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Clinical Implications of Conflicting Variant Interpretations in the Cancer Genetics Clinic

THESIS

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by

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ABSTRACT OF THE THESIS

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By

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Master of Science in Genetic Counseling

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Various commercial laboratories are used in cancer genetics practice, which can lead to clinicians receiving reports with conflicting categorizations of genetic variants. Such discrepancies can have significant clinical implications and can potentially lead to different counseling approaches for different patients with the same variant. In this study, we describe the frequency of this occurrence, analyze genetics providers' awareness of conflicting interpretations, and make comparisons of medical management recommendations provided to patients with discrepant classifications of the same variant. A cohort of 2,000 patients was recruited from three cancer genetics clinics from 2014 to 2016. All patients underwent the same hereditary cancer panel by one major commercial genetics laboratory. A review of ClinVar archives was performed to identify clinically significant conflicts between ClinVar and the test report, defined as either a variant of uncertain significance (VUS) on the test report with a pathogenic/likely pathogenic (P/LP) classification by major lab in ClinVar, or a P/LP variant on the test report with a VUS classification by a major lab in ClinVar. We demonstrate that 2.5% of patients had a variant with a clinically significant conflict in ClinVar when the test report was issued, including 19 patients with a P/LP variant reported in *APC* or *MUTYH*, and 31 patients with a VUS reported in *CDKN2A*, *CHEK2*, *MLH1*, *MSH2*, *MUTYH*, *RAD51C*, or *TP53*. For

patients with a VUS on their report who had a clinically significant conflict, analysis of available study case report forms and original results disclosure clinic notes revealed that only 10/28 (36%) of patients appeared to be counseled by a provider who was aware of the conflict. Patients in this cohort with clinically significant conflicts were then compared to patients outside the cohort who had been tested by the same providers utilizing different laboratories. A detailed case analysis led to the finding that discrepant counseling strategies were utilized for different patients with the same variant, within the same institution and even by the same counselor. The results of this study provide evidence that variant interpretation discrepancies have implications in medical management decisions. This highlights the importance of clinician awareness for possible conflicts in variant interpretation. Initiatives to harmonize variant classifications are critical to resolving discrepant interpretations and supporting clinicians in providing accurate risk assessment.

I. INTRODUCTION

1.1 Background and Significance of the Research

1.1.1 Overview of cancer and cancer formation

Cancer is one of the leading causes of death worldwide. In 2019, an estimated number of 1,762,450 new cases of cancer were diagnosed in the United States, and an estimated number of 606,880 individuals died of cancer. According to 2014 to 2016 data, approximately 39.3% of individuals will be diagnosed with cancer at some point in their lifetime, with a higher rate of diagnosis in males than females. The average five-year survival rate, based on data from 2009 to 2015, is 67.1%. However, the survival rate differs significantly depending on specific cancer type and ranges from approximately 9% to 98%. The most common types of cancer include those of the female breast, lung, prostate, and colon/rectum (SEER Program 2019).

Cancer is defined as an uncontrolled proliferation of cells. This can occur when there is a disruption of the body's normal processes to control cell division, which during adulthood typically only occurs as needed to replace damaged or dying cells. A tumor forms when proliferation of cells persists, and a tumor can become malignant, or cancerous, if it has already invaded or has the potential to invade surrounding tissue. Local nodal disease involves the cancer spreading to nearby lymph nodes, and metastatic disease involves the spread to distant organs, such as the lungs, liver, bone, or brain. All cancers are caused by an accumulation of new genetic alterations, also referred to as mutations or pathogenic variants, that disrupt the function of those genes. New genetic alterations that occur throughout an individual's lifetime are referred to as somatic mutations, which can be distinguished from the germline mutations that are present in an individual from the time of conception. Some of these somatic mutations, such as those in tumor suppressor genes, may inhibit or turn off the regulatory processes that control cell growth. Other mutations, such as those in oncogenes, promote cell growth. Mutations can accumulate in cells

over time as a result of errors in cell division and DNA damage caused by environmental exposures, including ultraviolet rays from the sun, radiation, tobacco, alcohol, a high-fat diet, occupational exposures, and certain viral, bacterial, or parasitic infections (National Cancer Institute, About Cancer).

All cancer is caused by genetic mutations; however, most cancer is not inherited. Cancer can be classified as sporadic, familial, or hereditary. The majority of cancers occur sporadically and are believed to be caused by environmental factors and random chance events, leading to an accumulation of acquired genetic mutations. These cancers tend to occur at older ages, most often over age 50.

In contrast, hereditary cancer syndromes account for approximately 5 to 10% of all cancers (Garber and Offit 2005). When an individual inherits a germline mutation in a gene that is associated with cancer development, the mutation will be present in every cell of their body. These individuals therefore have a greater lifetime risk of developing certain types of cancer, often at younger ages than are typically seen for the specific cancer type. Features suggestive of an inherited predisposition to cancer include younger ages at cancer diagnosis, multiple primary tumors in a single individual, bilateral primary tumors in paired organs, rare types of tumors, and multiple family members with cancer, such as two or more first-degree relatives and/or multiple generations with cancer. Additionally, since many hereditary cancer syndromes are associated with multiple types of cancer, a family history of types of cancer that align with a specific syndrome would further increase suspicion for an inherited mutation (National Cancer Institute, About Cancer).

The remaining 10 to 20% of cancers are considered to be familial. Familial cancers appear to cluster in families and are thought to be caused by an accumulation of environmental factors and multiple inherited factors that each have a weak effect individually, but may

moderately increase cancer risk when combined. In contrast to families with hereditary cancer, increased cancer risk in families with familial cancer cannot be attributed to a single cancer-predisposing mutation. Despite negative results on extensive genetic testing for hereditary cancer syndromes, these families are still considered to be at a modestly increased risk of the cancers that are present in family members.

1.1.2 Hereditary causes of cancer

The discovery of the mechanism of inherited forms of cancer began with Dr. Alfred Knudon's "two-hit" model of tumorigenesis in 1971. On the basis of observed and published reports of retinoblastoma, the most common eye tumor in children, Dr. Knudson hypothesized that two mutational events occurred in the development of retinoblastoma, and that those with the hereditary form were born with one germline mutation and had a second mutation in the other allele that occurred sporadically. He hypothesized that those with the sporadic form acquired biallelic somatic mutations. This model was proposed as an explanation for the observed earlier onset of retinoblastoma in children with a positive family history, as well as the occurrence of bilateral disease in all children with a family history (Knudson 1971). The *RB1* gene, responsible for hereditary retinoblastoma, was the first cancer predisposition gene to be identified in 1986 (Friend et al. 1986). It is now known that many hereditary cancer syndromes follow this "two-hit" model, in which those with an inherited predisposition are at increased risk to develop cancer due to a germline mutation in a tumor suppressor gene. While most adult-onset hereditary cancer syndromes follow an autosomal dominant inheritance pattern, the cancer itself develops in a recessive manner requiring mutations, whether inherited or sporadic, in both alleles.

The first identified breast cancer predisposition genes, *BRCA1* and *BRCA2*, were discovered in 1994 and 1995, respectively. Although mutations in these genes were first identified in families with hereditary breast cancer, they are now known to also be associated with inherited predispositions to ovarian, pancreatic, and prostate cancers, in addition to melanoma (Petrucelli et al. 1998-2016). Approximately 1 in 400 to 1 in 500 individuals in the general population carries a mutation in *BRCA1* or *BRCA2*, with a higher frequency in certain ethnic groups, and these individuals have lifetime cancer risks of up to 87% in females and 20% in males (Anglian Breast Cancer Study Group 2000, Breast Cancer Linkage Consortium 1999, Ford et al. 1994, Kote-Jarai et al. 2011, Leongamornlert et al. 2012, Moran et al. 2011, van Asperen et al. 2005, Whittemore et al. 2004). Among the first mutations discovered were three with a high frequency in the Ashkenazi Jewish population (Roa et al. 1996). To date there have been over 3,500 deleterious mutations identified that cause *BRCA1*- and *BRCA2*-associated hereditary breast and ovarian cancer (Kobayashi et al. 2013).

Several other syndromes are associated with inherited predisposition to breast cancer, including Li-Fraumeni syndrome, Cowden syndrome, Peutz-Jeghers syndrome, and hereditary diffuse gastric cancer syndrome, associated with heterozygous mutations in the *TP53*, *PTEN*, *STK11*, and *CDH1* genes, respectively. Individuals with Li-Fraumeni syndrome (LFS) have an increased risk for a wide spectrum of childhood and adult-onset cancers; the most commonly seen include sarcomas of the bone and soft tissue, breast cancer, central nervous system tumors, and adrenocortical carcinomas. Other prevalent cancers in LFS include leukemia, lymphoma, lung, and gastrointestinal cancers (Schneider et al. 1999-2019). However, individuals with LFS can develop virtually any type of cancer, with lifetime cancer risks of at least 70% in men and 90% in women (Guha and Malkin 2017, Mai et al. 2016). Cowden syndrome (CS), also known as *PTEN* hamartoma tumor syndrome, causes increased risks of multiple tumor types, most

notably benign and malignant disease of the breast, thyroid, and endometrium. Individuals with CS often develop hamartomatous and mixed gastrointestinal polyps, which increase the risk of colorectal cancer, and have distinct clinical features including macrocephaly and mucocutaneous stigmata (Eng 2001-2016). Peutz-Jeghers syndrome is characterized by increased risks of colorectal, gastric, small intestine, breast, ovarian, pancreatic, and other cancers, in addition to mucocutaneous pigmentation on the buccal mucosa and other areas, and Peutz-Jeghers-type hamartomatous polyps in intestinal and extraintestinal sites (McGarrity et al. 2001-2016). Hereditary diffuse gastric cancer syndrome confers an increased risk of diffuse gastric cancer and lobular breast cancer (Kaurah and Huntsman 2002-2018).

Hereditary cancer syndromes that confer significantly increased risks of gastrointestinal cancers include Lynch syndrome, familial adenomatous polyposis (FAP), and *MUTYH*-associated polyposis (MAP), in addition to those described above. Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer (HNPCC), is caused by heterozygous mutations in *MLH1*, *MSH2*, *MSH6*, or *PMS2*, or a heterozygous *EPCAM* deletion, and affected individuals have increased risks of colorectal, endometrial, gastric, and ovarian cancers, in addition to other types of cancer (Kohlmann and Gruber 2004-2018). FAP, which occurs due to heterozygous mutations in the *APC* gene, is characterized by the development of hundreds to thousands of gastrointestinal polyps, beginning at an average age of 16, which lead to a diagnosis of colon cancer by an average age of 39. All affected individuals will develop colon cancer without surgical intervention via a colectomy, and also have increased risks of cancers of the small bowel, pancreas, thyroid, central nervous system, bile ducts, and stomach (Bulow 2003, Bussey 1976, Gardner 1951, Petersen et al. 1991). Individuals with lower penetrance mutations in the *APC* gene have attenuated FAP, and tend to develop fewer colon polyps (average of 30) and develop colon cancer at later ages (Spirio et al. 1993, Neklason et al. 2008). MAP is an

autosomal recessive polyposis condition, in which affected individuals have germline mutations in both *MUTYH* alleles. Total lifetime number of colon polyps ranges from ten to a few hundred, and there is an 80-90% lifetime risk of colon cancer without surveillance (Al-Tassan et al. 2002, Jones et al. 2002, Lubbe et al. 2009, Sieber et al. 2003, Wang et al. 2004). There are also increased risks of duodenal, ovarian, and bladder cancers in affected individuals (Nielsen et al. 2012-2019). Heterozygous carriers have been found to have a two- to three-fold increased risk of colorectal cancer over that in the general population (Jenkins et al. 2006, Jones et al. 2009).

Another condition that confers an increased risk of pancreatic cancer is familial atypical multiple mole melanoma (FAMMM) syndrome, which occurs due to heterozygous mutations in the *CDKN2A* gene. Affected individuals often have multiple melanocytic nevi, and a 58-92% lifetime risk of melanoma. The risk of pancreatic cancer is approximately 17%. However, precise cancer risks are difficult to estimate due to variable expressivity within families and among various geographic regions, especially for melanoma risk as it relates to degree of sun exposure (Czajkowski et al. 2004, Eckerle Mize et al. 2009, Garber and Offit 2005).

The previously described hereditary cancer syndromes are due to mutations in high penetrance cancer risk genes, meaning that they confer cancer risks that are significantly increased (exact risk threshold is not well-defined, but generally greater than 4 to 5 times) above the general population risk (Couch et al. 2017, Easton et al. 2015, Hollestelle et al. 2010, Tung et al. 2016). Mutations in moderate penetrance cancer risk genes confer risks of cancer that are increased above the general population (approximately 2 to 5 times relative risk), but not as high as risks associated with high penetrance genes such as *BRCA1*, *BRCA2*, and the other genes detailed above (Couch et al. 2017, Easton et al. 2015, Hollestelle et al. 2010, Tung et al. 2016). Examples of moderate penetrance cancer risk genes include *ATM*, *BARD1*, *BRIP1*, *CHEK2*, *NBN*, *RAD50*, *RAD51C*, and *RAD51D,* mutations in which are associated with an increased risk

of breast and/or ovarian cancer in addition to other cancers. Additionally, *MUTYH* heterozygous carriers and individuals with a specific mutation in *APC*, c.3920T>A (p.I1307K), have moderately increased risks of colorectal cancer (Tung et al. 2016). *PALB2* has previously been referred to as a moderate risk gene for breast cancer; however, recent studies have shown that mutations are actually more likely to be associated with high risks of breast cancer, ranging from 5.3 to 14.41 times the general population risk (Couch et al. 2017, Easton et al. 2015, Shimelis et al. 2018, Yang et al. 2019). Exact risk associated with *PALB2* mutations has been shown to vary depending on family history (Antoniou et al. 2014), and it is frequently reported as both a moderate and high-risk gene for breast cancer.

1.1.3 Clinical cancer genetic testing

Following the discovery of the *BRCA1* and *BRCA2* genes, a genetic testing laboratory called Myriad Genetics Laboratories, Inc. obtained patents on both genes, associated mutations, and diagnostic testing. Myriad began offering clinical diagnostic tests for *BRCA1* and *BRCA2* mutations in 1996. Three diagnostic tests were available: (1) Comprehensive BRACAnalysis, involving full sequencing of both genes (deletion/duplication analysis had not yet been developed), (2) Single Site BRACAnalysis test, for testing of a single known *BRCA* mutation that had previously been identified in a family member, and (3) Multisite three BRACAnalysis, which detected three founder mutations in the Ashkenazi Jewish population (Gold and Carbone 2010). Myriad was the only laboratory offering *BRCA1/2* testing until 2013, when the Supreme Court ruled that genes, as products of nature, could not be patented, and that gene patents would limit scientific research and progress (Association for Molecular Pathology v. Myriad Genetics, Inc. [June 13, 2013]).

Other laboratories had already begun offering genetic testing for hereditary cancer syndromes such as Lynch syndrome and other highly penetrant syndromes. With the development of next-generation sequencing (NGS), massively parallel sequencing allowed for the rapid, efficient, and accurate sequencing of multiple genes simultaneously (Tucker et al. 2009, Walsh et al. 2010, Walsh et al. 2011). Using NGS technology, numerous commercial laboratories developed cancer gene panels, which analyze two to 125, or more, genes associated with inherited cancer risk (Ambry Genetics, Fulgent Genetics, GeneDx, Invitae, Myriad Genetics). Some panels only include genes associated with a specific type of cancer, such as breast cancer, while others are more comprehensive and cover genes associated with multiple types of cancer. Among the first commercial laboratories to offer hereditary cancer panel testing were Ambry Genetics, Fulgent Genetics, GeneDx, Illumina, Invitae, Myriad Genetics, and University of Washington (Easton et al. 2015). Numerous other genetics laboratories have since entered this market and currently offer hereditary cancer panel testing. Large companies such as Quest Diagnostics and LabCorp, which have traditionally offered an array of non-genetics laboratory tests, now also offer numerous genetic tests including hereditary cancer panels.

When any alteration is identified on genetic testing, there are five categories used to classify the variant by its degree of clinical significance, as recommended jointly by the American College of Medical Genetics (ACMG) and the Association for Molecular Pathology (AMP): (1) pathogenic, (2) likely pathogenic, (3) variant of uncertain significance (VUS), (4) likely benign, and (5) benign (Richards et al. 2015). These categories are listed in order of decreasing clinical significance. For most types of genetic testing, three possible overall test results can be received based on which, if any, variants are identified: “positive”, “negative”, and VUS. In the context of hereditary cancer panel testing, a positive result indicates that a pathogenic or likely pathogenic variant was identified and that this variant is believed to confer

an increased risk of cancer. When a pathogenic or likely pathogenic variant is identified, individuals with the variant may consider additional cancer screening and prevention measures such as risk-reducing surgeries and imaging. The identification of the variant often leads to cascade testing of family members. A negative result on genetic testing indicates that no clinically significant or uncertain variants were identified on the specific test ordered. A negative result may or may not be informative depending on the personal and family history and the specific test ordered. An individual may receive a negative result because the cancer(s) in their personal or family history are not due to a single genetic cause, the cancers in their family are due to a single genetic cause but the individual did not inherit that mutation, they may have a mutation in a gene that was not evaluated on the specific panel that was run, or they may have a mutation in a gene that was evaluated, but their specific mutation is not detectable with the laboratory technology that was used for the test. Negative results may include the identification of benign or likely benign variants. An uncertain result occurs when a variant of uncertain or unknown significance (VUS) is identified. A VUS is an alteration in a gene for which there is currently insufficient evidence to determine whether it causes disease or is part of benign human variation. Because VUSs are not clinically significant, it is advised that providers not modify medical management recommendations on the basis of a VUS result. Therefore, these individuals' cancer risk assessment and management must be based on their personal and family history. Over time, a significant proportion of VUSs are reclassified as laboratories gather more evidence about the variants' pathogenicity, with occasional variants upgraded to pathogenic or likely pathogenic, but the majority downgraded to benign variants (Macklin et al. 2017, Mersch et al. 2018).

1.1.4 Impact of variants of uncertain significance

Variants of uncertain significance are frequently identified on multigene panel testing. In the initial stages of hereditary cancer panel testing via NGS, one major clinical laboratory reported a 2.04% VUS rate per gene tested and inconclusive results in 15.1% to 25.6% of tests ordered, depending on the specific panel (LaDuca et al. 2014, Stuenkel et al. 2012). More recently, another major clinical laboratory reported 20.5% of patients undergoing a hereditary cancer panel were found to have at least one VUS (Macklin et al. 2017). The more genes assessed, the greater likelihood of identifying a VUS (Tucker et al. 2009). With the use of increasingly large gene panels, sometimes up to 100 or more genes, it has been well-established that a VUS is found in a considerable proportion of patients who undergo testing (Idos et al. 2019, Kapoor et al. 2015, Kurian et al. 2014, LaDuca et al. 2014, Macklin et al. 2017, Slavin et al. 2015, Tung et al. 2016).

Since much of the research and many reference databases thus far have focused predominantly on populations of European descent, VUS rates are disproportionately higher in non-European populations (Abul-Husn et al. 2019, Caswell-Jin et al. 2017, Domchek and Weber 2008, Hall et al. 2009, Kurian et al. 2018, Ricker et al. 2016). The frequency of VUS identification in *BRCA1/2* testing is approximately 12% in African American populations, 11% in East/Southeast Asian populations, and 8.5% in Hispanic populations, compared to approximately 4% in individuals of European descent (Abul-Husn et al. 2019). Earlier studies reported VUS rates in the *BRCA* genes as high as 46% in African Americans and 22% in Hispanics (Domchek and Weber 2008), suggesting that the rate of VUS identification is likely to be higher in genes that are not as well-studied. Additionally, the rate of identification of novel uncharacterized *BRCA1/2* variants in non-European populations is increased (Abul-Husn et al. 2019). When studying individuals with breast cancer undergoing cancer gene panel testing, one

study demonstrated that 44.5% of African Americans and 50.9% of Asians were found to have a VUS, compared to 23.7% of Caucasians and 23.8% of Hispanics (Kurian et al. 2018). Another study not limited to patients with breast cancer showed that non-Whites are significantly more likely to have a VUS identified on hereditary cancer panel testing than Whites (36% vs. 27%), and that although rate of VUS identification increases for all ethnicities as the number of genes increases, the impact is more substantial for non-Whites (Caswell-Jin et al. 2017). When undergoing hereditary cancer panel testing, the odds of VUS identification is increased in Hispanic, Asian, and African American populations compared to non-Hispanic Whites, and the identification of two or more VUSs is significantly more likely in Hispanic and African American populations (Ricker et al. 2016).

Despite the increasing use of gene panel testing and high frequency of VUS identification, there are limited guidelines on how and when providers should utilize VUSs in counseling and medical management of patients in a cancer genetics setting. While it is generally recommended not to use VUSs to drive medical management decisions, organizations such as the National Comprehensive Cancer Network (NCCN) recognize that there are instances in which providers may consider using a VUS, such as when there are discrepant interpretations of the variant among laboratories. NCCN acknowledges there are not clear protocols in place for when and how to consider the utilization of a VUS, and recommends deferring to a provider with genetics expertise (NCCN Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic Version 1.2020).

