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Psychosocial Experiences And Genetic Knowledge In Individuals With Pathogenic And Uncertain Tp53 Variants In The Prospective Registry Of Multiplex Testing (prompt) Cohort

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Psychosocial experiences and genetic knowledge in individuals with pathogenic and uncertain *TP53* variants in the Prospective Registry of Multiplex Testing (PROMPT) cohort

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Abstract

Germline mutations in the *TP53* gene confer increased risk of diverse malignancies in children and adults in the Li Fraumeni cancer susceptibility syndrome, a rare condition with estimated prevalence of about 1 in 3,555 in the population. The Prospective Registry of Multiplex Testing (PROMPT) study is a registry of individuals undergoing genetic testing for inherited cancer susceptibility using multi-gene panels regardless of test results and agreeing to participate in questionnaire studies about their experience. The purpose of this study was to investigate how the psychosocial experience of genetic testing and level of genetic understanding and knowledge differs for those who have a variant of uncertain significance (VUS) compared to those with a pathogenic *TP53* variant. We evaluated data from 69 eligible participants from the PROMPT Study. Using an electronic survey, we assessed their *TP53* knowledge and understanding with the KnowGene instrument, as well as their psychosocial experiences with the FACToR subscales. Differences in instrument performance were evaluated by variant class. Respondents with a pathogenic variant (those with Li-Fraumeni syndrome, "LFS") had significantly higher levels of distress compared to those with a VUS. Respondents with a pathogenic variant had moderately higher levels of genetic knowledge and understanding compared to those with a VUS. This exploratory project demonstrates the need for more comprehensive work assessing the burden of LFS on individuals testing positive for pathogenic variants in the *TP53* gene on germline genetic evaluation.

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Introduction

Li-Fraumeni syndrome (LFS) is a hereditary cancer predisposition syndrome that results from a germline mutation in *TP53*, a gene sometimes referred to as the "guardian of the genome". *TP53*'s gene product, p53, plays a major role in tumor suppression via regulation of cell division and DNA damage repair. In normal conditions, p53 responds to cell stress by activating proteins responsible for DNA repair, halting the cell cycle at an early checkpoint to allow for DNA repair when damage has been identified, and either restarting the cell cycle and allowing the cell to divide or initiating apoptosis if the DNA damage is deemed irreparable [1]. The loss of these regulatory functions in p53 allows unchecked cell growth that can lead to malignancy. Accordingly, acquired somatic mutations in *TP53* are some of the most frequently occurring mutations in human cancers [2]. The normal state is for people to have two copies of the *TP53* gene in all of their nucleated cells with normal/"wild type" sequence. Individuals with LFS have only one normally functioning germline *TP53* gene; the second copy is either partially or completely inactivated by a mutation in all cells. This condition confers an increased risk of diverse cancers beginning from an early age and increases the lifetime risk of developing multiple primary malignancies [3]. Cancers commonly seen in LFS ("core cancers") include adrenocortical carcinomas, breast cancer, central nervous system tumors, osteosarcomas, and soft-tissue sarcomas [3].

The prevalence of germline *TP53* mutations in the general population is not well-known, though a 2019 study reported an estimate of one carrier of a pathogenic variant in every 3,555 people [4]. Classic criteria for LFS include a patient with a sarcoma diagnosed before 45 years of age, a first-degree relative diagnosed with any cancer before 45 years of age, and a first- or second-degree relative diagnosed with any cancer before 45 years of age or a sarcoma diagnosed

at any age [3]. Historically, LFS patients have been identified through family history or individual cancer diagnoses, but the emergence of multigene panel testing has resulted in an increasing number of patients becoming aware of their LFS status despite no striking family history of cancer.

The LFS spectrum has expanded to include not only those who meet classical criteria, but also those who meet Chompret criteria. Chompret criteria, which were derived from a systematic study of individuals and families with pediatric malignancies in France [5], include a patient with a tumor belonging to the LFS tumor spectrum (e.g., premenopausal breast cancer, soft-tissue sarcoma, osteosarcoma, central nervous system tumor, adrenocortical carcinoma) before 46 years of age AND at least one first- or second-degree relative with an LFS tumor (except breast cancer if the proband has breast cancer) before 56 years of age or with multiple tumors, OR a patient with multiple tumors (except multiple breast tumors), two of which belong to the LFS tumor spectrum and the first of which occurred before 46 years of age, OR a patient with adrenocortical carcinoma, choroid plexus tumor, or rhabdomyosarcoma of embryonal anaplastic subtype, irrespective of family history OR a female patient with breast cancer before 31 years of age [6].

