hereditary cancer predisposition, this phenotype information was not available during the variant review process. Therefore, the molecular analysts utilized an incidental or secondary variant analysis approach such that a high threshold for pathogenicity must be met for variant result. The medical and family history presented in Table 2 was obtained from medical record review after variant analysis. Following stringent review, variants classified as Likely Pathogenic or Known Pathogenic were identified as eligible for return to patients. Prior to results return, these variants will be confirmed through analysis of a new sample via an orthogonal method (e.g. Sanger sequencing) and verified by an American Board of Medical Genetics and Genomics (ABMGG)-certified molecular pathologist. The confirmation step was ongoing at the time of submission. Once confirmed, the hereditary cancer predisposing variants will be returned to the patients through a board certified genetic counselor experienced in hereditary cancer. When medical record review documented a clinically known hereditary cancer predisposing variant, no additional steps for confirmation and results return were performed.

Results

To assess the frequency of pathogenic variants opportunistically identified in a group of unselected cancer patients undergoing tumor sequencing, we analyzed germline variants from 439 patients ascertained through the UNCseq™ study(5). Although all 247 genes of

the UNCseq™ panel were sequenced, we specifically investigated germline variants only in 36 genes strongly associated with hereditary cancer syndromes that were present on the somatic sequencing panel. Based on current knowledge about the spectrum of cancers associated with these hereditary cancer syndromes, 24 were considered concordant with the cancer types of the patients being analyzed (Table 1). These cancers included colorectal, ovarian, breast, musculoskeletal, lung, kidney, brain/CNS, melanoma, hematologic, and pancreatic cancers(18–25). Of all cases examined, 19/439 (4.3%) had germline variants classified as Pathogenic/ Likely Pathogenic (P/LP) in a hereditary cancer predisposing gene. Of these, 12 were in genes concordant with the presenting cancer at enrollment and 7 were in other hereditary cancer genes (Fig. 1, Table 2). The discrepancy in pathogenic/likely pathogenic variants listed in Table 2 and Figure 1 is due to a number of cases having pathogenic variants in discordant genes (e.g. *BRCA1* NM_007294.3:c.594-2A>C in patient 11 with colorectal cancer, *BRCA2* NM_000059.3:c.5233_5233delA, p.(Met1745fs) in patient 15 with AML, and others). The majority of these findings occurred in patients with colorectal, ovarian, breast, and pancreatic cancers; very few such findings occurred in patients with musculoskeletal, lung, kidney, brain, skin, or hematologic malignancies.

Overall, *BRCA1* and *BRCA2* harbored 11/19 (57.9%) of the pathogenic variants, the majority of which were classified Likely Pathogenic because they were novel variants expected to result in an early truncation or for which existing evidence suggested a pathogenic role based on classification guidelines(17) (Fig. 2). As might be expected, we discovered that a portion of the P/LP variants had been previously identified through prior clinical genetic assessment for hereditary cancer predisposition. Medical record review following variant classification revealed that the *BRCA1/2* variants identified in breast and ovarian cancer patients in this study had all been previously identified through routine clinical genetic testing, indicated based on medical and family history(26). However, we also discovered P/LP variants in patients whose prior clinical genetic testing was negative because it was focused on only *BRCA1/2* genes. None of the *ATM* and *CDKN2A* pathogenic variants identified in breast cancer patients were previously known (Table 2), reinforcing the idea that additional P/LP variants may exist in breast cancer patients that would be missed in individuals whose clinical testing was restricted to *BRCA1* and *BRCA2* (27,28).

Some patients had a prior history of cancers consistent with the variants identified in the germline analysis, but had been enrolled for cancers that were presumably unrelated (Table 2). For example, Patient 11 was previously diagnosed with breast cancer at age 41 and was enrolled in UNCseq™ when diagnosed with colorectal cancer at age 49. She was found to have a pathogenic canonical splice site variant in *BRCA1* (NM_007294.3:c.594-2A>C) that provides a very good explanation for her breast cancer, but there is debate about whether *BRCA1* mutations predispose individuals to colon cancer (29). Similarly, Patient 17 was previously diagnosed with breast cancer at age 52, but was enrolled in the UNCseq™ study for non-small cell lung cancer. She was found to have a pathogenic nonsense variant in *ATM* (NM_000051.3:c.352C>T, (p.Gln118Ter)) that provides a plausible explanation for her breast cancer. There is limited evidence to support the contribution of *ATM* to lung cancer(30).