Because a significant number of VUSs are reclassified over time (Macklin et al. 2017, Mersch et al. 2018), it is important to counsel patients with VUSs appropriately. Previous research has shown that VUSs in the *BRCA* genes cause patient confusion regarding surgical decisions, risk perception, and cancer distress, and that patients with a VUS find genetic

counseling less informative than those with an uninformative *BRCA*-negative result (Culver et al. 2013). When counseling patients with a VUS, most genetic counselors are confident in their understanding of and ability to communicate the results; however, they feel less confident that their patients comprehend the counselor's explanation of the results (Scherr et al. 2015). Patient misunderstanding was documented in a study of patients who received a VUS in one of the genes associated with Lynch syndrome, in which half of the patients believed their VUS was pathogenic, and the majority of participants were unaware of the possibility of finding a VUS prior to receiving their test results (Solomon et al. 2017). VUSs are also commonly misunderstood by non-genetics providers, a considerable proportion of whom have managed VUS carriers in the same manner as carriers of a pathogenic variant, resulting in unnecessary prophylactic surgeries, patient distress, and familial testing (Brierley et al. 2010, Brierley et al. 2012, Kurian et al. 2017, Richter et al. 2013).

1.1.5 Variant classification and reclassification

The joint 2015 ACMG-AMP statement recommends that clinical laboratories use the standard terminology described in section *1.1.3* above to classify a variant by its degree of clinical significance (Richards et al. 2015). The statement also provides guidelines for determining a variant's classification into one of the five categories, using evidence on a specific variant such as population, computational, functional, and segregation data. The guidelines define the term "likely" to represent variants for which the laboratory has at least 90% certainty of the variant being pathogenic or benign, although many laboratories have higher thresholds. Specific criteria are outlined in the full classification scheme recommended by the ACMG and AMP. Given that pathogenic and likely pathogenic variants are considered to be clinically "actionable" to providers, these stringent guidelines were created in an attempt to reduce the

number of variants misclassified as pathogenic without sufficient evidence, thus limiting the modification of treatment and/or surveillance when it may not be clinically indicated. Many clinical laboratories follow this classification scheme and some have developed their own classification schemes, often based on these widely utilized guidelines. Each laboratory uses their own internal data on a variant as well as publicly available literature and computational algorithms to classify it into one of these categories. In the cancer genetics setting, data from tumor testing may also be integrated into the interpretation of a germline variant (Walsh et al. 2018). The use of standardized sequence variant nomenclature (http://varnomen.hgvs.org/) by the Human Genome Variation Society (HGVS) is recommended for effective communication of variant information across organizations (Dunnen et al. 2016). The ACMG-AMP statement additionally recommends that all clinical molecular genetic testing be performed in a Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory due to the complexity of genetic variant analysis and interpretation.

Prior to the 2015 ACMG-AMP statement, many clinical laboratories had developed their own classification schemes and begun using classification categories similar to those described in the five-tier system, although standardized criteria for classification into each category had not been defined. At that time, available guidelines for variant classification included a statement by the UK Clinical Molecular Genetics Society and the Dutch Society of Clinical Genetics Laboratory Specialists in 2007, which proposed four categories: (1) certainly not pathogenic, (2) unlikely to be pathogenic, (3) likely to be pathogenic, and (4) certainly pathogenic (Bell et al. 2007, Moghadasi et al. 2016). In 2008, a statement by ACMG proposed six categories: (1) sequence variation is previously reported and is a recognized cause of the disorder; (2) sequence variation is previously unreported and is of the type which is expected to cause the disorder; (3) sequence variation is previously unreported and is of the type which may or may not be causative

of the disorder; (4) sequence variation is previously unreported and is probably not causative of disease; (5) sequence variation is previously reported and is a recognized neutral variant; and (6) sequence variation is previously not known or expected to be causative of disease, but is found to be associated with a clinical presentation (Richards et al. 2008).

As genetics laboratories test an increasing number of individuals and collect more data on a particular variant, the classification may change over time based on available evidence. It is rare for variants initially classified as pathogenic, likely pathogenic, benign, or likely benign to be reclassified into a separate clinical category (i.e. pathogenic to benign, or vice versa). VUSs are commonly reclassified over time, although this may take months to years (Macklin et al. 2017, Mersch et al. 2018, Slavin et al. 2018). Rarer variants and those in lower penetrance genes are predicted to take a longer time to correctly classify, as larger sample sizes are needed (Shirts et al. 2014). The majority of VUS reclassifications, often greater than 90%, are downgrades to the benign or likely benign categories (Mersch et al. 2018, Slavin et al. 2019). Consistent with differences in VUS identification rates in individuals of different ancestries, variant reclassification rates have been shown to vary by ancestry. African, Ashkenazi, Chinese, Middle Eastern, and Native American individuals have had an elevated annual rate of *BRCA1/2* variant reclassification when compared to individuals of non-Chinese Asian, Hispanic, and non-Hispanic European ancestries. In cancer risk genes other than *BRCA1* and *BRCA2*, reclassification rates are highest in African, Ashkenazi, and Hispanic individuals; modestly elevated in Middle Eastern and Chinese individuals; and lowest in non-Chinese Asian, Native American, and non-Hispanic European individuals (Slavin et al. 2018).

Reclassification of a variant may occur when clinical laboratories reevaluate the evidence on a particular variant according to their classification scheme. Reevaluation may be prompted by periodic review, identification of the variant in a new individual tested at that laboratory,

variant tracking studies within a family conducted by the laboratory, or at the request of the ordering physician or genetic counselor. The ACMG recommends that clinical laboratories have documented policies and protocols for variant-level reevaluation, respond to external requests in a timely manner, and regularly submit reclassification data to public databases such as ClinVar (discussed in the next section). Variants in which reclassification is most likely to have significant clinical implications should be prioritized for reevaluation (Deignan et al. 2019).

1.1.6 ClinVar

ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) is a publicly available online database of variant interpretations at the National Center for Biotechnology Information (NCBI). It was created in 2013 with the goal of sharing variant evidence and establishing consensus interpretations (Landrum and Kattman 2018). Organizations including clinical laboratories, research laboratories, expert panels, locus-specific databases, and professional societies that provide practice guidelines may submit interpretations of the clinical significance of a variant (Landrum et al. 2015). ClinVar has semi-automatic data flows in place to obtain variant data from GeneReviews, a peer-reviewed online NCBI resource with information on inherited conditions, and Online Mendelian Inheritance in Man (OMIM), an online catalog of human genes and genetic disorders created by Johns Hopkins University (ClinVar, Sources of data in ClinVar). ClinVar is widely utilized by a number of major commercial laboratories and other organizations. In 2018, ClinVar had accrued greater than 600,000 submissions for 430,000 unique variants, from at least 1,000 submitters (Landrum and Kattman 2018).

ClinVar aggregates submissions with respect to the specific variant as well as a particular variant-disease association. All submitted data pertaining to a particular variant is organized under a variation accession number with the prefix VCV ("variant ClinVar"). To ensure proper

identification of a variant and aggregation of submissions pertaining to the same variant, submitters utilize HGVS nomenclature and variants are mapped to specific reference sequences. This information is reviewed and verified by ClinVar staff; however, the interpretation of the clinical significance of a variant is reported directly from the submitter (Landrum et al. 2015). Each submission to ClinVar, which is assigned an accession number with the prefix SCV ("submission to ClinVar"), includes the interpretation of a variant, the condition for which the variant was interpreted, and any supporting evidence for the interpretation. Mode of inheritance as well as germline or somatic origin may be provided, but these are not required (Landrum et al. 2018). All SCVs submitted for a particular variant-disease association pair (i.e. a particular variant AND breast cancer) are aggregated and assigned an accession number with the prefix RCV ("reference ClinVar"). VCV (variant records), SCV (submission records), and RCV (variant-disease association records) accessions are all given version numbers, as SCVs may be updated by submitters and new SCVs are added over time. An aggregate overall interpretation is calculated for VCVs and RCVs on the basis of the submissions provided, and for each type of record (VCV, RCV, SCV), a review status of zero to four stars is given to indicate the weight of the submitted information, which often correlates with the confidence of the interpretation (Landrum and Kattman 2018, Landrum et al. 2016, Landrum et al. 2018). For example, zero stars indicates that no interpretation was provided or no evidence to support the interpretation was provided, three stars indicates that the interpretation is provided by an expert panel, and four stars indicates that the interpretation is accepted as part of a practice guideline. When there are conflicting interpretations among multiple submitters providing assertion criteria, two stars are assigned unless the interpretation is part of a practice guideline or has been reviewed by an expert panel (ClinVar, Review Status in ClinVar). Additionally, each type of record states the

date of the most recent submission and the date that the interpretation of the variant was last evaluated.

ClinVar uses standard terms for representation of clinical significance. When a submitter provides an interpretation of a variant to ClinVar, the clinical significance of the variant may be described using one of the five terms recommended by the 2015 ACMG-AMP guidelines: benign, likely benign, uncertain significance, likely pathogenic, and pathogenic. Several other non-standard terms may be used when the interpretation does not fall into one of the five categories, including "risk factor" to describe a variant that increases risk for but does not cause a disorder. The interpretation term provided by the submitter is available as part of the SCV accession (ClinVar, Representation of clinical significance in ClinVar and other variation resources at NCBI).

An overall interpretation of clinical significance is calculated for each RCV and VCV accession. If all of the submitted interpretations for a particular RCV or VCV record are consistent, then this interpretation will also be the overall interpretation. If there are conflicting interpretations among submitters, the interpretation(s) from the submitter with the highest review status will be used. If there are conflicting interpretation values from submitters with the same review status, the overall interpretation captures the type of conflict. Conflicts between pathogenic and likely pathogenic, or benign and likely benign, will be reported as "Pathogenic/Likely pathogenic" or "Benign/Likely benign," respectively. Any other conflict between standardized ACMG terms, such as a conflict between uncertain significance and pathogenic, will be reported as "Conflicting interpretations of pathogenicity" (ClinVar, Representation of clinical significance in ClinVar and other variation resources at NCBI).

ClinVar maintains a monthly archive of all data through a publicly available FTP site that can be accessed from ClinVar's website. Archives are generated the first Thursday of each

month and saved in XML files aggregated by RCV record. Each RCV record in the archive contains the overall RCV interpretation and review status, as well as the details of each SCV record for that particular RCV record, i.e. the submitter name, the submitter's interpretation value, and any evidence used to support that interpretation that was included in the ClinVar submission (Landrum et al. 2016). In 2017, ClinVar began utilizing an additional archive aggregated by VCV records, while continuing to maintain the RCV-centered archive (Landrum et al. 2017).

1.1.7 Importance of accurate variant classification

Identification of patients at increased risk for hereditary or familial cancer has significant implications on patients' clinical management. In the cancer genetics setting, medical management recommendations given to patients are driven by genetic test results and family history. Medical management recommendations may include type and frequency of cancer screening, discussion of prophylactic surgeries, and/or a recommendation for a cancer genetics evaluation for a patient's family members (Riley et al. 2011). Research has shown that increased screening and prophylactic surgeries in individuals with hereditary cancer syndromes correlate with increased survival (Domchek et al. 2010, Lindor et al. 2006). For some hereditary cancer syndromes, genetic test results may also inform surgical and/or chemotherapeutic/chemopreventive treatment decisions.

NCCN regularly releases up-to-date guidelines for the management of individuals with hereditary and familial cancer (NCCN Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic Version 1.2020, NCCN Genetic/Familial High-Risk Assessment: Colorectal Version 3.2019). Guidelines are currently available for individuals with pathogenic or likely pathogenic variants in high penetrance breast and ovarian cancer susceptibility genes, pancreatic

cancer susceptibility genes, several moderate penetrance breast and other cancer risk genes, and genes associated with Li-Fraumeni syndrome, Cowden syndrome, Lynch syndrome, Peutz-Jeghers syndrome, and several polyposis syndromes. Other professional societies have released guidelines for rare syndromes such as von Hippel Lindau Syndrome. When disclosing "positive" genetic test results (i.e. a pathogenic or likely pathogenic variant is identified), the physician and/or genetic counselor will review management recommendations for that syndrome with the patient as per available guidelines. Additionally, notification of at-risk relatives is recommended, as they may benefit from genetic counseling and testing (Riley et al. 2011).

Patients who receive an uninformative result on genetic testing (i.e. a negative result in the absence of a known familial mutation, or a variant of uncertain significance) or choose not to undergo genetic testing may be provided management recommendations on the basis of their personal and/or family history. Current NCCN guidelines also include screening practices for the prevention of apparently familial breast, pancreatic, and colorectal cancers (NCCN Breast Cancer Screening and Diagnosis Version 1.2019, NCCN Colorectal Cancer Screening 1.2020). For example, there are NCCN guidelines for women whose lifetime risk of breast cancer is predicted to be $\geq$20% based on cancer risk prediction models and for individuals with at least one first-degree relative with colorectal cancer. Individuals who do not meet NCCN criteria for either familial or hereditary cancers are generally recommended to abide by general population cancer screening guidelines. In individuals with uninformative results, genetic testing of other family members is generally not recommended, unless 1) there is a more informative relative to test (i.e. if the patient is unaffected but has an affected relative), or 2) in the case of identification of a VUS, family cosegregation studies may be considered to see if the variant tracks with the affected individuals, as this may provide the laboratory with evidence to contribute to

reclassification of the variant in the future, but this does not impact the family's clinical care unless the variant is reclassified (Garrett et al. 2016, Riley et al. 2011, Zuntini et al. 2018).

Therefore, when a variant is identified on genetic testing, the classification of the variant often has significant implications on the specific recommendations provided to that individual. In a study that evaluated medical management of patients with breast cancer and of unaffected patients, affected patients with a VUS in either *BRCA1* or *BRCA2* made similar surgical choices to average-risk patients. Additionally, unaffected VUS carriers were shown to have rates of prophylactic bilateral mastectomy that were significantly associated with first-degree family history and risk score calculated by the Tyrer-Cuzick/IBIS model for breast cancer risk (Welsh et al. 2017). Since management recommendations often depend on the classification of a variant, they may change over time as variants are reclassified. One study found that 7.8% of patients with a reclassified variant had a change in actionability of that variant, 64% of which were actionable upgrades (benign, likely benign, or VUS to pathogenic or likely pathogenic) and 36% of which were actionable downgrades (pathogenic or likely pathogenic to benign, likely benign, or VUS) (Slavin et al. 2019). Implications of a delayed upgrade or downgrade may have included surgical, treatment, and surveillance decisions, which may have led to missed opportunities to reduce cancer risk in high-risk individuals or unnecessary prophylactic surgeries in average-risk individuals. For the 25 probands in the study who were identified to have one of the variants in which the reclassification resulted in a change in actionability, there were 150 living first-degree relatives that may have also been impacted by the reclassification. When also considering second- and third-degree relatives, the clinical impact of changes in variant classification can be profound.

1.1.8 Conflicting variant interpretations

The 2015 ACMG-AMP guidelines for variant interpretation introduced a standardized classification scheme, which attempted to establish consistency of variant classification among different genetics laboratories. Soon after the implementation of the guidelines, assessment of discrepancies between laboratories' interpretation using their own individual classification criteria (which was used prior to the introduction of the guidelines) and using the ACMP-AMP criteria found a discrepancy rate of 21%, in which 5% of variants had a discrepancy between the two criteria that could potentially impact medical management (Amendola et al. 2016). Although the guidelines standardized some aspects of variant interpretation, discrepant classifications among laboratories remains an issue due to factors such as the subjectivity of determining when criteria are met, some laboratories using their own specific classification scheme, and differences in each laboratory's internal clinical data corresponding to patients tested at that particular laboratory (Amendola et al. 2016, Balmaña et al. 2016, Gradishar et al. 2017, Harrison et al. 2017, Harrison et al. 2018, Pepin et al. 2015).

Studies have reported discordant interpretation rates ranging from 11.7% to 66% (Amendola et al. 2016, Balmaña et al. 2016, Gradishar et al. 2017, Harrison et al. 2017, Harrison et al. 2018). Where in this range the discordance rate falls may vary depending on the year of the study, date the variants were last evaluated, specific genes evaluated (including differences in penetrance and clinical area of the genes), availability of data in public databases such as ClinVar, and types of laboratories and ClinVar submissions included in the study (Harrison et al. 2018, Yang et al. 2017). For example, well-studied high penetrance genes such as *BRCA1* and *BRCA2* have a lower reported frequency of variant interpretation discrepancies (Lincoln et al. 2017). In a study comparing classification of variants in cancer genes excluding *BRCA1/2* between CLIA-certified commercial laboratories and ClinVar submissions, 26% (155 variants)

were found to have conflicting interpretations between laboratories (Balmaña et al. 2016). The genes that were most likely to have conflicting interpretations included *CHEK2, ATM, RAD51C, PALB2, BARD1, NBN*, and *BRIP1*. While most conflicting interpretations involved a discrepancy that ranged from benign or likely benign to VUS (one that would not be considered clinically significant), 11% (56 individuals) had variants with interpretations that ranged from pathogenic or likely pathogenic to VUS. Since VUSs are most often treated as uninformative results, this type of discrepancy has significant clinical implications. However, many of these conflicts were among submissions in ClinVar that were not from clinical laboratories; when research laboratories, research databases, and databases such as Online Mendelian Inheritance in Man (OMIM) are excluded, the proportion of patients with clinically significant discrepant classifications is reduced (Nussbaum et al. 2017).

Multiple efforts have been made to resolve discrepancies in variant classification (Amendola et al. 2016, Harrison et al. 2017, Harrison et al. 2018, Lebo et al. 2018). After the implementation of the ACMG-AMP guidelines, one study found that consensus discussions between laboratories increased the rate of concordant classifications from 34% to 71% (Amendola et al. 2016). Another study showed that 87.2% (211/242) of variant classification discrepancies between four major commercial laboratories could be resolved with collaboration among the laboratories, which involved internal data sharing and reassessment with current criteria (Harrison et al. 2017). Utilizing an outlier approach to contact laboratories with classifications that had a medically significant discrepancy from the majority consensus classification in ClinVar, another study found that 62.3% (127/204) of discrepancies were resolved by this method, 35.4% of which had been previously resolved but were not updated in ClinVar, and the other 64.6% were reassessed and resolved due to prompting by the study (Harrison et al. 2018). The success of these efforts and continued prevalence of discrepant

classifications emphasize the importance of collaboration among laboratories through data sharing avenues such as ClinVar.

According to one study, the vast majority of cancer genetic counselors have encountered variants with conflicting interpretations, and this creates challenges when counseling patients (Zirkelbach et al. 2017). In this study, cancer counselors were surveyed on their practices and concerns regarding discrepant variant classifications. Most cancer counselors (96%) reported that they research variants even when they have no knowledge of a discrepant interpretation, and this most often involves reviewing variant databases. Respondents indicated that most discrepancies were discovered through review of variant databases, and others were discovered when an individual's relative was tested for a known familial variant at a different laboratory or when two unrelated individuals were found to have discrepant classifications of the same variant at different laboratories. The majority of counselors (83%) reported that when there is a discrepant interpretation, their confidence in the interpretation depends on the reporting laboratory. Some stated that they are more likely to trust certain laboratories' classifications—and therefore, use them as the basis for determining medical management recommendations—than other laboratories' classifications. Nearly all counselors who completed the survey (99%) expressed concerns surrounding counseling patients with variant interpretation discrepancies, specifically relating to a lack of data sharing, lack of a centralized database, lack of educational resources, and lack of communication among laboratories. Most counselors desired resources to aid in determining a counseling strategy for patients with these variants, especially support from the laboratories involved with the discrepancy and practice guidelines from a major society or organization. This study further reinforces the prevalence of conflicting variant interpretation discrepancies in a cancer genetics setting and explores the difficulty of handling these discrepancies in clinical practice.

1.2 Purpose of Study and Specific Aims

In cancer genetics practice, different commercial laboratories are frequently used, even within a single clinical practice, which can lead to clinicians receiving discrepant categorization of genetic variants. Discrepancies in variant classification by different genetics laboratories can have significant clinical implications. For example, the same variant may be categorized as likely pathogenic by one laboratory and as a VUS by another laboratory, potentially leading to two different counseling approaches for different patients with the same variant. While the presence of clinically significant conflicting variant interpretations has been established, limited research has been done to assess how variants with conflicting interpretations are utilized by clinicians and how they might impact patient care. Additionally, the prevalence of conflicting interpretations has not been studied in a defined population that underwent the same gene panel, and the prevalence of conflict among major commercial clinical laboratories is not well-studied. This study aims to take a clinical approach to describe the issues surrounding conflicting variant interpretations, including awareness of genetics providers when a variant identified in a patient has conflicting interpretations across major commercial laboratories and the possibility for discrepant medical management recommendations to be provided to patients with the same variant. Through a review of ClinVar archives, abstraction of patient data from a Progeny database, and a retrospective chart review, this study aims to:

1. Quantify the proportion of patients found to have a variant with a clinically significant conflicting interpretation across major commercial laboratories, (a) at the time of the report date, and (b) currently, among a cohort of 2,000 patients undergoing the same cancer gene panel between 2014 and 2017.