The introduction of multigene panel testing led to the identification of germline *TP53* mutations in individuals whose family histories do not make criteria for LFS by classic or Chompret criteria, suggesting that there may be bias in the early study of LFS [7]. This is especially true for women with breast cancer or breast cancer family histories, who still comprise the largest group of individuals undergoing genetic testing. As a result, some *TP53* carriers will be identified without a diagnosis or family history indicative of LFS. These carriers make up an interesting population to be studied to identify areas for improvement in the education and delivery of care that is required following cancer genetic testing.

As a result of *TP53's* ubiquitous role in cell cycle regulation/tumor suppression, variants in *TP53* confer an increased risk of a diverse array of tumors. As such, LFS patients experience an exceptionally large screening burden to manage their heightened cancer risk. Current screening guidelines from the National Comprehensive Cancer Network (NCCN) for adults who have LFS include a clinical breast exam every 6-12 months, annual breast MRI, comprehensive physical exam including neurologic examination every 6-12 months, colonoscopy and upper endoscopy every 2-5 years starting at 25 years of age or 5 years before the earliest known colon/gastric cancer in the family, annual dermatologic exam, annual whole body MRI, and annual brain MRI either as a part of the whole body MRI or as a separate exam [8]. Women with LFS may be further advised to undergo invasive risk-reducing surgeries such as mastectomies.

The Prospective Registry of Multiplex Testing (PROMPT, ClinicalTrials.gov identifier: NCT02665195) is an online registry for individuals and their families who have undergone multigene panel testing for cancer susceptibility. A collaboration among researchers from Memorial Sloan Kettering Cancer Center, Dana-Farber Cancer Institute, Mayo Clinic, and the University of Pennsylvania, the purpose of PROMPT is to identify families with hereditary cancer predisposition syndromes to further understand how genetic variants affect cancer risk. It was initiated in part to provide an opportunity for women who were not receiving care in academic institutions to participate in research that could provide data with implications for their care and the care of at-risk family members. Using these data, study leaders aim to improve the quality of care delivered to patients with inherited risks in less-studied genes. Five commercial genetic testing laboratories (Ambry Genetics, GeneDx, Myriad Genetics, Pathway Genomics, and Quest Laboratories) partner with the PROMPT investigators at the aforementioned research institutions to gather eligible participants by including study recruitment materials with an

individual's genetic testing results. Individuals may then access the PROMPT registry online and self-enroll. At the end of 2022, there were 10,233 participants including 1,227 partial respondents who could not be contacted. There are 58 unique genes under study.

Although knowledge continues to grow on LFS and its disease spectrum, there remains a gap in understanding the effects of an LFS diagnosis on individuals beyond clinical outcomes. This thesis attempts to narrow that gap by providing novel data from LFS patients reporting on their lived experiences. Through an electronic survey ("PROMPT-LFS survey") including both previously validated and investigator-developed items, this thesis assessed participant demographics, family cancer history, genetic information, related experiences, knowledge/understanding of inherited cancer risk, and psychosocial responses participants have to learning of their *TP53* variant. We hypothesized that participants with pathogenic mutations would experience higher levels of distress, *TP53* knowledge and genetic understanding while those with a VUS would experience higher levels of uncertainty.

Methods

Participant recruitment. Participants were recruited from the Prospective Registry of Multiplex Testing, an online registry created to reach and learn more about patients and families who have a genetic variant that may confer an increased risk of cancer [9]. This study was approved by institutional review boards at the University of Pennsylvania and Dana-Farber Cancer Institute. E-consent was obtained from participants for their initial registration in PROMPT, including an agreement to be contacted for relevant future studies. PROMPT participants who reported a *TP53* variant (classified as either pathogenic/likely pathogenic or variant of uncertain significance) were considered eligible for this study. Conducting this study

in the PROMPT cohort allowed us access to a larger, although still relatively small number of individuals with a *TP53* variant. Data were collected using REDCap software, through which an electronic cross-sectional survey was distributed to participants via email, including three follow-up reminders for non-responders. This survey was estimated to take approximately 15 minutes to complete. Along with the final reminder email, in an attempt to increase the response rate, an incentive in the form of a $20 Amazon gift card was offered to respondents for their completion of the survey. This incentive was also distributed to participants who had previously completed the survey.

