UNIVERSITY OF CALIFORNIA,
IRVINE

Family History: Effectiveness in Identifying Families at High Risk for Pediatric Onset Cancer Predisposition Syndromes

THESIS

submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Genetic Counseling

by

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2020

DEDICATION

To

my parents for their endless support

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ACKNOWLEDGEMENTS

I would like to thank my entire committee, Dr. Maureen Bocian, Kathryn Singh, and Dr. Catherine Goudie. I am thankful for Dr. Bocian's critical eye and insightful feedback, she always managed to provide a wealth of knowledge with a splash of humor. I would like to thank Katie, who has challenged me to think critically both throughout this process and during my graduate school training. I am inspired by her compassion, exemplary clinical skills, and her desire to constantly learn. I am grateful to Dr. Goudie for allowing me to be involved with the MIPOGG project, and for providing her expertise in pediatric cancer.

I am grateful to Lara Reichman for connecting me to the MIPOGG project, and for the countless hours she spent with me brainstorming and developing this project. I am very thankful for our friendship.

I would also like to thank my program director Pamela Flodman for her dedication to students, and her constant support over the last two years.

I am thankful for Greg's endless patience, kind-heartedness, and selflessness. He continues to be a source of encouragement when I need it the most and has been my rock throughout this process.

To my classmates thank you for the late-night work sessions, the many trips to coffee and boba shops, and the wonderful memories over these past two years. I look forward to our continued friendship and being colleagues.

Lastly, I would like to thank my amazing family and friends for their unconditional love and support. This would not have been possible without you.

ABSTRACT OF THE THESIS

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Master of Science in Genetic Counseling
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Family history is an important screening tool that can highlight features suggestive of a cancer predisposition syndrome (CPS). In collaboration with the McGill Interactive Pediatric OncoGenetic Guidelines (MIPOGG) project through McGill University and the Genome 4 Kids (G4K) study through St. Jude Children's Research Hospital, a retrospective analysis of an existing data set of pediatric oncology patients compared aspects of family cancer histories in participants with and without a CPS. MIPOGG is an app that generates a recommendation for or against a genetics referral based on the presence or absence of personal and family history features associated with a high risk for a CPS. Analysis of the features in MIPOGG indicated that personal history features alone were significantly associated with identifying a CPS in participants while family history features alone were not. Although the yield of identifying participants with a CPS using family history features was low, one participant with a CPS was only classified as high-risk for a CPS due to a family history feature. Factors such as a patient's age and cancer type did not have any clear associations with the degree of relationship or ages of

relatives with cancers in a family history. This study highlighted the importance of detailed characterization of personal history features and the low yield of family history as a screening tool for CPSs in the pediatric oncology setting. However, an important subset of pediatric oncology patients with a CPS will only have features concerning for a CPS in their family history; if only personal history features are evaluated, patients such as these may be missed as being at high risk for a CPS. Recognizing the power and limitations of family history as a screening tool for CPS identification can aid in the effectiveness of a healthcare provider's risk assessment for a CPS at the time of a child's cancer diagnosis.

I. INTRODUCTION

1.1 Pediatric Cancer Predisposition Syndrome

Up to 10% of pediatric cancers are due to a cancer predisposition syndrome (CPS), and up to nearly a third of cases warrant a genetics referral for evaluation for a CPS (Zhang *et al.* 2015; Knapke *et al.* 2011; Narod *et al.* 1991). In this study, a CPS is defined as a genetic condition that is associated with an increased risk for malignancy in childhood or adolescence. Currently, there are over 125 genes associated with more than 50 defined CPSs with a risk for pediatric cancer reported in the literature (Broder *et al.* 2017). As genetics continues to evolve and genetic testing technologies improve, the number of CPSs continues to increase.

Pediatric CPSs are complex and can be difficult to diagnose due to the wide range of variability in clinical features, modes of inheritance, and methods of detection. In addition to being associated with an increased risk for malignancy in childhood, many of these disorders also include other clinical features. One example is Noonan syndrome and other associated Rasopathies with similar features, which involve a characteristic pattern of short stature, distinctive facial features, congenital heart defects, varying degrees of developmental delay, and other physical features (e.g., widely spaced nipples, broad or webbed neck, unusual chest shape, etc.). Individuals with Noonan syndrome have an increased risk for certain types of cancer, including blood cancers (acute lymphoblastic leukemia (ALL) and juvenile myelomonocytic leukemia (JMML)), neuroblastic tumors, and embryonal rhabdomyosarcoma. One study estimates an eight-fold increased risk for cancer in individuals with Noonan syndrome compared to those without the condition (Kratz *et.al.* 2015). Case reports of individuals with other

Rasopathies have suggested a risk for cancer types similar to those seen in Noonan syndrome, such as JMML; however, the exact risks are not well established.

Another group of CPS's in which affected individuals have a distinctive pattern of physical features comprises the *WT1*-related syndromes. *WT1*-related syndromes are a group of disorders that have an increased risk for cancers, including Wilms tumor and, in some cases, gonadoblastoma. *WT1*-related syndromes are also associated with other features, including genitourinary abnormalities, aniridia, and intellectual disability (Dome *et al.* 2016). While not all CPSs associated with pediatric cancer have distinctive physical features, the combination of characteristic malignancies along with specific physical features can help make a CPS diagnosis when genetic testing is not possible or inconclusive.

In addition to the variability in clinical features, pediatric CPSs can be due to various genetic mechanisms, including chromosome abnormalities, autosomal dominant, recessive and X-linked single gene variants, or imprinting defects. Many children with a CPS are diagnosed by cytogenetic or molecular testing that identifies a chromosome abnormality or single gene disorder. However, not all pediatric CPSs are identifiable through genetic testing. This may be due to limitations in genetic sequencing technology, presence of an identifiable mutation only in tissues that are not typically tested, mosaicism for a mutation where the number of mutant cells is too low to be detectable in the tested tissue, incomplete testing (for example, gene sequencing without deletion/duplication analysis), or a current lack of scientific knowledge regarding the genetic mechanism causing the condition. Additionally, genetic testing is not always necessary to diagnose a CPS in clinical practice. Some pediatric CPSs, such as Neurofibromatosis Type 1 (NF1), can be diagnosed by using well established clinical criteria alone (Ferner *et al.* 2007). Genetic testing for individuals with NF1 is usually not necessary to establish a diagnosis but can

be considered for the purpose of confirming a diagnosis in childhood, when clinical manifestations may overlap with features present in other conditions (Wu-Chou *et al.* 2018), identification of at-risk family members, and family planning (Radtke *et al.* 2007).

The complexities of diagnosing a CPS in a pediatric patient with cancer have been extensively studied and documented in the literature. In 2017, the Society for Pediatric Oncology and Hematology published guidelines for when to suspect a CPS in a pediatric patient with cancer (Ripperger *et al.* 2017). These guidelines specify the features of family history and cancer history that are most suggestive of a CPS in a patient with a pediatric cancer. Some conditions are strongly associated with specific cancer types, such as adrenocortical tumors in Li-Fraumeni syndrome (Else *et al.* 2011) and uveal melanoma in BAP1 tumor predisposition syndrome (Masoomian *et al.* 2018), that can guide a clinician's approach to testing. Family history features are an important component when assessing risk for a CPS, especially when there are multiple affected family members and/or if the CPS is one that does not present with any distinctive physical manifestations apart from cancer.

1.2 Family History as a Screening Tool to Identify Risk for a CPS

A three-generation family history can highlight patterns of cancer, age of diagnosis, or presence/absence of other features suggestive of a CPS. A standard family history often includes information about the health history of first-, second-, and third-degree relatives, including age (current or age at death), cause of death, sex at birth, cancer diagnoses, congenital anomalies, intellectual disability, chronic illnesses, and miscarriages (Bennett *et al.* 1995). Additional health history information is often obtained if the patient has an indication for specific types of examination. For example, for an individual who is being evaluated for Peutz-Jeghers syndrome

(PJS), an autosomal dominant condition characterized by the association of gastrointestinal polyposis, mucocutaneous pigmentation, and cancer predisposition, it is important to obtain details about colonoscopy and endoscopy history and the number of polyps identified during these procedures, mucocutaneous pigmentation of the lips, mouth, nose, genitalia and fingers, and gastrointestinal complications (e.g., small bowel obstruction, gastrointestinal bleeding, and intussusception [one segment of intestine "telescopes" inside of an adjoining segment, causing an intestinal blockage]) (Beggs *et al.* 2010; NCCN: Genetic/Familial High-Risk Assessment: Colorectal 2019). Information about maternal and paternal ethnic backgrounds and consanguinity is routinely obtained because this may be relevant to determine the risk for certain conditions (e.g., increased risk for autosomal recessive conditions if there is consanguinity and higher prevalence of some genetic conditions in certain populations). The clinical validity of obtaining a family history has been well established, and professional societies have published position statements regarding the potential applications (National Society of Genetic Counselors (NSGC) Position Statement 2015; American College of Obstetrics and Gynecologists ACOG Committee Opinion 478, 2011). When performed at the right time and by an experienced healthcare professional, a thorough family history can aid in guiding diagnostic, screening, genetic and non-genetic testing options, interpretation of test results, and identification of at-risk family members. When appropriately utilized, family history is a tool that can potentially identify pediatric patients at high risk for a CPS prior to a cancer diagnosis, rendering it the ultimate prevention tool. This information can be important for the medical management of a patient *and* their family members. The power of family history in risk assessment for a CPS depends on a variety of factors, including the characteristics of the CPS and the accuracy and the comprehensiveness of the family history obtained, among others.

General features in a family history can be suggestive of a CPS, such as earlier than average age of diagnosis of cancers (e.g. breast cancer diagnosed at age 30 when breast cancer is most often diagnosed after age 50), multiple generations of family members with the same or related cancer (e.g. grandmother, father, and son diagnosed with colon cancer), more than one cancer diagnosis in an individual (e.g. a woman diagnosed with thyroid and breast cancer), cancer occurring in paired organs (e.g. cancer in both kidneys), and the presence of rare cancers (e.g. male breast cancer) (Sijmons 2010). While these general features can be useful when evaluating the risk for a CPS in a family, there are many factors that impact the utility of family history, including the accuracy of cancers reported and the age of family members. Family histories are typically reliant on the information reported by a patient or legal guardian of the patient. The accuracy of cancer reported in a family history has been shown to depend on characteristics of the individual reporting the information (e.g. age, ethnicity, education), cancer type, age at diagnosis, degree of relationship, and history in maternal vs. paternal relatives (Ozanne *et al.* 2012; Mai *et al.* 2011). Cancers affecting the breast or colon are more likely to be accurately reported, while cancers affecting other organs are often inaccurately reported or the type is unknown. (Sijmons *et al.* 2000). Family dynamics can also play a role in the ability to obtain accurate family history information. Cultural taboos surrounding discussion about health (Tehranifar *et al.* 2015), small number of individuals in a family, or limited contact with family members may result in a truncated family history (Kelly *et al.* 2015). Age of onset of cancers can also be a contributing factor. Adult-onset cancers in a family with a CPS may not have emerged due to the young ages of the individuals in the family. In general, first- and second-degree relatives of pediatric patients are likely to be younger than the expected age of an adult-onset cancer, and the lack of cancer in the family history of a child can potentially mask an underlying

CPS. Providers may not update family histories after the initial consultation, but updates to family history are essential because new cancers may emerge as the family members age. Characteristics of a CPS, including the inheritance pattern, penetrance (the likelihood for someone with a pathogenic mutation to have signs and/or symptoms of the condition), and variable expressivity (the number and severity of clinical features of the condition) can make the family history more or less useful in CPS risk assessment. CPSs with different patterns of inheritance (autosomal recessive, autosomal dominant, X-linked, etc.) typically have distinctive patterns of relationship among affected individuals in the family history. The vast majority of CPSs have autosomal dominant inheritance; however, a substantial number are also autosomal recessive conditions.

Autosomal dominant conditions are caused by a heterozygous pathogenic variant in one of the two copies of a gene and typically have multiple generations of affected individuals in a family. Unlike carriers of an autosomal recessive disorder, individuals with a pathogenic variant in only one copy of a gene associated with an autosomal dominant disorder have signs and symptoms of the disorder. Each first-degree relative (e.g. sibling or parent) of an affected individual has a 50% chance to have the same pathogenic variant. A classic example is hereditary retinoblastoma, which is a CPS that is caused by heterozygous pathogenic variants in the *RB1* gene. Such individuals present with retinoblastoma (eye cancer beginning in the retina) in early childhood and may also have risks for adolescent or adult onset of non-ocular cancers, including pineoblastoma (a rare brain cancer), osteosarcoma (bone cancer), leiomyosarcoma (soft tissue cancer), and melanoma (Dimaras *et al.* 2015). Typically, family histories of individuals with hereditary retinoblastoma will have multiple generations of individuals with retinoblastoma. However, there are cases where the family history of an individual with

hereditary retinoblastoma is unremarkable, either because a relative with the pathogenic variant may only have an undetected retinoma (a benign retinal tumor that has not progressed into a malignant retinoblastoma) or because of non-penetrance of the variant (does not cause clinical symptoms in the individual; see below), and in such cases it may appear that only one individual in the family has an RB1 mutation.

Autosomal recessive conditions are caused by biallelic pathogenic variants (present in both copies of a gene), and affected relatives are typically in the same sibship and/or the patient in question is from a consanguineous relationship (both parents are descended from the same ancestor). Individuals who are heterozygotes (have a pathogenic variant in only one of the two copies of a gene) are called carriers of the condition. Carriers are either asymptomatic or have less severe clinical features compared to individuals with the condition. Parents of an individual with an autosomal recessive disorder are typically carriers. Siblings of an individual with an autosomal recessive disorder have a 25% chance to inherit the same variants as the affected individual, a 50% chance to be a carrier of one of the variants, and a 25% chance to inherit neither of the variants. For example, Xeroderma Pigmentosum (XP) is a condition caused by biallelic variants in any of at least 9 genes (*DDB2, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, POLH, XPA,* or *XPC*). Individuals with XP have photophobia (sensitivity to light), a progressive neurologic disorder in 25%, an extreme sensitivity to sun exposure in 60% that causes sunburn with blistering, and, in all individuals, excessive skin pigmentation and a very high risk for skin cancers beginning in the first decade. However, carriers of a heterozygous mutation in any of the 9 genes associated with XP are asymptomatic. For conditions like XP, where carriers are asymptomatic, a family history may not be informative outside of a sibship or a consanguineous family because of the limited number of individuals expected to be affected and exhibit

symptoms and to reveal patterns of inheritance. However, some autosomal recessive conditions are associated with cancer risks in heterozygotes, and in these a family history may highlight features suggestive of a CPS. For example, a majority of individuals with Fanconi Anemia are identified to have biallelic mutations in at least 21 known genes (Mehta *et al.* 2018). When in the heterozygous state, some of the genes known to cause Fanconi Anemia are also associated with an increased risk for breast cancer in adulthood such as *BRCA2, PALB2,* and *BRIP1.* Individuals who are diagnosed with Fanconi Anemia due to biallelic pathogenic variants in *BRCA2, PALB2,* or *BRIP1* may have a family history of breast cancer or other related cancers suggestive of a CPS. There are limitations of family history, as discussed previously, that may obscure family history features suggestive of a CPS.

An unremarkable family history in an individual with a CPS can also be due to the presence of a *de novo* pathogenic variant or to germline mosaicism. An estimated 30% of individuals with Familial Adenomatous Polyposis (FAP) have a *de novo* (new) pathogenic variant in the *APC* gene, meaning they are the first person in their family to have that variant (Genetic/Familial High-Risk Assessment: Colorectal 2019). Additionally, some CPSs have a high rate of *de novo* pathogenic variants in their associated genes. One example is *SMARCA4*-related syndrome, which is almost always due to a *de novo* pathogenic variant in the *SMARCA4* gene. Pathogenic variants in *SMARCA4* are associated with an increased risk for cancer in a condition known as rhabdoid tumor predisposition syndrome (RTPS). RTPS is characterized by an increased risk for two types of tumor: rhabdoid tumors, which are rare, aggressive soft-tissue tumors that occur primarily in the organs of the central nervous system but also can occur in any location in the body, and small cell carcinoma of the ovary, hypercalcemic type (SCCOHT), which is a rare, aggressive, malignant rhabdoid tumor of the ovary (Foulkes *et al.* 2017*).*

Although the offspring and other descendants of individuals with a *de novo* pathogenic mutation would have a 50% chance to inherit the same pathogenic variant and subsequently to be diagnosed with the associated CPS, neither the individual's parents nor his or her other antecedent relatives would carry the variant.

One rare exception is germline mosaicism, where a parent of an individual with a CPS carries the pathogenic variant in his/her germ cells (egg or sperm), but either none of the somatic cells in the parent's body has the variant or there are so few somatic cells with the variant that the parent does not develop the disorder and testing on a blood sample cannot detect the variant. The variant would be new in the parent's germ cells and would not be present in the same generation (e.g., the parent's siblings or cousins) or previous generations (e.g., parents, aunts, uncles, grandparents). Each child of a parent with germline mosaicism would have a chance of *up to* 50% to inherit the variant, and the chance of this occurring would depend on the number of germ cells that carry the variant. Germline mosaicism and *de novo* mutations are just two reasons why the family history of an individual with a CPS may not present with the predicted features associated with the disorder's known mode of inheritance.

Other factors, such as reduced penetrance and variable expressivity, also may contribute to inconsistency in the presentation of family histories of individuals with well-defined CPSs by making it difficult to identify some of the affected individuals in the family (Taeubner *et al.* 2018). Reduced penetrance is a common feature in many of the autosomal dominant CPSs, where some of the individuals with a pathogenic variant have symptoms of the disorder while others do not. One example is Hereditary Paraganglioma-Pheochromocytoma (PGL/PCC), which is an autosomal dominant condition caused by pathogenic variants in the *MAX, SDHA, SDHAF2, SDHB, SDHC, SDHD*, or *TMEM127* genes. Individuals with Hereditary PGL/PCC have an

increased risk to develop paragangliomas and pheochromocytomas (rare tumors that arise from neuroendocrine tissues). While the risk to develop these tumors begins in childhood, the degree of penetrance of pathogenic variants in a Hereditary PGL/PCC-related gene is generally not well established. One study found that pathogenic variants in *SDHA* have a penetrance of approximately 10% to 50% by age 70, depending on the sample population (van der Tuin *et al.* 2018). ALK-related neuroblastoma is an autosomal dominant condition caused by mutations in the *ALK* gene. Individuals with ALK-related neuroblastoma have an increased risk to develop neuroblastic tumors (cancer affecting the adrenal glands or nerve tissues), typically in infancy through childhood. Depending on the specific pathogenic variant in *ALK*, up to half of individuals with that variant will develop a neuroblastic tumor, while the remainder of those with the variant are asymptomatic (Bourdeaut *et al.* 2012).

