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MANAGING VARIANT DISCREPANCY IN HEREDITARY CANCER: CLINICAL PRACTICE, BARRIERS, AND DESIRED RESOURCES

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Variants are changes in the DNA whose phenotypic effects may or may not be definitively understood. Because variant interpretation is a complex process, sources sometimes disagree on the classification of a variant, which is called a variant discrepancy. This study aimed to determine the practice of genetic counselors regarding variant discrepancies and to identify the barriers to counseling a variant discrepancy in hereditary cancer genetic testing. This investigation was unique because it was the first to address variant discrepancies from a clinical point of view. An electronic survey was sent to genetic counselors in the NSGC Cancer Special Interest Group. The vast majority of counselors (93%) had seen a variant discrepancy in practice. The most commonly selected barriers to counseling a variant discrepancy were lack of data sharing (90%) and lack of a central database (76%). Most counselors responded that the ideal database would be owned by a non-profit (59%) and obtain information directly from laboratories (91%). When asked how they approached counseling sessions involving variant discrepancies, the free responses emphasized that counselors consider family history and psychosocial concerns, showing that genetic counselors tailored the session to each individual. Variant discrepancies are an ongoing concern for clinical cancer genetic counselors, as demonstrated by the fact that counselors desired further resources to aid in addressing variant discrepancies, including a centralized database (89%), guidelines from a major organization (88%), continuing education about the issue (74%) and functional studies (58%).

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INTRODUCTION

Evolving technologies have made it possible to examine the genome more thoroughly than ever before. With these technologies, such as next generation sequencing (NGS), challenges have arisen, including an increased need for variant annotation and interpretation. Variants are changes in the DNA whose phenotypic effects may or may not be definitively understood. For example, a variant may be found that is known to cause an increased lifetime risk of cancer. Alternatively, a variant may not be well understood, making it difficult to predict if it affects the lifetime risk of cancer for the patient.

The previously mentioned NGS technology led to the development of a style of genetic analysis called panel testing, which first became available in 2012 in cancer genetics (Kurian et al., 2014). Many providers are turning to panel testing using NGS since it is a cost-effective way to test many genes at once (Gallego et al., 2015). It has been shown that the number of variants detected increases with the number of genes sequenced (Lincoln et al., 2015). Therefore, the use of panel testing increases the chance of discovering variants in genes associated with greater risk of cancer. In fact, when 29 genes were sequenced in 1062 people, 41% had at least one variant and 11.4% had two or more variants (Lincoln et al., 2015). When 20,000 genes were analyzed using whole exome sequencing (WES), variants of uncertain clinical significance (VUS) were found in 95% of the study population (Maxwell et al., 2016). The increasing rate of variant discovery has brought to light the importance of understanding the effects of these gene changes.

When a variant is identified, its pathogenicity must be determined. In the 2015 Standards and Guidelines for the Interpretation of Sequence Variants, the American College of Medical Genetics and Genomics (ACMG) recommended a 5-tiered system for indicating pathogenicity: pathogenic (P), likely pathogenic (LP), variant of uncertain

significance (VUS), likely benign (LB), and benign (B) (Richards et al., 2015). According to these guidelines, laboratory classification of a variant should be based on multiple lines of evidence, including population data, disease databases, segregation data, scientific and medical literature, and *in silico* predictors (Richards et al., 2015). Some experts attest that the most useful sources of information include allele frequency, conservation data, co-segregation, and the mutation type (Amendola et al., 2015). Population data can be found in databases such as the Exome Aggregation Consortium (ExAC), 1000 Genomes, and the Exome Variant Server (EVS) (Richards et al., 2015). From these databases, the prevalence of the allele in the population can be ascertained. In general, the more rare the allele, the more likely it is to be pathogenic. Conservation data can be found through Genomic Evolutionary Rate Profiling (GERP) and Combined Annotation Dependent Depletion (CADD). The scores obtained through these services indicate similarity between species. A lower CADD or GERP score means less conservation between species, which is considered less likely to be pathogenic. Co-segregation, another tool for analyzing variants, can determine if a variant tracks with the phenotype within a family. If the variant is present in family members with a disorder, it cannot be ruled out as benign. Lastly, certain mutation types are associated with an increased risk for pathogenicity. These include truncation of the gene, which implies an abbreviated protein product, and a *de novo* condition occurring with a *de novo* variant, which means both the condition and the gene change are new to the proband (Amendola et al., 2015). Based on the information obtained from many sources, the pathogenicity of a variant can be estimated, with varying degrees of certainty.