2. Assess if there is evidence that the genetics team had suspicion of pathogenicity when counseling the patient, for each patient found to have a VUS that has a clinically significant conflict by another major laboratory.
3. Determine the proportion of patients with a VUS with a clinically significant conflicting interpretation, where the family matches the phenotype associated with pathogenic variants in that gene.
4. Describe and compare the medical management recommendations within an institution among patients who have clinically significant discrepant classifications of the same variant.

I hypothesize that genetics providers are generally not incorporating knowledge of variant interpretation discrepancies in clinical counseling, and that recommendations for cancer screening and genetic testing of family members are more likely to correspond with the classification of the variant on the report even when there are conflicting interpretations by other laboratories. I hypothesize that there will be differences in clinical recommendations given to patients within the same clinical practice with the same variant who are tested at different laboratories and have discrepant classifications of that variant. The overall goals of this study are to raise awareness of conflicting variant interpretations among genetics and non-genetics providers, encourage providers to research patients' variants in ClinVar and other sources of data, and encourage genetics laboratories to participate in data sharing through databases such as ClinVar.

II. METHODS

2.1 IRB Approval

The data used for this study was taken from two research studies at the University of Southern California (USC). Both studies were reviewed and approved by the USC Institutional Review Board, under protocols 0S-13-1 (HS-13-00431) and 0S-12-4 (HS-12-00758). The involvement of personnel in this research who are not on the USC protocols was determined to constitute non-human subjects research by the Institutional Review Board of the University of California, Irvine (UCI). This determination was received on March 4, 2020. Documentation of the determination is available in Appendix A.

2.2 Study Sample

2.2.1 Hereditary Cancer Panel (HCP) Study

Under protocol 0S-13-1 (HS-13-00431), participants were enrolled through the Cancer Genetics clinics at the USC Norris Comprehensive Cancer Center, Los Angeles County + University of Southern California Medical Center (LAC+USC), and the Stanford University Cancer Institute. All patients who met study criteria were invited to enroll in the study during their genetics appointments at each of the three centers. Study criteria included having a personal or family history of cancer that conferred at least a 2.5% risk of identifying a genetic mutation by a validated cancer mutation probability prediction model (such as BRCAPro, Tyrer-Cuzick, BOADICEA, etc.), or met standard testing guidelines such as the National Comprehensive Cancer Network (NCCN) guidelines. Individuals with a known genetic mutation in their family were not eligible to participate unless there was an additional personal or family history of cancer not accounted for by the known mutation. Participants reviewed a study information sheet and provided written informed consent to enroll in the study.

A total of 2,000 participants were recruited between July 2014 and November 2016. All participants had the myRisk multi-gene hereditary cancer panel performed by Myriad Genetic Laboratories (Salt Lake City, Utah). Participants enrolled from the initiation of the study until July 2016 (1,665 participants, 83.3%) underwent a 25-gene panel, which included testing for the genes *APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, MLH1, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, SMAD4, STK11*, and *TP53*. Participants enrolled between July 2016 and the end of the enrollment period (335 participants, 16.8%), after the myRisk panel was updated, underwent a 28-gene panel, composed of the previously mentioned genes in addition to *GREM1, POLD1*, and *POLE*. Full sequencing and deletion/duplication analysis were performed for all genes, except only sequencing was performed for *POLD1* and *POLE*, and only deletion/duplication analysis was performed for *EPCAM* and *GREM1*.

After each participant was enrolled in the study, the genetics provider(s) completed a case report form (Case Report Form I, Appendix B). Information recorded in Case Report Form I included patient demographic information, specific study eligibility criteria met, physical examination information, if available, and the providers' differential diagnosis in order of suspicion. When participants' genetic test results were received, they were disclosed via a phone call or an in-person follow-up appointment. Medical management recommendations for cancer screening and prevention, as well as recommendations for genetic testing of family members, where indicated, were provided to the patients. The genetics providers' interpretation of the test results and recommendations for each patient were recorded in a second case report form (Case Report Form II, Appendix C).

Additional data collected through this study included participants' demographics, medical and family history, genetic test results, and responses to surveys that were administered as part of

the study. All data collected from the study was entered by researchers and stored in a secure Progeny database.

2.2.2 Cancer Genetics Registry

Under protocol 0S-12-4 (HS-12-00758), participants were recruited through the Cancer Genetics clinics at the USC Norris Comprehensive Cancer Center and the Los Angeles County Medical Center (LAC+USC) between April, 2013 and September, 2019. All patients seen in the Cancer Genetics clinics, regardless of probability of a genetic mutation being identified, were invited to enroll in the registry during their appointment. Participants provided written informed consent, which allowed their medical history information, family history information, genetic test results, and medical records to be used for future cancer genetics research. Participant information was stored in a secure Progeny database. As of September, 2019, over 4,500 participants were enrolled, 1,450 of whom were also enrolled in the HCP study. As will be discussed in subsequent section *2.3.5*, the only Cancer Genetics Registry participants included in this analysis were those who had the same variants with clinically significant conflicting interpretations identified in Hereditary Cancer Panel study participants.

2.3 Data Collection

2.3.1 Collection of HCP participant information

Data for all HCP study participants was extracted from the Progeny database. Collected data included demographic information (such as age, gender, race/ethnicity, education level, and all cancer diagnoses), all genetic test results, all dates seen in the Cancer Genetics clinic (including the genetic counselor and/or physician involved for each visit), differential diagnosis

fields in CRFI, and fields in CRFII corresponding to provider interpretation of results and medical management recommendations.

2.3.2 Ascertainment of past conflicting variant interpretations

A list was generated from the Progeny database of all variants identified in individual patients in the HCP study and the variant classifications on the original test reports. On 11/19/2019, data was automatically pulled from ClinVar for each variant, including the overall ClinVar classification and all submissions available in ClinVar that were last reviewed prior to the patient's report date. For variants in which the data was unable to be automatically pulled from ClinVar, the ClinVar records were manually reviewed on 12/23/2019 and 12/24/2019. The data was analyzed in Microsoft Excel to identify variants for which there was a clinically significant discrepancy between the classification on the report and the overall classification in ClinVar. A clinically significant discrepancy was defined as a discrepancy between benign, likely benign, or VUS and likely pathogenic or pathogenic. If the overall ClinVar classification was "conflicting interpretations of pathogenicity," the breakdown of classifications was reviewed to determine whether at least one had a clinically significant discrepancy from the report classification.

For variants with a clinically significant discrepancy (subsequently referred to as a "discrepancy") between the classification on the patient's report and at least one classification currently in ClinVar, ClinVar archives were reviewed to determine if there was discrepancy at the time of the patient's report between the classification of the laboratory the patient was tested at and at least one other major commercial laboratory. One variant with a discrepancy was excluded due to a mosaic inconclusive result that was recorded as a VUS in the Progeny database due to the uncertain significance of the mosaicism, even though several laboratories including

the testing laboratory unanimously interpret the variant as pathogenic. For the other variants with a discrepancy between the report classification and at least one classification currently in ClinVar, the ClinVar archive from the month of the patient's report (the first Thursday of the month) was manually reviewed. Each ClinVar archive XML file was reviewed using Mac terminal commands to identify all RCV records for the particular variant. The RCV record submissions were then evaluated to determine whether there were any clinically significant discrepant interpretations at that time by a major commercial laboratory, which was defined as a commercial laboratory in the United States that provides clinical testing, is CLIA (Clinical Laboratory Improvement Amendments) certified and CAP (College of American Pathologists) accredited, and has at least one thousand submissions to ClinVar. Examples of laboratories that were included in this category are Ambry Genetics, Color, Counsyl, Fulgent Genetics, GeneDx, Invitae, Prevention Genetics, Quest Diagnostics, and the University of Washington Department of Laboratory Medicine, among others. Additionally, submissions from ClinVar-determined expert panels were included. Submissions from research laboratories, GeneReviews, OMIM, and other laboratories were not included in order to focus on conflicts that have the potential to impact clinical care when the variants are identified in patients.

If there was no conflict in ClinVar during the month of the patient's report, ClinVar archives for the subsequent 12 months were reviewed to determine if there were any major laboratories with classifications last reviewed prior to the patient's report date, indicating that the laboratory was reporting a conflicting interpretation at the time of the patient's report but had not yet submitted it to ClinVar. For all variants determined to have clinically significant conflicts at the time of the patient's report, classifications by each laboratory at the time of the report were collected using the information available in ClinVar archives in addition to any classifications available in ClinVar on 11/19/2019 that were last reviewed prior to the patient's report date.

Pedigree charts of all patients with discrepant interpretations were reviewed in the Progeny database to determine the proband's total number of male and female first- and second-degree relatives. Total numbers of male and female living first-degree relatives were also counted. For relatives in which the sex was unspecified, 50% were counted as male and the other 50% as female. There were three families in which multiple relatives were part of this group of study participants. In these families, relatives were only counted for the older family member.

2.3.3 Evaluation for suspicion of VUS pathogenicity

Of the patients with variants that had discrepant interpretations at the time of the patient's report, those in which the variant was classified as a VUS on the report were evaluated to determine if the genetics provider(s) had knowledge of other laboratories' classification of pathogenic or likely pathogenic or suspicion that the VUS could potentially be pathogenic. This was assessed by reviewing fields related to provider interpretation of the report and recommendations for medical management and genetic testing of family members in Case Report Form II. Specific fields evaluated included "Current Molecular Diagnosis," "Current Clinical Diagnosis," medical management recommendations for cancer screening and prevention that pertain to the cancers associated with pathogenic variants in that gene, and whether genetic testing was recommended for any family members. If genetic testing was recommended for family members, the specific family members and recommended test were reviewed. If targeted variant testing was recommended, CRFII noted whether this was for identification of a deleterious variant or for VUS tracking studies. For patients in which CRFII had not been completed, clinical documentation of the phone call or in-person appointment in which results were disclosed was reviewed to extract the corresponding information. There were three patients for which CRFII was not completed and the clinic note from the results disclosure was

unavailable; these patients were excluded from this analysis. Provider interpretations and recommendations were interpreted in the context of patient and family history. For each discrepant variant, general and/or gene-specific criteria (shown in Table 1) were used to determine if there was evidence of provider suspicion of pathogenicity. Of note, only six genes were relevant for this analysis so only these genes are listed in Table 1.

Table 1. **Criteria used for ascertaining provider suspicion of pathogenicity**

	Evidence of Suspicion of Pathogenicity	No Evidence of Suspicion of Pathogenicity
General	• Mention of other labs' classifications as pathogenic or likely pathogenic • Molecular diagnosis mentions that variant is possibly pathogenic or likely pathogenic • Option for "genetic testing recommended for deleterious mutation" marked (if no additional known deleterious mutation is identified) • Molecular diagnosis describes variant as "suspicious" • Clinical or molecular diagnosis mentions possible influence of variant	• Molecular diagnosis of "[gene name] VUS" without evidence of suspicion • No additional screening beyond general population guidelines recommended for associated cancers • No genetic testing recommended for family members • Molecular diagnosis of "[gene name] mutation" when a known pathogenic variant was also identified, without mention of other variant • When a known pathogenic variant was also identified, genetic testing recommended only for known pathogenic variant
CDKN2A	• Pancreatic cancer screening recommended without personal or family history of pancreatic cancer • Dermatology exam recommended without personal or family history of skin cancer	• Clinical diagnosis of "sporadic melanoma" or "sporadic pancreatic cancer" • Dermatology exam and VUS tracking studies recommended in context of family history of melanoma, without evidence of suspicion • Dermatology exam recommended in context of personal history of melanoma, without evidence of suspicion
CHEK2	• Molecular diagnosis describes variant as "moderate risk" • Earlier or more frequent colonoscopies compared to general population in absence of personal or family history of colon cancer	• General population colonoscopy guidelines recommended • Clinical diagnosis of "familial breast cancer" without evidence of suspicion

		• Clinical diagnosis of "sporadic colon cancer" without evidence of suspicion
MLH1	• Earlier or more frequent colonoscopies compared to general population in absence of personal or family history of colon cancer	• Clinical diagnosis of "unexplained pancreatic cancer" • General population colonoscopy guidelines recommended
MUTYH	• Earlier or more frequent colonoscopies compared to general population in absence of personal or family history of colon cancer	• General population colonoscopy guidelines recommended
RAD51C	• Ovarian cancer screening and/or oophorectomy discussed or recommended	• Ovarian cancer screening and/or oophorectomy not discussed or recommended
TP53	• Whole body MRI recommended without a clinical diagnosis of LFS	• Clinical diagnosis of "sporadic breast cancer"

2.3.4 Determination of family phenotype

All patients in which the variant with discrepant interpretations had a report classification of VUS were also evaluated to determine whether each patient's personal and family history was consistent with the phenotype associated with pathogenic variants in that gene. This was accomplished by reviewing family history, pre-test differential diagnosis, and when possible, pre-test probability of a mutation in that gene or whether clinical diagnostic criteria for that syndrome was met.

Pedigree charts were reviewed to determine whether at least one individual in the family had a phenotype (history of cancer or polyps) that is associated with pathogenic variants in the particular gene. Associated cancers and phenotypes included:

- *CDKN2A*: melanoma, pancreas
- *CHEK2*: breast, colorectal
- *MLH1*: colorectal, endometrial, gastric, ovarian, pancreas, small bowel, ureter and renal pelvis, brain, biliary tract, small intestine
- *MUTYH*: colorectal cancer, colorectal or small bowel polyps

- *RAD51C*: ovarian
- *TP53*: adrenocortical carcinoma, brain, breast, gastrointestinal, genitourinary, leukemia, lymphoma, sarcoma

Pre-test differential diagnosis was determined by reviewing data from CRFI. Each patient's CRFI was reviewed to determine whether the gene with the variant of interest was considered on the genetics provider(s)' differential diagnosis, and if so, the specific ranking.

Patients were separated by whether the variant of interest was in a high penetrance gene (genes included were *CDKN2A, MLH1, MSH2,* and *TP53*) or a moderate penetrance gene (genes included were *CHEK2*, monoallelic *MUTYH*, and *RAD51C*). The patients with variants in high penetrance genes were assessed to determine pre-test probability of a mutation in that gene using risk model statistical software, or whether clinical criteria were met for the syndrome. For patients with a variant of interest in *CDKN2A*, the MelaPRO model (BayesMendel Lab, Harvard University) was used to calculate the probability of identifying a pathogenic variant in *CDKN2A* based on the patient's personal and family history. For patients with variants in *MLH1* or *MSH2*, the MMRpro model (BayesMendel Lab, Harvard University) and the PREMM1,2,6 model (Kastrinos et al. 2010; PREMM, Dana-Farber Cancer Institute) were used to estimate cumulative risk of identifying a mutation in *MLH1, MSH2, or MSH6*. For the patients with variants in *TP53*, pedigree charts were evaluated to determine whether the patient's personal and family history met classic Li-Fraumeni syndrome criteria, which is used as clinical diagnostic criteria (Li et al. 1988, Mai et al. 2012), and the most recent version of the Chompret criteria, which is used to identify families that are suspicious for Li-Fraumeni syndrome and should be offered *TP53* genetic testing (Bougeard et al. 2015).

2.3.5 Identification and analysis of discrepant classifications of the same variant

All unique variants identified to have discrepant interpretations at the time of an HCP participant's original report were queried in the Cancer Genetics Registry database to determine whether there were any patients who had been tested at other laboratories and received a discrepant result. For example, if a patient in the HCP study had a variant that was reported as a VUS, the Cancer Genetics Registry was searched to identify patients who were found to have that same variant, but classified as pathogenic or likely pathogenic by their testing lab. Similarly, if a patient in the HCP study had a variant that was reported as pathogenic or likely pathogenic, the Registry was searched to identify individuals with that variant classified as a VUS. This query was performed in January 2020. Four variants in *CDKN2A*, *CHEK2*, and *MUTYH* were identified for which there were patients with an original VUS classification and patients with an original pathogenic or likely pathogenic classification of that same variant.

All patients from the HCP and Cancer Genetics Registry studies with these four variants who were seen either at the USC Norris Comprehensive Cancer Center or the Los Angeles County + University of Southern California Medical Center were combined into a single dataset that included 57 patients with four variants. For HCP participants, recommendations provided in CRFII for medical management, cancer surveillance, and genetic testing of family members were assessed. For individuals not in the HCP study or for which CRFII was unavailable, genetics clinic notes documenting the results disclosure were reviewed and the corresponding information was extracted. There were five patients for which the genetics clinic note was unavailable.

Examples of cases with the same discrepant variant were used to evaluate for different counseling strategies utilized by the same genetic counselor. For three of the four variants, there were patients with discrepant classifications that were seen by the same counselor. Three unique counselors were involved in counseling patients with discrepant classifications of the same

variant. Case examples of discrepant classifications seen closest together in time were selected to be compared. For the fourth variant, there was only one patient with a VUS classification and one with a pathogenic classification, seen by two different counselors, so the counseling strategy for these two patients was evaluated.

For the selected case examples, pedigree charts for each patient were reviewed and pertinent personal and family history was recorded. A family history score was calculated to describe the proportion of the proband's first- and second-degree relatives who fit the phenotype associated with pathogenic variants in the specific gene. For *CDKN2A*, individuals with melanoma or pancreatic cancer were considered to fit the phenotype. For *CHEK2*, individuals with breast or colon cancer were included. Individuals with colorectal cancer or colorectal polyps were included for *MUTYH*. Number of first- and second-degree relatives with associated phenotypes was divided by the total number of first- and second-degree relatives. Of note, this was not limited to adults, and therefore included individuals who had not yet lived through their years of cancer risk.

2.3.6 Ascertainment of present conflicting variant interpretations

A list was generated from the Progeny database of all variants identified through the Hereditary Cancer Panel study and their current classifications by the reporting laboratory as of 3/24/2020. The data was sorted in Microsoft Excel to identify variants for which there was a discrepancy between the current laboratory classification and the overall classification in ClinVar (which was extracted on either 11/19/2019, 12/23/2019, or 12/24/2019). A discrepancy was again defined as a discrepancy between either benign, likely benign, or VUS and either likely pathogenic or pathogenic. If the overall ClinVar classification was "conflicting interpretations of pathogenicity," the breakdown of classifications was reviewed to determine

whether at least one had a discrepancy from the reporting laboratory classification. The variants with clinically significant conflicts were manually reviewed in ClinVar on 4/19/2020 to determine if the conflict involved a major commercial laboratory, which was defined as described in section *2.3.2*.

2.4 Data Analysis

Data were analyzed using IBM SPSS Statistics software version 26. Descriptive statistics, chi-square analyses, and Fisher's exact tests were performed. A p-value of less than 0.05 was considered statistically significant. In all chi-square analyses, d.f. refers to degrees of freedom. All Fisher's exact tests were two-sided.

Qualitative analysis was utilized to determine provider suspicion of pathogenicity for HCP participants with a variant classified as a VUS on their report that was reported as pathogenic or likely pathogenic by another laboratory at the same time. Criteria are outlined in Table 1 and were created based on standard genetic counseling practices, well-established cancer risks associated with specific genes, and generally recommended screening practices depending on family history and genetic test results.

Case examples illustrating counseling strategy for patients with discrepant classifications of the same variant were compared. Personal and family history, recommendations for cancer surveillance, and recommendations for genetic testing of family members were described and interpreted to assess whether the provider's counseling was driven by the variant classification and/or the personal and family history.

III. RESULTS

3.1 Demographic Characteristics of the Study Population

A total of 2,000 participants were recruited between July 2014 and November 2016. The majority of participants (80.7%) were female. The most frequently reported race/ethnicities were non-Hispanic White (40.6%), Hispanic (39.0%), and Asian (11.7%); 6.8% of participants had known Ashkenazi Jewish ancestry. The majority of participants (72.1%) were affected with at least one primary cancer excluding non-melanoma skin cancer, and 8.3% had been diagnosed with multiple primaries. Approximately half of participants (51.2%) had a negative result, meaning that no pathogenic/likely pathogenic variants or VUSs were reported by the laboratory. Of the participants, 12.2% had a positive result, in which at least one pathogenic or likely pathogenic variant was identified. The remaining 36.6% had an overall result of VUS, meaning that at least one VUS was identified, and no pathogenic or likely pathogenic variants were identified. In 40.5% of participants, at least one VUS was identified (Table 2).