Variable expressivity (when individuals with the same condition have different degrees of severity of features or different manifestations of the disorder) is a common feature of many CPSs, regardless of the mode of inheritance. Nevoid basal cell carcinoma syndrome (NBCCS), also known as Gorlin syndrome, is a rare autosomal dominant condition caused by mutations in the *PTCH1* or *SUFU* genes. Individuals with NBCCS can have a wide range of features with varying degrees of severity. The features can include large head size, congenital anomalies (e.g., cleft lip or palate and skeletal abnormalities affecting the skull, ribs, sternum, spine, hands, and feet), multiple cysts in the jaw, and an increased risk for cancer in childhood, such as medulloblastoma (a tumor of the brain, now often called primitive neuroectodermal tumor)and for multiple basal cell carcinomas (skin cancer) beginning in adolescence or early adulthood (Bala Subramanyam *et al.* 2015). Recognition and diagnosis of individuals with NBCCS and

other CPSs with reduced penetrance and variable expressivity can be difficult due to the wide range of variability in the severity of symptoms and age of onset of the condition.
There are many limitations to interpreting a family history, but the benefits can be important for both identifying a risk of a CPS and for the effective management of patients and their families after they are known to have a CPS.

1.3 Impact and Importance of Identifying a CPS in Pediatric Patients

Early identification of a CPS can provide important information to patients and their families about risks for future malignancies and other health conditions. A recent study reviewed the potential applications of this information, including modified treatment strategies, surveillance and early detection of future malignancies in the patients and their family members, and discussion with a prenatal genetic counselor regarding recurrence risk of the disorder in future pregnancies and options for possible prenatal genetic diagnosis (Jongmans *et al.* 2016). Identification of a CPS in pediatric oncology patients may have important implications for their cancer treatment. One example is BAP1-tumor predisposition syndrome, a condition caused by heterozygous pathogenic variants in the *BAP1* gene. BAP1-tumor predisposition syndrome is associated with various cancer types, including melanoma affecting the eyes and skin, other types of skin cancer (basal cell carcinoma and squamous cell carcinoma), malignant mesothelioma in the tissues that line the thorax and abdomen and cover the lungs and abdominal organs, clear cell renal cell carcinoma (kidney cancer), and, less commonly, other cancer types. Individuals with BAP1-tumor predisposition syndrome may have treatment modifications to avoid radiation exposure, given the possible increased risk for cancer. There is limited data regarding the effect of radiation exposure on individuals with a BAP1-tumor predisposition

syndrome; however, the increased risk for cancer is suspected because of the impaired ability of cells with a pathogenic variant in *BAP1* to perform cellular repair and apoptosis (cell death that occurs as a normal and controlled part of an organism's growth or development) (Kittaneh *et al.* 2018).

Multiple Endocrine Neoplasia Type 2B (MEN2B), which has a strong genotype-phenotype correlation (the association between specific germline mutations and the resulting spectrum of disease expression) and is associated with very early onset and aggressive medullary thyroid cancer (MTC) (as well as increased risk for several other types of cancer). Currently, no curative therapy exists for extensive medullary thyroid cancer, and surveillance is not effective at identifying small tumors (NCCN Thyroid Carcinoma 2019; Yasir *et al.* 2019). Due to the aggressiveness of the thyroid malignancy associated with MEN2B, prophylactic surgical removal of the thyroid is the most effective method to prevent the development and progression of thyroid cancer. The National Comprehensive Cancer Network (NCCN) guidelines recommend surgery as early as one year of age for individuals with specific pathogenic variants in the *RET* gene (mutations in codons 918 and 883) because such individuals are likely to develop metastatic MTC at an early age (NCCN: Thyroid Carcinoma 2019). Detection, early surveillance and treatment for individuals with MEN2B and other CPSs can have life-saving consequences. Another example of a condition where surveillance is essential is Familial Adenomatous Polyposis (FAP). FAP is a CPS that is associated with the development of hundreds to thousands of polyps throughout the colon and rectum. Individuals with FAP who do not undergo any intervention to manage the polyp burden have a risk of 93% for colorectal cancer by age 50. Due to the extensive number of polyps and high risk for colon cancer, individuals with FAP are recommended to undergo screening by colonoscopy with polypectomy (procedure to remove

polyps) beginning at age 10 to 15 years (NCCN: Genetic/Familial High-Risk Assessment: Colorectal 2019). Prophylactic colectomy (surgical removal of the colon to prevent the development of cancer) is currently the only effective method to reduce the essentially 100% lifetime risk for colon cancer in individuals with FAP (Tudyka *et al.* 2012). While the most prevalent features in FAP are colon polyps and colon cancer, extracolonic manifestations (symptoms affecting organs other than the colon) are also seen in individuals with FAP, including cancer in a part of the small intestine (duodenum), hepatoblastoma, a rare type of papillary thyroid cancer (cribriform-morular variant), stomach cancer, desmoid tumors, and benign, pigmented lesions of the retina (CHRPE).

Surveillance protocols in patients with Li-Fraumeni syndrome have been shown to be effective in early detection of asymptomatic tumors and in improving long-term survival (Villani *et al.* 2011; Villani *et al.* 2016). Li-Fraumeni syndrome is an aggressive CPS, caused by heterozygous pathogenic or likely pathogenic variants in the *TP53* gene and characterized by childhood-onset malignancies and an overall high lifetime risk of cancer (approximately 73% in males and 93% in females) (Villani *et al.* 2011; Chompret *et al.* 2000). An 11-year study of individuals with Li-Fraumeni syndrome found that the 5-year overall survival in the surveillance group was 89% and in the non-surveillance group was 60%. A significant finding in this study was that the early detection of aggressive malignancies ultimately led to less exposure to cytotoxic therapies and avoided the need for surgical resection (Villani *et al.* 2016). However, uncertainty still remains regarding phenotype-genotype correlations in individuals with a *TP53* variant, and there are psychological impacts associated with having this diagnosis and the extensive screening protocols implemented in these patients. The benefits and limitations of surveillance protocols should be assessed for all individuals with a CPS.

Identification of a CPS in a relative provides an opportunity to educate family members about their risks for developing cancer or other medical concerns apart from cancer. Consideration of genetic testing for family members of an individual with a CPS may be warranted, depending on a variety of factors, including the typical age of onset of the condition, age of the individual being tested, mode of inheritance, family and patient "wishes," and psychological factors. Identification of a CPS in an individual with a childhood cancer can be beneficial to family members for risk assessment, cancer treatment, cancer surveillance and early detection, and family planning purposes. In the U.S., prenatal genetic counseling is available for all individuals with a personal or family history of a CPS. This service can provide information regarding the risk to future offspring as well as education about prenatal and preconception testing options.

Obtaining a comprehensive family history is an important component of appropriate risk assessment and relies on the ability of a healthcare provider to elicit adequate and accurate information related to various CPSs. There are many challenges to non-geneticist healthcare providers' ability to obtain a complete family history with a clinical impact. These challenges will be explored further in the following section.

1.4 Pediatricians' and Pediatric Oncologists' Roles in Identifying Patients at High Risk for a CPS

Pediatric providers, such as pediatricians and pediatric oncologists, play an important role in the medical management of patients with a CPS and are also essential to the assessment and identification of patients who are at a high risk for a CPS. Pediatricians, pediatric nurse-practitioners, and family practitioners have the unique opportunity to routinely evaluate all children and are often the first medical providers to identify and address their clinical concerns.

A standard of care includes assessing a child's physical, emotional, and family health history to screen for potential illness (Committee on Hospital Care, 2013). During an evaluation, the pediatrician should obtain a child's family history and assess risk for conditions, such as cancer, that may have implications for surveillance, prevention and early detection in the child. The American Academy of Pediatrics (AAP) highlights the importance and the limitations of obtaining a family history in a pediatric primary care setting (Trotter *et al.* 2007). Some benefits of obtaining a family history include the collection of health information about family members that may guide testing and evaluation of a child, the identification of possible patterns of inheritance, and the use of family history as a tool to educate patients. A common limitation in the pediatric primary care setting is time. Pediatricians have relatively little time during a wellness visit to assess a child's physical and emotional health; this time restraint can pose a challenge to the collection of an accurate and clinically useful family history (Tarini *et al.* 2013). One study found that a majority of pediatricians (60.5%) reported insufficient time as the predominant barrier to taking an adequate family history (Saul *et al.* 2017). In addition to limited time, pediatricians' frequency of eliciting a family history and their level of detail are variable. In general, physicians in the primary care setting have expressed a lack of comfort with providing care to patients related to genetics and report inadequate access to resources regarding genetic information (Rinke *et al.* 2013). Similar studies have yet to be performed in the pediatric primary care setting, so it is unclear if levels of comfort or awareness of resources would be similar. Current recommendations from the AAP suggest obtaining a family history with a targeted approach surrounding the child's symptoms. While this targeted approach may aid efficiency in a pediatric clinic, there exists a possibility to miss potential health conditions in the family if the child is asymptomatic or if the spectrum of manifestations of a possible condition

are not recognized by the pediatrician. In the presence of a symptomatic child, a targeted approach could be effective in a specialty clinic such as pediatric oncology.

Pediatric oncologists play an essential role in the evaluation, management, and early detection (and, rarely, prevention) of a malignancy in a child. These aspects of care can be modified if a CPS is identified in a child. As stated previously, physicians utilize published guidelines regarding specific cancer types and associated features to determine the likelihood of a CPS. A pediatric oncology visit can be an opportune moment to elicit a family history, since this information is relevant to the patient's diagnosis, and the family may be more attuned to having an extensive history taken. However, similar to pediatricians, pediatric oncologists face challenges surrounding time restrictions and knowledge about features suggestive of a CPS that may hinder their ability to elicit family history information that could lead to the suspicion or identification of a CPS.

The ability of non-geneticist healthcare providers to obtain an accurate and comprehensive family history has proven to be a difficult task. There are many challenges to understanding all features associated with CPSs, and non-geneticist healthcare providers are often inadequately trained to provide a comprehensive genetic risk assessment (Rinke *et al.* 2013). Additionally, a push for efficiency in the pediatric primary care and pediatric oncology settings can make it difficult to elicit a comprehensive family history and assess for risk of a CPS. The McGill Interactive Pediatric OncoGenetic Guidelines (MIPOGG) is an eHealth decision-support tool developed to bridge the gap in efficiency and to rationalize accurate risk assessments for non-geneticist healthcare providers.

1.5 McGill Interactive Pediatric OncoGenetic Guidelines

The McGill Interactive Pediatric OncoGenetic Guidelines (MIPOGG) is an eHealth tool (an app) developed at McGill University that consists of over 140 tumor-specific decisional algorithms centered around information available soon after a pediatric cancer diagnosis. Using simple yes/no questions, MIPOGG generates a recommendation for or against a genetics referral for patients based on their likelihood of having an underlying CPS (Goudie et al. 2018). The MIPOGG app is freely available for download on smartphones and tablets.

The MIPOGG app is geared towards healthcare providers, such as pediatricians and pediatric oncologists, to aid in efficient and accurate identification of patients with features suggestive of a CPS. The app uses two sets of criteria, tumor-specific and universal criteria, to determine if an individual is advised to have a genetics evaluation.

The tumor-specific criteria include all malignant solid tumors, leukemias and lymphomas, and benign tumors known to be associated with a CPS. Some tumors are deemed so suggestive of a CPS that they are considered "direct referrals," and there are no tumor-specific questions asked (Appendix A); an example is retinoblastoma, for which each affected child is advised to have a genetics evaluation independently of family history or other personal features. The remaining tumor types each have corresponding tumor-specific algorithms modeled after the 2016 World Health Organization classification.

The MIPOGG app universal criteria are applied to all individuals for whom there are no tumor-specific criteria and comprise both personal and family history criteria. The personal history criteria focus on the number of tumors and the presence of other features that may be associated with the cancer (e.g., dysmorphic features or congenital abnormality). The family history criteria include the presence of any of the following features in a close relative: multiple

primary tumors, the same cancer type or organ affected as in the patient, and/or a close relative with cancer diagnosed at an early age. The specifics of the criteria utilized in this study are detailed in the methodology section.

The MIPOGG project was initially developed as a way to create a validated, comprehensive, and regularly updated set of pediatric guidelines that were accessible and easy to utilize by pediatric oncologists. The MIPOGG algorithm was developed over a five-year period with extensive literature and expert panel reviews for each tumor type, all of which was placed into an electronic format known as the MIPOGG app. A retrospective study was performed using the MIPOGG neuroblastic tumor algorithm on a set of pediatric patients with neuroblastic tumors. Clinical information was analyzed by the algorithm and properly identified all molecularly confirmed CPS-positive patients as individuals to be referred for a genetics evaluation. Compared to physicians, the algorithm identified 15 more patients as requiring a genetics evaluation. This study suggests that the algorithm can improve the current practice of detecting CPSs in patients with neuroblastic tumors (Goudie *et al.* 2017). Additionally, MIPOGG correctly recommended a genetics referral in 419 of 422 children (99%) from across Canada who had both a cancer diagnosis and a CPS diagnosis (this data was presented at the Annual Congress of the International Society of Paediatric Oncology (SIOP) 2019 but is unpublished at the moment). The three children who were missed had unique presentations/combinations of their cancer-CPS diagnoses. Notably, there were 17 children in this group of 422 who developed a second cancer before their physicians referred them for a genetics evaluation. All 17 of these children were identified for genetics referral by MIPOGG at the time of the first cancer and could have benefitted from earlier screening and, therefore,

earlier detection of their second cancer, perhaps with more opportunities for treatment in less advanced disease. These initial retrospective analyses are promising.

The integration of the tumor-specific criteria with the universal criteria has shown to be effective (i.e., sensitive and more rapid than physicians) at identifying patients at highest likelihood for a CPS. However, it is unclear how well the universal criteria alone can identify individuals at high risk for a CPS if they are asymptomatic or have a tumor type not typically associated with a CPS. Additionally, it is unclear if the universal criteria contribute to additional children being identified or if their tumor-specific features are sufficiently suggestive of a CPS. Understanding the underlying cause of cancer in pediatric oncology patients continues to be a topic of interest for many researchers, such as the Genome 4 Kids (G4K) study through the Saint Jude Children's Research Hospital in Memphis, TN.

1.6 Genome 4 Kids

The goal of the Genome 4 Kids (G4K) study through St. Jude Medical Center is to identify mutations that are constitutional (located in all cells in an individual's body) or somatic (located only in tumor cells) in children diagnosed with cancer and to correlate this information with clinical presentation, treatment response, and clinical outcome (Nichols 2015). This study utilizes next-generation sequencing technology to analyze the DNA of both tumor and germline samples from pediatric patients.

The development of next generation sequencing (NGS) technology has revolutionized the ability of clinicians and researchers to identify somatic and constitutional genetic changes that can lead to tumor development. NGS technology allows for the simultaneous analysis of multiple genes for small genetic changes, which has had a major impact on decreasing the turn-around

time for results and the costs of genetic testing. Genomic approaches to tumor profiling have played an important role in the classification of tumors and identification of pathways that can be targeted by therapeutic treatments (Surrey *et al.* 2019). Due to the potential clinical impact of identifying a pathogenic somatic or constitutional variant, genetic testing through NGS is routinely offered to many pediatric oncology patients.

In addition to extensive tumor DNA sequencing, all individuals enrolled in the G4K study are offered constitutional DNA sequencing through a panel of at least 150 genes associated with a CPS. The results of constitutional genetic testing may provide information about the cause of a tumor or cancer in a child or a teen. The genetic test results are discussed with the patient and his or her family in the context of the patient's clinical features. The study team also focuses on providing genetic information that may be important to patients and their families through genetic counselors or other study team members. Both the value and limitations of genetic testing should be considered when evaluating a patient at risk for a CPS.

1.7 Identification of Patients with a CPS using NGS

NGS has become a very common genetic testing methodology for tumor profiling and identification of patients with a CPS. Through the rapid analysis of multiple genes associated with monogenic (genetic conditions caused by changes in a single gene) CPSs, NGS technology has increased the efficiency of diagnosing patients with a CPS and, in turn, the identification of at-risk family members. Individuals can receive three types of results from the analysis of the patient's germline: positive, negative, or variants of uncertain significance (VUS).

A positive NGS result indicates that the patient's germline was identified to have one or more disease-causing pathogenic variants in a gene(s) associated with an increased risk for cancer. Depending on the gene, a positive result can impact treatment options and clinical management

for the child and can have health implications for family members. A positive result does not always lead to a diagnosis and may provide information about a condition that is unrelated to the patient's clinical features. The identification of pathogenic gene variants in adult-onset CPSs is a potential complication associated with genetic testing that does not have an immediate impact on the affected child's medical care. For example, genetic testing for the mismatch-repair genes (*MLH1, MSH2, PMS2, MSH6*) is important to identify individuals with Constitutional Mismatch Repair Deficiency (CMMRD) but can also identify variants that do not affect cancer risk during childhood. CMMRD is caused by biallelic (both copies of a gene) disease-causing variants in a mismatch-repair gene. (Mismatch repair is a system for recognizing and repairing errors that can arise during DNA replication and recombination and for repairing some forms of DNA damage.) CMMRD is a pediatric CPS associated with skin pigmentation differences and an increased risk for brain tumors, lymphoma, leukemia, gastrointestinal tract cancers, and other rare pediatric cancers (Wimmer *et al.* 2014). However, an adult-onset CPS called Lynch syndrome is an autosomal dominant condition caused by heterozygous pathogenic variants in one of the same mismatch repair genes. Lynch syndrome causes an increased risk for adult-onset cancers affecting the colon, uterus, urinary tract, ovary, small bowel, pancreas, and brain (Lynch *et al.* 2017), but childhood cancer is uncommon in Lynch syndrome. In a majority of cases, identification of a heterozygous pathogenic variant in a mismatch repair gene in a child would not explain their clinical features and would not impact their medical management until adulthood (e.g., screening for Lynch-related tumors usually does not start until age 20-25 years).

A negative NGS result indicates that a clinically significant variant was not reported in any of the genes analyzed. This could be due to limitations of NGS technology to detect all types of genetic change that may lead to a CPS, the presence of a pathogenic variant in a CPS-

associated gene that was not analyzed, or a non-hereditary cause for the patient's cancer. In the absence of a known familial pathogenic variant, a negative result does not exclude the possibility of an underlying genetic condition and should be considered an inconclusive result.