Although relatively few patients had clearly pathogenic variants, 178/439 (40.5%) had a germline Variant of Uncertain Significance (VUS) (Fig. 1, 3). In 24 patients, a VUS was found in a gene relevant to the presenting cancer type, while 143 patients had a VUS in hereditary cancer genes unrelated to their cancer type. Not surprisingly, 11 patients had a VUS in both pertinent and non-pertinent genes (Fig. 3).

Discussion

This manuscript explores the yield of clinically relevant findings from germline analysis in patients undergoing tumor-germline dyad sequencing. Consistent with the range reported in previous hereditary genetic disease studies(3,31,32), we found that 4.3% of patients had incidental pathogenic germline variants. This frequency is lower than the approximately 12% recently reported by Schrader et al. 2016(10). While 4.3% of our patients harbored pathogenic germline variants, analysis of the same 36 genes in the study by Schrader and colleagues(10) indicated 116/1,566 (7.4%) of their cases to have pathogenic variants in these genes (Table 1, this study; eTable 7, Schrader et al. 2016(10)). Further, if the data by Schrader is limited to the same cancer types included in our current analysis, only 61 of the 116 cases remain. Thus, when given the same restrictions, Schrader et al. report a pathogenic variant in 3.9% of cases, which is consistent with our findings. Therefore, it could be presumed that in a larger population of unselected cancers run on a larger gene capture, the UNCseq™ data may have revealed the presence of additional pathogenic germline variants. Regardless, both studies suggest that a small but clinically meaningful number of patients undergoing tumor-germline sequencing will harbor germline pathogenic findings in hereditary cancer susceptibility genes.

Interestingly, the majority of pathogenic or likely pathogenic variants identified in this study were in patients with breast or ovarian cancer, even though these diagnoses made up only ~1/3 of the total cohort studied. It is somewhat surprising that no patients were identified with Lynch syndrome, although this may simply be due to the relatively small number of colorectal cancer cases analyzed (N=53). The lack of findings in patients with other tumor types is not unexpected, given the relatively small contribution of monogenic cancer predisposition in those conditions.

Another explanation for the small proportion of patients with pathogenic variants in our study could be the utilization of a tumor-germline sequencing panel created for therapeutic rather than genetic diagnosis of hereditary cancer predisposition. For instance, the next-generation sequencing capture panel in our study lacks several important hereditary cancer predisposition genes such as *PALB2, BARD1, BRIP1,* and *PMS2.* While these genes are implicated in hereditary cancer predisposition, they may not necessarily be used to guide cancer treatment decisions and therefore were not on the therapeutic-focused UNCseq™ panel. Further, our germline analysis did not include CNV detection. Therefore, we cannot exclude the possibility that additional pathogenic germline variants may exist within this cohort.

In our series, half of the pathogenic variants had previously been identified through clinical genetic evaluation. The other half, representing about ~2% of our patients, were not

associated with any prior clinical hereditary cancer evaluation. For this subset of patients, the opportunistic germline analysis provides critical information for both the individual and their family, enabling potentially lifesaving interventions(9,33). Identifying pathogenic germline variants could also provide important prognostic information, guiding surgical procedures or targeted therapeutic options for the individual cancer patient, thereby providing immediate treatment applications(2,34). Further long-term follow-up is needed to assess whether this information ultimately benefits patients and/or their family members. We recognize that unexpected germline susceptibility information may be unwelcome to some patients depending on their personal situation or preference for information. Providers who obtain tumor-germline sequencing on their patients should thus be aware of the potential for germline findings and prepare their patients for this possibility.