Eligibility criteria. To be considered eligible for inclusion in this study, participants must have already been enrolled in PROMPT and have a *TP53* variant reported in their genetic test result. Prospective participants must have completed the PROMPT-LFS survey and reported their genetic testing result information to be included in these data. As outlined in the inclusion schema in Figure 1, three participants were excluded overall, one because she reported a benign variant and two because their variant was diagnosed as likely clonal hematopoiesis of indeterminate potential/aberrant clonal expansion (CHIP/ACE) rather than a germline *TP53* variant.

Classifying variants. We asked participants to report their understanding of the classification of their specific variant (pathogenic, VUS, negative, test results unclear to me) and its implications for cancer risk (e.g., LFS), as well as their personal history of cancer. If there was disagreement between a participant's genetic testing report and their self-reported data (e.g., self-reported a pathogenic mutation but the laboratory report classified the mutation as a VUS), self-reported data was used since their perception of their condition seemed more likely to affect their emotional responses as measured in this survey. *TP53* variants are classified using the

American College of Medical Genetics criteria [10] and are available for review in an online database called ClinVAR, maintained by the NHGRI [11]. The study investigators review the discrepant cases to ensure that participants have correct information about their variant, its interpretation, and its implications for their care.

Measures of knowledge and understanding. The KnowGene scale is a 16-item scale developed and validated at the Dana-Farber Cancer Institute that can be used to effectively measure an individual's knowledge/understanding after undergoing multigene panel testing and cancer genetic counseling [12]. For this study, we used an abbreviated 6-item version of the scale. Participants answered "agree", "disagree", or "I don't know" to each of the following six statements: 1) knowing about inherited risk (passed down within a family) can affect choices about cancer treatment (for example, cancer medications or surgery); 2) people with an inherited risk for cancer (and their at-risk relatives) are more likely to develop more than one type of cancer; 3) cancers in women, such as breast and ovarian cancer, can generally be passed on from either the father or the mother; 4) some gene mutations mean a larger increase in the risk for cancer while others mean a smaller increase in the risk for cancer; 5) a variant of uncertain significance (VUS) does not influence cancer treatment recommendations; and 6) multi-gene panel testing could find a mutation in a gene that is not clearly associated with the pattern of cancer in the family. Each question was assigned either a "0" for an incorrect response or a "1" for a correct response, the sum of which was then taken as a score out of 6 to quantify knowledge and understanding of genomic testing results. Higher scores indicate greater levels of knowledge and understanding.

Measures of emotional response to genetic testing results. To measure participants' emotional response to their genetic testing results, we asked them to complete the Feelings

About genomiC Testing Results (FACToR) questionnaire. Developed and validated by a team at the University of Washington in 2018, FACToR is a 12-item questionnaire with four subscales – negative emotions, positive feelings, uncertainty, and privacy concerns – designed to measure the psychosocial impact of receiving genomic findings [13]. Questions were posed as how often a feeling was experienced in the past week and participant responses were coded from 0 to 4 (0 = not at all, 1 = a little, 2 = somewhat, 3 = a good deal, 4 = a great deal). Summary scores were then calculated for each subscale by adding scores from individual items such that a higher score indicated greater functional impairment. The range of total scores was 0-16 on the positive feelings subscale, 0-12 on the negative emotions and uncertainty subscales, and 0-8 on the privacy concerns subscale. Scoring was done in accordance with the instrument's guidelines for subscale interpretation: higher scores indicate greater functional impairment. As directed for the positive feelings subscale, in order for a higher score to reflect greater functional impairment, scores are inverted, with responses originally coded as 0 counted as 4 and responses originally coded as 4 counted as 0 [13].

Analysis. To analyze the results of the KnowGene instrument, a simple linear regression was done to examine the relationship between variant class and *TP53* knowledge and understanding. To analyze the results of the FACToR scale, a generalized linear model was used to measure differences in subscale scores between variant classes.