The last type of NGS result is a variant of uncertain significance (VUS), which indicates that a genetic change was identified for which there currently is limited information regarding the clinical impact of the variant. The American College of Medical Genetics and Genomics (ACMG) published guidelines clearly stating that changes to clinical management should not be made based on the presence of a VUS (Richards *et al.* 2015). However, identification of a VUS in a gene associated with the patient's clinical features can pose a challenge to the healthcare team. With time and the accumulation of new data, many VUSs are reclassified, either to benign or to pathogenic. Multiple studies have analyzed the rate of reclassification of VUSs identified in hereditary cancer genetic testing panels in adult patients. Among reclassified VUSs (approximately 5-8%), the majority are downgraded to a benign variant that becomes reinterpreted as a negative result (72.5%-91.2%), while a small proportion are upgraded to a pathogenic or likely pathogenic variant (7.5%-8.7%) (Macklin *et al.* 2018; Mersch *et al.* 2018). Currently, there is limited data about reclassification rates among pediatric oncology patients identified to have a VUS in a gene associated with a CPS. The identification of VUSs may be a limitation to the diagnosis/confirmation of CPSs in the pediatric oncology patients analyzed in this study. Individuals may have a VUS that is causative of their clinical features and associated with a CPS, but there may not be sufficient clinical evidence to support upgrading the classification of the variant. Additional research and data collection will be required to clarify the number of patients with a CPS identified by the genetic testing panel in the G4K study.

1.8 Purpose of Study and Specific Aims

Using a retrospective analysis of an existing data set of pediatric oncology patients through the G4K study and MIPOGG project, this investigation aims to analyze which family history features of the MIPOGG algorithm are consistently met by individuals identified to be at high or low risk for a CPS. By establishing a generalized scoring system for the features reported in the family history portion of MIPOGG, this study will identify the patients for whom a family history would be indispensable (i.e., the only feature leading to a genetics referral by MIPOGG) in assessing risk for a CPS. The features most commonly reported in the family history of a pediatric oncology patient will be quantified to determine which questions may have the largest impact when identifying patients at high risk for a CPS; conversely, there may be questions that never contribute to the identification of a CPS in the studied G4K cohort.

I hypothesize that the majority of patients who are identified to be at high risk for a CPS will not meet universal family history criteria and will only meet personal history criteria. In the patients with a family history of cancer who are identified as being at high risk for a CPS, I hypothesize the most common family history criterion met will be a parent/sibling/half-sibling with cancer onset under the age of 50 and that this will also have the highest yield of identifying individuals with a CPS.

Identifying which questions have the highest yield for CPS identification could provide a short framework of family history questions for pediatricians to ask at well-child visits. Ideally, a family history can identify family members at high risk for a CPS prior to a cancer diagnosis, especially in a child. Early identification of a CPS can lead to appropriate surveillance, early cancer detection, and possibly prevention for the child and other family members. Understanding

which CPSs are most commonly identified using family history features, and which are not, can help to highlight the power and limitations of using family history as an effective screening tool.

II. METHODS

2.1 IRB Approval and Data Transfer Agreement

This study was determined to be non-human subjects research by the University of California, Irvine (UCI) Institutional Review Board (IRB) on October 25, 2019. A copy of the original determination is available with the lead researcher. This study involves collaboration with outside institutions McGill University and St Jude Children's Research Center, and a data transfer agreement was established between the University of California Irvine and St Jude Children's Research Center on December 30, 2019. A separate data transfer agreement was established between the University of California Irvine and McGill University on April 4, 2020. A data transfer agreement was previously established between McGill University and St Jude Children's Research Center under the MIPOGG study protocol.

2.2 MIPOGG and G4K Data Set

Using a REDCap Database, the MIPOGG study has collected de-identified phenotype and genotype information on pediatric oncology patients from multiple healthcare centers around the world, including participants in the G4K study through St. Jude Children's Research Center. No Protected Health Information (PHI) was available to the study team. Prior to this study, researchers and clinicians at St. Jude Children's Research Center assigned each participant a unique identifier and performed de-identified data entry into the MIPOGG REDCap database; the key that links participant PHI with the identifier is only accessible by the G4K study team. Features reported in the database were collected by healthcare providers at St. Jude Children's Research Center, and family histories were elicited by a genetic counselor at St. Jude Children's

Research Center. Family histories were obtained at the time of the participant's first cancer diagnosis; some of the participants' ages of first cancer diagnosis were not known, and family histories were obtained at the time of their enrollment into the G4K study. Family histories of participants who were diagnosed prior to the G4K study but in whom the age of cancer diagnosis was known excluded any family history information that occurred after the time of diagnosis of their first cancer.

The MIPOGG database includes information for each participant, such as cancer type, age at diagnosis, tumor pathology, laterality of tumor, genetic test result status, features of family history, and specific characteristics depending on cancer type. Cancer diagnosis in this study refers to the presence of a benign or malignant tumor in an individual.

The information collected in the MIPOGG database is based on features assessed by the MIPOGG algorithm. As discussed previously, the MIPOGG algorithm assesses an individual's risk for a CPS depending on their cancer type and features in their personal and/or family history. The MIPOGG database assessed for features in the MIPOGG universal criteria for all individuals regardless of their cancer type. Additional personal and family history features were assessed occasionally, based on an individual's cancer type (Figure 1).

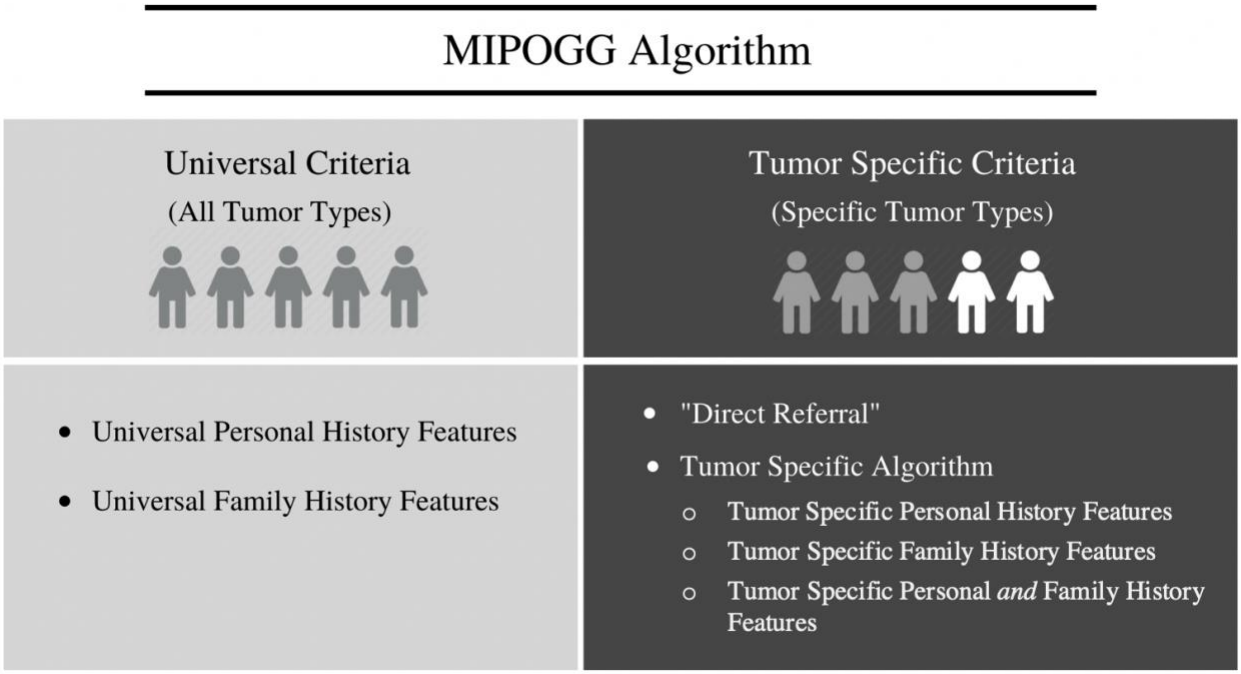

Figure 1: MIPOGG Algorithm Criteria. Two sets of criteria in the MIPOGG algorithm, Universal criteria (applied to participants with all tumor types except "direct referrals") and tumor-specific criteria (applied to participants with certain tumor types). Universal personal history features: questions asked about a participant's personal history, regardless of their cancer type. Universal family history features: questions asked about a participant's family history, regardless of their cancer type. "Direct Referral": Cancer types automatically considered high-risk for a CPS. Tumor-specific algorithm: a specific set of questions asked for participants with certain tumor types, excluding "direct referral" tumor types. Tumor-specific personal history features: questions that only pertain to the personal history of participants with tumor types in the tumor-specific algorithm. Tumor-specific family history features: questions that only pertain to the family history of participants with tumor types in the tumor-specific algorithm. Tumor-specific personal *and* family history features: questions that pertain to the participant and/or relatives of the participant with a tumor type in the tumor-specific algorithm.

Universal personal history features that were assessed for in all individuals regardless of tumor type include:

- More than one primary tumor (asynchronous or synchronous)
- Dysmorphism or congenital anomaly that, according to the clinician's judgment, may be related to the cancer

Universal family history features assessed for all individuals regardless of tumor type include:

- A parent or sibling diagnosed with cancer under the age of 50
- An aunt/uncle/first cousin or grandparent with cancer diagnosed under the age of 18

- A close relative diagnosed with the same cancer type or in the same organ
- A close relative with multiple primary tumors (excluding non-melanoma skin cancer) diagnosed under the age of 60
- A close relative was defined as a parent, sibling, aunt/uncle, first cousin, or grandparent to the child.

Additional information regarding personal and/or family history features was only collected for specific cancer types according to the tumor-specific criteria for each tumor type (Figure 2). For example, personal history features assessed for individuals with neuroblastic tumors include the presence of three or more café-au-lait spots, unexplained significant constipation starting in the newborn period, or overgrowth features (macroglossia, macrosomia, hemihyperplasia, or macrocephaly). The database also collected information regarding features present in the patient and/or their family members depending on the individual's tumor type. Some examples of questions assessed in the participant and/or their family member or exclusively in family members are detailed in Table 1.

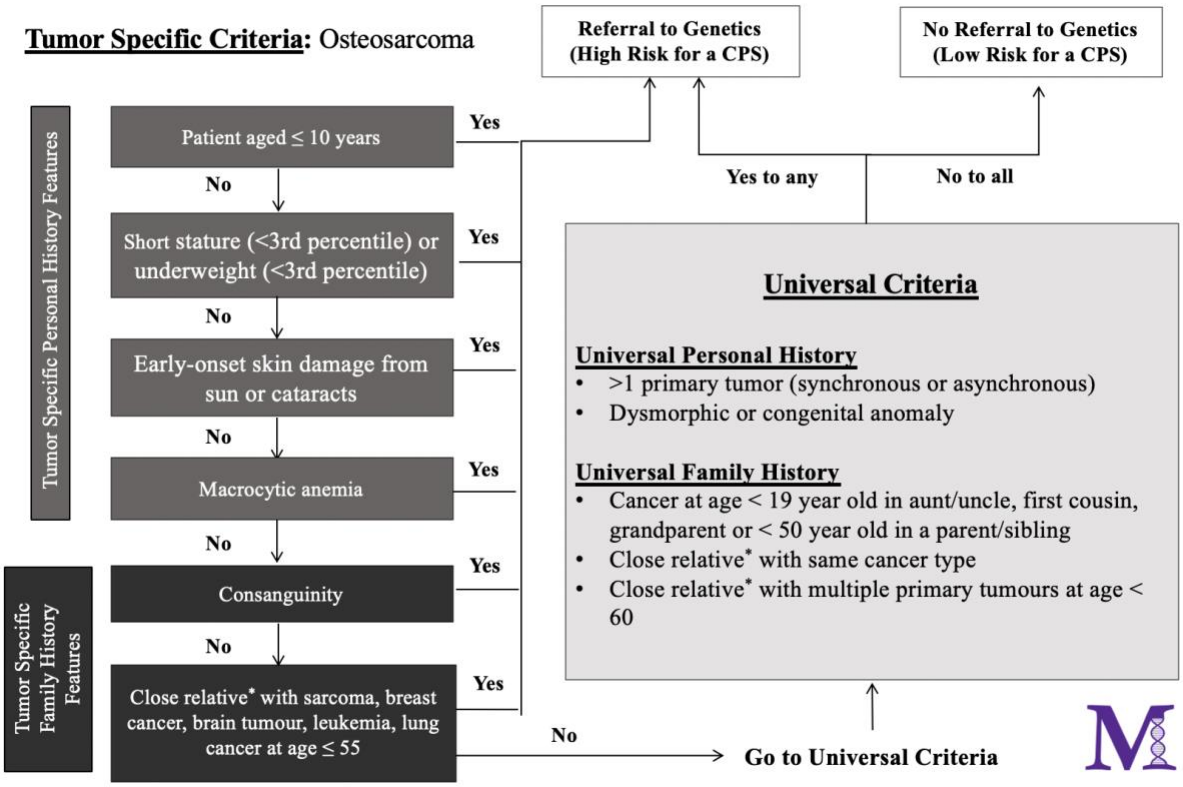

Figure 2: **Example of the MIPOGG Algorithm: Osteosarcoma**. Adapted from the MIPOGG eHealth tool. CPS: Cancer predisposition syndrome. Consanguinity: Both parents are descended from the same ancestor. Universal criteria (applied to participants with all tumor types except "direct referrals"). Tumor specific criteria (applied to participants with certain tumor types). Universal personal history features: questions asked about a participant's personal history regardless of their cancer type. Universal family history features: questions asked about a participant's family history regardless of their cancer type. "Direct Referral": Cancer types automatically considered high risk for a cancer predisposition syndrome (CPS). Tumor-specific algorithm: specific set of questions asked for participants with certain tumor types, excluding "direct referral" tumor types. Tumor specific personal history features: questions that only pertain to the personal history of participants with tumor types in the tumor specific algorithm. Tumor-specific family history features: questions that only pertain to the family history of participants with tumor types in the tumor specific algorithm.

Table 1: Examples of Features in the Tumor-Specific Criteria by Cancer Type

Cancer Type	*Tumor-Specific Personal History Features*	*Tumor-Specific Personal and Family History Features*	*Tumor-Specific Family History Features*
Acute Lymphoblastic Leukemia (ALL)	○ 3 or more café-au-lait spots ○ Microcephaly ○ Ataxia or oculocutaneous telangiectasia	○ Personal or family history of unexplained low platelets, immune thrombocytopenia, and/or low white blood cells	○ Family history of consanguinity ○ First- or second-degree relative with sarcoma, breast cancer, brain tumor, leukemia, adrenocortical carcinoma, or lung cancer before age 56 ○ Close relative with colorectal cancer, endometrial (uterine) cancer and/or ovarian cancer before age 56
Ependymoma	○ Tumor in the spine or craniocervical junction ○ Cataracts or hearing loss	○ Personal history of, or close relative with, schwannoma, meningioma, or neurofibroma	
High Grade Glioma	○ 3 or more café-au-lait spots ○ Axillary/inguinal freckling and/or a neurofibroma ○ Abnormal staining for one of the 4 mismatch repair genes		○ Family history of consanguinity ○ First- or Second-degree relative with sarcoma, breast cancer, brain tumor, leukemia, adrenocortical carcinoma, or lung cancer before age 56 ○ Close relative with a colorectal cancer, endometrial (uterine) cancer and/or ovarian cancer before age 56

Table 1 (Cont.): Examples of Features in the Tumor-Specific Criteria by Cancer Type

Neuroblastic Tumor	○ Bilateral tumors (adrenal glands) ○ Presence of 3 or more café-au-lait spots ○ Overgrowth features (macroglossia, macrosomia, hemihyperplasia, or macrocephaly)	○ Personal history of, or close relative with Hirschsprung disease ○ Personal history of, or close relative with a hypoventilation condition	
Non-Medullary Thyroid Cancer	○ Macrocephaly ○ Penile freckling ○ Cribriform/morular histologic variant of papillary thyroid carcinoma ○ Adenomatous thyroid nodules/ microadenomas ○ Second primary cancer (in an area not exposed to radiation) within five years of the first cancer diagnosed	○ Personal history of, or sibling with one or more gastro-intestinal polyps ○ Personal history of, or close relative with, renal cysts (cystic nephroma), lung cysts (pleuropulmonary blastoma), past lung surgery or ovarian sertoli-leydig cell tumor	○ Thyroid cancer in two or more first degree relatives ○ Close relative with gastro-intestinal polyposis, colectomy and/or colorectal cancer before age 50 or a desmoid tumor at any age ○ Cancer (excluding thyroid) occurring before age 50 in a parent/sibling or before age 19 in an aunt/uncle/first cousin/grandparent

Tumor-Specific Personal History Features: Questions asked that pertain to a patient's personal history. Tumor-Specific Personal *and* Family History: Questions asked that pertain to a patient's and their family members' personal history. Tumor-Specific Family History: Questions asked that pertain to a family members' personal history. Note, this is only a small subset of the cancer types assessed in the tumor specific criteria.

2.3 Study Cohort

A subset of individuals from the MIPOGG dataset analyzed in this study included 300 patients from the G4K study with a tumor or cancer diagnosed between June 17, 2003 and March 30, 2018. These individuals' ages at tumor/cancer diagnosis ranged from 0 to over 20 years, and participants were entered into the database regardless of cancer type or family history features.

The majority of participants in the G4K study had germline genetic testing with an NGS panel of over 150 CPS-related genes curated by the G4K study team and performed at the Clinical Genomics Laboratory at St. Jude Children's Research Hospital. A comprehensive list of the genes included on the NGS Panel used during the G4K study was not available to review by the study team. A total of 35 individuals from the original subset of 300 patients were considered to have a CPS. An individual was determined to have a CPS if he or she was found to have a pathogenic or likely pathogenic variant in a CPS-related gene on the NGS Panel developed by the G4K study team or a chromosomal abnormality associated with pediatric cancer risk (e.g., Down syndrome). For the purpose of this study, specific exclusion criteria were established:

- Individuals diagnosed with a first tumor/cancer over the age of 18 years were excluded because this study is focused on the pediatric oncology population.
- Individuals who were diagnosed with a CPS prior to their cancer diagnosis would not benefit from the MIPOGG algorithm. Five individuals with Down syndrome and one individual with Gardner syndrome were diagnosed prior to their cancer diagnoses. One individual was identified to be a heterozygous carrier of a pathogenic *MUTYH* mutation. *MUTYH* is typically associated with an autosomal recessive condition called *MUTYH*-associated polyposis (MAP), and adult cancer risks for carriers of a heterozygous pathogenic variant in *MUTYH* are not well established. For the purposes of this study, this individual was not considered to have a CPS but was included in the study. Three individuals were identified with adult-onset CPSs (Hereditary Breast and Ovarian Cancer and Lynch syndrome). Individuals with adult-onset CPSs were considered to be CPS-positive, but larger analyses allowed distinguishing between CPSs associated with pediatric-onset cancers and CPSs considered to be adult-onset conditions.

