Clinical Evaluation of Germline Sequencing for Hereditary Cancer Risk Assessment

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INTRODUCTION

Cancer is a leading cause of death worldwide, with an estimated 1,665,540 new cases in 2014[1]. In the past decade, major advances in DNA sequencing technologies coupled with dramatic reductions in cost have allowed next-generation sequencing to become a routine part of clinical care. In particular, cancer researchers and clinicians have benefitted greatly from information provided by sequencing technologies with regards to diagnosing tumors and determining effective courses of action for cancer patients. Tumor and germline sequencing can be carried out using whole-exome sequencing (WES) or with gene panels of known oncogenes and tumor-suppressor genes[2–4]. Often, genetic variants found in the blood (germline variants) are subtracted out from those found in the tumor, thus negating the possibility of discovering germline variants that could have profound implications for patients and their family members[5] (Figure 1).

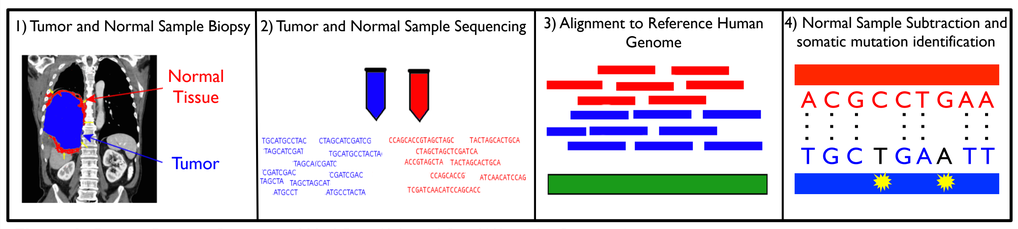

Figure 1: Cancer Genome Sequencing Workflow (*Adapted from Wikimedia Commons*).

While the majority of cancer cases arise sporadically, hereditary cancer syndromes such as Lynch Syndrome, Hereditary Breast and Ovarian Cancer Syndrome, and Li-Fraumeni Syndrome account for roughly 3-15% of cancer cases[6–10]. Previously, germline genetic testing is done only on those patients whose family history indicate an evident genetic predisposition to cancer[11]. In this study, we seek to ascertain the frequency of pathogenic or potentially pathogenic germline variants in multiple unselected cancer cases.

METHODS

Patients were recruited as a part of the Lineberger Comprehensive Cancer Center UNCSeq study. The analysis included a total of 328 patients of various cancer subtypes, including colorectal, breast, ovarian, musculoskeletal, lung, kidney, and brain/CNS. The cancer types were classified based on strong or weak genetic etiology. A list of susceptibility genes for each subtype was curated by searching through previous literature (Table 1).

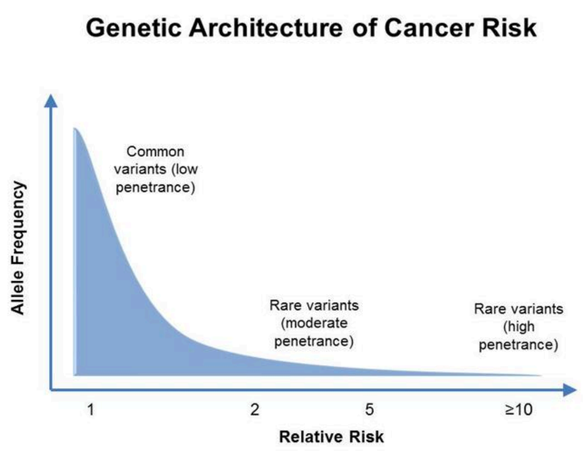

Figure 2: Genetic Architecture of Cancer Risk (*adapted from www.cancer.gov*).

After collection and shearing of genomic DNA, the entire coding region of specific genes were captured using the SureSelectXT Custom kit (Agilent Technologies) and sequenced on an Illumina HiSeq system. The sequencing reads were analyzed and aligned to the Human Genome Feb. 2009 Assembly (version GRCh37/hg19). Individual single nucleotide variants were curated according to curation guidelines set forth by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology[12]. Variants were curated according to their frequency in the general population, whether or not they have been reported previously, and through various in-silico methods. In this study, only rare, high penetrance variants with low minor allele frequencies were considered (Figure 2). All positive variants were confirmed by a genetic counselor from the UNC Department of Genetics.