Results

Sample characteristics. Table 1 presents select demographic and medical characteristics of the PROMPT-LFS survey respondents by variant class. Of 10,233 individuals enrolled in PROMPT, 157 reported the identification of a *TP53* variant in their multigene panel testing. Of

those 157 eligible individuals, 143 invitations were successfully delivered (14 invitations were undeliverable) and 72 PROMPT-LFS surveys were completed after 4 email solicitations for a 50.3% response rate. As noted in the inclusion schema shown in Figure 1, three participants did not meet the eligibility criteria, which resulted in a final cohort size of 69. A pathogenic *TP53* variant was reported by 37 (53.6%) participants and 32 (46.4%) participants reported a *TP53* variant of uncertain significance. Among those individuals who received the PROMPT-LFS survey but did not respond, 24 (33.8%) had reported a pathogenic *TP53* variant, 36 (50.7%) reported a *TP53* variant of uncertain significance, and 11 (15.5%) did not have variant classifications available. A large majority of respondents were female [65 (94.2%)] with a small number of male respondents (4). For non-responders the trend was similar with 65 (91.5%) female and 6 male participants. The average age of survey respondents in the overall cohort was 53; respondents with a pathogenic variant tended to be slightly younger with an average age of 47 compared to those with a variant of uncertain significance with an average age of 57 ($p = 0.022$). Non-responders had an average age of 52. All eligible participants had similar race/ethnicity distributions, with most participants identifying as non-Hispanic white. All participant classes had a reported majority of cancer history as opposed to having no cancer history, with the number of cancer diagnoses further represented in table 1.

KnowGene scores. Respondents with a pathogenic variant and those with a variant of uncertain significance were scored separately. As shown in tables 2 and 3, participants with a pathogenic variant generally had higher percentages of correct answers compared to those with a variant of uncertain significance. The mean KnowGene score for respondents with a P/LP variant was 4.62 out of a possible 6 and 4.13 out of a possible 6 for respondents with a VUS. Respondents with a P/LP variant had slightly higher scores on average (by 0.497 points, SE =

0.280) compared to those with a VUS (p = 0.08). Question-by-question performance is shown in table 3 and further described here: the first question was answered correctly by 94.6% of respondents with a pathogenic variant and 93.8% of respondents with a variant of uncertain significance. The second question was answered correctly by 91.9% of respondents with a pathogenic variant and 68.8% of respondents with a variant of uncertain significance; respondents with a pathogenic variant had significantly higher understanding than those with a VUS (p = 0.014). The third question was answered correctly by 89.2% of respondents with a pathogenic variant and 62.5% of respondents with a variant of uncertain significance; respondents with a pathogenic variant had significantly higher understanding than those with a VUS (p = 0.008). The fourth question was answered correctly by 67.6% of respondents with a pathogenic variant and 75% of respondents with a variant of uncertain significance. The fifth question was answered correctly by 40.5% of respondents with a pathogenic variant and 25% of respondents with a variant of uncertain significance. The sixth and final question was answered correctly by 78.4% of respondents with a pathogenic variant and 87.5% of respondents with a variant of uncertain significance. Both groups of respondents had low understanding of how a variant of uncertain significance could influence cancer treatment recommendations. Gender, race/ethnicity, history of cancer, medical specialty of the primary caregiver, whether the variant was known to be shared by other family members, and whether a participant had seen a cancer genetic counselor were all found to have no significant effect on *TP53* knowledge or understanding (data not shown).

FACToR results. Subscale scores are shown by variant class in table 4. Respondents of all variant classifications had the highest levels of functional impairment as it related to positive feelings: the pathogenic cohort had an average subscale score of 10.86 and the VUS cohort had

an average subscale score of 10.40. The pathogenic cohort had a significantly higher score on the negative emotions subscale, 3.38, compared to the VUS cohort, 1.20 ($p = 0.001$). The rates of uncertainty were similar in the two groups. Pathogenic respondents had an average uncertainty score of 2.76 and VUS respondents had an average score of 3.00. Interestingly, the final subscale, privacy, also showed a significant difference between variant classes. Pathogenic respondents had an average score of 1.78 and VUS respondents had an average score of 0.50 ($p = 0.013$).

Discussion

LFS is a rare inherited cancer predisposition syndrome in which individuals have only one normally functioning germline *TP53* gene. This condition increases the risk of developing a diverse array of malignancies, including those seen in childhood. Understandably, a diagnosis of LFS can cause distress and has implications not only for an individual but also for their family members, particularly their children. This can be especially shocking for people who have the expectation of identifying a single breast cancer gene variant, for example, compared to those who grow up in LFS families. Even among those in classic LFS families, it is not possible to know exactly what each individual's risks are; such a diagnosis inevitably carries some uncertainty.