- Germline genetic testing using the NGS panel through the G4K study was necessary to establish a diagnosis of a CPS for individuals in this study. Any individuals who did not undergo germline genetic testing for this panel of CPS-related genes were excluded from this study.
- Family history features were the primary focus of this study; therefore, individuals with an unknown family history were excluded. One patient with type 1 neurofibromatosis was excluded for this reason.
- Any individuals without sufficient information to determine a MIPOGG referral recommendation were excluded. Insufficient information included unknown personal history features or unknown tumor type.

Within the original cohort of 300 patients, 24 were excluded due to age over 18 at cancer diagnosis, the presence of a CPS at the time of cancer diagnosis, lack of germline genetic testing for a panel of CPS-related genes, or insufficient personal or family history information required to run the MIPOGG algorithm. The final number of individuals included in this study was 276.

2.4 Data Collected from MIPOGG Database

A data collection spreadsheet was curated that included information extracted from the MIPOGG REDCap database. Information was collected for each study participant who met inclusion criteria, including type of first cancer diagnosis, age at cancer diagnosis, tumor characteristics, and personal and family history features, in order to answer all MIPOGG tumor-specific questions and universal criteria. Only the information prior to/at the time of their first cancer diagnosis was used to answer questions in MIPOGG. Seventeen participants were diagnosed with a subsequent cancer at the time of enrollment into the G4K study. Three participants' subsequent cancer type was different from their first diagnosis, while fourteen had

the same cancer type, likely a recurrence of the first cancer. Features that presented after the participant's first cancer diagnosis were not applied to the question in MIPOGG, with the exception of family history information for participants with an unknown age at first cancer diagnosis (N=12). A determination for or against a genetic referral was then made using the MIPOGG algorithm by two independent study members (one with an oncology background and one with a genetic counseling background). Importantly, both evaluators were blinded to the genetic sequencing results for each patient.

Family history information extracted from the existing dataset included the presence of a first-degree relative (FDR), second-degree relative (SDR), and/or third-degree relative (TDR) or more distantly related relative with cancer. The number of family members diagnosed prior to age 19 and their degrees of relationship to the individual were assessed. The MIPOGG algorithm data for each individual was used to determine which inclusion criteria the individual met, such as personal history features (tumor direct referrals, personal history features in the tumor-specific criteria and/or universal criteria), tumor-specific family history features (family history questions only asked for individuals with certain tumor types), or universal family history features (family history questions asked for individuals regardless of tumor type).

2.5 Data Analysis

Microsoft Excel for Mac 2019 (version 16.22) and VassarStats: Website for Statistical Computation through Vassar College (Lowry 1998) were used to calculate descriptive statistical analyses. Microsoft Excel for Mac 2019 was utilized to summarize study participant characteristics using means and standard deviation for age and using counts and percentages for features such as cancer type, mutation status, recommendation for or against a referral, and family history features. Differences between two independent proportions with 95% confidence

intervals were calculated using VassarStats and the Wilson procedure with a correction for continuity (Newcombe *et al.* 1998; Wilson *et al.* 1927). These calculations were used to determine strength of association between the two factors of interest, and the estimated range of values that the true difference in proportion lies within. Differences in proportions and 95% confidence intervals were applied to various analyses in the study such as the proportion of participants with a CPS compared by features in the MIPOGG algorithm. The difference between these two proportions were assessed with the 95% confidence interval to determine the statistical significance of any associations identified. Confidence interval values that excluded the value zero were considered statistically significant. Other analyses of interest included participants identified with a CPS who have general family history features that are concerning for a CPS, the presence of certain features in the MIPOGG algorithm by CPS status, and degree of relationship of cancer reported compared by age of ascertainment of a family history.

III. RESULTS

3.1 Patient Demographics

3.1.1 Age and Cancer Type

A total of 276 MIPOGG study participants qualified for this study; all of the participants were diagnosed with cancer before age 19 and were evaluated at St. Jude Children's Research Hospital between June 17, 2003 and March 30, 2018. The mean age of first cancer diagnosis of participants was 6.6 years. The participants' ages of first cancer diagnosis were categorized into two-year age ranges from birth to age 18, with the exception of a three-year age range from 16 to 18 (Table 2) and an unknown category. The unknown category consisted of twelve individuals who had a previous cancer diagnosis (i.e., prior to their G4K study entry) and whose ages at their first cancer diagnosis were not available. The age categories with the highest frequencies were 0-1 (20%) and 2-3 (19%). Due to the large variety of cancer types first diagnosed in the study participants, the cancer types were organized into four categories: blood cancers, brain and central nervous system (CNS) tumors, retinoblastoma, and other solid tumors (Figure 3). The frequencies of subtypes of cancer are characterized in Table 3. The most common cancer type was blood cancer, which made up approximately 41% (N=112) of all first cancers diagnosed in the study cohort (N=276). The most common type of cancer diagnosed in participants was ALL (N=82). Additional demographic information, including sex and ethnicity, were not collected in the original REDCap MIPOGG database and were unavailable to the study team.

Table 2: Age of Participants at First Cancer Diagnosis

Mean (Standard Deviation)	6.6 (5.0)
Age Range (Years)	N=276
0-1	56 (20%)
2-3	51 (19%)
4-5	35 (13%)
6-7	25 (9%)
8-9	23 (8%)
10-11	27 (10%)
12-13	14 (5%)
14-15	18 (7%)
16-18	15 (5%)
Unknown	12 (4%)

Individuals in the unknown category were diagnosed with their first cancer prior to the G4K study, and the exact age of diagnosis was not reported.

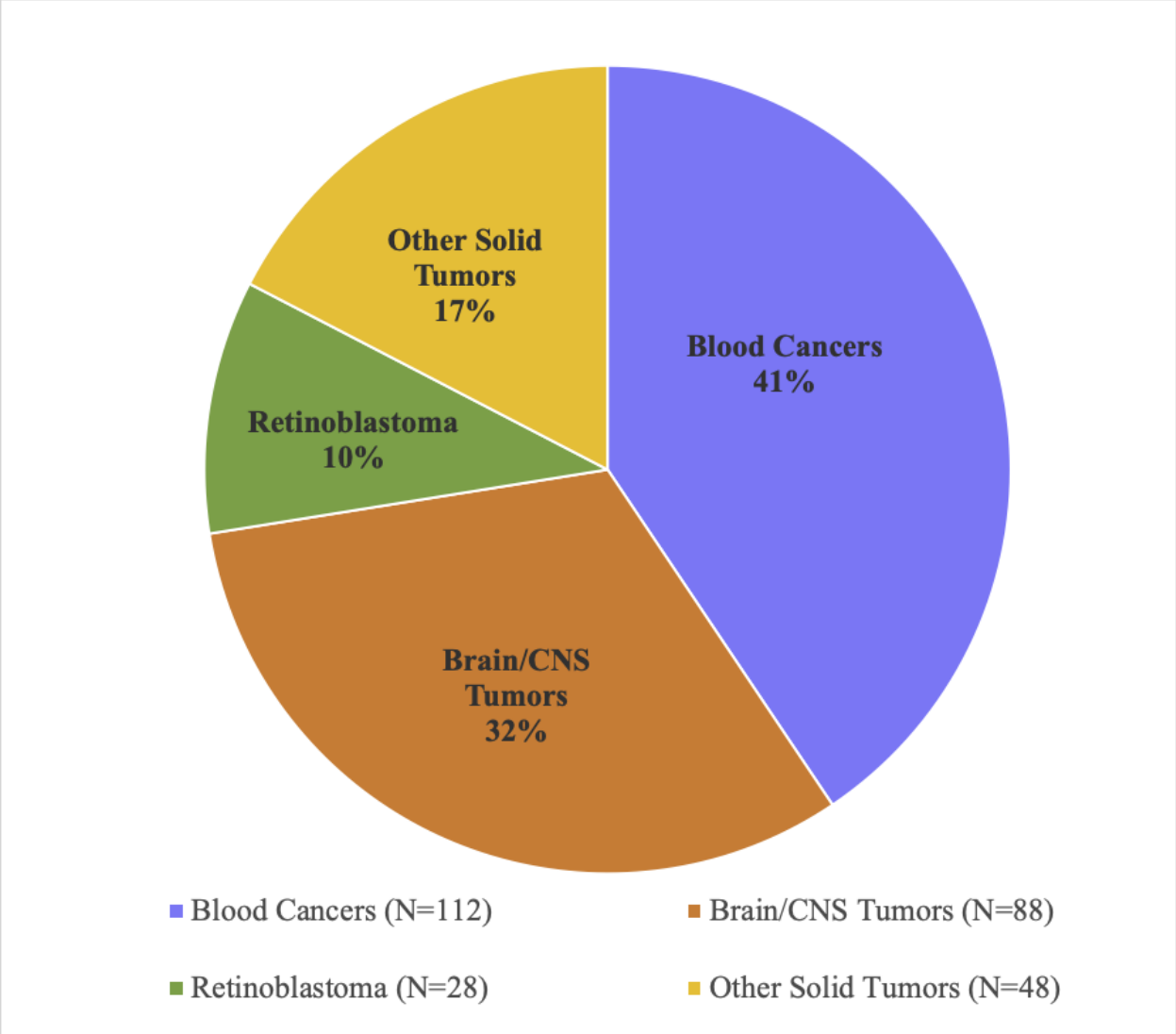

Figure 3: Distribution of first cancer types. 276 participants in the study were diagnosed with a large range of cancer subtypes at first cancer diagnosis, and the cancer subtypes were organized into four cancer categories (blood cancers, brain/CNS tumors, retinoblastoma, and other solid tumors).

Table 3: Frequency of First Cancer Subtypes Diagnosed in Participants

Blood Cancers	N=112
Acute Lymphoblastic Leukemia (ALL)	82 (73%)
Acute Myeloid Leukemia (AML) / Myelodysplastic Syndrome (MDS)	29 (26%)
Lymphoma	1 (1%)
Brain/CNS Tumors	N= 88
Craniopharyngioma	26 (30%)
High Grade Glioma	16 (18%)
Low Grade Glioma	15 (17%)
Medulloblastoma	15 (17%)
Ependymoma	10 (11%)
Atypical Teratoid Rhabdoid Tumor (ATRT)	2 (2%)
CNS Embryonal Tumors/ PNET-NOS	1 (1%)
Chordoma	1 (1%)
Embryonal Tumor with Multilayered Rosettes (ETMR)	1 (1%)
Meningioma	1 (1%)
Retinoblastoma	N=28
Retinoblastoma	28 (100%)

Table 3 (Cont.): Frequency of First Cancer Subtypes Diagnosed in Participants

Other Solid Tumors	N=48
Neuroblastic Tumor	13 (27%)
Wilms Tumor	6 (13%)
Ewing Sarcoma/ Askin Tumor/ PNET	5 (10%)
Rhabdomyosarcoma	5 (10%)
Osteosarcoma	3 (6%)
Germ Cell Tumor	3 (6%)
Hepatoblastoma	2 (4%)
Non-medullary Thyroid Carcinoma	2 (4%)
Sarcoma NOS	2 (4%)
Clear Cell Sarcoma of the Kidney	1 (2%)
Desmoid type Fibromatosis	1 (2%)
Gastrointestinal Carcinoma	1 (2%)
Low-Grade Fibromyxoid Sarcoma	1 (2%)
Melanoma	1 (2%)
Renal Cell Carcinoma	1 (2%)
Desmoplastic Small Round Cell Tumor	1 (2%)

CNS: Central nervous system; PNET: Primitive neuroectodermal tumor; NOS: Not otherwise specified.

The age distribution of each cancer type is characterized in Figure 4. Blood cancers and retinoblastoma typically occur in a very young aged population, which explains the age distribution and the peak frequency in the 2-3 and 0-1 age categories, respectively. The age distribution of the brain/CNS tumor and other solid tumor categories did not exhibit a clear pattern, which may be attributed to the large variety of cancer types in these categories. Characterization of the cancer subtypes within these categories may reveal a pattern in the age distribution, but this analysis was not performed due to the low number of participants with many of the cancer subtypes.

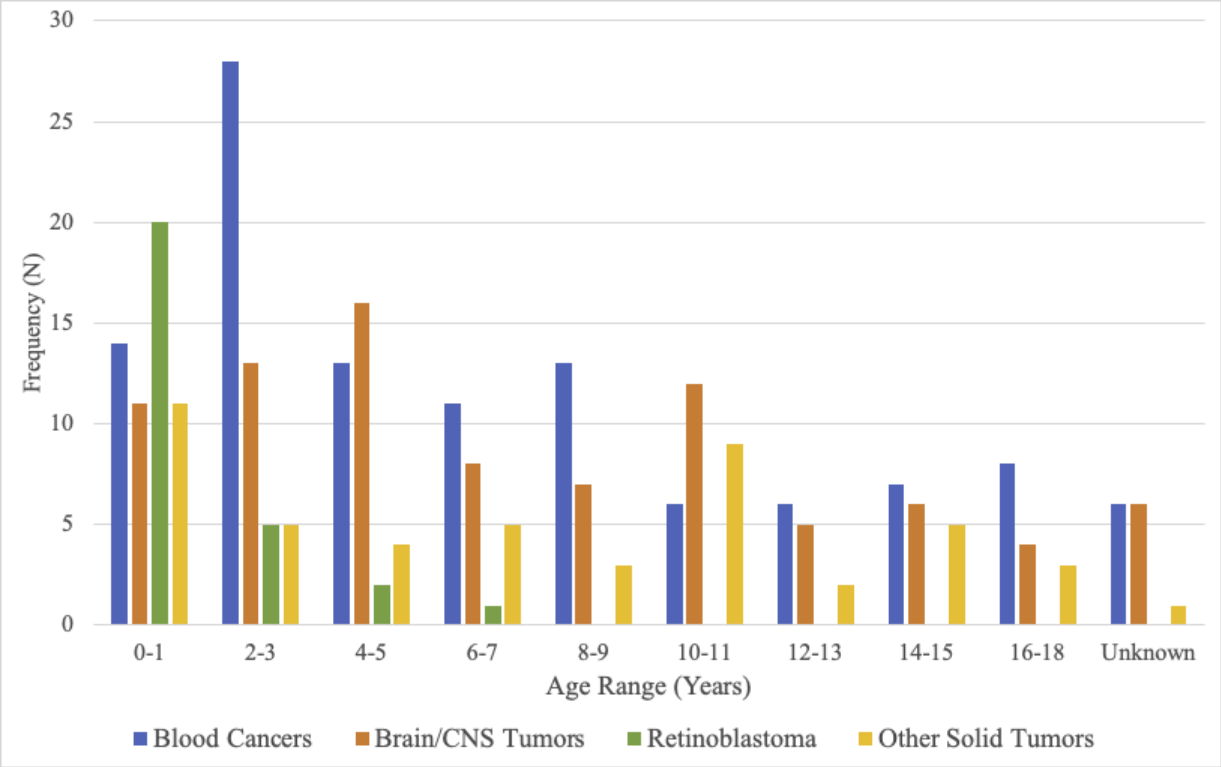

Figure 4: Age distribution of first cancer types. Ages of diagnosis were stratified by the four cancer types (blood cancer, brain/CNS tumors, retinoblastoma, and other solid tumors)

3.1.2 MIPOGG Risk Classification and CPS Status

Participants were categorized by first cancer type, and each individual was assigned a risk classification depending on the presence or absence of criteria included in the MIPOGG algorithms. The tumor-specific criteria and universal criteria in the MIPOGG algorithms were used to determine if a participant was at high risk or low risk for a CPS. A high risk for a CPS by MIPOGG is defined as a greater than 10% likelihood for the patient to have an underlying CPS. Individuals with features in the tumor-specific and/or universal criteria were classified as high-risk for a CPS, and a genetics referral was advised. Conversely, individuals without features in the tumor-specific and/or universal criteria were classified as being at low risk for a CPS, and a genetics referral was not advised. A total of 130 (47%) individuals were classified as high-risk for a CPS, and 146 (53%) individuals were classified as low-risk for a CPS (Table 4A).

The features used to determine a risk classification in MIPOGG were dependent on the participant's tumor type. In Table 4A slightly over one-third to one-half of participants were classified as high-risk in each cancer type, and participants with retinoblastoma were direct referrals. Certain cancer types are highly associated with a CPS and are automatically classified as high-risk for a CPS by MIPOGG; participants with these cancer types were considered direct referrals. Cancer types that were considered direct referrals in this study included retinoblastoma, atypical teratoid rhabdoid tumor, hepatoblastoma, gastrointestinal carcinoma, meningioma, and renal cell carcinoma (Table 4B). The cancer subtypes that were not considered for direct referral were characterized to determine the proportion of participants in each CPS risk classification (Table 4C).

Table 4A: Distribution of CPS Risk Classification by First Cancer Type

Cancer Type		*High Risk for a CPS*	*Low Risk for a CPS*
	N=276	N=130 (47%)	N=146 (53%)
Blood Cancers	112	47 (42%)	65 (58%)
Brain/CNS Tumors	88	30 (34%)	58 (66%)
Retinoblastoma	28	28 (100%)	0 (0%)
Other Solid Tumors	48	25 (52%)	23 (48%)

Percentages of each CPS risk classification were calculated by cancer type. High risk for a CPS refers to participants with the presence of one or more features assessed in the tumor specific and/or universal referral criteria. Low risk for a CPS refers to the absence of all features assessed in the tumor specific and/or universal criteria. All individuals in the three cancer categories (excluding Retinoblastoma) were assessed for features in the universal criteria, but only those with certain tumor types were assessed for features in the tumor-specific criteria.

Table 4B: Frequency of Cancer Subtypes Among Direct Referrals

Direct Referral	N=35
Retinoblastoma	28
Atypical Teratoid Rhabdoid Tumor	2
Hepatoblastoma	2
Gastrointestinal Carcinoma	1
Meningioma	1
Renal Cell Carcinoma	1

Direct Referral: Individuals with cancer types that are classified as high-risk for a CPS by MIPOGG. Independent of other features in the personal and family history, patients diagnosed with these tumor types are directly referred to genetics as per the MIPOGG.