Table 2. **Characteristics of 2,000 study participants**

		N	%
Gender	Female	1614	80.7%
	Male	386	19.3%
Age	<30	100	5.0%
	30-39	283	14.2%
	40-49	533	26.7%
	50-59	503	25.2%
	60-69	411	20.5%
	70-79	139	7.0%
	>79	31	1.6%
Race/Ethnicity	American Indian/Alaska Native	5	0.3%
	Asian	234	11.7%
	Black or African American	75	3.8%
	Hispanic	779	39.0%
	Native Hawaiian/Pacific Islander	5	0.3%

	Non-Hispanic White	811	40.6%
	Unknown/More than one	91	4.6%
Ashkenazi Jewish Ancestry	Yes	136	6.8%
	No	1759	87.9%
	Unknown/Unsure	105	5.3%
Education Level	Elementary school	223	11.2%
	High school	382	19.1%
	Trade/Vocational School	80	4.0%
	Some college	268	13.4%
	Junior college	89	4.5%
	College degree	437	21.9%
	Graduate degree	361	18.1%
	Unknown	160	8.0%
Cancer Status	None	558	27.9%
	Breast	611	30.6%
	Colon/Rectum	245	12.3%
	Gastric	41	2.1%
	Ovary	98	4.9%
	Pancreas	32	1.6%
	Prostate	22	1.1%
	Uterus	51	2.6%
	Other	176	8.8%
	Multiple primary types	166	8.3%
Original Overall Result	Positive	243	12.2%
	VUS	732	36.6%
	Negative	1025	51.2%
Any VUS Identified	Yes	810	40.5%
	No	1190	59.5%

Cancer Status reflects whether an individual has been diagnosed with cancer. Breast, Colon/Rectum, Gastric, Ovary, Pancreas, Prostate, Uterus, and Other include individuals with only one primary cancer type excluding non-melanoma skin cancer. Individuals with multiple primary types are only reflected in Multiple primary types.

3.2 Prevalence of Clinically Significant Discrepancies

Among the 2,000 participants, 975 had at least one variant identified, which was classified as an original overall result of positive (at least one pathogenic or likely pathogenic variant) or VUS (no pathogenic or likely pathogenic variants). There were a total of 1,326

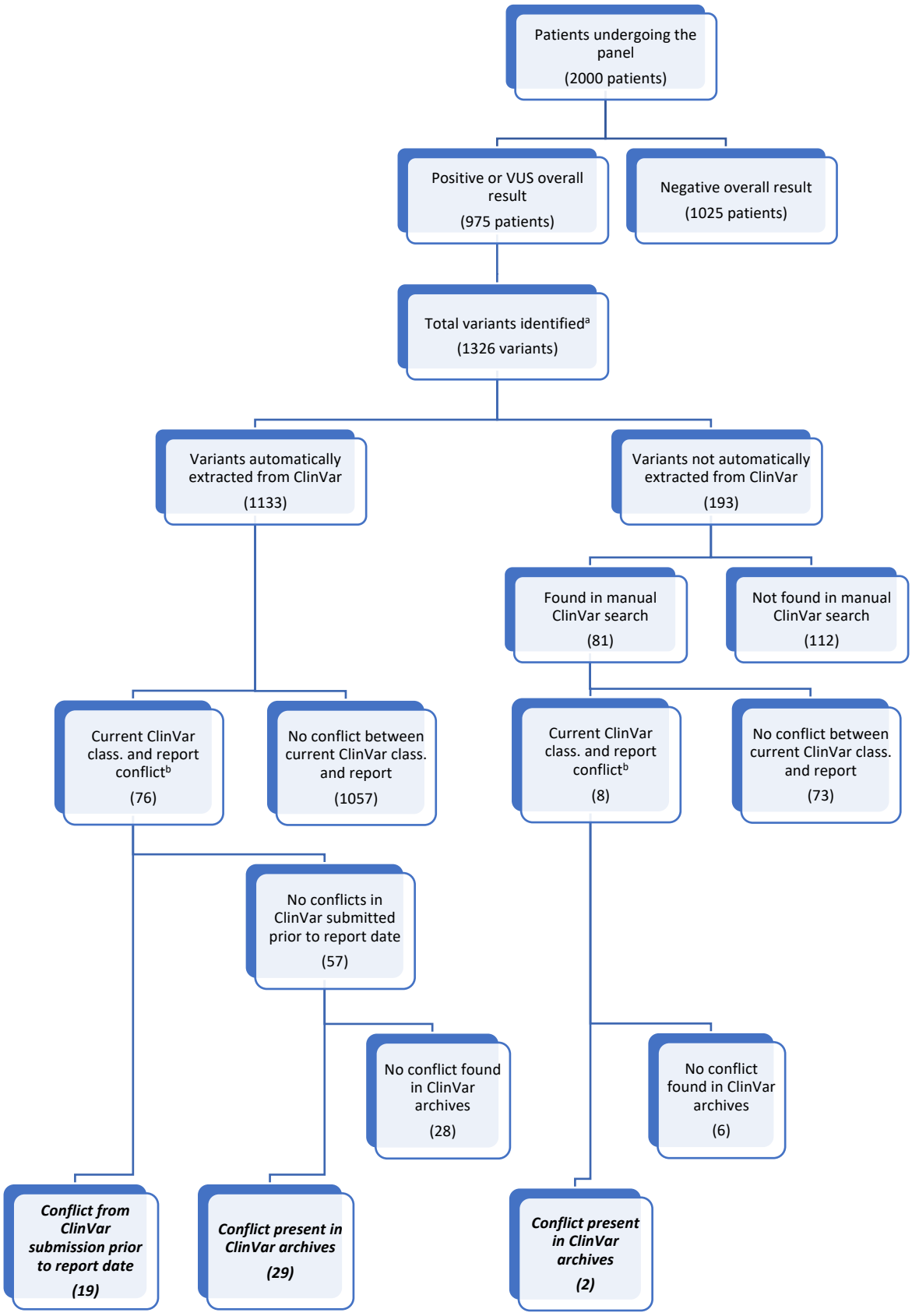
variants reported, which included 943 unique variants (i.e. if a variant was seen in multiple patients, it was included in the list once for each patient in which it was seen). A flowchart describing how the variants were evaluated is displayed in Figure 1.

Of the 1,326 variants, data were automatically extracted from ClinVar for 1,133 of them. There were 76 variants (6.7%) that were found to have a clinically significant discrepancy between the current ClinVar classification and the classification by the testing laboratory on the patient's original report. A clinically significant discrepancy was described as either a discrepancy between pathogenic/likely pathogenic and VUS/likely benign/benign, or an overall ClinVar classification of "conflicting interpretations of pathogenicity," in which at least one submission had a clinically significant discrepancy from the report classification (between pathogenic/likely pathogenic and VUS/likely benign/benign).

The variants were then assessed for clinically significant conflicts among major commercial laboratories at the time of the patient's original report. Prior to reviewing ClinVar archives, there were 19 variants in which current ClinVar data reflected a clinically significant conflict at the time of a patient's report because the most recent classification by a laboratory was last reviewed prior to the patient's report date. The remaining 57 variants were reviewed in ClinVar archives to determine whether there was a conflict present.

Figure 1. **Variant evaluation flowchart.** A total of 1,326 variant reports were identified in the study population. Variant reports were evaluated individually to determine whether there was a clinically significant conflict among major commercial laboratories at the time of the specific patient's report. Final conflicts are shown in bold and italics in the final row and represent conflicts among major laboratories as outlined by criteria in the Methods.

a. There were 943 total unique variants identified. Variants that were identified in multiple patients were counted each time they were identified, for a total of 1,326 variant reports. All of the following boxes represent number of variants, rather than patients.
b. A conflict represents a clinically significant conflict, which is defined as a discrepancy between pathogenic/likely pathogenic and VUS/likely benign/benign. Variants with an overall ClinVar classification of "conflicting interpretations of pathogenicity" are included if at least one of the submissions has a clinically significant conflict with the report classification.


Patients undergoing the panel
(2000 patients)
Positive or VUS overall result
(975 patients)
Negative overall result
(1025 patients)
Total variants identified[a]
(1326 variants)
Variants automatically extracted from ClinVar
(1133)
Variants not automatically extracted from ClinVar
(193)
Found in manual ClinVar search
(81)
Not found in manual ClinVar search
(112)
Current ClinVar class. and report conflict[b]
(76)
No conflict between current ClinVar class. and report
(1057)
Current ClinVar class. and report conflict[b]
(8)
No conflict between current ClinVar class. and report
(73)
No conflicts in ClinVar submitted prior to report date
(57)
No conflict found in ClinVar archives
(28)
No conflict found in ClinVar archives
(6)
Conflict from ClinVar submission prior to report date
(19)
Conflict present in ClinVar archives
(29)
Conflict present in ClinVar archives
(2)

Of the variants that were unable to be automatically extracted from ClinVar, 81 were found through a manual ClinVar search. There were 8 that had a clinically significant conflict using the current ClinVar classification, and 2 had the conflict confirmed in the ClinVar archives.

A total of 50/1,326 (3.8%) variant reports were found to have a clinically significant conflict at the time of the patient's original genetic test report. This represented 50/2,000 (2.5%) of the patients who underwent the panel, and 50/975 (5.1%) of the patients who had at least one variant identified on the panel.

There were 14 unique variants in which a conflict was identified. *CHEK2* was the most frequently identified gene with a conflicting variant (present in 17 patients) and had the greatest number of unique variants with conflicts (5). The other genes with conflicting variants were *APC* (with a conflict in the well-studied p.I1307K variant), *CDKN2A*, *MLH1*, *MSH2*, *MUTYH*, *RAD51C*, and *TP53*. The majority of genes only had one unique conflicting variant, except for *CHEK2*, as previously discussed, as well as *MUTYH* and *TP53*, each of which had two variants with conflicts (Figure 2).

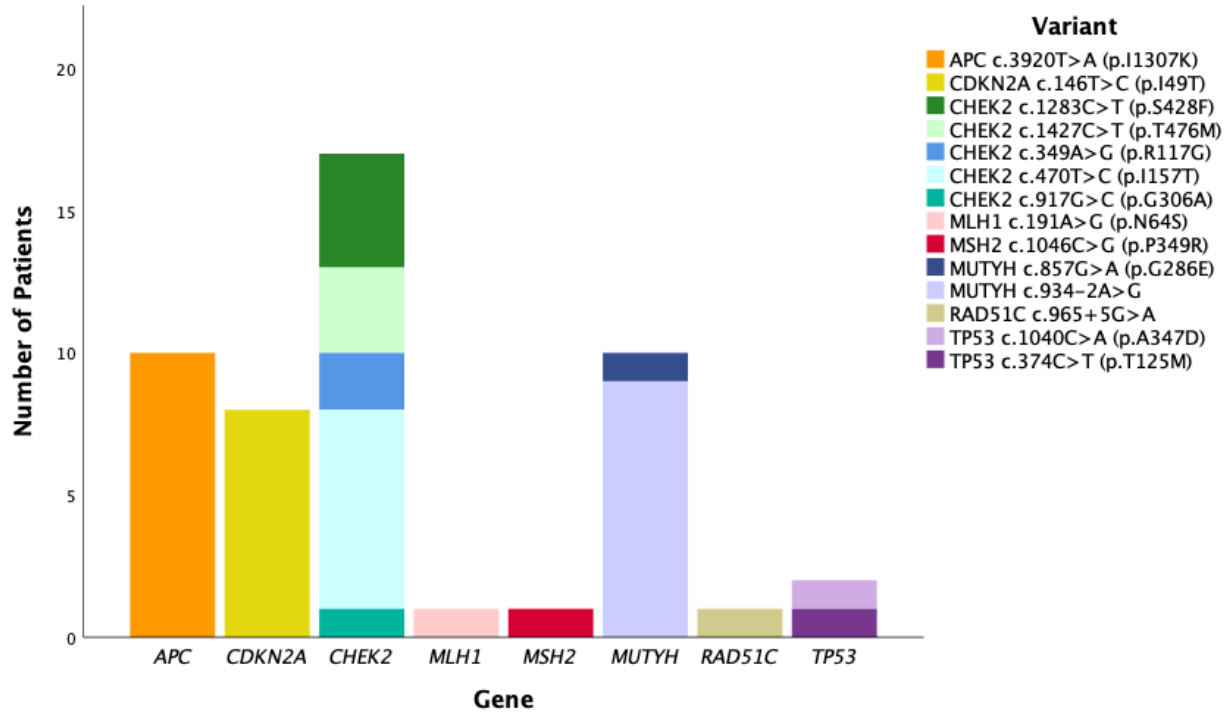


Figure 2. **Distribution of variants with clinically significant conflicts.** The 50 patients with variants that had clinically significant discrepant classifications are distributed by unique variant and aggregated by gene. Variants are noted using HGVS nomenclature. The order of variants in the key corresponds to the vertical order of variants in the graph.

Of the 50 patients with these conflicting variants, 19 individuals (38%) had a pathogenic or likely pathogenic classification, and 31 individuals (62%) had a VUS classification. Among these individuals, 72% were female and 50% were in their 50s or 60s. Half of the individuals (50%) were Non-Hispanic White, 26% were Hispanic, and 22% were Asian (Table 3). Of note, the proportion of the cohort with a discrepancy by race/ethnicity was 4.7% (11/234) for Asians, 1.7% (13/779) for Hispanics, and 3.1% (25/811) for Non-Hispanic Whites. In individuals with Ashkenazi Jewish ancestry, 9.6% (13/136) had a variant with a discrepancy. Nine of these 13 discrepancies were the *APC* c.3920T>A (p.I1307K) variant.

Table 3. **Characteristics of 50 patients with variants that have clinically significant discrepant classifications**

		N	%
Race/Ethnicity	Asian	11	22.0%
	Hispanic	13	26.0%
	Non-Hispanic, White	25	50.0%
	Unknown/More than one	1	2.0%
Ashkenazi Jewish Ancestry	Yes	13	26.0%
	Unsure	2	4.0%
	No	35	70.0%
Cancer Status	None	15	30.0%
	Breast	9	18.0%
	Colon/Rectum	9	18.0%
	Gastric	1	2.0%
	Ovary	4	8.0%
	Pancreas	1	2.0%
	Prostate	2	4.0%
	Uterus	2	4.0%
	Other	6	12.0%
	Multiple primary types	1	2.0%
Original Overall Result	Positive	23	46.0%
	VUS	27	54.0%
Lab Report Classification of Variant with Conflict	P/LP	19	38.0%
	VUS	31	62.0%

P/LP refers to a pathogenic or likely pathogenic classification.

Through a review of ClinVar archives and data currently available in ClinVar, classifications were captured for each individual's variant by each major laboratory and expert panel during the month that the patient's original genetic test report was issued (Table 4). Of note, Lab 3 reclassified the *APC* c.3920T>A variant from likely benign to risk factor between 11/2014 and 12/2015. For *CDKN2A* c.146T>C, Lab 1 was the only major commercial laboratory that classified the variant as likely pathogenic, while the other three classified it as VUS or likely benign. Lab 3 reclassified this variant from VUS to likely benign between 8/2015 and 11/2015.

For *CHEK2* c.1283C>T, all five major commercial laboratories with submissions in ClinVar classified the variant as pathogenic, while the reporting laboratory classified it as VUS.

Table 4. **Distribution of laboratory classifications of variants with conflicts**

Variant	Date	Count	Testing Lab	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Expert Panel
APC **c.3920T>A**	11/2014	1	LPV	P	RF	LB						
	12/2015	3	LPV	P	RF	RF	RF	VUS				
	3/2016	1	LPV	P	RF	RF	RF	VUS	VUS			
	4/2016	1	LPV	P	RF	RF	RF	VUS	VUS			
	6/2016	1	LPV	P	RF	RF	RF	VUS	VUS			
	8/2016	1	LPV	P	RF	RF	RF	VUS	VUS			
	11/2016	2	LPV	P	RF	RF	RF	VUS	VUS		LP	
CDKN2A **c.146T>C**	11/2014	1	VUS	LP	VUS	VUS						
	7/2015	1	VUS	LP	VUS	VUS						
	8/2015	1	VUS	LP	VUS	VUS						
	11/2015	2	VUS	LP	VUS	LB						
	1/2016	1	VUS	LP	VUS	LB						
	3/2016	1	VUS	LP	VUS	LB						
	4/2016	1	VUS	LP	VUS	LB						
CHEK2 **c.1283C>T**	12/2014	1	VUS	P	P							
	1/2015	1	VUS	P	P							
	10/2016	1	VUS	P	P	P	P			P		
	11/2016	1	VUS	P	P	P	P			P		
CHEK2 **c.1427C>T**	5/2015	1	VUS	LP	P							
	3/2016	2	VUS	LP	P	VUS	VUS					
CHEK2 **c.349A>G**	8/2014	1	VUS	LP	LP							
	9/2015	1	VUS	LP	LP							
CHEK2 **c.470T>C**	9/2014	1	VUS	P	LP							
	3/2015	1	VUS	P	LP					P		
	5/2015	1	VUS	P	LP					P		
	7/2015	1	VUS	P	LP					P		
	3/2016	1	VUS	P	LP	P	LP			P	LP	
	8/2016	1	VUS	P	LP	P	LP	VUS		P	LP	
	11/2016	1	VUS	P	LP	P	LP	VUS	LP	P	LP	
CHEK2 **c.917G>C**	11/2015	1	VUS	VUS	LP							
MLH1 **c.191A>G**	7/2016	1	VUS	LP	VUS	VUS						LP
MSH2 **c.1046C>G**	1/2015	1	VUS									LP
MUTYH **c.857G>A**	12/2014	1	VUS		P							

MUTYH							
c.934-2A>G	5/2015	2	LP	VUS	P	LP	
	6/2015	1	LP	VUS	P	LP	
	7/2015	2	LP	VUS	P	LP	
	8/2015	1	LP	VUS	P	LP	
	10/2015	1	LP	VUS	P	LP	
	6/2016	1	LP	VUS		LP	
	11/2016	1	LP	VUS	P	LP	
RAD51C							
c.965+5G>A	9/2016	1	VUS	LP		VUS	
TP53							
c.1040C>A	9/2015	1	VUS	LP			
TP53							
c.374C>T	2/2016	1	VUS	LP			LP

Count equals the number of individuals found to have the variant in which their report was issued in the particular month. Total count equals 50. Testing Lab is the laboratory at which the patient was tested. Labs 1 through 8 are other major commercial laboratories that had classifications submitted to ClinVar reflecting the classification at the time of the patient's report, and Expert Panel is a ClinVar-defined expert panel that had a classification submitted to ClinVar reflecting the classification at the time of the patient's report. P refers to a pathogenic/deleterious classification. LP refers to a likely pathogenic/suspected deleterious classification. LB refers to a likely benign/favor polymorphism classification. RF refers to a classification of "risk factor," and LPV refers to a classification of "low penetrance variant." Both terms are used to describe low penetrance pathogenic variants.

The proportion of patients with variants with clinically significant discrepancies was analyzed to determine whether it differed by year. The proportion of patients with at least one variant identified on the panel (N=975) that had a discrepancy was 4.5% in 2014, 4.9% in 2015, and 5.7% in 2016. The year 2017 was not included in this analysis because there were only three reports issued. There was no statistically significant difference found in the proportion of patients with a conflict based on report year (χ^2 (2 d.f.)=0.364, p=0.833).

The proportion of variant reports (N=1,326) with a discrepancy was analyzed to determine whether there was a difference based on the classification of the variant (Figure 3). Variants reported by the testing laboratory as pathogenic or likely pathogenic were significantly more likely to have a conflict than variants reported as VUS (χ^2 (1 d.f.)=12.725, p<0.001). However, the pathogenic/likely pathogenic variant reports with a conflict represented only two unique variants, *APC* c.3920T>A, common in Ashkenazi Jewish individuals, and *MUTYH* c.934-

2A>G, common in Asian individuals. Reports with a VUS classification that had a conflict in ClinVar represented 12 unique variants.

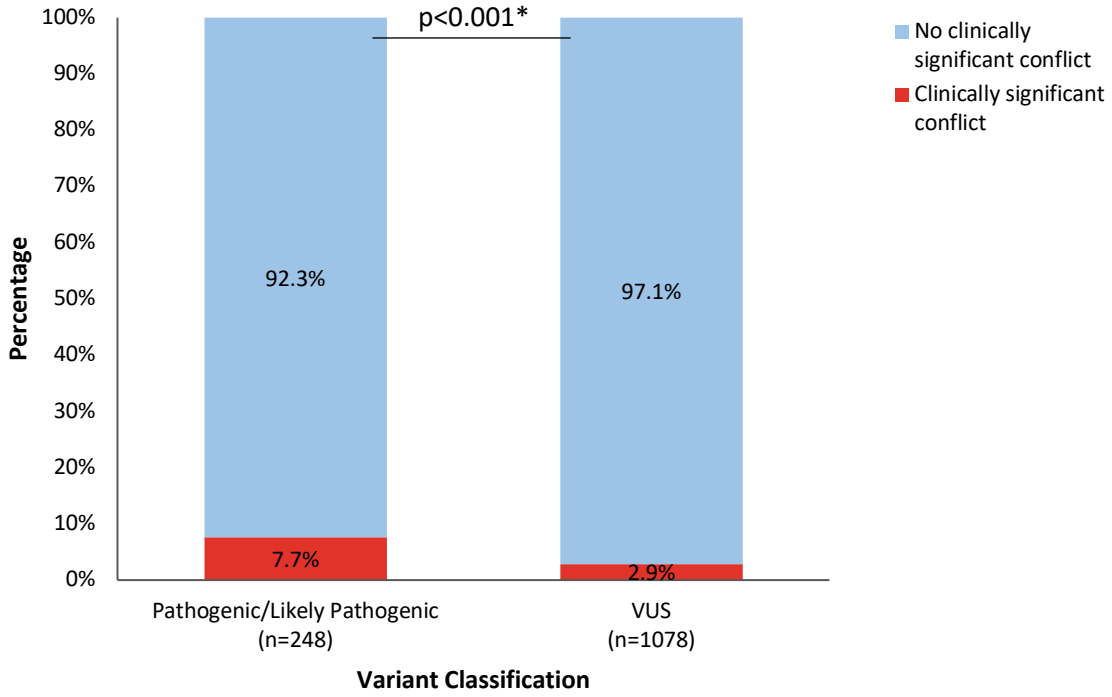


Figure 3. **Distribution of conflicting interpretations of variants by report classification.** N=1,326 total variant reports. Percentage reflects the total percentage of variant reports with and without a clinically significant conflict, separated by report classification of pathogenic/likely pathogenic or VUS. Variants categorized as pathogenic/likely pathogenic were more likely to have a conflict in ClinVar than variants categorized as VUS (χ^2 (1 d.f.)=12.725, p<0.001).