Once pathogenicity has been gauged, clinicians, including oncologists and genetic counselors, must determine the clinical utility of the results. The ACMG states that variants classified as either pathogenic or likely pathogenic are clinically actionable as the terms imply a greater than 90% certainty that the variant in question is truly disease-

causing (Richards et al., 2015). In such circumstances, management decisions can be made based on pathogenicity, including prophylactic surgery and increased surveillance for tumors. This idea is oversimplified, however, because the classification of variants is not always straightforward. It has been shown that sources are not always concordant in variant interpretation. For example, one laboratory may call a variant a VUS, while another classifies it as likely pathogenic. Differences between laboratories may be due to varying testing techniques, such as use of unlike cell lines or different assays (Karbassi et al., 2016). The differences may also be due to discrepancies in variant analysis, such as weighing evidence differently and setting varying thresholds for pathogenicity. Another source of discrepancies may be that some researchers have access to information that others do not. For example, one laboratory may have an internal database of test results that are not shared with other laboratories (Amendola et al., 2015).

Many previous studies have analyzed the frequency of variant discrepancies. When nine Clinical Sequencing Exploratory Research (CSER) laboratories analyzed 97 variants, the classification was concordant between all laboratories in only 19% of cases (Amendola et al., 2016). This number, however, may not be applicable to the clinical genetic counselor since it is very unlikely that a patient would be tested at nine different laboratories. Other more recent studies comparing annotations found in the ClinVar database to those from commercial genetic testing laboratories have found an 11.7% to 26.7% rate of discordant variant classifications (Gradishar et al., 2017, Harrison et al., 2017).

In order to analyze the prevalence of discordant classification in cancer genes specifically, one study looked at agreement between databases for variants found in their subjects via WES. Classifications were discordant in 16% of cases involving an autosomal dominant cancer gene and 23% of cases involving an autosomal recessive

cancer gene. There was a 4% rate of disagreement in clinical actionability for autosomal dominant conditions, and a 9% rate for autosomal recessive. The same study found a 17% discrepancy rate for all cancer and non-cancer genes, and an overall rate of 4% for disagreement on clinical actionability (Maxwell et al., 2015). Another study found that 26% of cancer variants found via recruitment into the Prospective Registry of Multiplex Testing (PROMPT) had conflicting interpretations when compared to the ClinVar database. In addition, 11% of variants had classifications that differed in clinical actionability (Balmaña et al., 2016). Having up to 26% discordance in variant calling implies an issue for clinicians in a cancer setting, who may receive different classifications depending on the source reporting the result. This is especially troubling in up to 11% of cases when the classification provided by one source implies clinical actionability, while the classification from another source does not.

Because variant classifications can differ between sources, clinicians may have difficulty applying discrepant variant results in a clinical setting. This study aims to identify the strategies that clinical genetic counselors use to understand variant results, to determine current counseling practice when there is a discordant result, and to understand the barriers to counseling when there is a variant discrepancy in hereditary cancer testing. The results may inform future guidelines for clinical application of inconsistent variant annotation.

METHODS

Study Population

This study surveyed practicing cancer genetic counselors, specifically members of the National Society of Genetic Counselors (NSGC) Cancer Special Interest Group (SIG). An email was sent to Cancer SIG members explaining the basis of the study and inviting them to complete the survey. Participation in the survey constituted consent to the study, and counselors could opt to discontinue at any point in the survey. The

inclusion criteria consisted of, 1; being a board certified or board eligible genetic counselor working primarily in oncology, 2; spending more than 50% of their time in a clinical setting, and 3; having attended an accredited genetic counseling master's program. These criteria excluded participants who had different experience and expertise than required for this study. Student members of the SIG were also excluded.