That being said, providers should also recognize that analysis of the germline as an ancillary part of a tumor sequencing assay does not substitute for clinical genetic evaluation and testing for cancer predisposition, when indicated. In clinical genetic testing for hereditary cancer susceptibility, the patient has a personal and/or family history suggestive of a hereditary cancer risk, and thus an elevated prior probability of having a causative genetic lesion. Germline testing in this setting is focused on the identification of variants in relevant genes and thus findings must be assessed for their potential causal/diagnostic role and communicated within the personal and familial context of the cancer history(35). Importantly, variants of uncertain significance (VUS) are frequently returned within the diagnostic evaluation of hereditary cancer risk(36) where clinical follow up could include testing additional family members to determine, for example, if the VUS segregates with the cancer risk in the family. Interestingly, when patients with features suggestive of a hereditary cancer predisposition undergo tumor-germline sequencing, the tumor data may aid the interpretation of germline VUS variants through assessment of loss of heterozygosity (LOH) in the tumor. Current efforts within the Clinical Genome Resource are focused on determining how LOH in the tumor can support pathogenicity of a germline variant(37).

On the contrary, when clinical laboratory assessment of tumor-germline pairs is performed for prognostic or therapeutic indications, the identification of germline variation would be considered an incidental or secondary finding. As such, only Pathogenic or Likely Pathogenic findings should be reported to patients per the recommendations of the American College of Medical Genetics and Genomics(31). This notion is supported by the finding of VUS results in almost half (40.5%) of patients in the current study and almost all patients in the Schrader et al study(10). This proportion was expected, given the large proportion of VUS results in other studies(32,38–40). The clinical relevance of these variants, by definition, remains to be determined. It is important to note that if this testing had been performed for suspicion of an underlying hereditary cancer predisposition, these VUS results would likely have been reported back to the ordering clinician. Further investigation into the VUS results, including segregation analysis and functional studies, might be necessary to provide additional evidence of pathogenicity(36,37). Although reporting and clinical correlation of VUS are appropriate through clinical genetics evaluation based on suspicion of a cancer predisposition, the sheer quantity of such findings in a germline analysis as demonstrated here and elsewhere(10) makes their routine clinical follow-up in all patients undergoing tumor-germline sequencing untenable. Because the identification of

germline variants in hereditary cancer genes is not the primary goal of tumor-germline dyad sequencing, these results are most consistent with the definition of incidental or secondary findings(41); given the low prior probability of clinical relevance, the majority of variants identified are likely to be inconsequential. In accordance with guidelines for evaluating incidental findings(31), VUS results were not considered appropriate to return to patients in this study.

While some studies report less or more VUS than our results here, these differences may stem from different thresholds of variant classification or the number of genes examined within each study. Ultimately, in a setting such as tumor-germline sequencing returning VUS results to patients would produce a significant clinical burden(33), may cause undue stress, and may result in potentially unnecessary surveillance, testing or procedures for the patient and family members erroneously presumed to be “at-risk”. Given the more stringent threshold for reporting of variants considered to be incidental or secondary to the testing indication, it is essential to recognize that the *absence* of a reported germline hereditary cancer variant on a tumor-germline test does not rule out the possibility that a pathogenic variant does, in fact, exist. This important distinction may need to be communicated to patients who incorrectly assume that their tumor genetic evaluation included comprehensive testing for hereditary cancer predisposition. It remains imperative for oncologists to ascertain whether their patients should be evaluated specifically for germline mutations due to personal, medical and family history indications including age at presentation, tumor phenotype, and ancestral background.

Whether next generation sequencing should be applied on a routine basis for tumor mutation profiling remains to be determined(42) though it is a major focus of precision medicine efforts. We demonstrate here that utilizing an incidental/secondary variant analysis approach for germline sequence data in unselected patients undergoing somatic sequencing may provide a small but important benefit with regard to the detection of clinically relevant, highly penetrant variants in hereditary cancer predisposition genes. Most of these findings can be ascertained through cancer genetics evaluation recommended on the basis of family history, age at presentation, ancestry or tumor phenotype. However, some of these patients may not be referred to a cancer genetic service(43–46) and a minority will be missed due to lack of typical clinical and/or family history indications(47,48).

Potentially unsuspected pathogenic variants have now been reported in a small, but not insignificant, proportion of cancer patients undergoing therapeutically indicated tumor-germline testing(10,11), and our data provide further support to this scenario. Disclosing the identification of a hereditary cancer predisposition would be highly relevant to the clinical care of these cancer patients and have important implications for their relatives’ medical guidance. Providers who obtain tumor sequencing will need to be cognizant of the implications of tumor-germline analysis with respect to potential incidental findings(49), understand the differences between tumor sequencing and clinical genetic testing for hereditary cancer susceptibility, and be able to effectively communicate these issues to their patients.