For each patient with a conflicting variant, the total number of male and female first- and second-degree relatives was counted to assess the broader impact of the conflicting variants on the family members of the individual who was tested (Table 5). Among 50 patients with conflicting variants, there were 291 first-degree relatives (215 living), 790 second-degree relatives, and 1081 total first- and second-degree relatives.

Table 5. **Summary of relatives potentially affected by discrepant classification**

		Mean	95% CI	Minimum	Maximum	Sum
First-degree relatives	Female	3.0	2.5 – 3.5	1	8	140
	Male	3.2	2.7 – 3.8	1	10	151
	Total	6.2	5.4 – 7.0	2	14	291
Second-degree relatives	Female	8.0	6.4 – 9.5	2	28	373
	Male	8.9	7.2 – 10.5	0	26	417
	Total	16.8	13.7 – 19.9	3	54	790
First- and second-degree relatives	Total	23.0	19.4 – 26.6	7	62	1081

The 50 patients with conflicting variants were from 47 unique families. When there were multiple individuals from the same family, only the older relative was reported.

3.3 Provider Suspicion of VUS Pathogenicity

Of the 50 total patients with a conflicting variant, 31 (62%) had a variant classified as VUS by the testing laboratory. These patients were seen by eight unique genetic counselors and five unique physicians. All patients were seen by a genetic counselor, and some were seen by a physician as well. For 28 of the patients, there was a CRFII or original results disclosure clinic note available to review. When reviewed, 64% (18/28) had no evidence of provider suspicion of pathogenicity. Proportion of patients in which there was provider suspicion varied by specific variant (Table 6, Figure 4). For example, for all six patients with *CDKN2A* c.146T>C, there was no evidence of provider suspicion. For *CHEK2* c.1283C>T, providers for all four patients were aware of the conflict. For the high penetrance genes (*CDKN2A*, *MLH1*, and *TP53*), only one out of nine had evidence that counseling was provided with knowledge of the discrepancy.

Table 6. **Suspicion of pathogenicity in patients with variants classified as VUS**

		Suspicion of pathogenicity		
		No N (%)	Yes N (%)	Total (N)
Variant	*CDKN2A* c.146T>C	6 (100%)	0 (0%)	6
	CHEK2 c.1283C>T	0 (0%)	4 (100%)	4
	CHEK2 c.1427C>T	2 (67%)	1 (33%)	3
	CHEK2 c.349A>G	1 (50%)	1 (50%)	2
	CHEK2 c.470T>C	4 (57%)	3 (43%)	7
	CHEK2 c.917G>C	1 (100%)	0 (0%)	1
	MLH1 c.191A>G	1 (100%)	0 (0%)	1
	MUTYH c.857G>A	1 (100%)	0 (0%)	1
	RAD51C c.965+5G>A	1 (100%)	0 (0%)	1
	TP53 c.1040C>A	0 (0%)	1 (100%)	1
	TP53 c.374C>T	1 (100%)	0 (0%)	1
Total		18 (64%)	10 (36%)	28

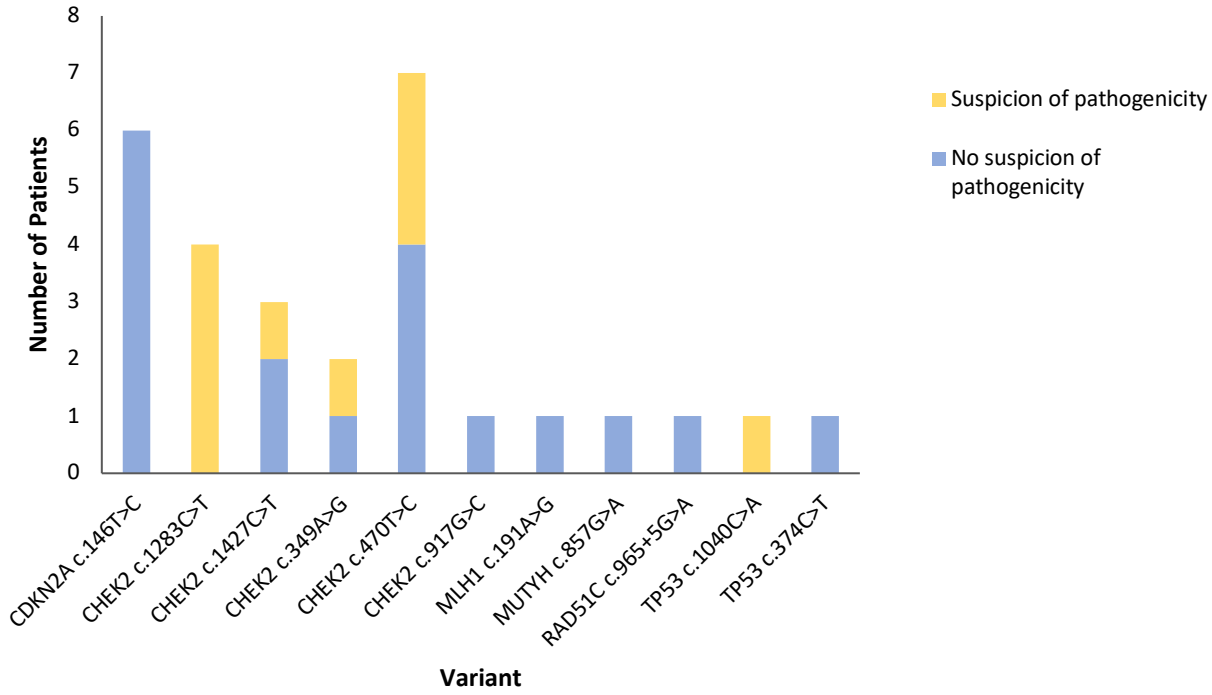


Figure 4. **Suspicion of pathogenicity in patients with variants classified as VUS.** N=28. The number and proportion of patients with each variant for which there was and was not evidence of provider suspicion of pathogenicity is shown.

Suspicion of pathogenicity was also evaluated by year to determine if providers may have been more or less likely to be aware of a conflict depending on the year each patient's original test report was issued (Figure 5). The year 2015 had the lowest proportion of patients (27.3%, 3/11) counseled with provider suspicion of pathogenicity, but there was no statistically significant difference found in proportion with suspicion of pathogenicity depending on report year (Fisher's exact p=0.876).

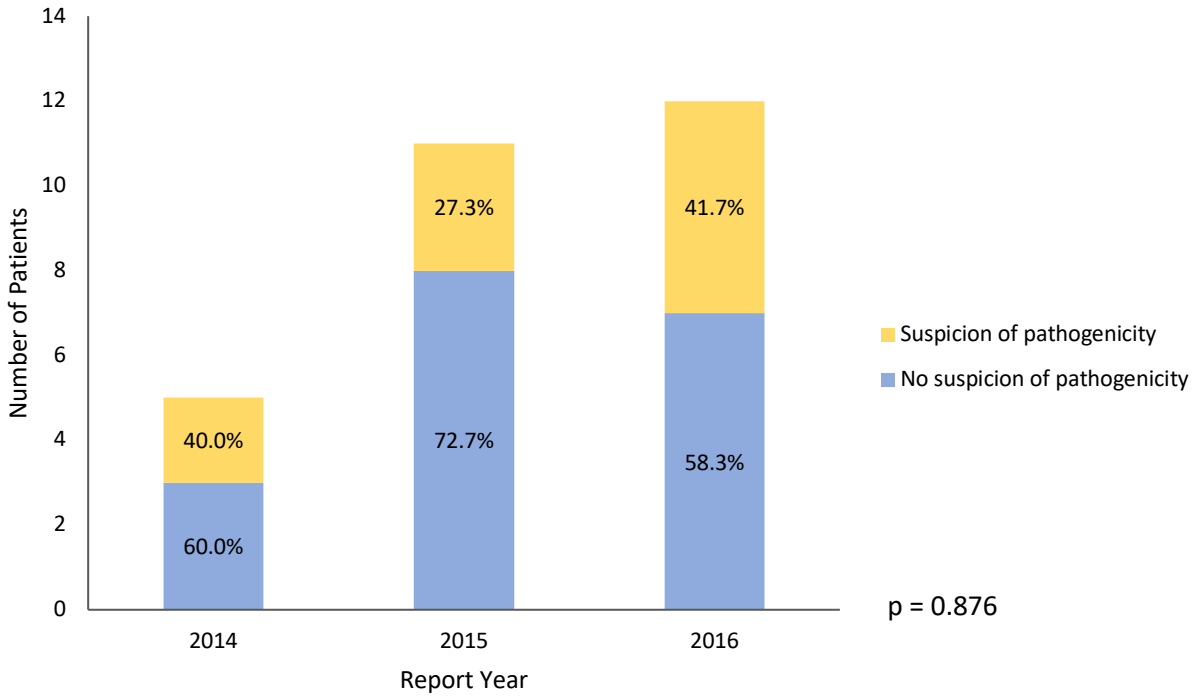


Figure 5. **Suspicion of pathogenicity by report year.** Report year represents the year that each patient's original genetic test report was issued.

Suspicion of pathogenicity was further analyzed by phenotype of the family (Table 7). For all patients without a personal or family history of specific cancer(s) well-established to be associated with the gene (or polyps for *MUTYH*), there was no evidence of provider suspicion of pathogenicity. In families in which there was an associated cancer (or polyps), 45.5% (10/22) did have evidence of provider suspicion. Additionally, we evaluated the clinician's pre-test

differential diagnosis on CRFI for all genes and specifically, high and moderate penetrance genes. 50% (7/14) of patients had evidence of provider suspicion when the VUS was in a gene included on the differential diagnosis. In contrast, only 21.4% (3/14) of patients had evidence that they were counseled with provider suspicion when the gene with the VUS did not appear on the differential diagnosis. For high penetrance genes, the one individual in which there was evidence of provider suspicion had a variant in a gene that was on the differential diagnosis. For moderate penetrance genes, 33.3% (3/9) of those not on the differential diagnosis had evidence of suspicion, and 60% (6/10) of those that were on the differential diagnosis had evidence of suspicion.

Table 7. **Suspicion of pathogenicity by family phenotype**

		Suspicion of pathogenicity		
		No N (%)	Yes N (%)	p-value
Associated cancer in family	No (N=6)	6 (100%)	0 (0%)	0.062
	Yes (N=22)	12 (54.5%)	10 (45.5%)	
All genes: differential diagnosis	No (N=14)	11 (78.6%)	3 (21.4%)	0.236
	Yes (N=14)	7 (50.0%)	7 (50.0%)	
HPGs: differential diagnosis	No (N=5)	5 (100%)	0 (0%)	0.444
	Yes (N=4)	3 (75.0%)	1 (25.0%)	
MPGs: differential diagnosis	No (N=9)	6 (66.7%)	3 (33.3%)	0.370
	Yes (N=10)	4 (40.0%)	6 (60.0%)	

Associated cancer in family reflects a personal or family history of cancers or polyps associated with pathogenic variants in the particular gene, as described in the Methods. Differential diagnosis indicates whether the gene was on the pre-test differential diagnosis recorded in CRFI. HPGs refers to high penetrance genes, and MPGs refers to moderate penetrance genes. Total N=28.

3.4 Provider Suspicion of VUS Classification

Individuals with the *MUTYH* c.934-2A>G variant, which was classified by the testing laboratory as likely pathogenic, were assessed to determine whether there was evidence that the provider was aware of the VUS classification by other laboratories. For all nine individuals, there was a CRFII or original results disclosure clinic note available to review. There was no evidence

for any of the nine individuals that they were counseled with provider knowledge of the VUS classification.

Of the ten individuals with *APC* c.3920T>A, which was classified by the testing laboratory as a low penetrance pathogenic variant, eight had a CRFII or original results disclosure clinic note available to review. There was no evidence for any of the individuals that they were counseled with provider knowledge of the VUS classification.

3.5 Assessment of Family Phenotype for VUSs in High Penetrance Genes

All 12 individuals with a VUS and a conflict in high penetrance genes were assessed to determine how closely the personal and family history matched the phenotype associated with pathogenic variants in the gene. In 58.3% (7/12), the gene was not on the pre-test differential diagnosis recorded on CRFI. For the genes that have associated risk models (*CDKN2A*, *MLH1*, and *MSH2*), pre-test mutation probability based on the personal and family history was calculated (Table 8). In the individual with *MSH2* c.1046C>G, the probability of a pathogenic variant in any Lynch syndrome gene was 97.1% by the MMRpro model, but only 7.3% by the PREMM1,2,6 model. Of note, this patient's original report was issued in January 2015 and the variant was upgraded to likely pathogenic by the testing laboratory in September 2016. For the individual with *MLH1* c.191A>G, whose initial report was issued in July 2016, this variant was eventually upgraded by the testing laboratory to likely pathogenic in December 2018 although the pre-test probabilities were only 0.1% and 5.0%. For all individuals with *CDKN2A* c.146T>C, the pre-test probability was less than 1%.

Table 8. **Risk model pre-test probability of a pathogenic variant**

	Mean	Minimum	Maximum	N
MelaPRO	0.1%	0.0%	0.4%	8
MMRpro	48.6%	0.1%	97.1%	2
PREMM1,2,6	6.2%	5.0%	7.3%	2

N represents the total number of individuals for which the model was run. The MelaPRO model was run for the eight individuals with the *CDKN2A* c.146T>C variant. The MMRpro and PREMM1,2,6 models were run for the two individuals with the *MLH1* c.191A>G and *MSH2* c.1046C>G variants. For MMRpro and PREMM1,2,6 the probability of a pathogenic variant in any Lynch syndrome gene is reflected.

For the two individuals with conflicting variants in *TP53*, pedigrees were assessed to determine whether the families met Chompret criteria and classic Li-Fraumeni syndrome criteria. Neither family met classic criteria; however, the family with *TP53* c.1040C>A met Chompret criteria. Of note, both variants are still classified as VUS by the testing laboratory.

3.6 Discrepant Classifications of the Same Variant within a Clinical Practice

The Cancer Genetics Registry allowed for identification of additional individuals with the same variants identified above in the HCP study. There were four unique variants found in which patients in the Registry had received discrepant classifications of the same variant identified in HCP patients from the USC Norris Cancer Center and the Los Angeles County + USC Medical Center. The four variants were *CDKN2A* c.146T>C, *CHEK2* c.349A>G, *CHEK2* c.470T>C, and *MUTYH* c.934-2A>G. There were 57 total patients (including those from HCP and the Registry) with these four variants. Demographic characteristics of these patients are summarized in Table 9. Results for these patients were received between April 2014 and June 2019.

Table 9. **Characteristics of 57 participants with discrepant classifications of four variants**

			N	%
Gender	Female		44	77.2%
	Male		13	22.8%
Age	<30		5	8.8%
	30-39		7	12.3%
	40-49		18	31.6%
	50-59		13	22.8%
	60-69		11	19.3%
	70-79		2	3.5%
	>79		1	1.8%
Race/Ethnicity	Asian		20	35.1%
	Black or African American		1	1.8%
	Hispanic		17	29.8%
	Non-Hispanic, White		18	31.6%
	Unknown/More than one		1	1.8%
Cancer Status	None		12	21.1%
	Breast		13	22.8%
	Colon/Rectum		7	12.3%
	Gastric		2	3.5%
	Ovary		5	8.8%
	Pancreas		1	1.8%
	Uterus		4	7.0%
	Other		7	12.3%
	Multiple Primary Types		6	10.5%
Variant/Classification	*CDKN2A* c.146T>C	Likely pathogenic	2	12.5%
		VUS	14	87.5%
	CHEK2 c.349A>G	Likely pathogenic	1	50.0%
		VUS	1	50.0%
	CHEK2 c.470T>C	Pathogenic	12	63.2%
		VUS	7	36.8%
	MUTYH c.934-2A>G	Pathogenic or Likely pathogenic	18	90.0%
		VUS	2	10.0%

For each of the four variants, medical management recommendations provided to patients with a pathogenic/likely pathogenic classification and a VUS classification were compared. For

CDKN2A c.146T>C, there were two patients with the variant classified as likely pathogenic and 14 with the variant classified as VUS (Table 9). There were three individuals with a VUS for which there was no CRFII or original results disclosure clinic note to review, so they were excluded from this analysis. Both patients with a likely pathogenic classification were recommended to undergo a skin exam with a dermatologist, and neither had a personal or family history of melanoma (Table 10). Of the patients with a VUS classification, two out of 11 were recommended to undergo a skin exam. One of these patients had a personal history of melanoma while the other had a family history of melanoma. The other nine were not recommended to undergo a skin exam and did not have a personal or family history of melanoma. There was a statistically significant association between report classification and skin exam recommendation when controlling for no personal or family history of melanoma by excluding those with a personal or family history (Fisher's exact p=0.018).

Pancreatic cancer screening was recommended for one of the individuals with a likely pathogenic classification, who did not have a personal or family history of pancreatic cancer. Of note, the original results disclosure clinic note discussed other laboratories' VUS classification, indicating that the provider was aware of the conflict. Although pancreatic cancer screening was discussed, it was recommended to begin at age 35 and the patient was in their 20s. The note discussed the possibility for recommendations to change due to the ambiguity of the variant, and it was recommended that the patient return to clinic in two years for updated management recommendations. The other individual with a likely pathogenic classification of the variant had a current diagnosis of pancreatic cancer, and therefore pancreatic cancer screening was not recommended. Knowledge of the classification of VUS by other laboratories was not apparent. Pancreatic cancer screening was not recommended for any of the individuals with a VUS classification.

Targeted variant testing was recommended for family members of one of the individuals with a likely pathogenic classification. For the other individual, targeted variant testing was not recommended. This is the same patient in which the provider displayed awareness of the conflict, and this awareness appeared to play a role in not recommending familial testing. Targeted variant testing in family members was not recommended for ten out of the 11 individuals with a VUS classification. In the other individual, VUS tracking studies were recommended due to a family history of melanoma.

Table 10. **Medical management recommendations for *CDKN2A* c.146T>C**

		Classification		
		LP N (%)	VUS N (%)	p-value
Skin exam recommended	No	0 (0%)	9 (81.8%)[a]	0.077[d]
	Yes	2 (100%)[b]	2 (18.2%)[c]	
	Total	2	11	
Pancreatic cancer screening recommended	No	1 (50.0%)[e]	11 (100%)[f]	0.154[h]
	Yes	1 (50.0%)[g]	0 (0%)	
	Total	2	11	
Targeted variant testing recommended	No	1 (50.0%)[i]	10 (90.9%)	0.295
	Yes	1 (50.0%)	1 (9.1%)[j]	
	Total	2	11	

LP represents a classification of likely pathogenic. Skin exam and pancreatic cancer screening recommendations are for the patient, and targeted variant testing refers to testing of family members for the *CDKN2A* c.146T>C variant. Fisher's exact test was used to calculate p-values. There were 13 individuals included in this analysis from 13 families.

a. None had a personal or family history of melanoma or other skin cancer.
b. Neither had a personal or family history of melanoma or other skin cancer.
c. One had a personal history of melanoma, and one had a family history of melanoma.
d. When controlling for no personal or family history of melanoma, Fisher's exact p=0.018*.
e. This individual had a current diagnosis of pancreatic cancer.
f. None had a personal or family history of pancreatic cancer.
g. There was no personal or family history of pancreatic cancer.
h. When controlling for no personal or family history of pancreatic cancer, Fisher's exact p=0.083.
i. Provider displayed awareness of conflict.
j. VUS tracking studies recommended for family history of melanoma.

There were 19 patients with the *CHEK2* c.470T>C variant, 12 classified as pathogenic and seven as VUS (Table 9). Recommendations for colonoscopy, breast MRI, and targeted variant testing were assessed (Table 11). The CRFII or original results disclosure clinic note was available for all 19 patients. When assessing colonoscopy recommendations, five were excluded from the analysis. One was excluded because the patient was deceased when the results were received, and no recommendations were provided. Another individual was excluded because they were recommended to begin colonoscopy early, but the frequency was not specified. The other three patients had active metastatic disease (two with a colon primary, one with a pancreatic primary), and no cancer screening was recommended until after completion of treatment. Of the nine included with a pathogenic classification, two either were recommended to undergo general population colonoscopy screening guidelines or colonoscopy was not discussed. The other seven were recommended to undergo colonoscopy screening every five years or more frequently. Of the five with a VUS classification, three were recommended to follow general population guidelines and two were recommended to have more frequent colonoscopies.