Survey

The survey was created by the authors using Qualtrics software (2015) available through the University of Texas Health Science Center at Houston. It was distributed via email to members of the NSGC Cancer SIG in June 2016. Two reminder emails were sent in July and August 2016. The survey was closed on August 31, 2016. The survey took approximately 15-20 minutes for each participant to complete, and all answers were anonymous.

The survey was a semi-structured questionnaire with 32 questions. There were 7 demographic questions that collected information about schooling and work setting. The next section evaluated the counselor's strategies for assessing variant results. In this section, participants were asked questions about researching variants, including the lines of evidence they used and how often they researched variants independently. Counselors were then asked about their current counseling practice regarding variant discrepancies, including how often they identified a variant discrepancy and how they managed them in a clinical setting. There were two scenarios involving discovery of variant discrepancies, followed by questions about how participants would handle these situations. Lastly, there were questions regarding barriers to counseling when there are discordant variant results. At the end of the survey, the counselors had the option to input their email address to enter a drawing for a gift card. All research protocols met the requirements of the University of Texas Health Committee for the Protection of Human Subjects, and this study was assigned approval number HSC-MS-16-0436.

Statistics

STATA 14 software was used to analyze statistics (StataCorp, 2015). Primary outcomes included descriptive analyses. Results were reported as frequencies with percentages. Secondary explorative analysis was performed, using Fisher exact tests or t-tests based on the nature of the data, to compare differences between groups. All comparative tests were considered significant at type I error rate of 5%. Free text responses were analyzed for similarities.

RESULTS

Demographics

There were 281 responses to the survey, which represented 33% of the largest mailing to 849 counselors. Twenty-five respondents did not complete the demographic information and therefore were excluded from the analysis. There were 24 respondents who met exclusion criteria, including current students and genetic counselors that did not work primarily in a clinical oncology setting. This left a total of 224 responses that were included in analysis. Of these, 60 (27%) were partially complete, and the answered questions were included in analysis. The demographic information of the respondents is reported in table 1.

Table 1: Demographics

	Number of Respondents	Percentage of Respondents
Year graduated from a genetic counseling master's program		
1971-1979	2	1
1980-1989	15	7
1990-1999	23	10
2000-2009	49	22
2010-2016	135	60
Experience in cancer genetics in number of years		
0 to 5	145	65
5+ to 10	39	18
10+ to 15	21	9
15+ to 20	12	5
20+	7	3
Specialties counseled regularly		
Pediatric	34	15
Breast	214	96
Gynecological	211	95
Endocrine	105	47
Gastrointestinal	214	96
Other*	12	5
Licensure available in participant's state		
Yes	102	45
No	120	54
Unsure	1	1

*Other responses: Nine counselors indicated that they counsel all specialties (4%). One counselor indicated each of the following: "prostate" (1%), "leukemia" (1%), and "head, neck, renal, CNS" (1%).

Strategies for Assessing Variant Results

The genetic counselors were asked a series of questions about how they approach a non-discrepant variant result provided by a laboratory. A large majority, 178 (96%) of counselors indicated that they conduct their own research on genetic testing results that report a variant. The most common line of evidence used in research was disease databases, with 148 counselors (83%) indicating that they use them "always" or "most of the time". Other commonly used lines of evidence were mutation type, with 94 (53%) "always" or "most of the time" responses, and conducting a primary literature search, with 87 (49%) "always" or "most of the time" responses. One line of evidence that was less commonly used was functional studies, with 64 (36%) responses. Participants also

ranked the lines of evidence that were most informative when researching variants. Counselors were most likely to use the lines of evidence that they found the most informative ($p=0.01$ for mutation type, $p<0.0001$ for all other lines of evidence).

To further explore the attitudes of counselors about variant results, they were asked if their confidence in the classification of a single variant depended on the laboratory providing it. Of 213 respondents, 178 (83%) of counselors affirmed that their confidence does depend on the performing laboratory. To clarify, one respondent stated, “Not all labs - or classification systems - are created equally. Whichever lab was ‘better’…is the classification I would use for medical management”.