Acknowledgements

Bryce A Seifert, Ph.D., Julianne M. O'Daniel, M.S., C.G.C, and Jonathan S. Berg, M.D., Ph.D. had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Financial Support: Support of all authors and this manuscript was provided by the University Cancer Research Fund and National Institutes of Health grant U01 HG006487.

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Statement of Translational Relevance

Tumor-germline next generation sequencing is rapidly advancing as a tool for personalized oncology. Typically, germline mutations are subtracted out from those in the tumor to identify somatic mutations. This exploratory research study sought to investigate the frequency of pathogenic germline mutations among patients unselected for indicators of hereditary cancer predisposition that were undergoing tumor-germline sequencing. With approximately 4% of the cases harboring pathogenic variants, diagnostic germline findings such as these could be beneficial for patients and their families.

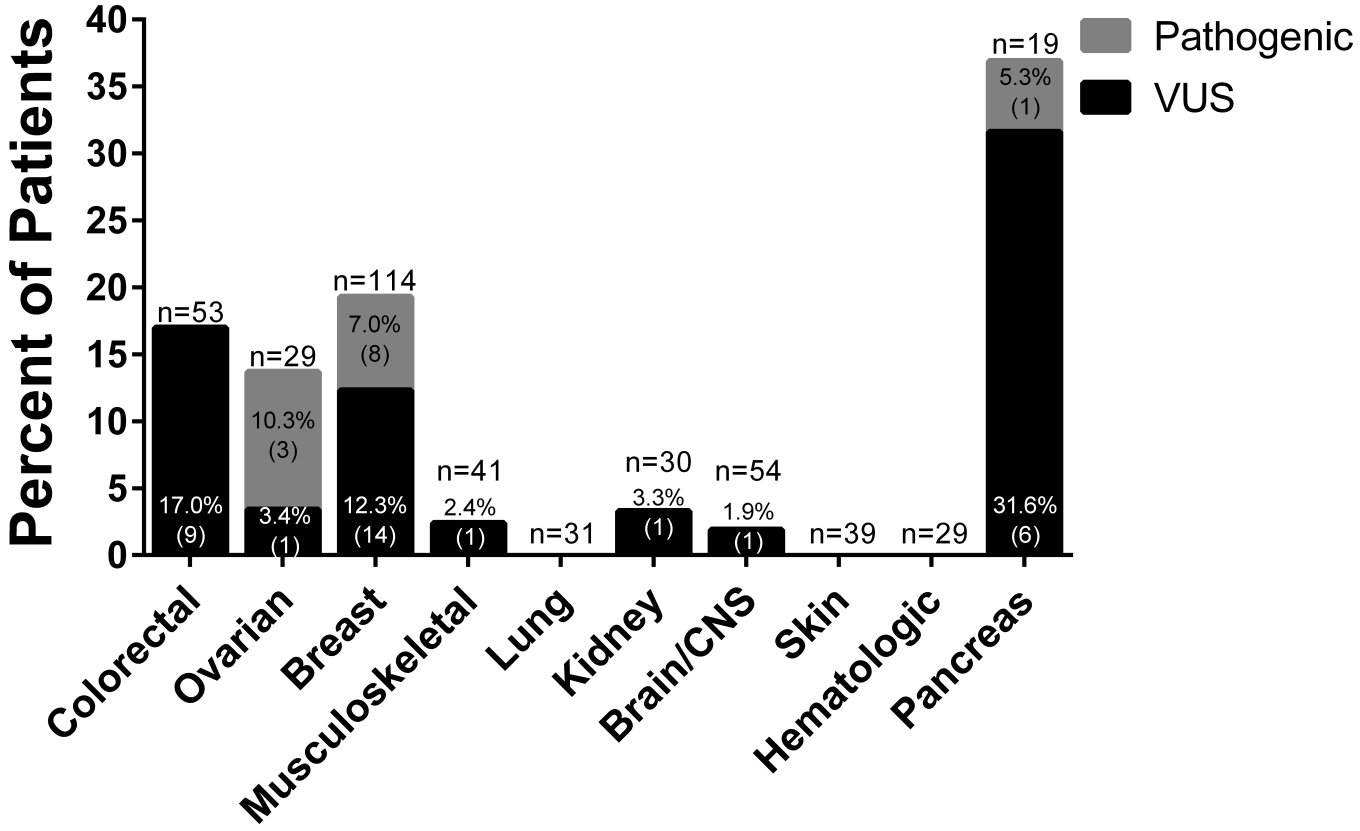

Figure 1. Germline findings across all UNCseq™ patients

The percentages of patients with Pathogenic variants (light shading) or Variants of Uncertain Significance (VUS, dark shading) in genes that are concordant with the tumor type are depicted as stacked bar graphs. Numbers above the bars represent the sample size for the specific tumor type. Numbers in parentheses represent the number of patients with Variants of Uncertain Significance or Pathogenic variants. Here, Pathogenic variants include both Likely Pathogenic (LP) and Known Pathogenic (KP) variants. In both the ovarian and breast cancer groups, one patient in each group had a Variant of Uncertain Significance and a Pathogenic variant. Hence, 8 cases were found to have pathogenic variants in genes concordant with breast cancer, 3 cases were found with pathogenic variants in genes concordant with ovarian cancer, and 1 case was found to have a pathogenic variant in a gene concordant with pancreatic cancer.