Historically it has been difficult to study LFS because most research institutions don't have many patients with LFS; the PROMPT cohort offers a unique opportunity to collect a larger number of LFS patients. This thesis provided novel data from LFS patients and individuals with uncertain *TP53* variants reporting on their lived experiences and knowledge following the receipt of genetic testing results. This was done using the KnowGene instrument and FACToR scale.

The results of this study suggest that individuals who are diagnosed with a pathogenic *TP53* variant experience greater levels of negative emotions than those with a VUS, and they exhibit slightly higher *TP53* knowledge and understanding than those with a VUS. These findings are in agreement with our previously stated hypotheses and may be leveraged in the future to support studies aiming to improve certain areas of care especially as it relates to the education of both healthcare providers and patients on LFS.

In particular, one question in the KnowGene instrument highlights the importance of *TP53* education (agree/disagree: a VUS does not influence cancer treatment recommendations). 40.5% of participants with a pathogenic variant answered this question correctly, and only 25% of respondents with a VUS answered correctly. While the same may not be true for every cancer syndrome, LFS patients in particular should avoid radiation in the treatment of cancer because the radiation increases the risk of sarcoma or other cancers in the radiation field. It is concerning that a majority of the VUS cohort answered this question incorrectly, as VUS carriers will likely have their variants downgraded to benign and should receive less intense screening and treatment recommendations.

Although participants with a pathogenic variant had a higher level of understanding overall, an interesting finding was that participants with a VUS answered the final question (agree/disagree: multi-gene panel testing could find a mutation in a gene that is not clearly associated with the pattern of cancer in the family) correctly at a higher rate than those with a pathogenic variant (VUS 87.5% correct vs. pathogenic 78.4% correct, $p = 0.326$). This may reflect the environment in which participants were notified of their mutation, since the advent of multigene panel testing has led to the identification of *TP53* variant carriers among those with no striking family history of LFS. Previously, individuals with LFS were identified primarily

through single-gene testing but *TP53* variants are increasingly being found in individuals who have undergone genetic testing without the specific suspicion of LFS [7]. This phenomenon may also be responsible for the decreased understanding among respondents with a VUS as it relates to cancer inheritance and inheritance risks. Individuals with a pathogenic *TP53* variant in the PROMPT cohort were significantly younger than those with a VUS, likely because they were aware of a relevant family cancer history or known variant as opposed to undergoing testing following cancer incidence, for example. This pattern of earlier variant identification may also be a reason for the higher levels of understanding among respondents with a pathogenic variant.

The psychosocial experience of individuals in the PROMPT cohort was assessed with the use of the FACToR scale. Respondents with a pathogenic and those with an uncertain variant scored similarly and highly on the positive feelings subscale (10.86 and 10.40, respectively, $p = 0.634$), reflecting an increased level of functional impairment. Undergoing cancer genetic testing and identifying a variant of interest is not a pleasant experience, as is reflected in that measure. The two groups differed significantly as it related to negative emotions: respondents with a pathogenic variant had significantly higher scores on the negative subscale than those with a VUS (3.38 vs. 1.20, $p = 0.001$). However, a recent study has suggested that as the time since initial receipt of genetic testing results increases, negative emotions can significantly decrease [14]. This may be because individuals adjust to their diagnosis over time or they become more comfortable and secure with screening/disease management. This study was limited in its cross-sectional nature and could be improved through the use of repeated measures at different times of follow-up.

Finding a significant elevation in negative emotions among the P/LP cohort encourages the formation of LFS support groups, with which some survey respondents reported

involvement. A recent systematic review of qualitative studies on cancer support groups found that participation in such groups conferred multiple benefits to participants, including informational support, shared experience, and learning from others [15]. Participating in such groups has the potential to increase *TP53* knowledge and understanding and decrease the emotional impairment seen among individuals who have identified a variant of interest in their genetic testing results.

Both groups of respondents experienced similar levels of uncertainty, (pathogenic = 2.76 and VUS = 3.00, p = 0.742). Even though LFS has been defined since 1969, there still remains ambiguity regarding the best methods for monitoring this syndrome – it is understandable that patients would experience moderate levels of uncertainty in such a setting.