Table 4C: Distribution of CPS Risk Classification by Cancer Subtype (Excluding Direct Referrals)

Blood Cancer	N=112	*High Risk for a CPS* *N=48*	*Low Risk for a CPS* *N=64*
ALL	82	31 (38%)	51 (62%)
AML / MDS	29	17 (59%)	12 (41%)
Lymphoma	1	0 (0%)	1 (100%)
Brain/CNS Tumors	N=85	*High Risk for a CPS* N=26	*Low Risk for a CPS* N=59
Craniopharyngioma	26	3 (12%)	23 (89%)
High Grade Glioma	16	5 (31%)	11 (69%)
Low Grade Glioma	15	8 (53%)	7 (47%)
Medulloblastoma	15	6 (40%)	9 (60%)
Ependymoma	10	4 (40%)	6 (60%)
Chordoma	1	0 (0%)	1 (100%)
CNS embryonal tumors/PNET-NOS	1	0 (0%)	1 (100%)
ETMR	1	0 (0%)	1 (100%)

Table 4C (Cont.): Distribution of CPS Risk Classification by Cancer Subtype (Excluding Direct Referrals)

Other Solid Tumors		*High Risk for a CPS*	*Low Risk for a CPS*
	N=44	N=21	N=23
Neuroblastic Tumor	13	4 (31%)	9 (60%)
Wilms Tumor	6	5 (83%)	1 (17%)
Ewing Sarcoma/ Askin Tumor/ PNET	5	1 (20%)	4 (80%)
Rhabdomyosarcoma	5	4 (80%)	1 (20%)
Germ Cell Tumors	3	0 (0%)	3 (100%)
Osteosarcoma	3	1 (33%)	2 (67%)
Non-medullary Thyroid Carcinoma	2	2 (100%)	0 (0%)
Sarcoma NOS	2	1 (50%)	1 (50%)
Clear Cell Sarcoma of the Kidney	1	1 (100%)	0 (0%)
Desmoid Type Fibromatosis	1	1 (100%)	0 (0%)
Desmoplastic Round Cell Tumor	1	0 (0%)	1 (100%)
Low Grade Fibromyxoid Sarcoma	1	0 (0%)	1 (100%)
Melanoma	1	1 (100%)	0 (0%)

High risk for a CPS refers to participants with the presence of one or more features assessed by MIPOGG. Low risk for a CPS refers to the absence of all features assessed by MIPOGG. Percentages of each CPS risk classification were calculated by cancer subtype. All individuals in the four cancer categories were assessed for features in the universal criteria, but only those with certain tumor types were assessed for features in the tumor-specific criteria. ALL: Acute lymphoblastic leukemia; AML/MDS: Acute myeloid leukemia/ myelodysplastic syndrome; PNET: Primitive neuroectodermal tumor; NOS: Not otherwise specified, ETMR: Embryonal tumor with multilayered rosettes.

Genetic testing was performed with an NGS panel (research basis) of over 150 CPS-related genes at St. Jude Children's Research Hospital, and of the 276 individuals, 27 (10%) were identified to have a CPS. Participants who were identified with a CPS were referred to as "CPS-positive," indicating the presence of a pathogenic or likely pathogenic variant on the NGS Panel. The 27 CPS-positive participants were stratified by cancer type (blood, brain/CNS tumor, retinoblastoma and other solid tumors) in Table 5A. The highest proportion of CPS-positive

participants was in the retinoblastoma group, 38% (N=10). Of all of the CPS-positive participants (N=27), the majority were identified with an autosomal dominant CPS (N=25), while two individuals were identified to have an autosomal recessive CPS, constitutional mismatch repair deficiency (CMMRD). Each CPS-positive participant was classified as high- or low-risk for a CPS, depending on the presence or absence of features in the tumor-specific and/or universal criteria at the time of his or her first cancer diagnosis (Table 5B).

Table 5A: Distribution of CPS Status by Cancer Type

First Cancer Type	N=276	*CPS Positive* N=27 (10%)	*CPS Negative* N=249 (90%)
Blood Cancer	112	7 (6%)	105 (94%)
Brain/CNS Tumor	88	8 (9%)	88 (91%)
Retinoblastoma	28	10 (36%)	18 (64%)
Other Solid Tumors	48	2 (4%)	46 (96%)

First cancer type diagnosed in a participant was specified because some participants had received a second cancer diagnosis at the time of genetic testing, and these were not included in this analysis. Percentages of each CPS risk classification were calculated by cancer type. CPS-Positive: The presence of a pathogenic or likely pathogenic variant in a CPS-associated gene; CPS-Negative: The absence of a pathogenic or likely pathogenic variant in a CPS-associated gene.

Table 5B: Distribution of Pediatric and Adult-Onset CPSs

CPS	*N*	*Gene*	*Risk Classification by MIPOGG*	*Cancer Type*
Pediatric-Onset CPSs (N=24)				
Hereditary Retinoblastoma	10	*RB1*	High	Retinoblastoma
Neurofibromatosis Type I (NF1)	1	*NF1*	High	ALL
	2		High	Low Grade Glioma
Constitutional Mismatch Repair Deficiency (CMMRD)	1	*PMS2*	High	ALL
	1		High	ALL† and High Grade Glioma‡
BAP1-tumor predisposition syndrome	1	*BAP1*	Low	Ewing Sarcoma
Familial Adenomatous Polyposis (FAP)	1	*APC*	High	Craniopharyngioma
Gorlin syndrome (Nevoid Basal Cell Carcinoma syndrome (NBCCS))	1	*PTCH1*	High	Medulloblastoma
Li-Fraumeni syndrome (LFS)	1	*TP53*	High	Astrocytoma
Noonan syndrome	1	*PTPN11*	High	ALL
Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia	1	*CBL*	Low	Germ Cell Tumor
SMARCA4-related syndrome	1	*SMARCA4*	High	Neuroblastic Tumor
SDHA Hereditary Paraganglioma/ Pheochromocytoma (PGL/PCC)	1	*SDHA*	Low	Low Grade Glioma
WT1-Related syndrome	1	*WT1*	High	ALL
Adult-Onset CPSs (N=3)				
Hereditary Breast and Ovarian Cancer (HBOC)	1	*PALB2*	Low	High Grade Glioma
	1	*BRCA2*	Low	ALL
Lynch syndrome	1	*MSH2*	High	High Grade Glioma

CPS: cancer predisposition syndrome. Pediatric-Onset CPSs: Cancer predisposition syndromes associated with an increased risk for cancer in children. Adult-Onset CPSs: cancer predisposition syndromes associated with an increased risk for cancer in adulthood. Risk classification by MIPOGG: *High risk* refers to the presence of features in the universal and/or tumor-specific criteria and *low risk* for a CPS refers to the absence of features in the universal and tumor-specific criteria. Cancer Type: Current and previous cancer types in the participant at the time of enrollment in the G4K study. ALL: Acute lymphoblastic leukemia. All of the CPSs identified were autosomal dominant conditions except CMMRD, an autosomal recessive disorder. Two individuals were diagnosed with CMMRD, and both were identified with compound heterozygous pathogenic variants in *PMS2*. †First cancer diagnosed. ‡Second cancer diagnosed.

3.2 MIPOGG Analysis

3.2.1 MIPOGG Criteria and CPS Status

Of the 276 participants in the study, 130 were classified as being at high risk for a CPS and 146 were classified as having a low risk for a CPS by MIPOGG (Table 6A). The proportion of CPS-positive participants who were identified in each category of risk classification by MIPOGG were compared, and a 95% confidence interval for the difference in proportion between the two groups was calculated. The confidence interval excluded zero, which demonstrated a statistically significant difference in the proportion of CPS-positive participants in each category; the high-risk group had a larger proportion of participants with a CPS than the low-risk group (17% vs 3%).

A similar characterization was performed with the exclusion of the three individuals with adult-onset CPSs (two individuals with Hereditary Breast and Ovarian Cancer (HBOC) and one individual with Lynch syndrome) (Table 6B). Adult-onset CPSs were excluded because these conditions are not typically associated with an increased risk for pediatric cancers and, thus, MIPOGG is not intended to identify those patients. The proportion of participants identified with a CPS, excluding the adult-onset conditions, was compared by MIPOGG risk classification, and a 95% confidence interval for the difference in proportion between the two groups was calculated. Similar to the analysis assessing all participants with a CPS, a statistically significant difference was identified in the proportion of participants with a pediatric-onset CPS in each category. The high-risk group had a larger proportion of CPS-positive participants than the low-risk group (16% vs. 2%).

In both groups—all participants and participants only with pediatric-onset CPSs—the positive predictive value of MIPOGG (17% and 16%, respectively) was significantly associated with identifying a CPS-positive participant when compared to the percentage of participants who

were CPS-positive and classified as low-risk rate in each group (3% and 2% respectively). Additionally, the proportion of participants classified as low-risk for a CPS and who were not identified to have a CPS (the negative predictive value (NPV)), was substantial in all participants (97%). This was further increased to 98% for pediatric-onset CPSs), which indicates the effectiveness of MIPOGG to identify patients at high and low risk of pediatric-onset CPSs in this study cohort.

These findings illustrate the association between individuals with a CPS and the presence of features assessed by MIPOGG. To further investigate the significance of association between CPS status and the features used for risk classification, the features were individually assessed.

Table 6A: Distribution of Risk Classification by MIPOGG in All Participants

Risk Classification by MIPOGG	N=276	*CPS Positive* N=27	*CPS Negative* N=249	*Difference in Proportion (95% CI)*
High	130	22 (17%)	108 (83%)	0.14 (0.06-0.22)*
Low	146	5 (3%)	141 (97%)	

Table 6B: Distribution of Risk Classification by MIPOGG in Pediatric-Onset CPSs

Risk Classification by MIPOGG	N=273	*CPS Positive* N=24	*CPS Negative* N=249	*Difference in Proportion (95% CI)*
High	129	21 (16%)	108 (84%)	0.14 (0.07-0.22)*
Low	144	3 (2%)	141 (98%)	

Tables 6A and 6B: Percentages of CPS status were calculated for each risk classification by MIPOGG. CPS: Cancer predisposition syndrome. CPS Positive: The presence of a pathogenic or likely pathogenic variant in a CPS-associated gene; CPS Negative: The absence of a pathogenic or likely pathogenic variant in a CPS-associated gene. Risk classification by MIPOGG: *High-risk* refers to the presence of features in the universal and/or tumor-specific criteria and *low-risk* for a CPS refers to the absence of features in the universal and tumor-specific criteria. Difference in Proportion: The proportion of participants identified with a CPS was compared by MIPOGG risk classification, and a 95% confidence interval for the difference in proportion between the two groups was calculated. *The confidence interval excluded zero, which demonstrated a statistically significant difference in the proportion of participants with a CPS in each category; the high-risk group had a larger proportion of participants with a CPS than the low-risk group, including (5A) All participants and (5B) Pediatric-onset CPSs. **5B**) Pediatric-Onset CPSs: Cancer predisposition syndromes associated with an increased risk for cancer in children

3.2.2 Categories in the MIPOGG Algorithm and CPS Status

Features in the MIPOGG algorithms fit into three categories: personal history criteria, tumor-specific family history criteria, and universal family history criteria (Figure 1). The personal history category includes features that were only present in the participant, and was assessed in the tumor-specific criteria and universal criteria (Table 1; Figure 2). Family history features in the tumor-specific and universal criteria were independently assessed (Table 1; Figure 2). Of the participants who were classified as being at high risk for a CPS (n=130), the majority of CPS-positive participants, 86% (n=98), had features in the personal history category (Table 7A). Fewer participants with a CPS had features in the tumor-specific family history (n=29)

and/or universal family history (n=37). Although many of the participants considered at high risk by MIPOGG were identified with features in one category (n=96, 74%) a notable proportion had features in more than one category (n=34, 26%). The CPS status of participants with the presence of features in a single category or a combination of the personal and/or family history categories was analyzed to determine if a significant association existed between the CPS-positive participants and personal and/or family history features (Table 7B).

Participants with features in the categories that only included family history were of particular interest. Each independent MIPOGG category (i.e., a single personal or family history feature, a combination of personal and/or family history features, or the absence of all features) was analyzed to determine if certain categories more often identified individuals who were CPS-positive. The proportions of participants with and without a CPS were compared within each MIPOGG category, and 95% confidence intervals for the difference in proportion between the two groups were calculated. The confidence intervals excluded zero, indicating a statistically significant difference by CPS status for three scenarios: 1) for the personal history *only,* 2) the personal history *and* universal family history, and 3) for participants without any features, but the other comparisons were not statistically significant. The participants in the personal history-only and the personal history *and* universal family history categories had a larger proportion of participants with a CPS than without a CPS, 52% vs. 24% and 19% vs. 5%, respectively. The low-risk category (participants with no features in the MIPOGG categories) had a larger proportion of participants without a CPS than with a CPS, 56% vs. 19%. Notably, the categories that included only family history features (tumor-specific family history *only*, universal family history *only*, and universal family history *and* tumor-specific family history), were not significantly associated with CPS status. The significant association between two categories, including personal history features and CPS-positive participants, suggests that personal history

features were often a predictor of a CPS in this study cohort. While family history features alone were not significantly associated with a CPS, there were participants with a CPS who were classified as high-risk for a CPS due only to features in their family history.

Table 7A: Distribution of CPS Status Among Participants Classified as High Risk for a CPS by MIPOGG Referral Category

MIPOGG Referral Category	N=130**	*CPS Positive* N=22**	*CPS Negative* N=108**
Personal History	98	19 (86%)	77 (71%)
Universal Family History	37	6 (27%)	31 (28%)
Tumor Specific Family History	29	3 (13%)	26 (24%)

Table 7B: Distribution of CPS Status Among All Participants by MIPOGG Referral Category

MIPOGG Referral Category	N=276	*CPS Positive* N=27	*CPS Negative* N=249	*Difference in Proportion (95% CI)*
Personal History *Only*	74	14 (52%)	60 (24%)	0.28 (0.07-0.47)*
Universal Family History *Only*	9	0 (0%)	9 (4%)	0.04 (-0.12-0.07)
Tumor Specific Family History *Only*	13	2 (7%)	11 (4%)	0.03 (-0.04-021)
Personal History *and* Universal Family History	18	5 (19%)	13 (5%)	0.13 (0.01-0.34)*
Personal History *and* Tumor Specific Family History	6	0 (0%)	6 (2%)	0.02 (-0.13-0.05)
Tumor Specific Family History *and* Universal Family History	6	1 (4%)	5 (2%)	0.01 (-0.03-0.19)
Personal History *and* Tumor Specific Family History *and* Universal Family History	4	0 (0%)	4 (2%)	0.01 (-0.14-0.04)
Low Risk (No features in the MIPOGG referral categories)	146	5 (19%)	141 (56%)	0.38 (0.17-0.51)*

Tables 7A and 7B: Percentages of CPS status were calculated for each MIPOGG referral category. Personal history: the presence of personal history features assessed in the tumor-specific (including tumor types automatically considered high-risk for a CPS) and/or universal criteria. Universal Family History: the presence of family history features assessed in the universal criteria. Tumor-Specific Family History: the presence of family history features assessed in the tumor-specific criteria. CPS: Cancer predisposition syndrome. CPS-Positive: The presence of a pathogenic or likely pathogenic variant in a CPS-associated gene; CPS-Negative: The absence of a pathogenic or likely pathogenic variant in a CPS-associated gene. **7A) ****The total of the frequencies and percentages in each column do not sum to N (100%) because some individuals had features in multiple referral categories. **7B)** Low-risk: the absence of personal and family history features assessed by MIPOGG. Difference in Proportion: The proportion of participants in each MIPOGG category was compared by CPS status, and a 95% confidence interval for the difference in proportion between the two groups was calculated. *The confidence interval excluded zero, which demonstrated a statistically significant difference in the proportion of participants with the MIPOGG category in with each CPS status; the personal history *only* and the personal history *and* universal family history categories had larger proportions of CPS-positive participants than CPS-negative, and the low-risk category had a larger proportion of CPS-negative participants than CPS-positive.

Three participants with a CPS (Li-Fraumeni syndrome, Noonan syndrome, and *WT1*-related syndrome) were classified as high-risk for a CPS, primarily due to family history features. Two participants had features in only the tumor-specific family history category, and one participant had a feature in both the tumor-specific and the universal family history categories. Two participants had a FDR or SDR with sarcoma, breast cancer, brain tumor, leukemia, adrenocortical carcinoma, or lung cancer before age 56, which was a criterion included in tumor-specific algorithms for individuals with ALL or a high-grade glioma. The participant with Noonan syndrome was diagnosed with ALL and had a paternal grandmother who died from breast cancer at age 32. The participant with Li-Fraumeni syndrome was diagnosed with a high-grade glioma and had a mother with breast cancer at age 26; this participant also had a feature assessed in the universal family history category (e.g. FDR and/or half-sibling with cancer before age 50). The participant with *WT1*-related syndrome was diagnosed with ALL and had a paternal grandfather with colon cancer diagnosed in his late 20's. This participant met the tumor-specific criteria for ALL due to the presence of a close relative with colorectal cancer, endometrial (uterine) cancer and/or ovarian cancer before age 56. These three participants were classified as high-risk for a CPS, primarily due to their family histories. Other participants with a CPS who were classified as high-risk for a CPS were identified due to features in both their personal history and family history. Both the tumor-specific and universal family history features were reported in participants with a CPS; however, the tumor-specific family history questions were not assessed for all participants. Characterization of the features assessed in the universal family history category was performed to determine if certain features were more commonly reported in participants with or without a CPS, regardless of tumor type.

3.2.3 MIPOGG Universal Family History Category Analysis

The features in the universal family history category were characterized to better understand the distribution of CPS status with each feature (Table 8). The universal family history category consists of four features: FDR (and half-sibling) with cancer before age 50, aunt/uncle/first cousin/grandparent with cancer before age 19, close relative with the same cancer type or same organ affected by cancer, or close relative with multiple primary tumors (excluding non-melanoma skin cancer) before age 60. A close relative was defined as a parent, sibling, half-sibling, aunt/uncle, grandparent or first cousin. The vast majority of participants in the study, 87% (n=240), did not have a feature in the universal family history category. The most common universal family history feature reported by participants was an aunt/uncle/first cousin/grandparent with cancer before age 19 (n=14). Of the participants with a CPS (N=27), the most common universal family history feature was a FDR and/or half-sibling with cancer before age 50, with three participants (11%) having this history. The CPSs identified in these three individuals included Li-Fraumeni syndrome, hereditary retinoblastoma, and CMMRD. The participant with Li-Fraumeni syndrome had a mother diagnosed with breast cancer at age 26, the participant with hereditary retinoblastoma had a mother diagnosed with unilateral retinoblastoma at age 18 months, and the participant with CMMRD had a mother diagnosed with cervical cancer in her 20's-30's. Some participants in the study met multiple family history criteria (eight participants met two criteria and one participant met three criteria). Only one out of the six participants with two universal family history features was identified to have a CPS. This was the same participant identified with hereditary retinoblastoma. Statistical significance was not assessed for the difference in proportion in each universal family history feature and CPS status due to the small number of participants meeting each of the family history features independently.