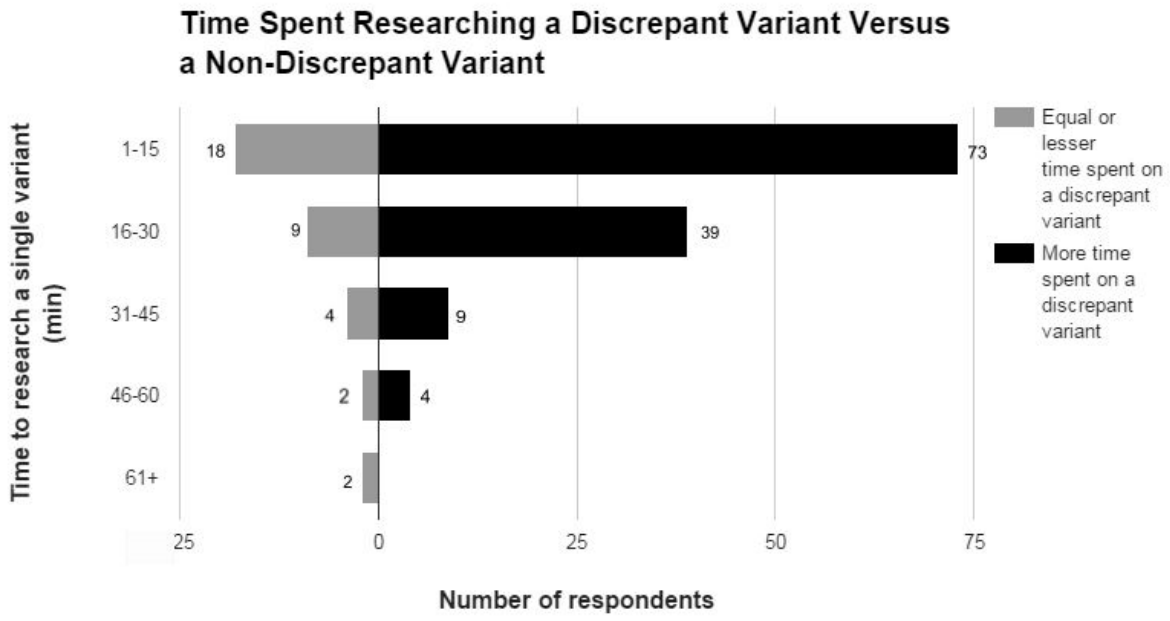
Current Variant Discrepancy Counseling Practice

Of 180 participants, 167 (93%) had seen a variant discrepancy in practice and 111 (62%) had come across a discrepancy on 3 or more occasions in the last 3 years. A total of 143 (78%) counselors found a discrepancy by searching the variant in a disease database. Eighty-five (46%), counselors indicated they discovered a discrepancy by using different laboratories to test relatives, 73 (40%) indicated testing two unrelated patients at different laboratories, and 29 (16%) indicated testing one patient at two different laboratories.

Those that indicated they refer to databases “always” or “most of the time” when researching variants were more likely to discover a discrepancy in a database ($p<0.0001$). However, this same group was not statistically more or less likely to discover discrepancies overall than those who refer to disease databases less often ($p=0.518$). There was also no statistical difference in the likelihood of identifying a variant discrepancy based on the type of testing most frequently ordered ($p=0.254$). Of our 213 respondents, 186 indicated that they order panel testing most often (87%), 4 indicated that they order syndrome-specific testing most often (2%), and 23 indicated that they order the two about equally (11%).