Recommendation for breast MRI screening was evaluated for the 13 females with this variant. Of the eight individuals with a pathogenic classification, breast MRI was recommended for six. Of the four individuals with a VUS classification, two were recommended to undergo breast MRI and the other two were not.

There were 13 unique families with this variant. When evaluating whether targeted variant testing was recommended for family members, one family was excluded due to the patient being deceased when the results were received, and no recommendations were provided. One other family was excluded because all at-risk family members had already been tested for the variant prior to presenting to Cancer Genetics. Of the five families with a pathogenic

classification, four were recommended to undergo targeted variant testing. It is unclear why targeted variant testing was not recommended for one of the families. It was not discussed in the clinic note, and it may be possible that it was recommended but not documented in the note. Of the six with a VUS classification, only one was recommended to undergo targeted variant testing.

Table 11. **Medical management recommendations for *CHEK2* c.470T>C**

		Classification		
		Pathogenic N (%)	VUS N (%)	p-value
Colonoscopy frequency[a]	General population	2 (22.2%)	3 (60.0%)	0.266
	Every 5 years or more frequently	7 (77.8%)	2 (40.0%)	
	Total	9	5	
Breast MRI recommended[b]	No	2 (25.0%)	2 (50.0%)	0.547
	Yes	6 (75.0%)	2 (50.0%)	
	Total	8	4	
Targeted variant testing recommended[c]	No	1 (20.0%)	5 (83.3%)	0.080
	Yes	4 (80.0%)	1 (16.7%)	
	Total	5	6	

Colonoscopy and breast MRI recommendations are for the patient, and targeted variant testing refers to testing of family members for the *CHEK2* c.470T>C variant. When colonoscopy was not discussed, general population recommendations were assumed. Fisher's exact test was used to calculate p-values.

a. Total n=14 patients.
b. Total n=12 females.
c. Total n=11 families.

There were 20 patients with the *MUTYH* c.934-2A>G variant, 18 classified as pathogenic or likely pathogenic and two as VUS (Table 9). Recommendations for colonoscopy and targeted variant testing or *MUTYH* sequencing were assessed (Table 12). The CRFII or original results disclosure note was available for 18 patients. When assessing colonoscopy frequency, one patient was excluded due to being deceased at the time that the results were received, and another patient was excluded because the recommended frequency of colonoscopy was not

specified. There was one patient for which there was no CRFII or original genetics results disclosure note, but another provider's note, which discussed the colonoscopy recommendations from the genetics provider, was available. Of the 15 with a pathogenic classification, one was recommended to undergo general population colonoscopy screening guidelines, and the other 14 were recommended to undergo colonoscopy every 5 years or more frequently. Both individuals with a VUS classification were recommended to follow general population screening guidelines. There was a statistically significant association between classification of this variant and frequency of colonoscopy recommendation (Fisher's exact p=0.022).

There were 15 unique families with this variant, excluding the two for which there was no available CRFII or original genetics results disclosure note. Of the 13 with a pathogenic or likely pathogenic classification, there were two in which targeted familial variant testing or *MUTYH* sequencing was not recommended and 11 in which targeted familial variant testing and/or *MUTYH* sequencing was recommended. For the two in which it was not recommended, clinic notes were reviewed to ascertain what drove not recommending familial testing. One patient's initial report was issued in April 2014, and from the results disclosure note, it appears that familial testing for monoallelic *MUTYH* mutations was not generally recommended at that time because "[the] gene was recently discovered in 2003" and "its full clinical picture [was] not yet fully understood." Of note, a letter was mailed to the patient in 2016, in which the patient was informed of updated recommendations including the recommendation for family members to undergo *MUTYH* sequencing. For the other patient with a pathogenic/likely pathogenic classification in which familial testing was not recommended, the clinic note suggested that familial *MUTYH* testing would not have changed management: "According to the most current guidelines with NCCN, enhanced colonoscopy screening is recommended for *MUTYH* carriers only if there is a personal or family history of colorectal cancer. In her case, there is no personal

or family history of colorectal cancer… At this point, we recommended she follow routine screening guidelines to begin colonoscopy screening by age 50 and repeat every 10 years." In both families with a VUS classification of the *MUTYH* variant, targeted variant testing and/or *MUTYH* sequencing was not recommended.

Table 12. **Medical management recommendations for *MUTYH* c.934-2A>G**

		Classification		
		P/LP N (%)	VUS N (%)	p-value
Colonoscopy frequency[a]	General population	1 (6.7%)	2 (100%)	0.022*
	Every 5 years or more frequently	14 (93.3%)	0 (0%)	
	Total	15	2	
Targeted variant testing or *MUTYH* sequencing recommended[b]	No	2 (15.4%)	2 (100%)	0.057
	Yes	11 (84.6%)	0 (0%)	
	Total	13	2	

Colonoscopy recommendations are for the patient, and targeted variant testing refers to testing of family members for the *MUTYH* c.934-2A>G variant. *MUTYH* sequencing refers to sequencing of the gene to assess either for the presence of biallelic mutations, which would result in *MUTYH* associated polyposis syndrome (MAP), or to assess carrier status of the patient's partner to ascertain risk of MAP in offspring. When colonoscopy was not discussed, general population recommendations were assumed. Fisher's exact test was used to calculate p-values.

a. Total n=17 patients.
b. Total n=15 families.

There were two patients with the *CHEK2* c.349A>G variant, one with a likely pathogenic classification and the other with a VUS classification (Table 9). A detailed analysis of the cases is summarized in Table 13. The patients were seen by different genetic counselors 13 months apart. For the patient with a VUS classification, the recommendations appeared to be driven by the patient's *PALB2* mutation and family history of colon cancer. There was no indication that the provider was aware of the *CHEK2* variant being reported by other laboratories as pathogenic or likely pathogenic, and targeted familial testing was not recommended. Of note, this variant was later upgraded to likely pathogenic by the testing laboratory, and the patient was counseled

at that time regarding the risks associated with this variant. In the patient with a likely pathogenic classification, counseling about the risks associated with this variant appeared to be provided, and targeted variant testing for family members was recommended.

Table 13. **Counseling strategy for patients with discrepant classifications of *CHEK2* c.349A>G**

	VUS (6/2017)	**Likely Pathogenic (7/2018)**
Gender	Female	Female
Age at Testing	49	63
Personal History	Breast cancer	Breast cancer
Family History of *CHEK2*-Related Cancers	First-degree relatives with colon cancer and high-grade dysplasia	Two first-, three second-, and two third-degree relatives with breast cancer; first-degree relative with colon cancer
Family History Score	2.0%	14.8%
Additional Variants	*PALB2* mutation	None
Mammogram	Continue/begin now, repeat yearly	Continue/begin now, repeat yearly
Breast MRI	Continue/begin now, repeat yearly	Continue/begin now, repeat yearly
Colonoscopy	Continue/begin now, repeat every 5 years	Continue/begin now, repeat every 5 years
Family Member Testing	Targeted *PALB2* variant testing for siblings; panel testing for individuals with colon cancer/dysplasia	Targeted *CHEK2* variant testing for siblings

3.7 Counseling Strategy for Discrepant Classifications by the Same Counselor

Three unique genetic counselors were involved in counseling patients with discrepant classifications of the same variant and were randomly assigned numbers. There were three physicians involved in providing recommendations to patients with these variants; however, there were no physicians who were involved in a patient with a pathogenic/likely pathogenic classification of a variant and also a VUS classification of the same variant. Therefore,

differences in the physicians' counseling strategy for patients with discrepant classifications of the same variant could not be assessed.

Counselor 1 and Counselor 2 both provided recommendations to patients with discrepant classifications of *CHEK2* c.470T>C, and case details are summarized in Table 14 and Table 15. For Counselor 1's patient with the variant reported as a VUS, the counselor did display awareness that the variant was "low moderate risk" and stated in the CRFII that the patient's cancer was "possibly influenced by *CHEK2*, but not completely attributable." Additionally, earlier and more frequent colonoscopy screening was recommended in the absence of a family history of colon cancer. However, breast MRI and targeted variant testing for family members were not recommended. It appeared that the variant played some role in the recommendations provided. The patient with a pathogenic classification appeared to be given recommendations driven by the variant.

Table 14. **Counseling strategy for patients with discrepant classifications of *CHEK2* c.470T>C seen by Counselor 1**

	VUS (11/2016)	Pathogenic (4/2018)
Gender	Female	Female
Age at Testing	39	36
Personal History	Breast DCIS	Gastric cancer
Family History of *CHEK2*-Related Cancers	None	One second-degree relative with colon cancer
Family History Score	0%	7.1%
Additional Variants	*NBN* VUS	*RAD50* and *RECQL4* VUSs
Mammogram	Continue/begin now, repeat yearly	Begin at age 40, repeat yearly
Breast MRI	Not recommended	Begin at age 40, repeat yearly
Colonoscopy	Begin at age 40, repeat every 5 years	Begin at age 40, repeat every 5 years
Family Member Testing	None	Targeted variant testing for first-degree relatives

Counselor 2 saw three patients with this variant within 13 months of one another. The first patient with a pathogenic classification appeared to be counseled on the basis of the variant. All at-risk family members had already been tested. This patient underwent testing due to the recommendation for targeted testing after a relative was found to have this variant. In contrast, family members of the individual with a VUS classification were not recommended to undergo testing, and none of them had testing previously. The second individual with a pathogenic classification also appeared to be counseled on the basis of the variant, as targeted variant testing was recommended due to the potential to change management for relatives.

Table 15. **Counseling strategy for patients with discrepant classifications of *CHEK2* c.470T>C seen by Counselor 2**

	Pathogenic (12/2015)	VUS (8/2016)	Pathogenic (1/2017)
Gender	Female	Male	Female
Age at Testing	47	66	58
Personal History	None	Colon cancer	Breast cancer and pancreatic cancer
Family History of *CHEK2*-Related Cancers	Two first-degree relatives with DCIS; three second-degree and one third-degree relative with breast cancer	First-degree relative with colon cancer; second-degree relative with colon cancer; second-degree relative with breast cancer	Third-degree relative with breast cancer; second-, third-, and fourth-degree relatives with thyroid cancer
Family History Score	31.3%	21.4%	0%
Additional Variants	None	*APC* VUS	VUSs in *GALNT12, NBN, and TYR*
Mammogram	Continue/begin now, repeat yearly	N/A	None[a]
Breast MRI	Continue/begin now, repeat yearly	N/A	None[a]
Colonoscopy	Continue/begin now, repeat every 5 years	None[a]	None[a]
Family Member Testing	None[b]	None[c]	Targeted variant testing recommended for first-degree relatives

a. No screening was recommended while undergoing treatment for metastatic cancer.
b. Other at-risk family members had previously undergone testing; patient's testing was recommended due to this variant being found in family members.
c. No additional relatives had previously undergone testing.

Counselor 3 provided recommendations to patients with discrepant classifications of *CDKN2A* c.146T>C, and case details are summarized in Table 16. Although the counselor did not display awareness of the conflict when counseling the patient with a VUS classification, the screening recommendations provided on the basis of the family history were similar to what would have likely been recommended if the variant were classified as pathogenic or likely pathogenic, except that pancreatic cancer screening was not recommended. The patient with a

likely pathogenic classification received similar recommendations for a skin exam and targeted testing of family members. Pancreatic cancer screening was not recommended due to the patient currently undergoing treatment for metastatic pancreatic cancer.

Table 16. **Counseling strategy for patients with discrepant classifications of *CDKN2A* c.146T>C seen by Counselor 3**

	VUS (1/2016)	Likely Pathogenic (12/2016)
Gender	Female	Female
Age at Testing	32	59
Personal History	Uterine cancer	Pancreatic cancer
Family History of *CDKN2A*-Related Cancers	First-degree relative with melanoma	First-degree relative with pancreatic cancer
Family History Score	8.3%	3.7%
Additional Variants	None	*NBN* VUS
Dermatology Exam	Continue/begin now, repeat yearly	Baseline evaluation now, repeat as indicated
Pancreatic Cancer Screening	None	None[a]
Family Member Testing	VUS tracking studies	Targeted variant testing for first-degree relatives

a. No screening was recommended while undergoing treatment for metastatic cancer.

Counselor 2 and Counselor 3 both saw patients with discrepant classifications of *MUTYH* c.934-2A>G, described in Table 17 and Table 18. The patients seen by Counselor 2 were counseled one month apart. Although neither patient had any family history of colon cancer or polyps, enhanced colonoscopy screening and targeted variant testing were recommended for the patient with a likely pathogenic classification and not for the patient with a VUS classification. The patient with a VUS classification did not appear to be counseled with knowledge of the conflict.

Table 17. **Counseling strategy for patients with discrepant classifications of *MUTYH* c.934-2A>G seen by Counselor 2**

	VUS (12/2016)	Likely Pathogenic (11/2016)
Gender	Female	Female
Age at Testing	70	47
Personal History	Ovarian cancer	None
Family History of Colon Cancer and Polyps	None	None
Family History Score	0%	0%
Additional Variants	None	*CDKN2A, CHEK2*, and *MSH2* VUSs
Colonoscopy	Not discussed[a]	Continue/begin now, repeat every 5 years
Family Member Testing	None	Targeted variant testing for siblings and children; panel for sister with additional cancer history

a. General population screening recommendations were assumed.

Results were received for the patients counseled by Counselor 3 two months apart, and the patients were three years apart in age. Enhanced colonoscopy screening was recommended for the individual with the likely pathogenic classification, but not the individual with the VUS classification. Additionally, targeted variant testing of family members was only recommended for the individual with a likely pathogenic classification. However, the patient with a likely pathogenic classification did have a family history of colon cancer, while the other did not.

Table 18. **Counseling strategy for patients with discrepant classifications of *MUTYH* c.934-2A>G seen by Counselor 3**

	VUS (2/2019)	Likely Pathogenic (12/2018)
Gender	Female	Female
Age at Testing	48	51
Personal History	Breast cancer	None
Family History of Colon Cancer and Polyps	None	Second-degree relative with colon cancer
Family History Score	0%	3.2%
Additional Variants	*DICER1* and *MSH6* VUSs	*BAP1* VUS
Colonoscopy	Not discussed[a]	Continue/begin now, repeat every 5 years
Family Member Testing	None	Targeted variant testing suggested for family members

a. General population screening recommendations were assumed.

3.8 Present Conflicting Variant Interpretations

Of the original cohort of 50 patients with variants with clinically significant conflicting interpretations, the conflict has since been resolved in five patients with four unique variants: *CHEK2* c.349A>G, *MLH1* c.191A>G, *MSH2* c.1046C>G, and *MUTYH* c.857G>A. Three variants (*CHEK2* c.349A>G, *MSH2* c.1046C>G, and *MUTYH* c.857G>A) were upgraded by the testing laboratory from VUS to pathogenic/likely pathogenic. *MLH1* c.191A>G was previously classified as likely pathogenic by another major commercial laboratory and an expert panel. The laboratory has since downgraded the classification to VUS, and the expert panel no longer has a submission in ClinVar.

Several patients have variants in which the conflict has now become more drastic, including those with *CHEK2* c.1427C>T, which is now classified as benign by the testing laboratory but still classified as likely pathogenic by several other major laboratories. Additionally, *CHEK2* c.917G>C is classified as likely benign by the testing laboratory and likely

pathogenic by several other major laboratories. Both variants are still classified by some laboratories as VUS. *MUTYH* c.934-2A>G remains as likely pathogenic by the testing laboratory and several others; however, it has been downgraded by at least two laboratories to likely benign. Among the original cohort of 2,000 patients in the study who underwent the same panel, 3.1% (62/2,000) currently have evidence of a clinically significant conflict among major laboratories according to reclassification data from the testing laboratory and data that is currently available in ClinVar (Table 19). There are 18 unique variants with conflicts in the genes *APC*, *BRCA2*, *CDKN2A*, *CHEK2*, *MLH1*, *MUTYH*, *PALB2*, *RAD51C*, and *TP53*. One patient has two of these variants, *BRCA2* c.7826G>T and *CDKN2A* c.146T>C. Nearly all of the variants with conflicts have an overall classification of "Conflicting interpretations of pathogenicity" in ClinVar. However, *MLH1* c.2048C>T, *MLH1* c.306G>T, and *TP53* c.1040C>A have overall ClinVar classifications of likely pathogenic, VUS, and likely pathogenic, respectively, due to classifications by expert panels.

Table 19. **Distribution of laboratory classifications of variants with current conflicts**

Variant	Count	Testing Lab	Lab A	Lab B	Lab C	Lab D	Lab E	Expert Panel
APC **c.3920T>A**	16	LPV	P	RF	RF	VUS	LP	
BRCA2 **c.7826G>T**	1	VUS	LP	VUS	VUS			
BRCA2 **c.8350C>T**	2	VUS	LP	VUS	LP	LP	VUS	
CDKN2A **c.146T>C**	9	VUS	LP	VUS	VUS	VUS	VUS	
CHEK2 **c.1283C>T**	4	VUS	P	P	P	LP	P	
CHEK2 **c.1427C>T**	3	B	LP	VUS	LP	LP	VUS	
CHEK2 **c.470T>C**	7	VUS	P	P	LP	P	P	
CHEK2 **c.592+3A>T**	1	LP	LP	VUS	VUS	VUS	VUS	
CHEK2 **c.707T>C**	3	LP	LP	LP	VUS	VUS	VUS	
CHEK2 **c.917G>C**	1	LB	LP	VUS	LP		LP	
MLH1 **c.2048T>C**[a]	1	LP	LP		VUS			LP
MLH1 **c.306G>T**	1	LP	P	LP	P		LP	VUS
MUTYH **c.934-2A>G**	9	LP	LB	LB	LP	VUS	LP	
PALB2 **c.3350+5G>A**	1	VUS	LP	LP				
RAD51C **c.965+5G>A**	1	VUS	LP	VUS			VUS	
TP53 **c.1040C>A**	1	VUS	LP	VUS	P	LP		LP
TP53 **c.374C>T**	1	VUS	LP	P	VUS	LP	LP	
TP53 **c.711G>A**	1	LP	P	VUS				

Key: P: Pathogenic; RF/LPV: Risk Factor/Low Penetrance Variant; LP: Likely Pathogenic; VUS; LB: Likely Benign; B: Benign

Count represents the number of individuals found to have the variant. Total count equals 63. Testing Lab is the laboratory at which the patient was tested. Labs A through E are the five major commercial laboratories which had ClinVar submissions for the greatest number of these variants, reflecting the classification in ClinVar in April 2020. Expert Panel represents multiple different ClinVar-defined expert panels that had a classification in ClinVar in April 2020. P refers to a pathogenic/deleterious classification. LP refers to a likely pathogenic/suspected deleterious classification. LB refers to a likely benign/favor polymorphism classification. B refers to a benign/polymorphism classification. "Risk factor" and "low penetrance variant" are both terms used to describe low penetrance pathogenic variants.

a. This variant's conflict was found incidentally due to data entry error, in which the Progeny database reflected the original testing laboratory classification of VUS but the upgrade to likely

pathogenic was not captured. The current overall ClinVar classification and current testing laboratory classification are both likely pathogenic. However, this variant was included in the count due to Lab C classifying it as VUS.

IV. DISCUSSION

In cancer genetics practice, the interpretation of genetic variants plays a major role in informing the medical management recommendations provided to patients. Therefore, discrepancies in variant interpretation can have significant clinical implications. This study aimed to identify the frequency of clinically significant conflicting interpretations among major commercial laboratories, assess evidence to determine whether providers are aware of these discrepancies, assess phenotype of families with these variants, and describe and compare the counseling strategies utilized with patients with conflicting interpretations of the same variant.

4.1 Prevalence of Past and Present Clinically Significant Discrepancies

Within the original cohort of 2,000 patients, 2.5% (50) were found to have a variant with a clinically significant conflict at the time that their report was issued. Of those with at least one variant identified, a clinically significant conflict was found in 5.1% of patients (50/975). When assessing all unique variants identified, 1.5% (14/943) had a clinically significant conflict. This rate of discrepancy is lower than previously published studies (Amendola et al. 2016, Gradishar et al. 2017, Harrison et al. 2017, Harrison et al. 2018), which aimed to quantify the number of unique variants with conflicting interpretations, rather than the number of patients impacted by these conflicts. Additionally, many previous studies either included all types of conflicts or all submitters in ClinVar. One patient-focused study found that 11% of patients with a variant identified on hereditary cancer panel testing had a clinically significant discrepancy (Balmaña et al. 2016). However, the study included all ClinVar submissions and was not limited to clinical laboratories. Exclusion of literature and research submissions has been shown to significantly reduce the rate of discrepancy (Yang et al. 2017). When the variants described in the Balmaña et al. 2016 study were reevaluated to only include submissions from clinical laboratories and

ClinVar-determined expert panels, only 5.5% of patients had a clinically significant conflict (Nussbaum et al. 2017). This is consistent with our finding that 5.1% (50/975) of those with non-negative results had a variant with a clinically significant conflict when their report was issued. By focusing on types of conflicts that have the potential to impact medical management and only including ClinVar submissions by laboratories that provide a considerable amount of clinical testing, our findings are likely to reflect the proportion of patients who may actually be impacted by these discrepancies. Additionally, an advantage of this study is that all 2,000 patients were tested through a laboratory that does not submit variant interpretations to ClinVar or other publicly available databases. This allowed additional conflicts to be captured that may have been missed if only evaluating laboratories that submit to ClinVar.