Interestingly, respondents with a pathogenic variant had significantly increased levels of privacy concerns compared to those with a VUS (1.78 vs. 0.50, p = 0.013). Despite the passage of the Genetic Information Nondiscrimination Act (GINA) in 2008, individuals with genetic conditions continue to experience distress and concern for their privacy and the impact their condition may have on their insurance and/or employment status [16]. GINA does not include protection for life insurance, and does not cover individuals who receive government- or military-sponsored healthcare. Further, insurance companies may still request and use genetic information when making payment decisions. These concerns may lead to decreased participation in research studies for individuals with genetic conditions, further contributing to decreased levels of genetic knowledge and understanding. As previously outlined, LFS has multiple screening guidelines that patients are recommended to undergo on a regular basis. These procedures can and do pose a financial burden for some individuals, which may result in decreased adherence. The finding of significant privacy concerns among respondents with a

pathogenic variant supports further work into improving access to relevant resources (e.g., whole body MRI for LFS patients) for individuals with genetic conditions.

Notable differences were seen between respondents with a pathogenic variant and those with a VUS on measures of understanding and psychosocial functioning. However, no clear predictors were identified in this cohort. Gender, history of seeing a genetic counselor, having previous cancer diagnoses, and having a family member share the variant did not significantly affect the model fit. Future work should be done focused on increasing the sample size to identify predictors that can be leveraged to increase levels of understanding and decrease levels of distress.

This study had several limitations. First, the sample was quite homogenous on the basis of race/ethnicity and gender according to available data so these findings may not be generalizable to a larger cohort. A larger sample size may help improve this. However, it is not surprising that a large number of the eligible PROMPT participants are women since many women were notified of their *TP53* variant after undergoing multigene panel testing following breast cancer diagnosis or notable breast cancer family history. The time between LFS diagnosis/receipt of genetic testing results and PROMPT-LFS survey completion was variable among participants. People who have had a longer period of time to come to terms with their diagnosis may have less extreme scores on the FACToR scale.

In this cohort, people with a pathogenic/likely pathogenic variant were significantly younger than those with a variant of uncertain significance which may be indicative of a known variant in the family. Being aware of a variant's existence and pursuing testing to clarify whether an individual has it may be indicative of a higher level of interaction with the healthcare system; repeated medical visits that individuals with LFS experience to maintain their screening regimen

likely increase their genetic knowledge and understanding. People who have been aware of their variant status for longer may have higher scores on the KnowGene than individuals who have recently undergone genetic testing.

The decentralized model of PROMPT is a unique feature that allows the study to expand its reach to individuals who may otherwise not have access to research institutions and identify individuals and families with less common genetic conditions. However, since the PROMPT cohort is not restricted to a single site there is likely variation in the delivery and receipt of care at the individual level as a result of the level of training a medical provider has gone. Areas of improvement for caring for patients with LFS identified in this study include educating both patients and providers about the risks of variants and their inheritability. More psychosocial support for these patients should be a priority of those most involved in their care. Moving forward, PROMPT remains a promising avenue to reach more LFS families and connect them with resources and research opportunities.

Table 1. Demographic and medical characteristics by variant classification[1].

	Pathogenic/likely pathogenic (n = 37, 25.9%)[2]	**Variant of uncertain significance (n = 32, 22.4%)[2]**	**Non-responders (n = 71, 49.7%)[2]**	***p*-Value[3]**
Age				
Average (± SD)	48.6 ± 11.0	57.3 ± 13.3	52.4 ± 13.0	*0.022*
Median (range)	46.8 (30.7–72.9)	59.1 (35.2–80.9)	51.8 (23.4–81.3)	
Sex				
Male	2 (5.4)	2 (6.3)	6 (8.5)	*0.823*
Female	35 (94.6)	30 (93.7)	65 (91.5)	
Race/ethnicity				
Non-Hispanic white	12 (85.7)	17 (85.0)	60 (89.6)	*0.769*
Non-Hispanic black	0 (0.0)	0 (0.0)	2 (3.0)	
Hispanic/Latino	1 (7.1)	1 (5.0)	1 (1.5)	
Asian/Pacific Islander	0.0 (0.0)	1 (5.0)	3 (4.5)	
American Indian or Alaska Native	1 (7.1)	1 (5.0)	1 (1.5)	
Cancer history				
Yes	34 (91.9)	19 (59.4)	50 (70.4)	*0.017*
No	3 (8.1)	13 (40.6)	19 (26.8)	
Unknown	0 (0.0)	0 (0.0)	2 (2.8)	
Number of previous cancer diagnoses				
0	3 (8.3)	13 (40.6)	19 (27.5)	*<0.001*
1	10 (27.8)	17 (53.1)	26 (27.7)	
2	13 (36.1)	2 (6.3)	17 (24.6)	
≥ 3	10 (27.8)	0 (0.0)	7 (10.1)	
Variant classification				
Pathogenic/likely pathogenic	37 (100.0)	0 (0.0)	24 (33.8)	
Variant of uncertain significance	0 (0.0)	32 (100.0)	36 (50.7)	
Unknown	0 (0.0)	0 (0.0)	11 (15.5)	