Cancer (tissue of origin)	Total Cases (%)	Susceptibility Genes
GI-Colorectal	46 (14.0)	MSH2, MLH1, MSH6, APC
Gynecologic-Ovarian	26 (7.93)	BRCA1, BRCA2, MRE11A, TP53, MSH6
Breast (Ductal, Lobular, NOS, Other)	104 (31.71)	BRCA1, BRCA2, ATM, CHEK2, CDH1, MRE11A
Musculoskeletal (Soft Tissue Sarcoma, Bone)	41 (12.50)	TP53
Lung (Non-small cell, Small cell, NOS, Other)	28 (8.54)	TP53
GU-Kidney	30 (9.15)	VHL
Brain/CNS (Astrocytoma, Glioma, Oligodendroglioma, Other)	53 (16.16)	NF1, TSC1, NF2, TSC2, TP53
Total	328 (100)	

Table 1. Total counts and percentages of patients from each cancer subtype along with susceptibility genes included in the UNCSeq gene capture panel.

RESULTS

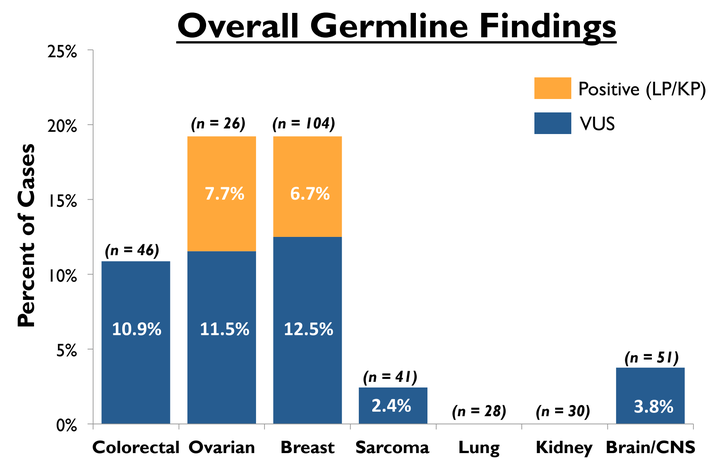

Figure 2: Overall germline findings for identified susceptibility genes across 7 major cancer types.

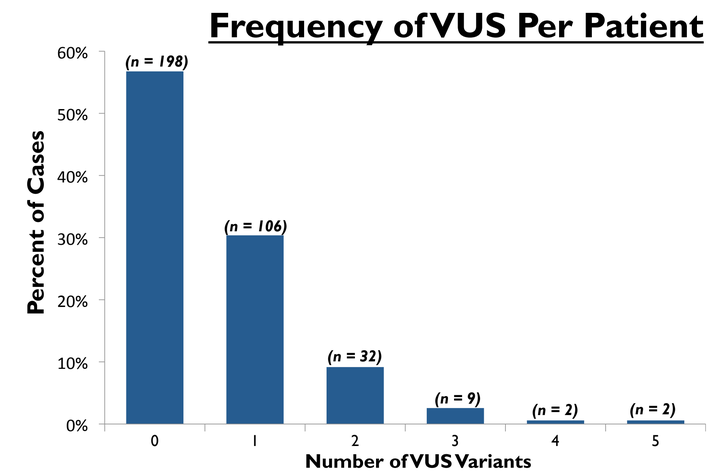

Figure 3: Frequency of variants of uncertain significance across 247 sequenced genes.

Figure 2 shows the total number of positive (likely pathogenic or known pathogenic) and VUS cases found in each cancer subtype. As expected, cancer subtypes with known genetic etiologies (colorectal, ovarian, and breast) had notably higher levels of VUS and positive variants – consistent with values found in the literature. Cancer subtypes with no or little known genetic etiologies, on the other hand, did not harbor a significant number of pathogenic variants. Figure 3 shows a frequency distribution of the number of variants of uncertain significance found per patient. A significant number of patients (43.3%) had one or more VUS. Currently, these variants would not be reported back to a patient – however, as knowledge of variants increases with time, some of these variants could potentially be clinically actionable in the future.