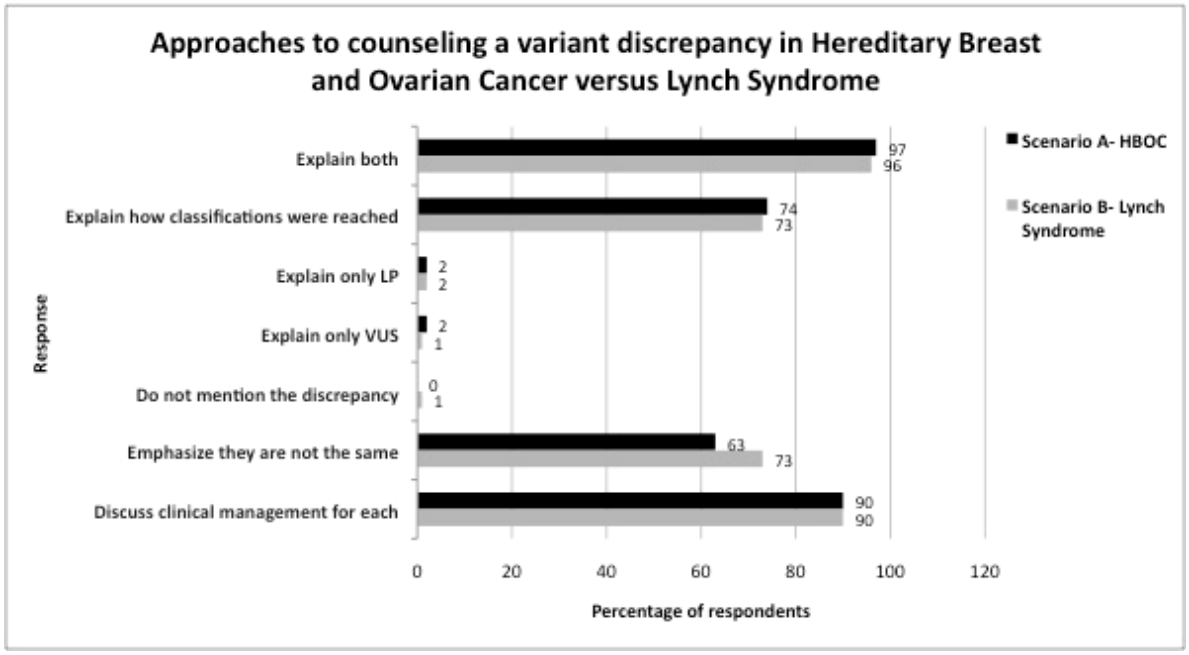
Of 181 counselors, 98 (54%) reported taking 1-15 minutes to research a *non-discrepant* variant result. When asked how long it takes to research a *discrepant* variant with discordant classifications, 40 (24%) counselors indicated that it takes 46-60 minutes, 39 (23%) counselors selected 16-30 minutes, and 31 (19%) selected 31-45 minutes. There was a significant difference in the time spent researching a non-discrepant variant versus a discrepant variant (p=0.001), with the majority of counselors spending more time researching a variant discrepancy than a non-discrepant variant (Figure 1). Of 160 respondents, 125 (78%) spent more time following up on a discrepancy than researching a single variant, and 4 (3%) spent less time. A total of 52 (33%) counselors spent 45 minutes or more of extra time researching a discrepancy compared to researching a single variant. The amount of time a counselor spent following up on a discrepancy was not dependent on the number of discrepancies they had previously discovered (p= 0.482).