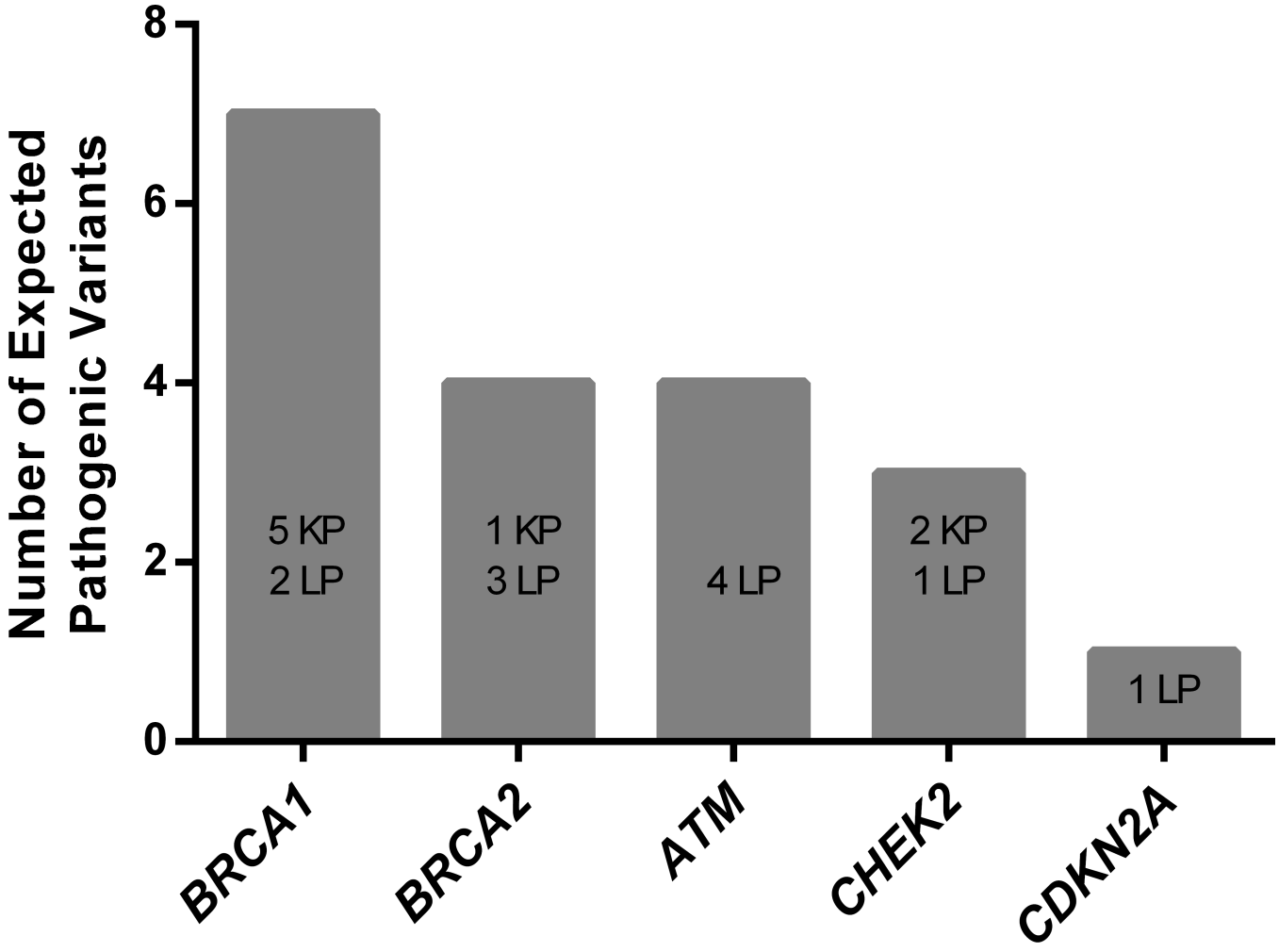

Figure 2. Germline Pathogenic/Likely Pathogenic variants identified in all UNCseq™ patients

The numbers of Known Pathogenic (KP) and Likely Pathogenic (LP) variants across all UNCseq™ patients analyzed are depicted as a bar graph, divided by gene.

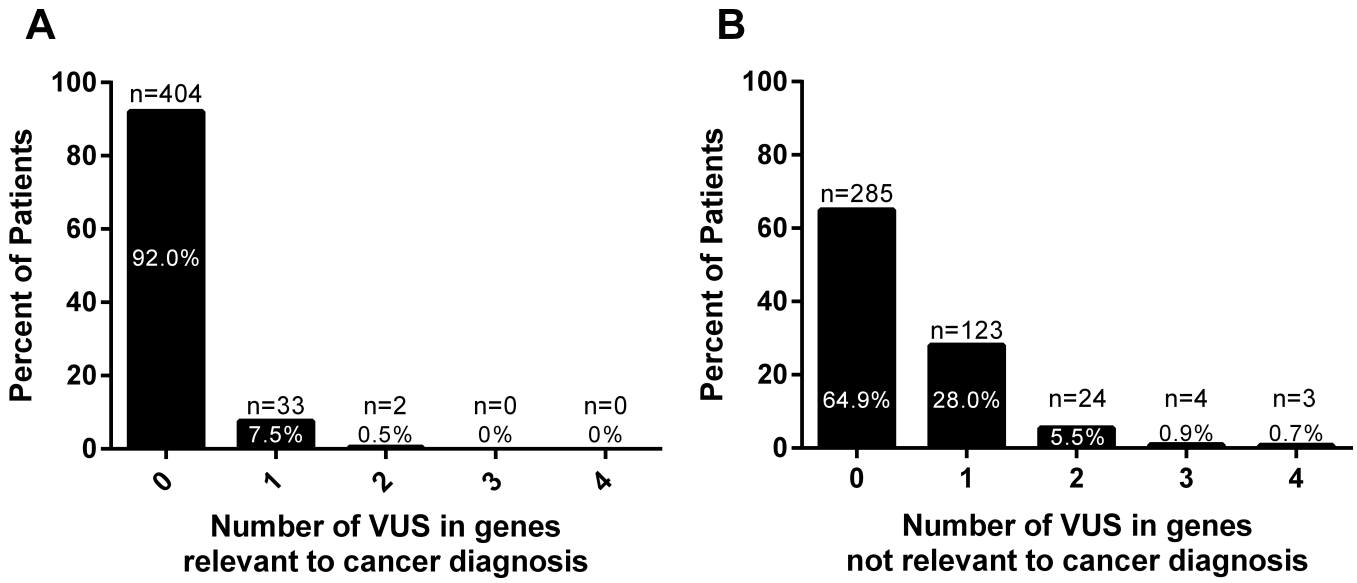

Figure 3. Germline Variants of Uncertain Significance across all UNCseq™ patients

A. Variants occurring in genes relevant to the patient's cancer diagnosis. **B.** Variants occurring in genes unrelated to the patient's cancer diagnosis. Numbers above the bars represent the frequency of patients with 0,1,2,3 or 4 variants. Percentages represent the percent of all UNCseq™ patients analyzed.