When evaluating the current prevalence of conflicting variants, 3.1% of patients (62/2,000) now have a clinically significant conflict. This is 6.4% (62/975) of the patients that had a non-negative result. The reason for this increase in prevalence is unclear. It may be that conflicting variant interpretations are more common now than they were when patients' reports were issued; however, it is also possible that older conflicts were more likely to be missed than current conflicts. One reason for this is because ClinVar archives were only reviewed if there was a clinically significant discrepancy between the current overall ClinVar classification and the original testing laboratory's classification. If the current overall ClinVar classification was concordant but the past overall ClinVar classification was discordant, the variant would have been missed. Additionally, there are many more submissions in ClinVar now than there were when the study began in 2014, as ClinVar was only released in 2013 (Landrum and Kattman 2018). If a laboratory was classifying a variant a certain way when a patient's report was issued but had not yet submitted the classification to ClinVar, that laboratory's classification would not

have been captured. Regardless of the reason, it is clear that this is an important issue in clinical genetic testing.

The genes and variants identified to have conflicts are consistent with previously published studies (Balmaña et al. 2016, Nussbaum et al. 2017). *CHEK2* had the greatest number of unique variants with conflicts and affected the greatest number of patients, both at the time that patients' reports were issued (5 unique variants among 17 patients) and currently (6 unique variants among 19 patients). In the Balmaña et al. 2016 study, 63.2% (36/57) of the variant reports with a clinically significant conflict were in *CHEK2*; clinically significant conflicts were present in 31% of *CHEK2* variant reports. Other genes with conflicts included *APC*, *BRIP1*, *CDKN2A*, *FH*, *MSH6*, *MUTYH*, *NBN*, *PALB2*, and *RAD51C*. Of note, *BRCA1* and *BRCA2* were excluded from the Balmaña study. Our study similarly identified current clinically significant conflicts in *APC*, *CDKN2A*, *MUTYH*, *PALB2*, and *RAD51C*, and additionally identified conflicts in *BRCA2*, *MLH1*, and *TP53*. Excluding *APC* c.3920T>A (p.I1307K), most of the variants (9/17) are in high penetrance genes, including *PALB2*. This finding is surprising, as one would expect more of the variants with conflicts to be in moderate penetrance genes since many individuals with pathogenic variants in moderate penetrance genes never develop cancer. Therefore, variants in moderate penetrance genes are generally more difficult to definitively classify as benign or pathogenic based on clinical findings. The high prevalence of discrepancies in high penetrance genes may suggest that if these variants are truly pathogenic, they are either rare, and therefore poorly understood, or could be common and may be moderate penetrance pathogenic variants. Another reason for discrepancies in these genes may be that many of these genes were not routinely tested until the development of panels, so data on these variants is limited.

As stated previously, 3.1% of patients in the original cohort (62/2,000) have a variant with a clinically significant conflict as of April 2020. Although this is a small number, the impact

on these patients can be substantial. Patients with these variants are likely to be currently following medical management recommendations that are discrepant from recommendations provided to patients tested at other laboratories, extending the degree of impact beyond these 62 patients to all patients found to have these variants through hereditary cancer panel testing. Some of these patients may be either at increased cancer risk and receiving inadequate surveillance, or at average risk and receiving excessive and unnecessary interventions. With 62 patients identified through only three cancer genetics clinics, there are likely to be thousands of other patients with these and other variants that have clinically significant conflicts. Expanding further, the scope of the problem is more profound, as the 50 patients with conflicts at the time of the report date had a total of over 1,000 first- and second-degree relatives, highlighting the vast number of individuals who could potentially receive inaccurate recommendations for cancer surveillance. The impact is likely to be even larger for patients whose testing was ordered by non-genetics professionals, as they may be less aware of variant reclassification in the future and may be less likely to consult public databases such as ClinVar and therefore be unaware of the discrepant classifications. Additionally, since three of the variants are now reported by some laboratories as benign or likely benign, these would likely not be mentioned on a genetic test report, and providers would not be aware that their patient has a variant classified as pathogenic/likely pathogenic by other laboratories. While this study focused on genes associated with inherited predisposition to cancer, variant interpretation discrepancies exist in all areas of clinical genetic testing and can have substantial implications on clinical care.

Even when providers are aware of a conflict, there may be profound clinical implications. For example, NCCN provides guidelines for cancer surveillance and risk-reduction in individuals with pathogenic or likely pathogenic variants in cancer predisposition genes (NCCN Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic Version 1.2020, NCCN

Genetic/Familial High-Risk Assessment: Colorectal Version 3.2019), and many insurance companies utilize NCCN guidelines to determine coverage of services (NCCN at a Glance, Rocque et al. 2018). Since these guidelines specify that individuals must have a pathogenic/likely pathogenic variant and not a VUS, insurance companies may deny procedures for risk-reduction or heightened surveillance in individuals whose test report classifies their variant as a VUS, even if the provider were to recommend the surveillance on the basis of a known conflict. This effect would be even more profound in individuals who do not even meet NCCN criteria for enhanced surveillance based on family history. Additionally, as many aspects of cancer treatment shift to precision medicine, in which an individual's germline or tumor mutation status can inform the specific cancer treatment drugs given, there are now FDA approvals and clinical trials for targeted treatments such as PARP (poly(ADP-ribose) polymerase) inhibitors, which are only available to individuals with a pathogenic/likely pathogenic variant in specific genes (ClinicalTrials.gov, NCCN Breast Cancer Version 4.2020, NCCN Ovarian Cancer Version 1.2020, NCCN Pancreatic Adenocarcinoma Version 1.2020, NCCN Prostate Cancer Version 2.2020). A variant interpretation discrepancy could potentially mean that among two patients with the exact same cancer type and germline variant, one patient would qualify for a drug on the basis of their variant while the other patient would not qualify for the drug.

Individuals with *APC* c.3920T>A (p.I1307K) made up 25.4% (16/63) of current conflicts, and individuals with *CHEK2* c.470T>C (p.I157T) made up 11.1% (7/63) of current conflicts. National guidelines and previous research have determined that both are low penetrance pathogenic variants (Boursi et al. 2013, Han et al. 2013, Liang et al. 2013, Liu et al. 2012a, Liu et al. 2012b, NCCN Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic Version 1.2020, NCCN Genetic/Familial High-Risk Assessment: Colorectal Version

3.2019, Siolek et al. 2015). However, these variants are still classified as VUSs by some laboratories. The high prevalence of these variants in the study sample is consistent with the fact that both are founder mutations and previous research which has shown that the prevalence of conflict is higher with low penetrance variants (Yang et al. 2017). A challenge in the classification of low penetrance variants is that they do not fall into any of the categories outlined in the ACMG-AMP guidelines (Dolinsky et al. 2017, Richards et al. 2015, Yang et al. 2017), and this challenge is further highlighted by the results of our study.

Of the other variants identified to have conflicts, several have been described in the literature (Balmaña et al. 2016, de Andrade et al. 2018, Evans et al. 2019, Landrith et al. 2020, Lara-Otero et al. 2019, Miyaki et al. 2005, Nussbaum et al. 2017, Takao et al. 2018, Tao et al. 2004, Tao et al. 2008, Toh et al. 2018), but a consensus interpretation has not been made.

4.2 Provider Knowledge of Classifications by Other Laboratories

This study revealed that only 36% of patients (10/28) with a VUS that was classified as pathogenic or likely pathogenic by another major commercial laboratory when their report was issued appeared to be counseled with knowledge of the conflict. To our knowledge, this study is the first to describe provider awareness, or lack thereof, of conflicts. This finding is not surprising, as hundreds of variants are identified in patients who undergo hereditary cancer panel testing. Additionally, genetics providers have limited time to research every VUS identified on their patients' testing, particularly when a family history does not fit the phenotype associated with that gene, and especially as clinically significant conflicts are a relatively infrequent occurrence when considering the vast number of patients who undergo hereditary cancer panel testing. A notable observation is that evidence of provider awareness of conflict was not necessarily more frequent in more commonly identified variants. For example, records for each

of the six patients with *CDKN2A* c.146T>C had no evidence of provider awareness of the conflict. For *CHEK2* c.470T>C, another commonly identified variant in this study, three out of the seven patients were counseled with provider awareness while awareness was not apparent in records of the other four. As discussed previously, this variant has been determined to be a low penetrance pathogenic variant by previous research and national guidelines. Since this variant is still classified as a VUS by the testing laboratory, the four patients apparently counseled without provider awareness are likely not aware that their variant is considered by multiple sources to be pathogenic, and are not likely to be currently following the recommended guidelines for breast and colon cancer screening for individuals with *CHEK2* mutations. All four patients with *CHEK2* c.1283C>T appeared to be counseled with knowledge of the conflict. For all nine individuals with *MUTYH* c.934-2A>G, which was classified by the testing laboratory as likely pathogenic and by another laboratory as a VUS, none appeared to be counseled with knowledge of the conflict. This variant is now classified by two major commercial laboratories as likely benign, raising the question of whether familial testing and enhanced colonoscopy screening that was recommended for most of these patients was truly warranted.

The frequency of awareness is likely to be even lower among non-genetics professionals. Although many hereditary cancer panels are ordered by genetics providers, it is not uncommon for oncologists, surgical oncologists, gynecologists or even primary care physicians, to order genetic testing for their patients. Non-genetics oncology providers have displayed limited understanding of VUSs (Kurian et al. 2017), and may therefore be more likely to misinterpret or less likely to be aware of a variant with discrepant interpretations. The results of this analysis demonstrate the limited awareness among genetics providers, and they also highlight the need for increased awareness among both genetics and non-genetics providers and eventually consensus interpretations across laboratories.

4.3 Assessment of Family Phenotype for VUSs with Conflicts

Family phenotype of individuals with conflicting variants reported as VUSs was assessed in several ways: whether an individual in the family had an associated cancer, pre-test differential diagnosis, pre-test probability of identifying a mutation by risk model calculation, and clinical diagnostic criteria. There were no statistically significant differences in suspicion of pathogenicity by whether there was an associated cancer in the family or whether the gene was on the pre-test differential diagnosis. However, this analysis was limited by a small sample size as well as the fact that clinicians may have done research on VUSs that was not documented in the CRFII or clinic note.

Although it could not be confirmed by this study, one may presume that providers would be less likely to research a VUS if the personal and family history were not suspicious for a mutation in the gene. Of the 28 patients for which suspicion of pathogenicity was assessed, half (14/28) did not have the gene on the differential diagnosis. Of all of the individuals with VUSs in high penetrance genes, seven out of 12 did not have the gene on the differential diagnosis. Additionally, all individuals with *CDKN2A* c.146T>C had less than a 1% pre-test mutation probability by MelaPRO, the individual with *MLH1* c.191A>G had a probability of 0.1% by MMRpro, the individuals with *MLH1* c.191A>G and *MSH2* c.1046C>G had probabilities of less than 10% by PREMM1,2,6, and neither of the individuals with *TP53* c.1040C>A or *TP53* c.374C>T met classic LFS criteria. For the variants which may be truly pathogenic, this is notable because if the providers were not aware of the conflict, these individuals are not likely to have been provided enhanced surveillance recommendations in the absence of a significant family history. Some of these variants have since been upgraded by the testing laboratory (*MLH1* c.191A>G, *MSH2* c.1046C>G) or had evidence of provider awareness of the conflict (*TP53* c.1040C>A), so recommendations for pathogenic/likely pathogenic variants have been provided

to these patients. However, patients with other variants (*CDKN2A* c.146T>C, *TP53* c.374C>T) are likely not following enhanced surveillance recommendations, which could be risky if their variants are truly pathogenic. Both variants are still classified as pathogenic/likely pathogenic by other laboratories. There were also patients with VUSs in moderate penetrance genes in which the conflict still exists and the provider did not display awareness of the conflict (*CHEK2* c.1427C>T, *CHEK2* c.470T>C, *CHEK2* c.917G>C, *RAD51C* c.965+5G>A). Patients with these variants whose personal and family history were not suspicious are also not likely to be following enhanced surveillance recommendations. Patients whose variants in moderate penetrance genes are truly pathogenic may also be missing opportunities for earlier cancer diagnosis, though the impact is likely not as profound as for the patients whose variants are in high penetrance genes. The significant number of patients whose personal and family histories do not align with the phenotype associated with the gene may be an indication that some of these variants are truly low penetrance pathogenic variants, and may not produce a phenotype similar to what would be expected for higher penetrance pathogenic variants in these genes. However, this also raises the question of whether individuals with these variants, even if pathogenic, should be provided the same recommendations as individuals with higher penetrance mutations in these genes, especially in the absence of a significant personal or family history.

4.4 Recommendations and Counseling Strategy for Discrepant Classifications of the Same Variant

When assessing recommendations provided to patients seen at the same institution (where all of the cancer genetics providers work closely together) with discrepant classifications of the same variant, there were cases in which discrepant counseling strategies were utilized, sometimes even among the same genetic counselor. For example, in individuals with *CDKN2A*

c.146T>C, a skin exam with a dermatologist was only recommended for individuals with a VUS if they had a personal or family history of melanoma, but was recommended for individuals with a likely pathogenic classification regardless of personal and family history. In patients with *MUTYH* c.934-2A>G, a statistically significant association was identified between laboratory classification and colonoscopy frequency recommended. Two patients with *MUTYH* c.934-2A>G, whose results were received just one month apart and neither of whom had a family history of colon cancer or polyps, were provided discrepant recommendations for colonoscopy frequency and targeted variant testing/*MUTYH* sequencing for family members by the same counselor. Additionally, there were patients with discrepant classifications of the same variant (for example, *CHEK2* c.349A>G and *CDKN2A* c.146T>C) who were provided identical or similar cancer surveillance recommendations; however, in the patient with a VUS classification, recommendations appeared to be driven by the family history and/or additional variants identified, and the recommendations may not have been concordant without these additional factors.

Many discrepancies in counseling strategy are likely explained by the providers' lack of awareness of the conflict. However, there were some cases in which even though the provider was aware of the conflict, the recommendations provided did not completely align with one classification, highlighting the challenges of counseling patients with these variants even when providers are aware. For example, for Counselor 1's patient with *CHEK2* c.470T>C classified as a VUS, enhanced colonoscopy screening was recommended in the absence of a family history of colon cancer. However, breast MRI and targeted familial variant testing were not recommended, even though both were recommended for Counselor 1's patient with a pathogenic classification who was almost the same age (both in their mid to late 30s) and had no personal or family history of breast cancer. Additionally, in one patient with *CDKN2A* c.146T>C classified as likely

pathogenic, the provider was aware of the conflict and recommended a skin exam and pancreatic cancer screening, but did not recommend targeted variant testing for family members. Some factors that may have contributed to these recommendations include a lack of insurance coverage (for the breast MRI), uncertainty about how relatives would be managed after undergoing familial testing for a variant with a known conflict, and the opportunity to reassess the variant in a few years to determine whether there is a more consistent interpretation and modify recommendations at that time. These findings demonstrate the complexity of counseling patients with these variants and the potentially profound implications on patients and their family members, who may be following excessive or inadequate cancer surveillance recommendations.

4.5 Limitations

The first limitation of this study is that there may be some variants with clinically significant conflicting interpretations that were missed by the analysis of ClinVar archives. Variants were only assessed in ClinVar archives in this study if there was a clinically significant conflict between the original lab report classification and the current (in November 2019) overall ClinVar classification. Variants in which the current ClinVar classification and original report classification are concordant, but at the time of the patient's report were discordant, were not captured. Additionally, because the overall ClinVar classification does not capture every submission (i.e. if a submitter with a higher review status calls a variant likely pathogenic, and a submitter with lower review status calls it VUS, the overall ClinVar classification will be likely pathogenic), additional variants may have been missed if the overall ClinVar classification and original testing lab classification were concordant, but there was another major commercial laboratory with a discordant submission. When assessing prevalence of current conflict, one variant that falls into this scenario was identified incidentally due to a data entry error. Therefore,

there may have been others that were missed. Additionally, laboratories are not required to submit to ClinVar or update submissions when a variant is reclassified. The results of this study are dependent on laboratory data that is currently in ClinVar and was in ClinVar at the time that patients' reports were issued. Although for most variants a laboratory's most recent ClinVar submission is likely to reflect the classification that the laboratory is/was reporting at the time, the classification reported by the laboratory at the time cannot be confirmed. Also, laboratories other than the testing laboratory that did not have a submission in ClinVar could not be captured. Additionally, there were 112 variant reports which could not be found in ClinVar, so it could not be assessed whether any of these variants had a conflict. Some variants cannot be found because they are not in ClinVar; however, occasionally there are variants in ClinVar that are unable to be manually searched, and can only be found when viewing all variants in a particular gene.

The second limitation is the subjectivity in assessing provider suspicion of pathogenicity in variants reported as a VUS by the testing laboratory and awareness of VUS classification in variants reported as pathogenic/likely pathogenic by the testing laboratory. Criteria were created to assess for suspicion of VUS pathogenicity based on standard cancer genetic counseling practices, including testing of at-risk family members for pathogenic variants, enhanced surveillance recommendations provided to individuals at increased risk of cancer due to a pathogenic variant and/or family history, and recommendations provided to individuals in the general population at average risk of cancer. Due to the complex nature of cancer genetics recommendations, criteria used for assessing provider suspicion varied by gene and other aspects of the clinical case, including family history and other variants identified on the panel. This limited the ability to create standardized criteria. Additionally, while CRFII or the original results disclosure clinic note did display awareness of the conflict in some cases, lack of evidence cannot definitively exclude a provider's awareness. There was no field in CRFII that

specifically inquired what research, if any, was performed on a VUS, or if there was a known conflict. Although unlikely, there may have been cases in which the provider was aware of the conflict by other laboratories but trusted the testing laboratory's classification more, and therefore did not change recommendations on the basis of the discrepancy or state this information in the CRFII or clinic note.

Furthermore, assessment of whether providers were aware of the classification of *MUTYH* c.934-2A>G, which was classified as likely pathogenic by the testing laboratory, as VUS by another laboratory could not be effectively determined in the same way as variants classified by the testing laboratory as VUS and pathogenic/likely pathogenic by other laboratories. In all nine individuals with the variant, none had documentation of awareness of the conflict. It is possible that providers would be more inclined to document and discuss another laboratory's classification of pathogenic/likely pathogenic when the testing laboratory classifies it as a VUS, rather than the other way around. This may be because the provider assumes that the laboratory classifying a variant as pathogenic/likely pathogenic has more data on the variant, since the ACMG-AMP guidelines outline strict criteria that must be met for a classification of pathogenic or likely pathogenic, and therefore is more trusting of the pathogenic/likely pathogenic classification. Additionally, since all VUSs are intended to eventually be reclassified as either benign or pathogenic, the provider may assume that the lab calling a variant a VUS may not have accumulated enough internal data yet, but would eventually reclassify the variant to be concordant with the laboratory that classifies it as pathogenic/likely pathogenic. Therefore, it is difficult to ascertain whether providers who counseled patients with a pathogenic/likely pathogenic classification of *MUTYH* c.934-2A>G were aware of the VUS classification by another laboratory and just did not discuss this awareness in the CRFII or clinic note.

A third limitation of this study is the small sample size when assessing factors associated with provider suspicion of the pathogenicity of a VUS and its impact on clinical recommendations. Larger and more focused studies are needed to determine if there is a true difference in clinical recommendations based on provider suspicion of a VUS. Furthermore, since patients were seen by multiple counselors, some patients were seen concurrently with a physician while some were not, and the two sites within the clinical practice have different patient populations, there may have been other factors that influenced the counseling approach utilized.

Also, because all patients included in this part of the analysis were seen through a single clinical practice, generalizability is limited, as different cancer genetics clinics have their own procedures and protocols. While limiting this part of the analysis to a single institution is helpful for making direct comparisons, as discrepant counseling strategies are more likely to be related to the classification of the variant when the clinical site is consistent, it would be interesting to see if similar patterns of discrepant counseling strategies are exhibited in other cancer genetics clinics.

4.6 Future Directions

The results of this study highlight the prevalence of conflicting variant interpretations and the limitations in awareness among providers. While the results of this study should encourage providers to review variants in public databases such as ClinVar, the awareness of conflicting interpretations brings additional challenges. With the exception of *APC* c.3920T>A (p.I1307K) and *CHEK2* c.470T>C (p.I157T), there are no currently existing guidelines on what recommendations should be provided to patients with these variants. Additionally, there are no guidelines detailing in general how providers should counsel patients with conflicting variants.