Figure 1



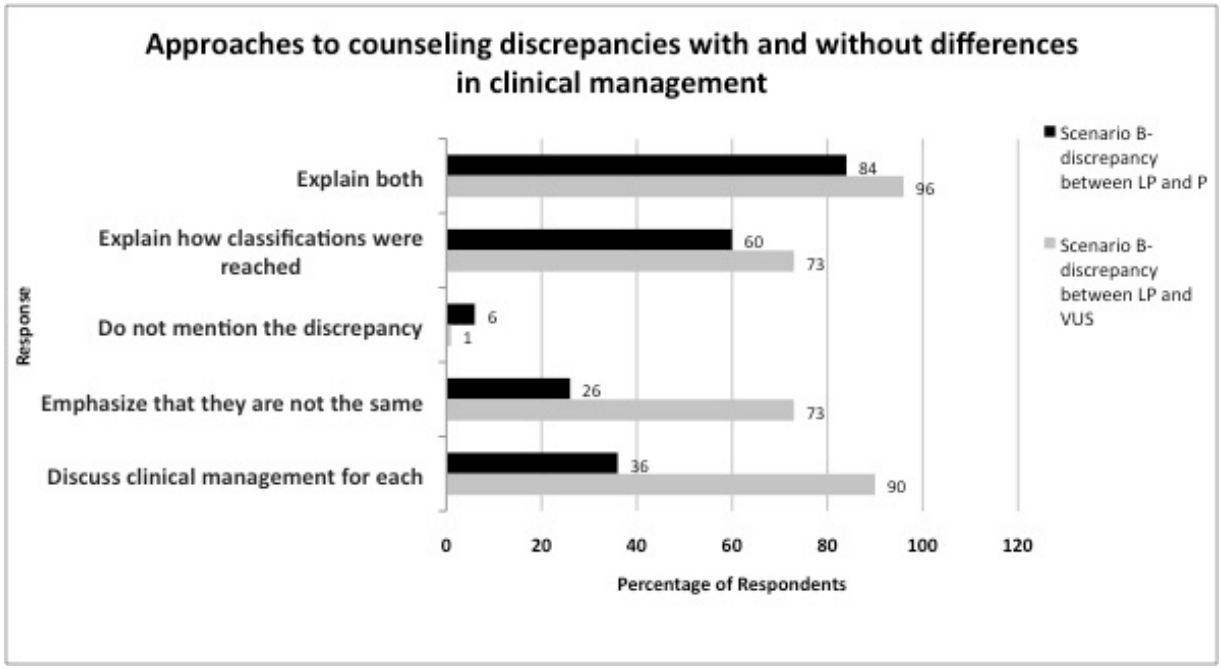
Scenarios were utilized to assess how respondents approach counseling sessions involving variant discrepancies. In scenario A, a variant in Hereditary Breast and Ovarian Cancer (HBOC) was classified by one laboratory as a VUS and by another as LP. Most counselors indicated that they would explain both classifications, explain how each classification was reached, emphasize that they are not the same, and discuss clinical management for each. In scenario B, a variant in a gene associated with Lynch Syndrome was classified by one laboratory as a VUS and by another other as LP. The same four responses were selected most often for this question (figure 2). For the majority of responses, those that selected a specific approach for the discrepancy in scenario A involving HBOC were statistically more likely to choose that same approach for the discrepancy in scenario B involving Lynch Syndrome. This is true for the following responses: explain both ($p=0.015$), explain how each classification was reached ($p<0.0001$), explain only VUS ($p<0.0001$), emphasize they are not the same ($p<0.0001$), and discuss clinical management for each ($p<0.0001$).

Figure 2



In scenario B, participants were also asked to select approaches to a counseling session involving a variant in a gene associated with Lynch syndrome that is classified as LP by one laboratory and P by another laboratory. Counselors were likely to respond similarly to this question as to the previous question in scenario B, mentioned above, where the variant is classified as LP and VUS (figure 3). This was the case for the following responses: explain both ($p=0.014$), explain how each classification was reached ($p<0.0001$), emphasize they are not the same ($p=0.002$), and discuss clinical management for each ($p=0.032$). In general, more counselors chose to emphasize that the classifications were not the same, with 121 (73%) respondents, and to discuss clinical management for each, with 148 (90%) respondents, when comparing LP and VUS. This is compared to 43 (26%) that chose to emphasize that the two classifications were not the same and 60 (36%) that chose to discuss clinical management when comparing LP and P (figure 3).

Figure 3



Both scenarios allowed counselors to write “other” responses to the questions. Many of these responses emphasized the importance of family history in deciding how to approach a counseling session involving a discrepancy and how to manage the patient’s care. For example, a respondent clarified that they “discuss the result in the context of family history and whether prophylactic surgery could be recommended based on family history alone.” The free responses also pointed out a focus on psychosocial support for the patient. One counselor explained that they “discuss psychosocial aspects of having discrepant test results and how that has impacted the patient.”