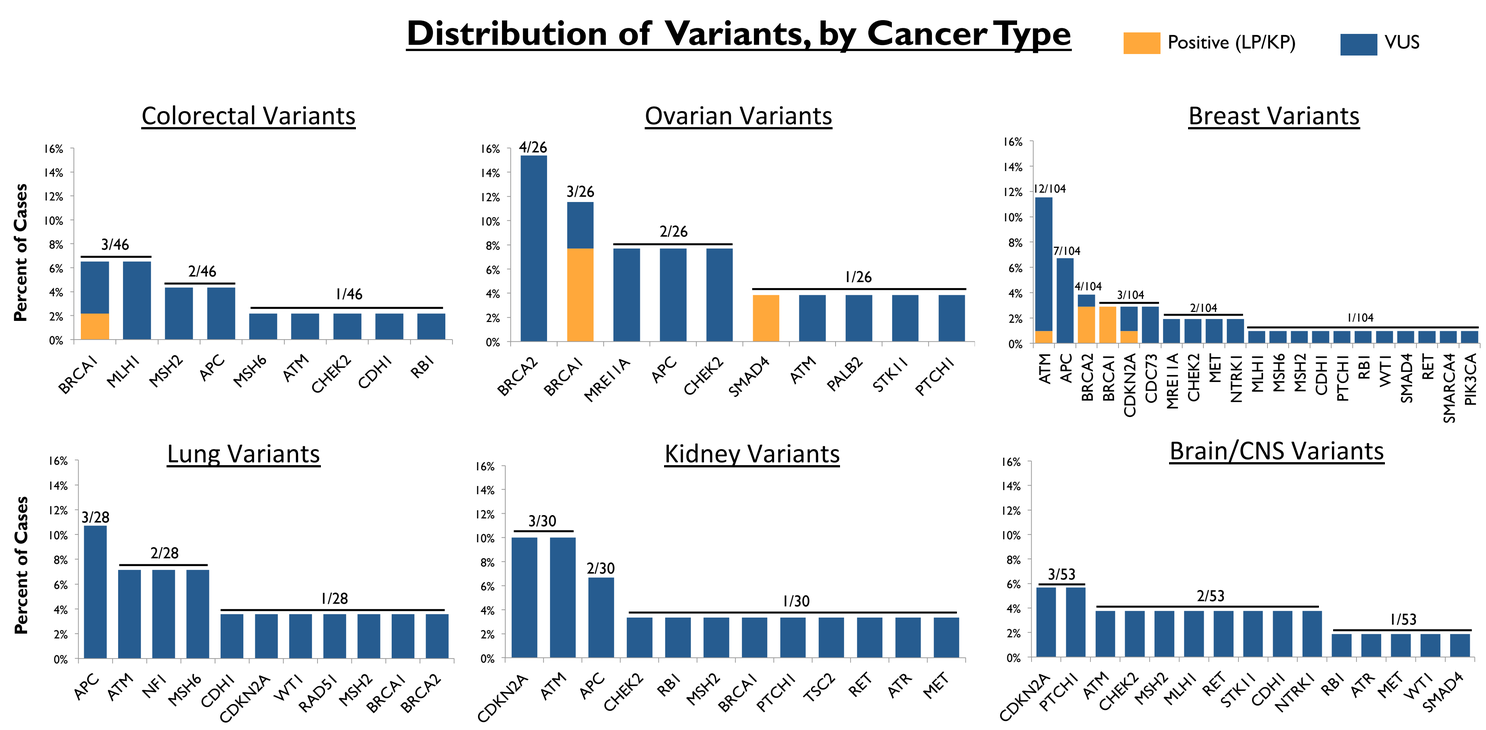

Figure 4: Distribution of variants across various cancer types. The top row shows cancers with known genetic etiologies (colorectal, ovarian, and breast) and the bottom row shows cancers with little or no know genetic etiology (lung, kidney, and Brain/CNS).