Table 8: Distribution of CPS Status by Universal Family History Feature

Universal Family History Feature		*CPS Positive*	*CPS Negative*
	N=276**	N=27**	N=249**
FDR and/or Half-Sibling with Cancer Before Age 50	13 (5%)	3 (11%)	10 (4%)
Aunt/Uncle/First Cousin/Grandparent with Cancer Before Age 19	14 (5%)	2 (7%)	12 (5%)
Close Relative with the Same Cancer Type or Same Organ Affected by Cancer	11 (4%)	2 (7%)	9 (4%)
Close Relative with Multiple Primary Tumors+ Before Age 60	7 (3%)	0 (0%)	7 (3%)
None	240 (87%)	21 (78%)	219 (88%)

**The total frequency of the column does not sum to N (100%) because some participants had more than one universal family history feature. FDR: First-degree relative. Close relative: Parent, sibling, half-sibling, aunt/uncle, grandparent, or first cousin. +Multiple primary tumors excluding non-melanoma skin cancer. CPS: Cancer predisposition syndrome. CPS-Positive: The presence of a pathogenic or likely pathogenic variant in a CPS-associated gene; CPS-Negative: The absence of a pathogenic or likely pathogenic variant in a CPS-associated gene.

Each feature in the universal family history category was stratified by cancer type and CPS status (Table 9A-9D). Among the participants with a feature in the universal family history category, the most common cancer type was blood cancer. Blood cancer was the most common cancer type in participants regardless of universal family history category, with the exception of an aunt/uncle/first cousin/grandparent with cancer before age 19, where equal numbers had blood cancers and brain/CNS tumors (n=5, 36%). Among participants with a blood cancer, 43% had a FDR and/or half-sibling with cancer before age 50, 36% had an aunt/uncle/first cousin/grandparent with cancer before age 19, 73% had a close relative with the same cancer type or same organ affected by cancer, and 71% had a close relative with multiple primary tumors (excluding non-melanoma). Regardless of universal family history category, blood cancer was the most common cancer type in participants who were CPS-negative (Table 9A).

Differences across universal family history categories in CPS-positive participants could not be assessed due to the small numbers of participants in each group.

Table 9A: Distribution of CPS Status by Cancer Type with a FDR and/or Half-Sibling with Cancer Before Age 50

Cancer Type		*CPS Positive*	*CPS Negative*
	N=14	N=3	N=10
Blood Cancers	6 (43%)	1 (33%)	5 (50%)
Brain/CNS Tumors	3 (21%)	1 (33%)	2 (20%)
Retinoblastoma	3 (21%)	1 (33%)	2 (20%)
Other Solid Tumors	1 (7%)	0 (0%)	1 (10%)

Table 9B: Distribution of CPS status by Cancer Type with an Aunt/Uncle/First Cousin/Grandparent with Cancer Before Age 19

Cancer Type		*CPS Positive*	*CPS Negative*
	N=14	N=2	N=12
Blood Cancers	5 (36%)	0 (0%)	5 (42%)
Brain/CNS Tumors	5 (36%)	1 (50%)	4 (33%)
Retinoblastoma	1 (7%)	0 (0%)	1 (8%)
Other Solid Tumors	3 (21%)	1 (50%)	2 (17%)

Table 9C: Distribution of CPS status by Cancer Type with a Close Relative with Same Cancer Type or Same Organ Affected by Cancer

Cancer Type		*CPS Positive*	*CPS Negative*
	N=11	N=2	N=9
Blood Cancers	8 (73%)	0 (0%)	8 (89%)
Brain/CNS Tumors	1 (9%)	1 (50%)	0 (0%)
Retinoblastoma	1 (9%)	1 (50%)	0 (0%)
Other Solid Tumors	1 (9%)	0 (0%)	1 (11%)

Tables 9 (A-C): FDR: First-degree relative. Close relative: parent, sibling, half-sibling, aunt/uncle, grandparent, or first cousin. +Multiple primary tumors excluding non-melanoma skin cancer. CPS-positive: individuals identified with a pathogenic or likely pathogenic variant in a gene associated with a cancer predisposition syndrome (CPS). CPS-negative: individuals who were not identified with a pathogenic or likely pathogenic variant in a gene associated with a CPS

Table 9D: Distribution of CPS status by Cancer Type with a Close Relative with Multiple Primary Tumors. Before Age 60

Cancer Type		*CPS Positive*	*CPS Negative*
	N=7	N=0	N=7
Blood Cancers	5 (71%)	0 (0%)	5 (71%)
Brain/CNS Tumors	2 (29%)	0 (0%)	2 (29%)
Retinoblastoma	0 (0%)	0 (0%)	0 (0%)
Other Solid Tumors	0 (0%)	0 (0%)	0 (0%)

Table 9D: Close relative: parent, sibling, half-sibling, aunt/uncle, grandparent, or first cousin. CPS-positive: individuals identified with a pathogenic or likely pathogenic variant in a gene associated with a cancer predisposition syndrome (CPS). CPS-negative: individuals who were not identified with a pathogenic or likely pathogenic variant in a gene associated with a CPS.

3.3 Age of Ascertainment and Family Cancer History

Details about each participant's family history of cancer were collected as part of the G4K study by genetic counselors at St. Jude Children's Research Center and organized based on the participant's age at ascertainment. The majority of patients had their family history elicited at the time of their first cancer diagnosis; however, some participants' ages at first cancer diagnosis were not known, and their family histories were obtained at the time of enrollment into the G4K study. Individuals diagnosed with their first cancer at age 12-13 were more likely than other groups to have any family history of cancer (100%, N=14 had a family history), and those diagnosed at age 4-5 were the least likely (83%, N=30 had any family history). The majority of participants in the age 16-18 group (77%, N=13) had a FDR and/or SDR with cancer, while only 39% of participants aged 0-1 (N=22) had a FDR and/or SDR with cancer (Table 10).

The proportion of participants with a family cancer history only in relatives beyond a first- and/or second-degree relationship was calculated for each age group. A 95% confidence interval was calculated for each proportion to account for the varying sample size in each age group. The proportion and confidence intervals were plotted to visually determine if significant

trends appeared and minimal overlap between confidence intervals was suggestive of a significant difference between proportions (Appendix B). There appeared to be a significant difference between the youngest age groups, 0-1 and 2-3, and the oldest age group, 16-18. Surprisingly, the 10-11 age group also appeared to differ significantly from the 16-18 age group. Because of these apparent differences, proportions of family cancer histories affecting relatives beyond a first- and/or second-degree relationship by age group were compared, and 95% confidence intervals for the difference in proportion were calculated for each age group with 16-18 as the reference. The confidence intervals excluded zero for comparisons between age groups, 0-1, 2-3, and 10-11 age groups. This demonstrated a statistically significant difference in the proportion of a family history of cancer only in relatives beyond a first- and/or second-degree relationship and age of the participant at ascertainment for these age groups. This suggested a possible association between age of the participant and the degree of relationship of relatives with cancer. Participant age at cancer diagnosis was already shown to be correlated with certain cancer types (Figure 4) and degree of relationship of family history reporting (Appendix B). Because of these two associations, the degree of relationship of family cancer history by participant cancer type was analyzed to see if trends were consistent with what was seen in the entire cohort of patients.

Table 10: Degree of Relationship of Family Cancer History Distributed by Participant Age at Ascertainment

Age Range (Years)	N=276	*Any Family Cancer History* N=247 (90%)	*FDR and/or SDR with Cancer* N=140 (51%)
0-1	56	50 (89%)	22 (39%)
2-3	53	46 (87%)	23 (43%)
4-5	36	30 (83%)	17 (47%)
6-7	26	23 (89%)	15 (58%)
8-9	24	21 (88%)	15 (63%)
10-11	28	27 (96%)	13 (46%)
12-13	14	14 (100%)	8 (57%)
14-15	22	21 (96%)	14 (64%)
16-18	17	15 (88%)	13 (77%)

Percentages of family cancer history were calculated for each age range. Age at ascertainment: The age of the participant when the family history was obtained; this was either at the age at first cancer diagnosis, or if unknown, at the age of their most recent cancer diagnosis. Any family cancer history: Individuals with a family member diagnosed with cancer regardless of degree of relationship, cancer type, or age of cancer diagnosis. FDR: First-degree relative. SDR: second-degree relative. FDR and/or SDR with cancer: individuals with a FDR (parent or sibling) and/or SDR (half-sibling, grandparent, aunt/uncle) diagnosed with cancer regardless of cancer type or age of diagnosis.

Similar to the data shown in Table 10, the proportion of participants with a family cancer history affecting relatives beyond a first- and/or second-degree relationship was characterized for each cancer type and stratified by age group. A 95% confidence interval was calculated for each proportion to account for the varying sample sizes in each age group. The proportions and confidence intervals were plotted to determine visually if significant trends appeared (Figure 5). Although no obvious trends appeared among the age groups, 95% confidence intervals for the difference in proportions were calculated for the age groups with the largest differences in proportion for each cancer type. All confidence intervals include zero, indicating the differences were not statistically significant between the two age groups. Additionally, the age categories previously identified with significant differences when assessing age at ascertainment regardless

of cancer type (16-18 compared to 0-1, 2-3, and 10-11) were not significant when accounting for cancer type. As expected, but not previously accounted for in Table 10, the family histories in the retinoblastoma group were only reported for participants in three age categories (0-1, 2-3, and 4-5) because the age of cancer diagnosis in the retinoblastoma group were exclusively in these categories.

Figure 5: Distribution of Family Cancer History Affecting Relatives Beyond a First- or Second-Degree Relationship by Age at Ascertainment and Participant Cancer Type

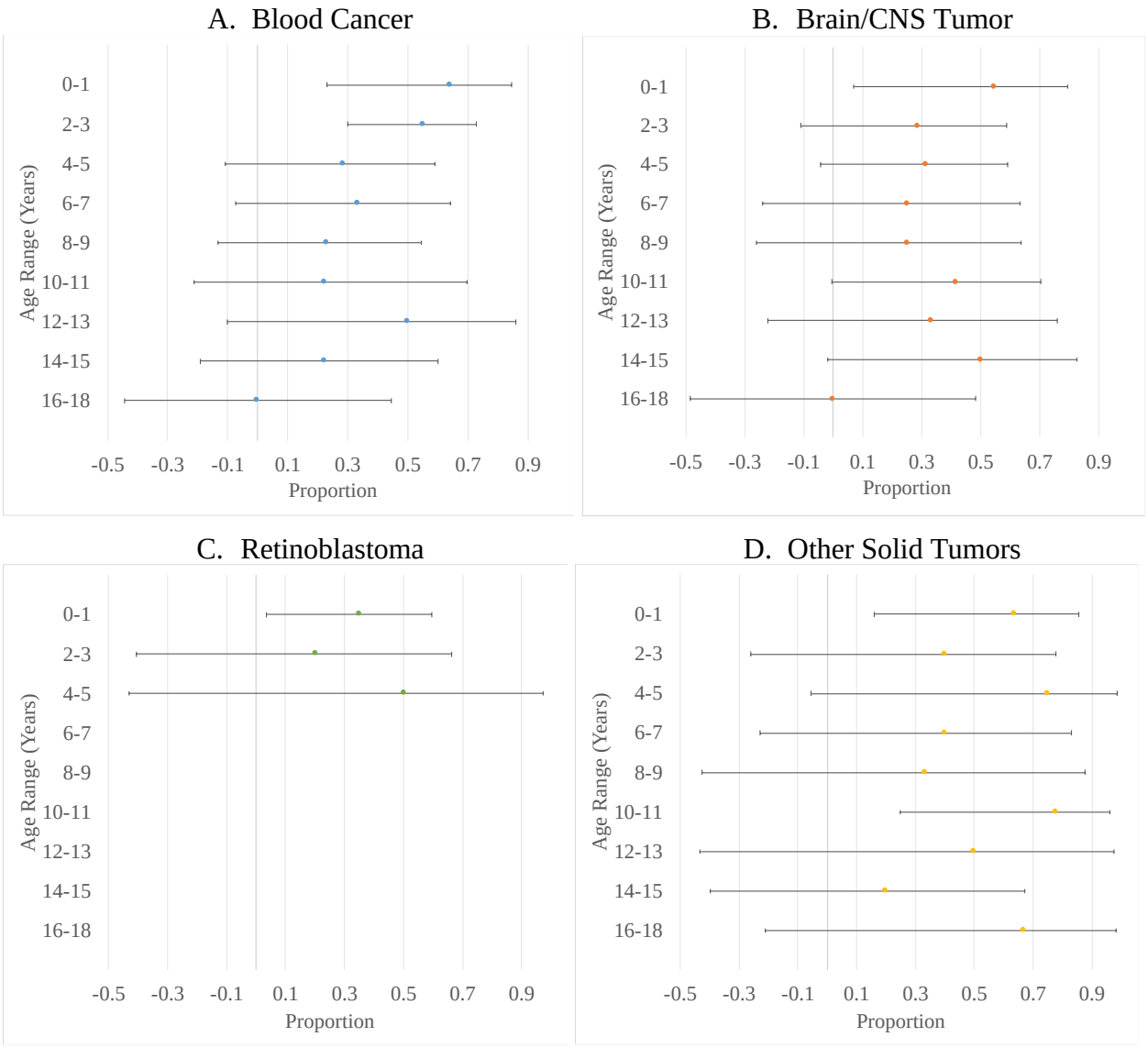

Figure 5(A-D): A: Participants with blood cancers. **B**: Participants with brain/CNS tumors. **C**: Participants with retinoblastoma. **D**: Participants with other solid tumors. Age at ascertainment: The age of the participant when the family history was obtained; this was either at the age at first cancer diagnosis, or if unknown, at the age of their most recent cancer diagnosis. Proportion: A 95% confidence interval was calculated for the proportion of participants with a family cancer history affecting relatives beyond a first- or second-degree relationship and stratified by age at ascertainment. Confidence intervals account for varying sample sizes in each age group and indicate the range of values within which the true proportion lies; the upper and lower limits of the confidence intervals are depicted with bars. Data points illustrate the proportion of participants in the study cohort with a family cancer history affecting relatives beyond a first- or second-degree relationship.

3.4 Analysis and Characterization of Types of Cancer History in a Family

Additional types of family history, not specifically assessed in the universal or tumor specific family history categories, were characterized to analyze association with CPS status (Table 11). The types of family history assessed did not exclude any cancer types and included individuals with a family history of cancer, regardless of age or degree of relationship. The types of family history assessed included a family history of cancer before age 19, cancer diagnosed in a family member before age 19 regardless of their degree of relationship, a FDR with cancer, regardless of age at diagnosis, and a FDR with cancer before age 19 (Table 11). The proportion of participants with a CPS was compared by the type of family history, and 95% confidence intervals for the difference in proportions were calculated to determine statistical significance in the two proportions. All confidence intervals include zero, which demonstrated that a statistically significant difference was not identified in the proportion of participants with a CPS within each category of family history. Although a significant association was not identified between the type of family history and CPS status, some types of family history are concerning for a CPS, such as a family history of cancer before age 19 and/or a FDR with cancer. Participants with these features in their family histories who were not identified with a CPS were of particular interest.

Table 11: CPS Status by Type of Cancer History in a Family

	N=276	*CPS Positive* N=27	*CPS Negative* N=249	Difference in Proportion (95% CI)
Any Family Cancer History				
Yes	247	24 (10%)	223 (90%)	0.01 (-0.08-0.19)
No	29	3 (10%)	26 (90%)	
Family Cancer History Before Age 19				
Yes	32	3 (9%)	29 (91%)	0.005 (-0.17-0.09)
No	244	24 (10%)	220 (90%)	
FDR with Cancer				
Yes	14	3 (21%)	11 (79%)	0.12 (-0.04-0.42)
No	262	24 (9%)	238 (91%)	
FDR with Cancer Before Age 19				
Yes	3	1 (33%)	2 (67%)	0.24 (-0.08-0.78)
No	273	26 (10%)	247 (91%)	

Any family cancer history: Individuals with a family member diagnosed with cancer regardless of degree of relationship or age of cancer diagnosis. CPS-Positive: The presence of a pathogenic or likely pathogenic variant in a CPS associated gene; CPS-Negative: The absence of a pathogenic or likely pathogenic variant in a CPS associated gene; FDR: first-degree relative; Difference in Proportion: The proportion of participants identified with a CPS was compared by type of cancer history in a family, and a 95% confidence interval for the difference in proportion between the two groups was calculated. All confidence intervals include zero, indicating that a statistically significant difference was not identified in the proportion of participants with a CPS in each category.

The degrees of relationship among family members with pediatric cancers were characterized by participant CPS status and family member cancer type (Table 12). In the group of participants who were CPS-negative, the most common type of pediatric cancer diagnosed in relatives was blood cancer (n=18), and 78% (n=14) of the blood cancers were reported in a relative beyond a first- and/or second-degree relationship. The vast majority (N=25, 74%) of family members with pediatric cancers, regardless of cancer type, were reported in a relative beyond a first- and/or second-degree relationship. Five CPS-negative participants had two relatives with pediatric cancers; these relatives were all beyond a first- and/or second-degree

relationship. Three CPS-positive participants reported a family cancer history before the age of 19; one was a FDR with retinoblastoma, and two were in relatives beyond a first- and/or second-degree relationship —one with ALL and the other with an unspecified cancer. The CPSs in these three participants were hereditary retinoblastoma, FAP, and *SMARCA4*-related syndrome. The participant with a mother with retinoblastoma was described in Table 9A due to the presence of a FDR and/or half sibling with cancer before age 50. The individual with FAP reported a maternal first cousin diagnosed with an unspecified cancer type at age 17, and the individual with *SMARCA4*-related syndrome reported a maternal first cousin diagnosed with ALL at age 13.

Table 12: Distribution of the Degree of Relationship of Pediatric Cancers in Family Members by Participant CPS Status and Family Member Cancer Type

Family Member Pediatric Cancer Type		*FDR*	*SDR*	*Beyond a FDR and/or SDR*
	N	N	N	N
Family Members of CPS Negative Participants (N=34)				
Brain	5	0	0	5
Blood	18	1	3	14
Retinoblastoma	1	0	1	0
Embryonal Rhabdomyosarcoma	1	0	0	1
Colon	1	0	0	1
Ovarian Teratoma	1	0	0	1
Thyroid	1	1	0	0
Polyposis§	1	0	1	0
Testicular	1	0	0	1
NOS	4	0	2	2
Total	**34**	**2**	**7**	**25**
Family Members of CPS Positive Participants (N=3)				
Blood	1	0	0	1
Retinoblastoma	1	1	0	0
NOS	1	0	0	1
Total	**3**	**1**	**0**	**2**

Pediatric cancer: Cancers diagnosed in before age 19. CPS Positive: The presence of a pathogenic or likely pathogenic variant in a CPS associated gene. CPS Negative: The absence of a pathogenic or likely pathogenic variant in a CPS associated gene. FDR: first-degree relative. SDR: second-degree relative. Beyond a FDR and/or SDR: A relative beyond a first- and/or second-degree relationship. NOS: cancer type was not otherwise specified. §Relative with >100 polyps and a clinical diagnosis of FAP. Percentages for the degree of relationship of relatives with a pediatric cancer were not calculated due to the small sample size.