[1] Table values are mean ± SD for continuous variables and n (column %) for categorical variables.

[2] Numbers may not sum to total due to missing data and percentages may not sum to 100% due to rounding.

[3] *P*-values are for analysis of variance (ANOVA) test for continuous variables and chi-square test for categorical variables.

Table 2A. KnowGene answers from participants with a pathogenic/likely pathogenic variant (n=37).

KnowGene item (% of correct answers)	**Agree**	**Disagree**	**I don't know**
Knowing about inherited risk (passed down within a family) can affect choices about cancer treatments (for example, medications or surgery). **(94.6%)**	35*	1	1
People with an inherited risk for cancer (and their at-risk relatives) are more likely to develop more than one type of cancer. **(91.9%)**	34*	2	1
Cancers in women, such as breast and ovarian cancer, can generally be passed on from either the father or the mother. **(89.2%)**	33*	2	2
Some gene mutations mean a larger increase in the risk for cancer while others mean a smaller increase in the risk for cancer. **(67.6%)**	25*	4	8
A Variant of Uncertain Significance (VUS) does not influence cancer treatment recommendations. **(40.5%)**	6	15*	16
Multi-gene panel testing could find a mutation in a gene that is not clearly associated with the pattern of cancer in the family. **(78.4%)**	29*	1	7

** indicates the correct answer.*

Table 2B. KnowGene answers from participants with a variant of uncertain significance (n=32).

KnowGene item (% of correct answers)	Agree	Disagree	I don't know
Knowing about inherited risk (passed down within a family) can affect choices about cancer treatments (for example, medications or surgery). **(93.8%)**	30*	1	1
People with an inherited risk for cancer (and their at-risk relatives) are more likely to develop more than one type of cancer. **(68.8%)**	22*	0	10
Cancers in women, such as breast and ovarian cancer, can generally be passed on from either the father or the mother. **(62.5%)**	20*	2	10
Some gene mutations mean a larger increase in the risk for cancer while others mean a smaller increase in the risk for cancer. **(75.0%)**	24*	1	7
A Variant of Uncertain Significance (VUS) does not influence cancer treatment recommendations. **(25.0%)**	13	8*	11
Multi-gene panel testing could find a mutation in a gene that is not clearly associated with the pattern of cancer in the family. **(87.5%)**	28*	0	4

** indicates the correct answer.*

Table 3. Question-by-question performance of respondents with a pathogenic/likely pathogenic variant compared to those with a variant of uncertain significance using the KnowGene instrument.

	P/LP mean (SD)[1]	VUS mean (SD)[2]	β (SE)[3]	*p*-Value
Question 1	0.946 (0.229)	0.938 (0.246)	0.008 (0.057)	*0.883*
Question 2	0.919 (0.277)	0.688 (0.471)	0.231 (0.092)	*0.014*
Question 3	0.892 (0.315)	0.625 (0.492)	0.267 (0.098)	*0.008*
Question 4	0.676 (0.475)	0.750 (0.440)	-0.074 (0.111)	*0.505*
Question 5	0.405 (0.498)	0.250 (0.440)	0.155 (0.114)	*0.177*
Question 6	0.784 (0.417)	0.875 (0.336)	-0.091 (0.092)	*0.326*

[1] Mean score for each question by respondents with a pathogenic/likely pathogenic variant. A score of 1.00 would result if every participant answered the question correctly.