Counseling patients with uncertain or inconclusive cancer genetic test results has numerous challenges, including patients' lack of understanding and distress (Medendorp et al. 2020). Previous research has shown that 99% of cancer genetic counselors have concerns about counseling patients with discrepant variant interpretations, and resources including a centralized database, support from the laboratories, practice guidelines, continuing education, and functional studies are desired (Zirkelbach et al. 2017). Working to develop these resources for genetics providers is an important next step.

Another important area for future research is determining whether the variants identified to have conflicting interpretations in this study and others are truly pathogenic or benign, and then establishing consistent interpretations among labs. This will allow clinicians to most effectively provide accurate and consistent test interpretations to their patients. Collaboration and data-sharing among laboratories have been successful in reducing the rate of discrepancies previously (Amendola et al. 2016, Garber et al. 2016, Harrison et al. 2017, Harrison et al. 2018, Lebo et al. 2018), and would be beneficial for these variants.

Additionally, consistent interpretations across laboratories are needed even for the variants *APC* c.3920T>A (p.I1307K) and *CHEK2* c.470T>C (p.I157T), which have been determined low penetrance pathogenic variants by national guidelines (NCCN Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic Version 1.2020, NCCN Genetic/Familial High-Risk Assessment: Colorectal Version 3.2019). Laboratories should participate in efforts to establish consistent interpretations of these variants.

Finally, development of guidelines for classification of low penetrance variants, with a structure similar to the ACMG-AMP guidelines (Richards et al. 2015), may be helpful in resolution of some of these conflicts. Additionally, as more low penetrance pathogenic variants

are discovered in the future, distinct surveillance guidelines for individuals with low penetrance variants in specific genes may become warranted.

4.7 Conclusions

In summary, the findings from this study support previously published literature describing the proportion of patients found to have clinically significant conflicting variant interpretations on hereditary cancer panel testing among major commercial laboratories (between approximately 5% and 6% of patients with a non-negative result) and the genes most frequently involved (Balmaña et al. 2016, Nussbaum et al. 2017). This study is the first to describe provider awareness of clinically significant conflicts when counseling patients with a VUS that was classified as pathogenic/likely pathogenic by other laboratories, and found that only 36% (10/28) of patients appeared to be counseled with provider awareness of the conflict. Many patients with a VUS in which there was a clinically significant conflict did not have a personal or family history that matched the phenotype associated with pathogenic variants in that gene, likely reducing the chance that providers would research the variant and potentially indicating that if these variants are truly pathogenic, they may be low penetrance. A detailed case analysis led to the finding that discrepant counseling strategies were utilized for different patients with the same variant, within the same institution and even by the same genetic counselor. Our findings provide evidence that variant interpretation discrepancies can have profound clinical implications and highlight the importance of clinicians evaluating variants in public databases such as ClinVar. Laboratories should also be encouraged to submit classifications to publicly available databases and collaborate to resolve discrepant interpretations to support clinicians in providing accurate test interpretations.

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APPENDIX A

The email correspondence below is from the UCI IRB, received March 4, 2020.

JASON ZELL
DOM HEMATOLOGY
RE: Activities that Do Not Constitute Human Subjects Research
The University of California, Irvine (UCI) Human Research Protections Program complies with all review requirements defined in 45 CFR Part 46, *Protection of Human Subjects*. 45 CFR 46.102(e) defines research as "a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge; and 45 CFR 46.102(f) defines a human subject as "a living individual about whom an investigator conducting research obtains (i) Obtains information or biospecimens through intervention or interaction with the individual, and uses, studies, or analyzes the information or biospecimens; or (ii) Obtains, uses, studies, analyzes, or generates identifiable private information or identifiable biospecimens.
Private information includes information about behavior that occurs in a context in which an individual can reasonably expect that no observation or recording is taking place, and information that has been provided for specific purposes by an individual and that the individual can reasonably expect will not be made public (e.g., a medical record).
The UCI Human Research Protections (HRP) staff reviewed the information you submitted pertaining to your project and concluded that the project, as described, does not qualify as human subjects research because the activities do not involve human subjects. Therefore, the research are not subject to UCI IRB review and approval. If your project changes in ways that may affect this determination, please contact the HRP staff for additional guidance.
Sincerely,
Jessica Sheldon, CIP
Alternate Member, Institutional Review Board

APPENDIX B

Study Title: Hereditary Cancer Panel Testing

PI Name:_____ Gregory Idos, MD__ Site Number: _____Norris_____ Protocol Number__0S-13-1_____

CASE REPORT FORM PART I
VERIFICATION FORM

Subject Number _____ Subject Initials:_____
F M L

The PI signature on this form should be obtained after ALL the Case Report Forms for this subject have been completed.

"I have reviewed the Case Report Form for the above subject and certify that they are accurate and complete."

_____ _____
Principal Investigator's Signature Date of Signature

_____
Name of Investigator Completing CRF

_____ _____
Investigator's Signature Date of Signature

Date eligibility determined _____/_____/_____ Eligible ☐ Yes ☐ No

Date consent signed _____/_____/_____

Subject Withdrew Consent ☐ Yes ☐ No If Yes, Date Withdrawn ____/____/____

Subject Demographics

Date of Birth ____/____/____

Race ☐ American Indian/Alaska Native ☐ Asian ☐ Native Hawaiian or Pacific Islander ☐ Black or African American ☐ White ☐ More than one Race ☐ Unknown

Ethnicity ☐ Hispanic or Latino ☐ Not Hispanic or Latino ☐ Unknown

Ancestry: Maternal Side_____ Paternal Side _____

Does the patient have Ashkenazi Jewish ancestry? ☐ Yes ☐ No

Primary Language _____

Preferred Follow-Up Method: ☐ Email (English only) ☐ Mail – English ☐ Mail – Spanish ☐ None/Other:_____

CASE REPORT FORM PART I
ELIGIBILITY FORM: SCREENING AND INCLUSION CRITERIA

Subject Number _____ Subject Initials:_____
F M L

Answers to at least one the following SCREENING QUESTIONS must be **YES** for the subject to be ELIGIBLE.

	YES	NO	
1)	_____	_____	History of multiple primary cancers
2)	_____	_____	Diagnosed with cancer under the age of 50
3)	_____	_____	Two or more first or second-degree relatives with cancer
4)	_____	_____	Families with at least one family member was diagnosed with cancer under age 50
5)	_____	_____	Individuals meeting a phenotypic diagnosis or clinical testing criteria of specific hereditary cancer syndromes

ANSWERS to at least one of the following INCLUSION CRITERA must be yes for the subject to be included in the study.

1)	YES	NO	Probability	
				Pre-test Mutation probability score of >2.5% based on published model as listed below(Please list probability calculation in percentages for each model as appropriate.)
	_____	_____	_____	BOADICEA
	_____	_____	_____	BRCAPro ER/PR/H2N status, if applicable:_____
	_____	_____	_____	IBIS (Tyrer-Cuzick)
	_____	_____	_____	PANCPro
	_____	_____	_____	PREMM1,2,6
	_____	_____	_____	PENN II
	_____	_____	_____	PTEN Cleveland Clinic Score
	_____	_____	_____	MELAPro
	_____	_____	_____	MMRPro
	_____	_____	_____	Myriad II
	_____	_____	_____	Other_____
				Clinical testing criteria met or clinical judgment over 2.5% probability. Describe reason for probability, for example meets Amsterdam 2 criteria, NCCN genetic testing criteria, >10 adenomas
	_____	_____	_____	_____

CASE REPORT FORM PART I
ELIGIBILITY FORM

Subject Number _____ Subject Initials:_____
F M L

2) YES	NO	Clinical criteria	Clinical diagnosis of the following recognized cancer genetic syndromes
_____	_____	_____	Li Fraumeni Syndrome
_____	_____	_____	Hereditary Breast and Ovarian cancer syndrome
_____	_____	_____	Lynch syndrome
_____	_____	_____	Familial or Attenuated Adenomatous Polyposis syndrome
_____	_____	_____	Hereditary Melanoma syndrome
_____	_____	_____	Hereditary Pancreatic syndrome
_____	_____	_____	Cowden Syndrome
_____	_____	_____	Hereditary Diffuse Gastric Cancer
_____	_____	_____	Peutz Jeghers Syndrome
_____	_____	_____	Juvenile Polyposis Syndrome
_____	_____	_____	Other

EXCLUSION CRITERIA

Answers to the following must be **No** for the subject to be ELIGIBLE.

	Yes	No	Patients meeting one of the following criteria will be excluded from the study
1)	_____	_____	Patient does not meet inclusion or screening criteria
2)	_____	_____	Prior genetic testing for germline cancer susceptibility
3)	_____	_____	Family member with known cancer syndrome mutation and patient has no risk factors for other genetic conditions [If eligible list relationship to patient /gene _____]
4)	_____	_____	Inability to provide written informed consent

CASE REPORT FORM PART I - PHYSICAL EXAMINATION

Subject Number _____ Subject Initials:_____ (F M L) Visit Date:_____ (MM DD YYYY)

☐ **Physical Examination Not Performed (please skip to next section)**

VITAL SIGNS

Temperature: _____ °C / °F	Pulse: _____ bpm
Respiration Rate:_____ per min	Blood Pressure: _____ / _____ (Systolic (mm Hg) / Diastolic (mm Hg))

Examine the following and place a ✓ in the appropriate column. If "Abnormal" is ✓'d then provide the condition(s) in the comments column as provided.

Body System	Normal	Abnormal (Check all that apply)	Not Done	Comments
Skin		☐ Sebaceous adenomas ☐ Lipoma ☐ Fibroma ☐ Epidermoid cyst ☐ Keratocanthoma ☐ Trichilenoma ☐ Café-au-lait macules ☐ Axillary freckling ☐ Inguinal freckling ☐ Other _____ ☐ Other _____		
HEENT		Head circumference _____ ☐ Mucosal cobblestoning ☐ Supernumery teeth ☐ Dentigerous cysts ☐ Odentomas ☐ Osteomas ☐ Fundal pigmentation ☐ Other ☐ Other		
Thyroid		☐ Mass ☐ Other ☐ Other _____		
Breast		☐ Fibrocystic changes ☐ Other ☐ Other _____		
Other				

CASE REPORT FORM PART I
PRE-HEREDITARY CANCER PANEL DIFFERENTIAL DIAGNOSIS

Subject Number _____ Subject Initials:_____
F M L

Lynch Syndrome tumor analysis
Has the patient had any previous tumor analysis? ☐ Yes ☐ No If YES check box of completed test(s) and summarize results: ☐ MSI ☐ IHC ☐ BRAF ☐ Hypermethylation
Results_____

If ordering clinical testing in a stepwise approach, would you include tumor analysis as a next step? ☐Yes ☐ No
If YES then, ☐Prior to germline testing ☐ Concurrent to germline testing ☐ Considering post germline testing
Comment_____

Differential Diagnosis
List differential diagnosis in order of suspicion, from highest to lowest. List syndromes here such as "HBOC, CS, LFS" or genes under strong consideration. List genes or conditions in the differential that are not included in the Myriad panel.

	CONDITION	**REASON (check all that apply)**	**SOURCE(S)**	**If ordering testing in a <u>stepwise gene by gene approach</u> would you order clinical testing?**
1)	☐ CS ☐ JPS ☐ FAMMM ☐ LFS ☐ FAP ☐ LS ☐ HBOC ☐ MAP ☐ HDGC ☐ PALB2 ☐ Other Br/ Ov ☐ PJS ☐ Other _____	☐Mutation probability ☐Clinical judgment ☐Clinical criteria ☐Personal/family history		☐Yes ☐No If Yes, in what rank order would you test? _____ (Enter number 1-8) Comments _____
2)	☐ CS ☐ JPS ☐ FAMMM ☐ LFS ☐ FAP ☐ LS ☐ HBOC ☐ MAP ☐ HDGC ☐ PALB2 ☐ Other Br/ Ov ☐ PJS ☐ Other _____	☐Mutation probability ☐Clinical judgment ☐Clinical criteria ☐Personal/family history		☐Yes ☐No If Yes, in what rank order would you test? _____ (Enter number 1-8) Comments _____
3)	☐ CS ☐ JPS ☐ FAMMM ☐ LFS ☐ FAP ☐ LS ☐ HBOC ☐ MAP ☐ HDGC ☐ PALB2 ☐ Other Br/ Ov ☐ PJS ☐ Other _____	☐Mutation probability ☐Clinical judgment ☐Clinical criteria ☐Personal/family history		☐Yes ☐No If Yes, in what rank order would you test? _____ (Enter number 1-8) Comments _____
	CONDITION	**REASON (check all that apply)**	**SOURCE(S)**	**If ordering testing in a <u>stepwise gene by gene approach</u> would you order clinical testing?**
4)	☐ CS ☐ JPS ☐ FAMMM ☐ LFS ☐ FAP ☐ LS ☐ HBOC ☐ MAP ☐ HDGC ☐ PALB2 ☐ Other Br/ Ov ☐ PJS	☐Mutation probability ☐Clinical judgment ☐Clinical criteria ☐Personal/family history		☐Yes ☐No If Yes, in what rank order would you test? _____ (Enter number 1-8)

	☐ Other _____			Comments _____
5)	☐ CS ☐ JPS ☐ FAMMM ☐ LFS ☐ FAP ☐ LS ☐ HBOC ☐ MAP ☐ HDGC ☐ PALB2 ☐ Other Br/ Ov ☐ PJS ☐ Other _____	☐Mutation probability ☐Clinical judgment ☐Clinical criteria ☐Personal/family history		☐Yes ☐No If Yes, in what rank order would you test? _____ (Enter number 1-8) Comments _____
6)	☐ CS ☐ JPS ☐ FAMMM ☐ LFS ☐ FAP ☐ LS ☐ HBOC ☐ MAP ☐ HDGC ☐ PALB2 ☐ Other Br/ Ov ☐ PJS ☐ Other _____	☐Mutation probability ☐Clinical judgment ☐Clinical criteria ☐Personal/family history		☐Yes ☐No If Yes, in what rank order would you test? _____ (Enter number 1-8) Comments _____
7)	☐ CS ☐ JPS ☐ FAMMM ☐ LFS ☐ FAP ☐ LS ☐ HBOC ☐ MAP ☐ HDGC ☐ PALB2 ☐ Other Br/ Ov ☐ PJS ☐ Other _____	☐Mutation probability ☐Clinical judgment ☐Clinical criteria ☐Personal/family history		☐Yes ☐No If Yes, in what rank order would you test? _____ (Enter number 1-8) Comments _____
8)	☐ CS ☐ JPS ☐ FAMMM ☐ LFS ☐ FAP ☐ LS ☐ HBOC ☐ MAP ☐ HDGC ☐ PALB2 ☐ Other Br/ Ov ☐ PJS ☐ Other _____	☐Mutation probability ☐Clinical j udgment ☐Clinical criteria ☐Personal/family history		☐Yes ☐No If Yes, in what rank order would you test? _____ (Enter number 1-8) Comments _____

APPENDIX C

CASE REPORT FORM PART II
VERIFICATION FORM

Subject Number _____ Subject Initials:_____
F M L

The PI signature on this form should be obtained after ALL the Case Report Forms for this subject have been completed.

"I have reviewed all the Case Report Forms for the above subject and certify that they are accurate and complete."

_____ _____
Principal Investigator's Signature Date of Signature

CASE REPORT FORM PART II
GENETIC TESTING

Date results disclosed:_____

Was tumor analysis performed concurrently with genetic testing? ☐ Yes ☐ No If YES, check box of completed test(s) and summarize results: ☐ MSI ☐ IHC ☐ BRAF ☐ Hypermethylation
Results_____
_____

Current Molecular Diagnosis (for example: *RAD51C* mutation and *BRCA2* VUS)
_____

Current Clinical Diagnosis (for example: Familial breast cancer, HBOC with *BRCA* mutation or sporadic colon cancer)
_____
_____

Was additional genetic testing or tumor analysis recommended for the patient? ☐ Yes ☐ No

If YES, list what testing and for what element of the differential diagnosis (for example tumor analysis for Lynch syndrome or RET germline testing for *MEN2*)
_____
_____
_____

MEDICAL MANAGEMENT RECOMMENDATIONS

Subject Number:____ Subject Initials:_____
F M L

☐ Medical management recommendations are modified for this patient based on advanced metastatic disease. Explain _____

Gastrointestinal

Screening:

Was an upper endoscopy recommended? ☐ Yes ☐ No

If "Yes"	☐ Continue/begin now or ☐ Initiate at age
	Frequency: ☐ Baseline and repeat as indicated ☐ Every 6 mo ☐ Yearly ☐ Every 2-3 yrs ☐ Other

Was small bowel screening recommended? ☐ Yes ☐ No

If "Yes"	☐ Continue/begin now or ☐ Initiate at age
	Method of exam: ☐ Capsule endoscopy ☐ Double balloon enteroscopy ☐ Other
	Frequency: ☐ Baseline and repeat as indicated ☐ Every 6 mo ☐ Yearly ☐ Every 2-3 yrs ☐ Other

Was pancreatic cancer screening recommended? ☐ Yes ☐ No

If "Yes"	☐ Continue/begin now or ☐ Recommended age of initiation
	Method of exam: ☐ MRCP ☐ Endoscopic ultrasound ☐ Other
	Frequency: ☐ Every 6 mo ☐ Yearly ☐ Every 2-3 yrs ☐ Other

Was colonoscopy screening recommended? ☐ Yes ☐ No

If "Yes"	General population screening guidelines? ☐ Yes ☐ No (USPSTF Guideline: begin at age 50, if normal repeat at 10 years) OR Recommend colonoscopy ☐ Continue/begin now or ☐ Initiate at age _____ Frequency: ☐ Baseline and repeat as indicated ☐ Every 6 mo ☐ Yearly ☐ Every 3 yrs ☐ Every 5 yrs ☐ Every 10 yrs ☐ Other

Chemoprevention:

Chemoprevention recommended ? ☐ Yes ☐ No Type_____

Surgery:

Colon surgery recommended? ☐ Yes ☐ No Type_____

Skin

Skin exam with a dermatologist recommended? ☐ Yes ☐ No

If "Yes"	☐ Continue/begin now or ☐ Initiate at age
	Frequency: ☐ Every 6 months ☐ Yearly ☐ Every 2-3 yrs ☐ More than 3 years ☐ Other

Urologic (Males Only):

Prostate cancer screening recommended? ☐ Yes ☐ No

If "Yes"	☐ Continue/begin now or ☐ Recommended age of initiation
	Frequency: ☐ Baseline and repeat as indicated ☐ Every 6 mo ☐ Yearly ☐ Every 2-3 yrs ☐ More than 3 years ☐ Other

OB/Gyn (Females Only):

Screening:

Ovarian cancer screening recommended? ☐ Yes ☐ No

If "Yes"	☐ Continue/begin now or ☐ Initiate at age
	Method of exam (check all that apply): ☐ CA-125 blood test ☐ transvaginal ultrasound:
	Frequency: ☐ Every 6 mo ☐ Yearly ☐ Other

Uterine cancer screening recommended? ☐ Yes ☐ No

If "Yes"	☐ Continue/begin now or ☐ Initiate at age
	Method of exam (check all that apply): ☐ transvaginal ultrasound ☐ endometrial biopsy ☐ Other ____
	Frequency: ☐ Every 6 mo ☐ Yearly ☐ Other

Chemoprevention:

Oral contraceptives recommended for cancer prevention? ☐ Yes ☐ No

Tamoxifen offered as an option for cancer prevention? ☐ Yes ☐ No

Evista (raloxifene) offered as an option for cancer prevention? ☐ Yes ☐ No

Surgery:

Hysterectomy recommended? ☐ Yes ☐ No At what age? _____

BSO recommended? ☐ Yes ☐ No At what age?_____

Breast:

Clinical breast exam (CBE) recommended? ☐ Yes ☐ No

If "Yes"		General population screening guidelines? ☐ Yes ☐ No (ACS Guideline: CBE about every 3 years for women in their 20s and 30s and every year for women 40+)
	OR	
		Recommend CBE ☐ Continue/begin now or ☐ Initiate at age _____ Frequency: ☐ Every 6 months ☐ Yearly

Mammography recommended? ☐ Yes ☐ No

If "Yes"		General population screening guidelines? ☐ Yes ☐ No (ACS Guideline: Yearly mammograms starting at age 40)
	OR	
		Recommend mammography ☐ Continue/begin now or ☐ Initiate at age _____ Frequency: ☐ Every 6 months ☐ Yearly ☐ Other_____

Breast MRI recommended? ☐ Yes ☐ No

If "Yes"	☐ Continue/begin now or ☐ Initiate at age
	Frequency: ☐ Yearly ☐ Every 2-3 years ☐ Other

Prophylactic breast surgery discussed as an option? ☐ Yes ☐ No

OTHER

Please list all other screening recommendations based upon genetic cancer risk assessment (please include organ system, type of screening and frequency)

_____
_____
_____

Genetic testing for family members

Was genetic testing recommended for any family members? ☐ Yes ☐ No
Check all that apply. Explain which family member(s) and what test was recommended:
☐ Testing for deleterious mutation(s) identified in proband_____
☐ VUS tracking studies _____
☐ Panel testing _____
☐ Single gene(s) _____