The age of diagnosis in FDRs with cancer was characterized by participant CPS status and FDR cancer type (Table 13). Among all participants, the cancer types in first-degree

relatives were somewhat evenly distributed, with cervical cancer (n=4) and breast cancer (n=3) being the most common. The other cancer types were present in only one or two FDRs. The cervical cancers were diagnosed primarily between ages 20 to 30 (n=3), and one mother was diagnosed at age 31. Three mothers of participants were diagnosed with breast cancer. Two were mothers of CPS-negative participants; one mother was diagnosed at age 44, and the other mother's age was not reported, and there was one mother of a CPS-positive participant who was diagnosed at age 26. This CPS-positive participant was diagnosed with Li-Fraumeni syndrome and was described in Table 6B due to the presence of a feature in both the tumor-specific family history *and* universal family history categories. FDRs of two other CPS-positive participants were diagnosed with cervical cancer and retinoblastoma. The participant with a FDR with cervical cancer was diagnosed with CMMRD and was previously described in Table 6B due a feature in the tumor-specific family history *only* category. The participant with a FDR with retinoblastoma was described in Table 9A due to the presence of a FDR and/or half sibling with cancer before age 50. Although the number of CPS-positive participants with a FDR with cancer was small, the ages of these relatives were stratified and revealed that the relatives of participants with a CPS were in the two younger age categories (diagnosed under age 19, and diagnosed between ages 20-30), while the ages of the FDRs of participants without a CPS were in all three age categories. Statistical analyses to analyze trends in the age of diagnosis in FDR with cancer were not performed due to the small number of participants in each age group.

Table 13: Distribution of Age of Diagnosis by Participant CPS status and Cancer Type in FDRs with Cancer

Cancer Type in Family Member		*Diagnosed Before Age 19*	*Diagnosed Between Age 19-30*	*Diagnosed Between Age 31-45*	*Not Reported*
	N	N	N	N	N
Family Members of CPS Negative Participants (N=11)					
Cervical	3	0	2	1	0
Blood	2	1	0	1	0
Breast	2	0	0	1	1
Skin	2	0	0	0	2
Brain	1	0	1	0	0
Thyroid	1	1	0	0	0
Total	**11**	**2**	**3**	**3**	**3**
Family Members of CPS Positive Participants (N=3)					
Retinoblastoma	1	1	0	0	0
Breast	1	0	1	0	0
Cervical	1	0	1	0	0
Total	**3**	**1**	**2**	**0**	**0**

FDR: first-degree relative. CPS-Positive: The presence of a pathogenic or likely pathogenic variant in a CPS associated gene. CPS-Negative: The absence of a pathogenic or likely pathogenic variant in a CPS associated gene. FDR: first-degree relative. SDR: second-degree relative. Not Reported: Age of cancer diagnosis in the FDR was not reported. Percentages for the age of cancer diagnosis in a FDR were not calculated due to the small sample size.

IV. DISCUSSION

The utility of family history as a screening tool for a CPS in the context of pediatric cancers is complex. Healthcare providers for pediatric patients with cancer are faced with the challenges of treatment and management of the cancer as well as recognition of patients who may be at risk for additional malignancies or clinical features associated with an underlying CPS. Family history is often utilized as a screening tool for both pediatric and adult patients who are at risk for a CPS; however, there are unique limitations to utilizing family history to help identify a CPS in the pediatric oncology setting. The aims of this study were (1) to determine the effectiveness of personal history or family history features as a risk assessment tool for identifying a CPS in a pediatric oncology patient, and (2) to analyze which universal family history features in the MIPOGG algorithm are consistently met by individuals identified to be at high or low risk for a CPS.

4.1 Association Between MIPOGG and CPS Status

The major findings in this study were related to CPS status and personal and/or family history features in MIPOGG. The positive predictive value (PPV) of MIPOGG for pediatric-onset CPSs was 16%, and the negative predictive value (NPV) was 98%. The vast majority of participants who were identified as high-risk were not identified with a CPS (83% all participants, and 84% excluding adult-onset CPSs) and participants with a CPS were statistically more likely to be considered high-risk than low-risk for a CPS by the MIPOGG algorithm. Only a very small percentage of patients classified as low-risk were found to have a CPS, and this decreased further when excluding adult-onset CPSs (3% vs 2%), demonstrating the negative

predictive value of the MIPOGG tool (for identifying a pediatric CPS) to be 98%. The exclusion of adult-onset CPSs aligns with the goal of identifying CPSs associated with pediatric cancers in pediatric oncology patients, and the high proportion of CPS-negative participants in the low-risk group highlights the ability of MIPOGG to correctly identify individuals who do not have features suggestive of a CPS. This very high NPV is important for MIPOGG, since it is primarily a screening tool for CPSs in the pediatric oncology population. The sensitivity for identifying a pediatric-onset CPS was 88% in our study population, and, importantly, the overall prevalence of a CPS was ~10%, which is consistent with what is reported in the literature (Brodeur *et al.* 2017; Jongmans *et al* 2016; Narod *et al.* 1991). Calculating the sensitivity of MIPOGG in this study population and for all pediatric oncology patients is difficult because this relies on knowing the overall prevalence of a CPS in both populations. While 22 participants were identified with a CPS in this study, there may be CPSs that were not identified due to various types of limitations of genetic testing such as not all genes associated with pediatric-onset CPSs were included on the NGS panel in the G4K study, CPSs may only be detectable on specific types of tests (e.g. methylation studies, chromosomal microarray, etc.), there are likely to be CPSs that are not yet discovered or available for genetic testing, and uncertain variant classification could later be upgraded to pathogenic. These same limitations apply to our understanding of the prevalence of CPSs in the general pediatric oncology population.

The presence of a personal history alone and/or personal history *and* universal family history features were significantly associated with CPS status, in this case, the presence of a CPS. Participants who had a feature in the personal history *only* or in the personal history *and* universal family history categories were more often CPS-positive than CPS-negative. This suggests that a detailed characterization of personal history features in pediatric oncology

patients is important because these features were present significantly more often in the CPS-positive participants than in those who were CPS-negative. Interestingly, the personal history *and* tumor-specific family history category and the personal history *and* tumor-specific family history *and* universal family history category were not significantly associated with CPS status. This could be attributed to the smaller sample size in the two categories (N=6 and N=4, respectively) compared to the personal history only (N=74) and the personal history *and* universal family history (N=18) categories. Similarly, the categories that only assessed family history features were not significant predictors of a CPS. However, the significance of both personal history *and* universal family history features in CPS-positive participants in this study suggests that family history is more often a predictor when there are also features suspicious for a CPS in a pediatric oncology patient's personal history.

While the presence of a family history feature alone was not a significant predictor of identifying a CPS, there were three CPS-positive participants who were recommended for a genetics referral because of the presence of family history features (without any personal features). One individual met both tumor-specific and universal family history criteria, and two individuals met only tumor-specific family history criteria. The individual who met both tumor-specific and universal family history criteria was identified to have Li-Fraumeni syndrome. The mother of this participant was diagnosed with breast cancer at age 26, which is consistent with the age of onset and cancer type typically associated with Li-Fraumeni syndrome. Family history was an essential component of this participant's risk assessment for a CPS because characterization of the tumor type alone (astrocytoma) did not provide sufficient evidence for a high-risk classification by MIPOGG.

Two individuals with ALL were identified with a CPS (Noonan syndrome and *WT1*-related syndrome, respectively) and met tumor-specific family history criteria for individuals with ALL. Notably, the ALL diagnosed in the participant with *WT1*-related syndrome is not a typical tumor type associated with this condition and is likely to be unrelated to the participant's *WT1*-related syndrome. The participant with Noonan syndrome had a paternal grandmother who died from breast cancer at age 32, and the participant with *WT1*-related syndrome had a paternal grandfather with colon cancer diagnosed in his late 20's. While the cancers reported in family members of both participants were not suggestive of the specific CPSs identified, their ages of onset raise suspicion for a CPS in these families. The family history features in all three individuals meeting family history criteria (universal and/or tumor-specific) were concerning for a CPS in the participants and/or their relatives. This highlights one of the complexities of risk assessment for a CPS. Features associated with a CPS can have overlapping features with the patient's personal and family history of cancer, which is what is expected. However, in some instances, the typical features of an identified CPS do not overlap with the family cancer history, yet the family history may be concerning for a different CPS. A genetics evaluation for patients and possibly for their family members in the presence of a concerning family history is essential and may highlight risk factors for different CPSs in the patients and their relatives.

Many participants in this study with tumor-specific and universal family history features were not identified by MIPOGG to have a CPS. The lack of identification of a CPS does not eliminate the possibility of a CPS in the participant, although the residual risk is likely to be very low. When a child with a malignancy is not diagnosed with a CPS, his or her family member(s) could have a CPS related to the cancers in the family history but unrelated to the child's cancer diagnosis. One example is the CPS-negative participant who had a second-degree relative with

over 100 intestinal polyps (Table 11). This participant was diagnosed with a high-grade glioma, and the maternal aunt was clinically diagnosed with FAP at age 18 due to her polyp history but never had genetic testing. While the aunt's features are highly suggestive of FAP, a diagnosis was not molecularly confirmed. The participant was not identified to carry a pathogenic variant in *APC* or *MUTYH,* two genes that are highly associated with extensive gastrointestinal polyp development at a young age. Although it is unlikely, there is a small possibility that the participant may have an unidentified CPS related to the personal and/or family history features. The more likely scenario is that the participant's maternal aunt carries a pathogenic variant in *APC* or a different gene associated with extensive polyp development and that the participant did not inherit the familial pathogenic variant and developed a glioma for a different reason. Regardless of the participant's genetic test results, management recommendations, in addition to genetic counseling and genetic testing, are important for all relatives of this aunt, given the potential risk for a CPS related to her clinical features. A different participant, who was diagnosed with ALL, had a family history of a maternal grandmother with bilateral breast cancer diagnosed at age 40, a maternal great-aunt who was diagnosed with and died from ovarian cancer at age 56, and a maternal great-grandmother who was diagnosed with breast cancer under the age of 50. This participant was not identified to have a pathogenic variant in any of the CPS genes analyzed. This participant's family history is highly suggestive of a CPS, and there could be a CPS in the family unrelated to the participant's cancer diagnosis, or, less likely, the panel used in the study may not have included all known genes associated with an increased risk for breast cancer because this panel was designed to analyze genes associated with an increased risk for pediatric cancers. In such a scenario, family members may be falsely reassured by the negative genetic test result and have the misconception that their own cancer risks are low due to the lack

of identification of a CPS in the participant. In both examples, the concerning features in the participant's family history have health implications for family members who have an increased risk for a CPS that may not be related to the participant's cancer diagnosis. The features in these participants' family histories were more concerning for adult-onset CPSs, but MIPOGG is designed to identify only CPSs associated with pediatric cancers and includes features in the universal family history category to identify those who are at high risk for a childhood-onset CPS. Currently the app is streamlined to ask the fewest number of questions necessary to determine if a genetics referral is recommended. This streamlined approach does not assess family history features of adult-onset CPSs. Although the goal of MIPOGG is not to identify adult-onset CPSs, in the future the app could potentially be modified to identify family histories suggestive of adult-onset CPSs and generate a separate report for the child's parents and/or relatives. These could be shared with a genetics professional who could provide information regarding the adults' risks for a CPS related to their history.

The features in the universal family history category were characterized to determine how often these features were present by CPS status and participant cancer type. An association between CPS status and reporting of features in the universal family history category was not observed, likely due to the small number of participants who were CPS-positive and who had reported a feature in the universal family history category (n=6). The most common features in the study cohort included a FDR and/or half-sibling with cancer before age 50 and an aunt/uncle/first cousin/grandparent with cancer before age 19 (n=13 and n=14, respectively). Less commonly, participants reported a close relative with the same cancer type or same organ affected by cancer and a close relative with multiple primary tumors (excluding non-melanoma skin cancer) before age 60 (n=11 and n=7, respectively). The small number of participants with

family history features in a close relative may be attributed to a variety of factors, including the rarity of these features in family members and features of the CPSs identified in this study population. Some of the participants' cancer types are very rare, with a low likelihood to present in a family member even in the presence of a CPS. A history of the same cancer type in a close relative may be important for certain CPSs, such as retinoblastoma, but not in other CPS types, since many conditions have wide variability in the types of cancers associated and/or modes of inheritance other than autosomal dominant (e.g., *de novo* mutations, autosomal recessive inheritance, etc). Additionally, the presence of multiple primary tumors in a family member is likely rare for most CPSs. Some CPSs, such as Li-Fraumeni syndrome, can cause an increased risk for multiple cancers to develop in an individual. Many CPSs have considerable variability, and even with an increased risk for multiple tumor development, many relatives will not have this feature or will not yet have presented with a second malignancy due to their young age. The types of CPSs identified in the study may also have contributed to the low frequency of this feature, because many of the CPSs associated with pediatric cancer risk are not highly associated with the development of multiple cancers in an individual. The features that are associated with CPSs vary widely, and additional studies with a larger cohort of patient's representative of a wider range of CPSs can better assess the power of the universal family history features in MIPOGG.

An additional characterization of the universal family history features was stratification by cancer type. The majority of participants with a universal family history feature, regardless of CPS status, had a blood cancer (Table 8A). This could be due to chance because of the high frequency of participants with blood cancer as compared to the other three main cancer types (brain/CNS tumors, retinoblastoma, or other solid tumors) combined with the relatively small

number of participants with each universal family history feature. Equal numbers of participants with brain/CNS tumors and blood cancers had an aunt/uncle/first cousin/grandparent with cancer before age 19 (N=5), which is also likely due to chance. If there truly were an association between brain/CNS tumors and an aunt/uncle/first cousin/grandparent with cancer before age 19, further characterization might show a higher association with certain cancer subtypes in this group. This characterization was not performed due to the small number of participants and the lack of power to draw any conclusions about cancer subtype and an aunt/uncle/first cousin/grandparent with cancer before age 19.

Generally, features concerning for an underlying CPS are not identified in family cancer histories of pediatric oncology patients, and that was also the case in this study cohort. This may be due to features of many of the more common CPSs (reduced penetrance, inheritance pattern, etc.) and/or to general limitations to obtaining/interpreting a family history (e.g. family dynamics, provider knowledge, age of relatives, number of relatives in a family, etc.) that were explored extensively in a previous section. Although these limitations could not be investigated in this study cohort, they likely contributed to the small number of CPS-positive participants with family history features concerning for a pediatric-onset CPS. All of the CPSs identified in this study were autosomal dominant conditions, with the exception of CMMRD, an autosomal recessive condition. This cohort of CPS-positive participants would have a higher likelihood to have a family cancer history concerning for a CPS compared to participants with conditions with other modes of inheritance (e.g., autosomal recessive conditions). Notably this does not account for the number of autosomal dominant *de novo* mutations, which was not determined in this study cohort, but patients with autosomal dominant *de novo* mutations would not be expected to have a family history of cancer related to their diagnosis. Patients with autosomal recessive

conditions are also less likely to have family history features concerning for a pediatric-onset CPS, except for certain recessive conditions where heterozygous mutations cause adult-onset CPSs, often with moderate penetrance (e.g., Fanconi anemia, where heterozygous mutations can cause hereditary breast cancer). The CPSs identified in this study cohort are a very small subset of all known pediatric-onset CPSs and do not represent the many known types of pediatric-onset CPSs. The frequency of patients with concerning family history features may be different in a study cohort with a representative distribution of patients with pediatric-onset CPSs.

4.2 Impact of Age at Ascertainment on Family Cancer History

In addition to analyzing the family history features in MIPOGG, the degree of relationship of relatives with a cancer history was examined by category of participant's age at cancer diagnosis. The participants' age at cancer diagnosis referred to their first cancer diagnosis or, if unknown, the age at their current cancer diagnosis. An individual's age at cancer diagnosis typically is associated with the age of relatives in the family; younger individuals would be more likely to have parents who are younger than parents of older individuals. Cancer risk generally increases with advancing age, and the age of participants' family members is very likely a factor in the frequency and the degree of relationship of relatives with cancer. When participants were analyzed based on the age of cancer diagnosis and degree of relationship of relatives with cancer, a lower proportion of FDR and/or SDR with cancer appeared to be reported in the youngest age groups, 0-1 and 2-3, compared to the oldest age group, 16-18. Analysis of family cancer history affecting relatives beyond a first-degree or second-degree relationship in each age group revealed significant differences between the 16-18 age group and the 0-1, 2-3, and 10-11 age groups. These significant differences indicate a lower proportion of cancer reporting in FDR and/or SDR

in each of the three age groups, as compared to the 16-18 age group. The reason for the lower reporting in the 10-11 age category was not clear but could be due to the types of cancers that occurred in participants in this age category, among many other limitations of screening by a family history that were detailed above.

An additional analysis was performed to compare family cancer history based on the participants' cancer types and the age at ascertainment of their family histories. When the data was organized in this way, no significant associations were identified between age groups and the proportion of family cancer histories affecting relatives beyond a first-degree or second-degree relationship. A likely confounder was the retinoblastoma group, because all participants with retinoblastoma were ascertained in the younger age groups (0-1, 2-3, and 4-5). While not significant, the blood cancer and brain/CNS tumor categories had higher proportions of a family cancer history affecting relatives beyond a first- or second-degree relationship in the 0-1 age group compared to the 16-18 age category. A larger cohort of patients with a more even distribution of participants' ages at ascertainment could reveal a significant difference in proportion of family cancer history between the oldest and youngest age groups. Additionally, the other solid tumors group did not have a clear pattern between age groups in the distribution of family cancer history affecting relatives beyond a first or second-degree relationship. This is likely related to the wide range of cancer types categorized in the other solid tumors group. Characterization of cancer subtypes in this group could reveal trends in family cancer history currently not seen, but this was not performed due to the small number of participants in each cancer subtype. Family cancer history could be dependent on the participant's cancer type, but additional characterization of cancer types as well as a larger and more evenly distributed cohort

of patients in each age group is necessary to determine if there is an association between age at ascertainment and family cancer history.

4.3 Types of Family Cancer History

No significant association was identified when comparing types of cancer history in a family (any family cancer history, a family member with a pediatric cancer (cancer diagnosed before age 19), a FDR with cancer, or a FDR with pediatric cancer) and participant CPS status, but some of the sample sizes were very small and limited the ability to assess for a difference. A larger group of CPS-positive patients would provide more evidence to support or refute the lack of significant association between CPS status and types of cancer history in a family.

Participants with a family member with a pediatric cancer (defined as diagnosed before age 19) or a FDR with cancer were characterized to assess the types of cancers in relatives of participants with and without a CPS, as well as degree of relationship (in relatives with cancer diagnosed before age 19). Blood cancer was the most common cancer type among relatives with a pediatric cancer, but it should also be noted that blood cancers are the most common type of pediatric cancer. Some participants in the group of individuals without a CPS had multiple relatives with pediatric cancers, and some of these individuals had at least one family member with pediatric blood cancer. The majority of relatives of participants, regardless of CPS status, who had a pediatric cancer were beyond a first- and/or second-degree relationship. This may not be replicated in other studies because family histories taken by non-genetics providers often do not include these more distantly related relatives. If this more distant family history is not obtained, there is a risk of missing some CPS diagnoses. Due to the small sample size, statistical analyses were not performed to determine if associations exist between the participants' cancer type and the frequency, degree of relationship, or cancer type diagnosed in relatives with

pediatric cancer. A larger sample of patients, with family histories obtained with the same level of detail as in this study, would allow for statistical analysis to determine if associations exist.