Table 1

UNCseq™ cancer cases and hereditary susceptibility genes analyzed

Cancer Type (Subtype)	Cases (% of Total)	Hereditary cancer susceptibility genes evaluated	Hereditary cancer susceptibility genes not analyzed[a]
Colorectal	53 (12.1%)	*MLH1,MSH2, MSH6, APC, PTEN, SMAD4, STK11*	*PMS2, MUTYH, EPCAM, BMPR1A*
Ovarian	29 (6.6%)	*BRCA1, BRCA2, MRE11A, TP53, MSH6, CHEK2, MLH1, MSH2*	*PALB2, BARD1, BRIP1 RAD51C, RAD51D, PMS2*
Breast (Ductal, Lobular, Other)	114 (26.0%)	*BRCA1, BRCA2, ATM, CHEK2, CDH1, MRE11A, PTEN, STK11, TP53*	*PALB2, BARD1, BRIP1*
Musculoskeletal	41 (9.3%)	*TP53*	
Lung (Non-small cell, Small cell, Other)	31 (7.1%)	*TP53*	
Kidney	30 (6.8%)	*VHL, MET*	*FH, FLCN*
Brain/CNS (Astrocytoma, Glioma, Oligodendroglioma, Other)	54 (12.3%)	*NF1, NF2, TSC1, TSC2, TP53*	
Skin (Melanoma, Non-melanoma, Other)	39 (8.9%)	*CDKN2A, PTCH1*[c] *, TP53*	*BAP1*
Hematologic (ALL, AML, CLL, Other)[b]	29 (6.6%)	*RUNX1, CEBPA, TP53*	*NBN, MRE11A, PTPN11*
Pancreas	19 (4.3%)	*BRCA1, BRCA2, CDKN2A, ATM, TP53, STK11, MLH1, MSH2, MSH6*	*PALB2, PMS2, EPCAM*
Total	439		
Other hereditary cancer genes	*AKT1, CDC73, CDKN1B, EGFR, MEN1 NTRK1, PIK3CA, RB1, RET, SMARCA4, SMARCB1,WT1*		

[a] These hereditary cancer susceptibility genes were not included on the targeted UNCseq™ capture panel.

[b] Hematologic cancer abbreviations: Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL).

[c] *PTCH1* variants were considered relevant only in skin cancer cases that were of the non-melanoma type(25).

Table 2

Clinical summary of UNCseq™ patients with germline pathogenic variants

Patient	Cancer type at Enrollment	Sex	Age[a]	Gene	cDNA change[b]	Protein change	Variant type	Classification	Clinical Genetics Evaluation	Personal and/or Family History	Prior Clinical Testing	Clinical Test Result[c]	Concordance with Cancer at Enrollment[d]
1	Ovarian	F	48	*BRCA1*	NM_007294.3: c.5266_5267insC	p.(Gln1756fs)	Frameshifting indel	KP	Yes	Ashkenazi Jewish F:Lung, Bladder MA: Pancreas, 72	Yes	(+)	Yes
2	Ovarian	F	41	*BRCA1*	NM_007294.3: c.5193+1G>T	N/A	Splice-site	KP	Yes	No Cancer History	Yes	(+)	Yes
3	Breast	F	37	*CDKN2A*	NM_000077.4: c.35C>A	p.(Ser12Ter)	Nonsense	LP	Yes	Personal: Melanoma, 21,31 Family: Adopted	Yes	(−)	No
4	Breast	F	55	*BRCA1*	NM_007294.3: c.2457_2457delC	p.(Asp821fs)	Frameshifting indel	KP	Yes	MMaR:Breast, Ovarian, Pancreas M:Breast, 36	Yes	(+)	Yes
5	Breast	F	29	*BRCA1*	NM_007294.3: c.211A>G	p.(Arg71Gly)	Missense	KP	Yes	S:Breast, 28; M:Breast,42 MA:Breast,33; MA:Breast,37	Yes	(+)	Yes
6	Breast	F	63	*ATM*	NM_000051.3: c.1561_1562delAG	p.(Glu522fs)	Frameshifting indel	LP	Yes	M:Breast,55; MA:Breast,30s & 40s 3MU:Blood MA:Cancer,60s	Yes	(−)	Yes
7	Breast	F	29	*BRCA2*	NM_000059.3: c.7538_7539insA	p.(Thr2515fs)	Frameshifting indel	LP	Yes	M: Breast, 39&49, and Brain/CNS, 58	Yes	(+)	Yes
8	Breast	F	35	*BRCA1*	NM_007294.3: c.131G>A	p.(Cys44Tyr)	Missense	KP	Yes	PA: Bilateral Breast, 45	Yes	(+)	Yes
9	Breast	F	37	*BRCA2*	NM_000059.3: c.8575delC	p.(Gln2859fs)	Frameshifting indel	KP	Unknown	Unknown (adopted)	Yes	(+)	Yes
10	Breast	M	53	*BRCA2*	NM_000059.3: c.5718_5719delCT	p.(Leu1908fs)	Frameshifting indel	LP	Yes	S:Breast,50; B:Colon,53 PA:Breast, 55; PC:Colon, 35 Known Familial Mutation	Yes	(+)	Yes