Figure 4 shows the percent of cases with a VUS or pathogenic variant in specific genes across multiple cancer types. As expected, ovarian and breast cancer had the most variants, particularly in *BRCA1* and *BRCA2*. A total of three pathogenic incidental findings were found: *BRCA1* in a patient from the colorectal cohort, *SMAD4* in an ovarian cancer patient, and *CDKN2A* in a breast cancer patient. Surprisingly, in all cancer types with the exception of the Brain/CNS cancer, *ATM* and *APC* consistently appeared to have a relatively high level of VUS variants. At present there is no evidence in literature to suggest that *APC* and *ATM* play a significant role in any other cancer other than familial adenomatous polyposis and hereditary breast cancer respectively. The frequency of their occurrence could be due to the sheer size of the genes, which is much larger than other genes on the capture panel.

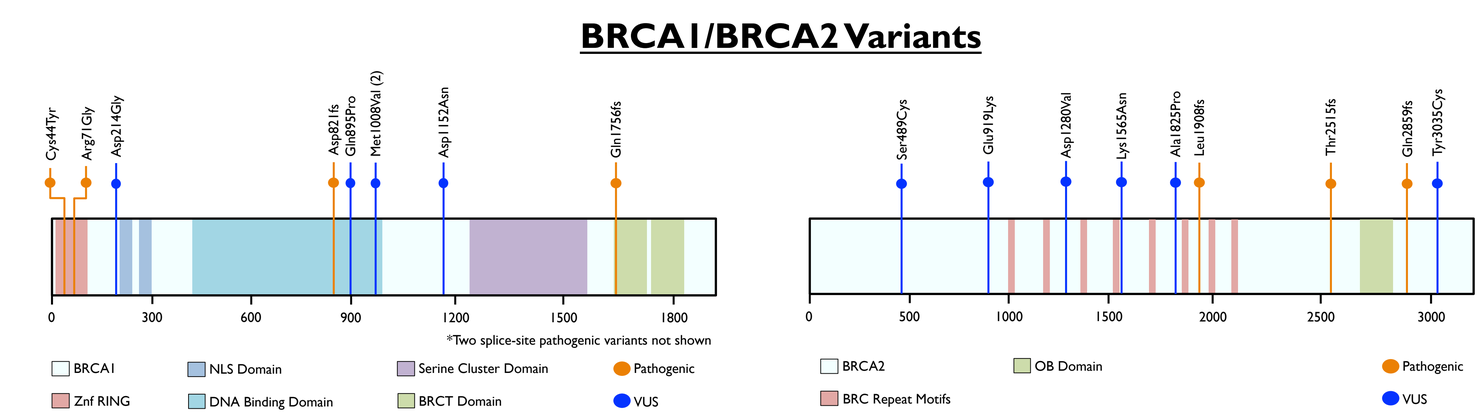

Figure 5: Lolliplot showing distribution of germline variants in *BRCA1*.

Figure 6: Lolliplot showing distribution of germline variants in *BRCA2*.

Figures 5 and 6 show the distribution of germline variants (from all cancer types) in two frequently mutated genes: *BRCA1* and *BRCA2*. The location of variants relative to domains in the resulting protein could have implications for determining the potential pathogenicity of VUS variants.

CONCLUSION & FUTURE DIRECTIONS

Germline sequencing of a cohort of 328 patients of various cancer types identified a total of 12 potentially pathogenic mutations and 132 variants of uncertain significance. Cancer types with known genetic etiologies contained mutations in identified susceptibility genes at a frequency that was agreeable with previous values found in literature. There were no pathogenic mutations identified in susceptibility genes of cancer types with weak genetic etiologies. These results suggest that germline sequencing may benefit appropriately selected patients, particularly those with an evident family history of a hereditary cancer syndrome. Additionally, 43.3% of cases contained at least one variant of uncertain significance, which does not contribute to risk assessment and may prompt anxiety or overtreatment. If germline sequencing is to reach its full potential in terms of clinical utility, it is critical that variants of uncertain significance are classified according to their clincical interpretation and that this information is communicated readily through open databases.

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