The ages of diagnosis of FDRs with cancer were characterized by participant CPS status and FDR cancer type. A total of 14 participants had a FDR with cancer; 11 participants were identified with a CPS, and three participants were not identified with a CPS. The age of FDRs with cancer was slightly younger in relatives of participants with a CPS compared to relatives of participants without a CPS. While this data suggests a possible association between the age of a first-degree relative with cancer and CPS status, statistical analysis was not performed to determine the strength of this observation due to the small number of CPS-positive participants. Additionally, three participants who were CPS-negative had a FDR with cancer without a reported age of diagnosis; the ages of these cancers could impact the distribution of ages of diagnosis of FDRs in CPS-negative participants. Analysis of a larger number of participants with a family cancer history including a pediatric cancer and/or FDRs with cancer is necessary to determine if associations exist between these family cancer histories and CPS status.

4.4 Limitations of Genetic Testing for a CPS in a Pediatric Patient

There are many benefits to identifying a CPS in a pediatric patient using a genetic testing panel, but there are also limitations. Some of the benefits of panel testing for CPS genes associated with pediatric cancer were discussed previously and include providing an explanation for the patient's clinical features, having information about associated features including the risk for development of additional malignancies, implementation of screening and early detection of malignancies, and the risks to family members and future children. The limitations to panel testing were also addressed and include the identification of adult-onset CPSs in pediatric

patients, identification of variants of uncertain significance, and the possibility of a CPS not identified because its associated gene was not among those in the 150 genes analyzed on the G4K study panel.

Three participants were identified to be heterozygous for pathogenic variants in genes associated with adult-onset CPSs (*BRCA2, PALB2, MSH2*), which is not the goal of MIPOGG. As discussed previously, biallelic mutations in these genes cause CPSs associated with pediatric cancer risk, but heterozygous pathogenic variants in these genes cause an increased risk for certain cancer types in adulthood. Heterozygous pathogenic variants in *BRCA2* and *PALB2* are typically associated with an increased risk for breast cancer, while such variants in *MSH2* are associated with an increased risk for colon and endometrial cancer. Additional cancer types are associated with pathogenic variants in these three genes, but the lifetime risk is typically lower compared to these primary cancer types.

The family histories of the participants with pathogenic variants in *BRCA2, PALB2, MSH2* were analyzed after the study to determine if the participants had concerning family histories for the conditions identified in them. The participant identified with a pathogenic variant in *BRCA2* had a paternal great-aunt with breast cancer diagnosed in her 40s and a paternal great-grandmother diagnosed with lung cancer in her 80's. The participant with a pathogenic variant in *PALB2* had a paternal great-aunt with two breast cancers, the first diagnosed at age 50 and the second diagnosed in her 60's, a paternal great-aunt with uterine cancer at an unknown age, a paternal great-great-uncle with colon cancer at an unknown age, and a maternal great-great-grandmother with breast cancer at age 80. The individual with a pathogenic variant in *MSH2* had a paternal great-grandmother with colon cancer and breast cancer diagnosed at unknown ages who died over age 80, a paternal great-grandfather with

lymphoma at age 84, and a maternal great-great-grandfather with bladder cancer at age 85. In these three families, a striking family history of cancer did not emerge, with the exception of the paternal great-aunt with breast cancer in her 40's. The lack of concerning family history features could be attributed to limitations of family history (unknown ages of family members, sex of family members [for sex-dependent cancers such as uterine], a limited number of relatives in each generation, or family dynamics), reduced penetrance of the condition, among other reasons.

Identification of an adult-onset CPS, while important for adult relatives, would not impact a pediatric patient's current medical management. In the setting of a healthy pediatric patient, genetic testing for adult-onset conditions is not typically recommended because of the lack of immediate clinical utility and the many ethical complexities, including timing of result disclosure to the patient, patient autonomy, and patient consent (NSGC 2017). As discussed previously, the benefits of identifying biallelic mutations in a gene associated with pediatric cancer (e.g. CMMRD or Fanconi Anemia) can have serious management implications, and these benefits are typically thought to outweigh the risk of identifying an adult-onset CPS in a pediatric patient.

In addition to the possibility of identifying an adult-onset CPS, genetic testing panels often identify VUSs. A VUS in one or more genes was identified in 117 participants without a CPS and in 10 individuals with a CPS; in the 10 individuals with a CPS, a VUS was identified in a gene different from the one associated with their CPS. While the majority of VUSs are reclassified to benign variants in adult cancer patients, the rate of VUS reclassification to benign variants in pediatric oncology patients is not well defined. A very preliminary assessment of the features associated with the genes that a VUS was identified in suggested an unlikely association with the participants clinical features, with the exception of one individual diagnosed with

retinoblastoma who was identified with a VUS in *RB1*. Although unlikely, there is a possibility that a VUS identified in a study participant may later be determined to be pathogenic and related to the participant's personal and family history features. The VUSs identified in this study were not investigated further to determine if any of the variants had been reclassified or if there are possible correlations between the VUS and features in the participant. Future studies could analyze the classification of the VUSs reported in participants and possible associations with personal and family history features.

A negative genetic test result in pediatric oncology patients can reduce the likelihood for many pediatric-onset CPSs. The G4K study panel included over 150 genes associated with a pediatric-onset CPS, many of which are well characterized pediatric-onset CPSs, but there may be unknown CPSs or very rare CPSs not evaluated by this panel. These limitations are important for this study and in the general pediatric oncology setting because patients who test negative for a panel of genes associated with pediatric-onset CPSs may have an underlying CPS that has not yet been identified. Follow up and continued characterization of personal and family history features of CPS-negative patients may lead to additional genetic testing in the future for rare or new conditions.

4.5 Limitations of the Study Cohort and Data Set

All pediatric cancer patients were enrolled in the G4K study at St Jude Children's Research Hospital regardless of cancer type or other factors that may have been concerning for a CPS; this allowed for minimal ascertainment bias. Certain characteristics related to cancer type and age at diagnosis were more prevalent in this study cohort. Large proportions of study participants were diagnosed with blood cancers (40.6%) or brain/CNS tumors (31.9%), and the

age of first cancer diagnosis was primarily in the youngest two categories, including the 0-1 (n=56, 20.3%) and 2-3 (n=51, 18.5%) age ranges. This relationship between cancer type and age at diagnosis is representative of what is typically identified in the U.S. pediatric oncology population (SEER 1975-2017). Age at first cancer diagnosis can be associated with certain cancer types, as demonstrated with the retinoblastoma group (Figure 4). Detailed analyses of associations between each tumor type and age at diagnosis were not performed because the frequency of each tumor type was small, and some tumor types only included one participant. This small frequency of tumor types greatly limited the ability to perform analyses related to specific tumor types.

The proportion of participants identified with a CPS in this study (10%) was consistent with the 10% that is generally cited throughout the literature for pediatric cancer patients (Zhang *et al.* 2015). Among the 27 participants who were CPS-positive, a total of 14 CPSs were identified. Hereditary retinoblastoma was the most common CPS and was diagnosed in 37% (N=10) of individuals with a CPS. Each of the remaining 13 other CPSs identified was present in only one or two participants, with the exception of the three individuals with NF1. All of the CPSs identified have autosomal dominant inheritance, with the exception of one autosomal recessive condition, CMMRD. Not all participants had personal or family history features consistent with the pediatric-onset CPS identified in them. For example, the participant with *WT1*-related syndrome had ALL, and the participant with SDHA Hereditary Paraganglioma/Pheochromocytoma had a low-grade glioma. Identifying pediatric-onset CPSs in these participants provided information about their risks to develop cancer and other features related to their conditions, but interestingly the cancer types in these participants are not typically associated with the conditions identified in them and are likely coincidental. Associations

between each condition and features in the MIPOGG algorithm were not assessed due to the small number of individuals identified with each condition. Future studies, including a larger number of participants with each CPS and a more diverse set of CPSs, may reveal associations not identified in this study.

This study was also limited to the information assessed by the MIPOGG algorithm and reported by the study participants' healthcare providers. Information such as patient's ethnic backgrounds and sex was not collected. Comparisons between individuals of different ethnic backgrounds and sex could reveal associations in VUS rates, cancer types, and/or family cancer histories that were not analyzed in this study. Additionally, the presence or absence of MIPOGG criteria in participants was not always clear, and the healthcare providers collecting the information in the database occasionally indicated that features were "unknown." For the purposes of this study, if a healthcare provider indicated that a feature was present, then the individual met the associated MIPOGG criteria. Conversely if a healthcare provider did not indicate that a feature was present (e.g., "unknown" or "no"), then the individual did not meet MIPOGG criteria. This meant that not all features were assessed for participants with "unknown" answers, and there possibly were individuals who would have met MIPOGG criteria if these features had been assessed. Additional analyses could be performed to determine if there are trends in frequency of features reported as unknown and the potential impact this lack of reporting may have had on MIPOGG risk classification.

4.6 Future Directions

Although many studies have investigated family histories of patients with specific features or conditions (Linabery *et al.* 2015, Mosse *et al.* 2008), a limited number have investigated the utility of family history for CPS risk assessment in the general pediatric

oncology population. One small study used a family history form in a pediatric oncology setting to identify patients at high risk for a CPS; a total of four out of 57 patients had a family history concerning for a CPS. Twelve patients had genetic testing, including the four with a concerning family history, and none were identified with a CPS, which was likely due to the small sample size (Hamilton *et al.* 2017). Similarly, this study did not show a significant association between family history and CPS identification in study participants. Future studies with a larger number and more diverse set of cancer types and CPSs are necessary to clarify trends and associations related to family cancer history. Additional follow up and continued characterization of personal and family history features is necessary for all patients, but especially CPS-negative patients because of the possibility of additional genetic testing for rare or new conditions becoming available in the future. Interestingly, a study of pediatric oncology patients, who were previously evaluated in a genetics clinic, identified family history as the most common reason for a follow up genetic referral. This study illustrated the importance of collection and review of a patient's family history as a part of their ongoing care (Knapke *et al.* 2011).

The MIPOGG app was an effective predictor of CPS status in this study cohort, with a PPV of 16% and NPV of 98%. A previous study performed a retrospective evaluation of the MIPOGG app in a group of patients with neuroblastic tumors (Goudie *et al.* 2018). The MIPOGG app identified 51 out of 209 patients as high risk for a CPS, and 6 out of 51 had a genetic or clinical confirmation of a CPS. The app correctly identified all patients with a CPS in the study cohort, indicating a 12% PPV and a 100% NPV in this study group. While these PPV and NPV are specific to the group of neuroblastic tumors, they are similar to the percentages identified in our study cohort. Additional studies are necessary to confirm these preliminary

results, but these two studies are very promising and show the power of MIPOGG to identify patients at high or low risk for a pediatric-onset CPS.

4.7 Conclusions

The results of this study found personal history features were a significant predictor of a CPS in participants, while family history features alone were not. These preliminary results suggest the importance of characterization of personal history features in a pediatric cancer patient to identify patients at high risk for a CPS. Additionally, family cancer history can be a predictor of CPS status in pediatric cancer patients when paired with personal history features, but independently does not have the same strength in association with CPS status. Factors including the participant's cancer types and age at ascertainment of family history did not have a clear influence on the frequency or degree of relationship of family cancer history. While the yield of CPS identification in this study cohort was not associated with features in a family history, one participant with Li-Fraumeni syndrome was identified to be high risk for a CPS solely due to features in their family history. This demonstrates the importance of eliciting a family history as a part of the risk assessment for pediatric cancer patients and their family members who may be at risk for a CPS. This study highlights the value of understanding the limitations of family history as a screening tool for CPS identification, and although only a small number of pediatric cancer patients will have a concerning family history, these patients have the potential to be missed as high risk for a CPS if only personal history features are evaluated.

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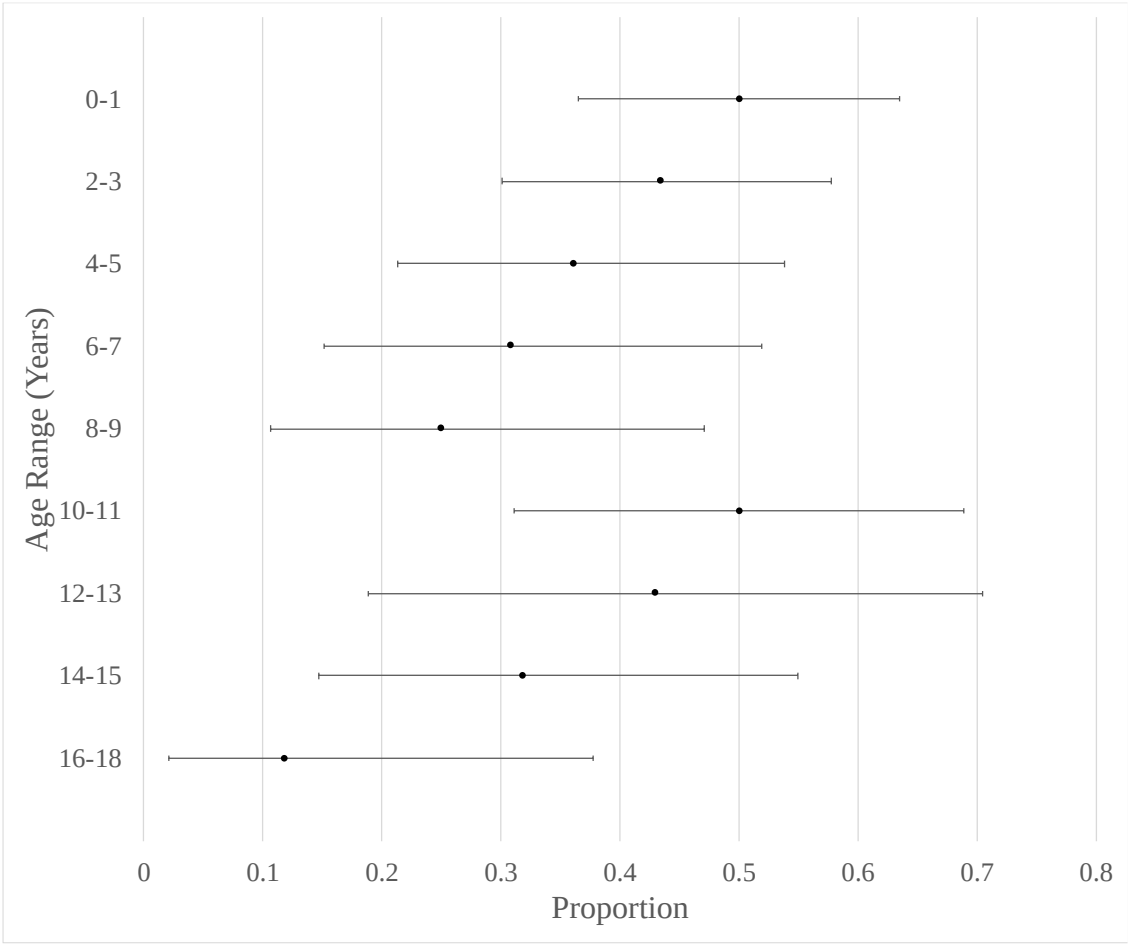
APPENDIX A: MIPOGG Direct Referral Cancer Types

Tumor Types	
Adrenal tumor	Hepatoblastoma
Adrenocortical carcinoma	Leiomyosarcoma
Pheochromocytoma / Paraganglioma	Lipomatous tumors
Anaplastic sarcoma of the kidney	Liposarcoma
Bone and soft tissue tumor	Atypical lipomatous tumor / Well-differentiated liposarcoma
Angiomyolipoma	Medullary thyroid carcinoma
Lymphangioleiomyomatosis	Mesothelioma
Chondrosarcoma	Nephroblastomatosis
Breast tumor	Nerve sheath tumor
Breast carcinoma	Acoustic Neuroma
Phyllodes tumor	Malignant peripheral nerve sheath tumor
Carcinoma of the uterus / vagina	Schwannoma
Cardiac tumor	Schwannomatosis
Cardiac myxoma	Malignant rhabdoid tumor
Cardiac rhabdomyoma	Ovarian tumor
CNS Tumors	Ovarian carcinoma
Atypical teratoid rhabdoid tumor	Sertoli-Lothereydig cell tumor (ovary)
Choroid plexus carcinoma	Sex cord tumor with annular tubules (ovary)
Dysplastic cerebellar gangliocytoma	Pancreatic tumor
Endolymphatic sac tumor	Pancreatic neuroendocrine tumor
Hemangioblastoma	Pancreatic carcinoma
Medulloblastoma - SHH/WNT	Pheochromocytoma / Paraganglioma
Medulloepithelioma	Bronchial neuroendocrine tumor
Meningioma	Mesothelioma
Meningiomatosis	Pleuropulmonary blastoma
Pituitary blastoma	Renal tumor
Pineal parenchymal tumor	Angiomyolipoma
Pineoblastoma	Cystic nephroma
Schwannomatosis	Malignant rhabdoid tumor (kidney)
Subependymal giant cell astrocytoma	Renal Cell Carcinoma
Fibroblastic and myofibroblastic tumor	Skin tumor
Gardner fibroma	Squamous cell carcinoma
Nuchal type fibroma	Fibrofolliculoma
Gastro-intestinal tract tumor	Mucosal neuroma
Gastrointestinal carcinoma	Trichodiscoma
Gastrointestinal polyp: Adenomatous polyp	Thymic neuroendocrine tumor
Gastro-intestinal stromal tumor	
Head and neck tumor	
Nasal chondromesenchymal hamartoma	
Medulloepithelioma	
Retinoblastoma	
Keratocystic odontogenic tumor	
Parathyroid tumor	
Squamous cell carcinoma	

APPENDIX B: Distribution of Family Cancer History Affecting Relatives Beyond a First- or Second-Degree Relationship by Age at Ascertainment



Age at ascertainment: The age of the participant when the family history was obtained; this was either at the age at first cancer diagnosis, or if unknown, at the age of their most recent cancer diagnosis. A 95% confidence interval was calculated for the proportion of participants with a family cancer history affecting relatives beyond a first- or second-degree relationship and stratified by age at ascertainment. Confidence intervals indicate the range of values in which the true proportion lies within; the upper and lower limits of the confidence interval are depicted with bars. Data points illustrate the proportion of participants with a family cancer history affecting relatives beyond a first- or second-degree relationship in the study cohort.