Patient	Cancer type at Enrollment	Sex	Age[a]	Gene	cDNA change[b]	Protein change	Variant type	Classification	Clinical Genetics Evaluation	Personal and/or Family History	Prior Clinical Testing	Clinical Test Result[c]	Concordance with Cancer at Enrollment[d]
11	Colorectal	F	49	*BRCA1*	NM_007294.3: c.594-2A>C	N/A	Splice-site	LP	Yes	Personal: Breast, 41 Family: PA: Breast, 29; PC: Breast, 50	Yes	(+)	No
12	AML	M	54	*BRCA1*	NM_007294.3: c.594-2A>C	N/A	Splice-site	LP	Unknown	Not Reported	No	N/A	No
13	GI-other	M	54	*ATM*	NM_000051.3: c.8545C>T	p.(Arg2849Ter)	Nonsense	LP	No	No Cancer History	No	N/A	No
14	Breast	F	59	*CHEK2*	NM_007194.3: c.1100delC	p.(Thr367fs)	Frameshifting indel	KP	No	Non-Contributory M:Lymph node, 80 U: Liver	No	N/A	Yes
15	AML	M	57	*BRCA2*	NM_000059.3: c.5233_5233delA	p.(Met1745fs)	Frameshifting indel	LP	No	F:Pancreas, 72	No	N/A	No
16	Pancreas	M	61	*ATM*	NM_000051.3: c.170G>A	p.(Trp57Ter)	Nonsense	LP	Yes	B:Pancreas, 52	No	N/A	Yes
17	NSCLC	F	66	*ATM*	NM_000051.3: c.352C>T	p.(Gln118Ter)	Nonsense	LP	No	Personal: Breast, 52 Family: S:Breast, F: Bone (myeloma), B:Amyloidosis	No	N/A	No
18	Musculoskeletal	F	57	*CHEK2*[e]	NM_007194.3: c.1100delC	p.(Thr367fs)	Frameshifting indel	KP	No	F:Kidney PGM: Lung, B:CNS (2)	No	N/A	No
19	Ovarian	F	52	*CHEK2*	NM_007194.3: c.1486C>T	p.(Gln496Ter)	Nonsense	LP	No	Personal: Melanoma, 57 Family: PU: Stomach	No	N/A	Yes

Abbreviations:

Cancer type: AML=Acute Myelogenous Leukemia; NSCLC= Non-small cell lung cancer.

Gender: F= female; M= male. Classification: KP= Known Pathogenic; LP= Likely Pathogenic; VUS= Variant of Uncertain Significance.

Family History: M=Mother; F=Father; S=Sister; B=Brother; MA= Maternal Aunt; MU= Maternal Uncle; MGM= Maternal Grandmother; MGF= Maternal Grandfather; MMaR=Multiple Maternal Relatives; PA= Paternal Aunt; PU= Paternal Uncle; PGF=Paternal Grandfather; PGM=Paternal Grandmother; PC= Paternal Cousin; U=Uncle.

[a]Age at the time of diagnosis

[b]Transcripts are listed according to the HGVS nomenclature recommendations or the commonly accepted transcript.

[c] Clinical Test Result: (+) = Same Mutation Reported, (−) = Gene was not included in the clinical genetic test and these negative results indicate new diagnostic results.

[d] Concordance with cancer at enrollment: Yes = Pathogenic variant is in a gene that is concordant with the presenting cancer at enrollment. No = Pathogenic variant is in a gene that is discordant with the presenting cancer at enrollment.

[e] CHEK2 has been implicated as a susceptibility gene for a Li-Fraumeni-like cancer syndrome. However, the current evidence for this association is disputed(50).