[2] Mean score for each question by respondents with a variant of uncertain significance. A score of 1.00 would result if every participant answered the question correctly.

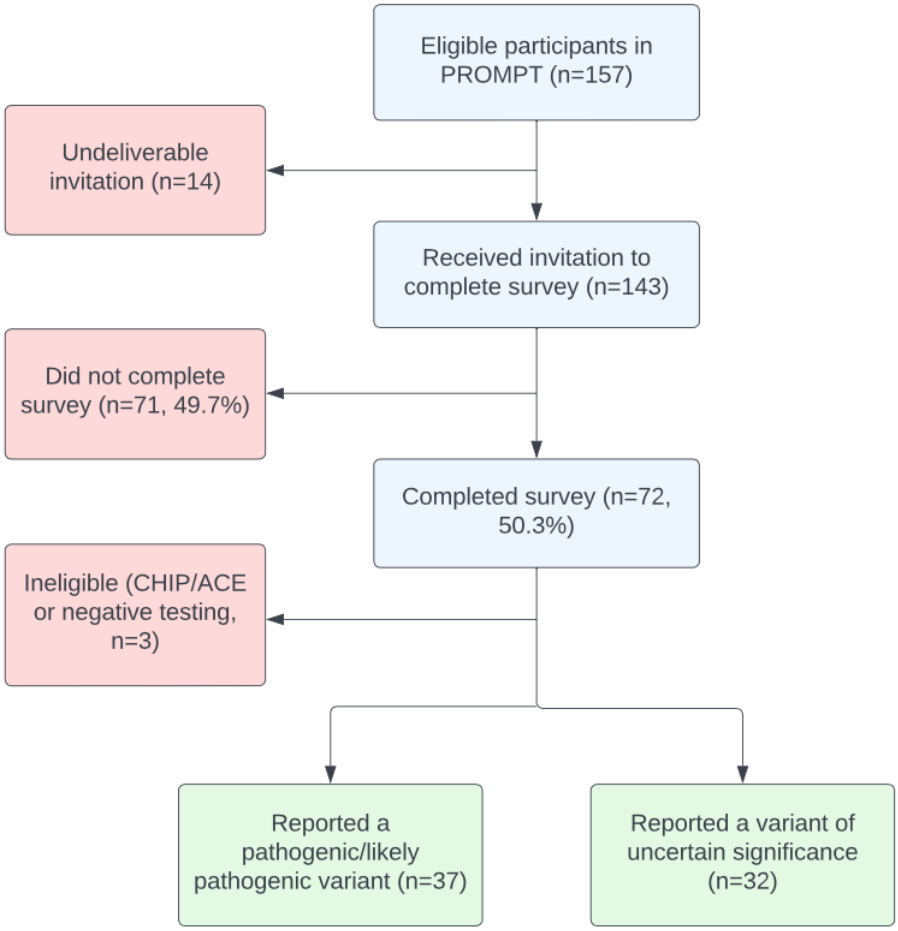
[3] VUS respondents were used as the reference. Beta estimates reflect the difference in performance between respondents with a pathogenic/likely pathogenic variant compared to those with a VUS.

Table 4. Generalized linear model assessing the relationship between psychosocial outcomes, as measured by the FACToR subscales, and variant class.

FACToR scales	**Pathogenic/likely pathogenic (n = 37)**	**Variant of uncertain significance (n = 32)**	***p*-Value[1]**
Positive subscale (0-16)			
Average (± SD)	10.86 ± 2.91	10.40 ± 4.62	*0.634*
Median (range)	11.0 (1.0–16.0)	11.0 (1.0–16.0)	
Negative subscale (0-12)			
Average (± SD)	3.38 ± 3.29	1.20 ± 1.92	*0.001*
Median (range)	3.0 (0.0-11.0)	0.0 (0.0-6.0)	
Privacy subscale (0-8)			
Average (± SD)	1.78 ± 2.48	0.50 ± 1.61	*0.013*
Median (range)	0.0 (0.0-8.0)	0.0 (0.0-8.0)	
Uncertain subscale (0-12)			
Average (± SD)	2.76 ± 2.75	3.00 ± 3.28	*0.742*
Median (range)	2.0 (0.0-10.0)	1.5 (0.0-12.0)	

[1] *p*-Values are for t-test.

Figure 1. Inclusion schema.



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