Barriers to Counseling

When asked to select barriers to the genetic counseling process regarding variant discrepancies, 162 of 164 (99%) counselors selected at least one barrier. The most frequently selected barrier with 147 responses was lack of data sharing (90%), followed by 118 for lack of a central database (72%), 94 for lack of educational resources (60%), and 88 for legal liability for clinicians and laboratories (54%).

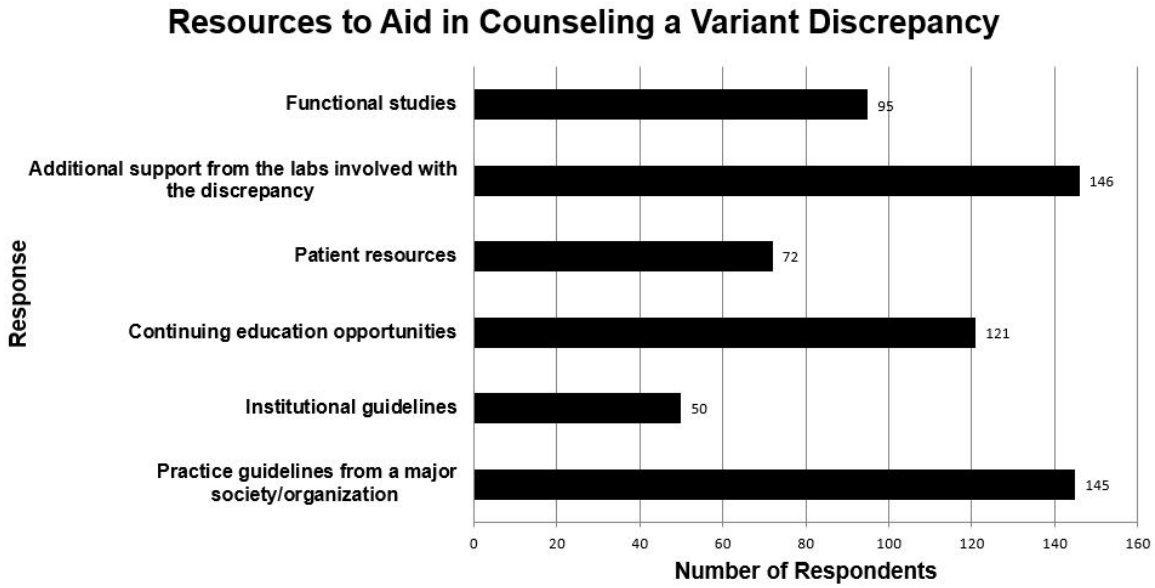
Participants were asked to indicate who should ideally input information into a centralized database, and 150 (95%) selected laboratories only or a combination of laboratories and another source. No participants chose patients as the sole source of information for a centralized database. A total of 96 respondents (59%) indicated that a non-profit should own such a database. Forty-four counselors (27%) chose government as the ideal owner of a centralized database.

Of 164 respondents, 115 indicated that they are concerned about legal liability when it comes to variant discrepancies (70%). Licensed genetic counselors were statistically more likely to indicate that they were concerned about legal liability than those that are not licensed ($p=0.004$). However, there is no statistical difference in the time that licensed and unlicensed counselors spend following up on variant discrepancies ($p=0.203$). In addition, there was no difference in follow-up time between

counselors who are concerned about legal liability and those that are not concerned about legal liability (p=0.205).

Lastly, counselors chose resources that would help in counseling when they come across a discrepancy (figure 4). The most common response selected by 148 (89%) counselors was additional support from the laboratories involved with the discrepancy, followed by 145 (88%) that chose practice guidelines from a major society/organization, 121 (74%) that chose continuing education opportunities, and 95 (58%) that chose functional studies.

Figure 4



DISCUSSION

Frequency and Time Considerations

Clinical cancer genetic counselors should expect to come across the issue of variant discrepancies in practice, given that 93% of respondents saw at least one discrepancy in clinic. There was no difference in the number of discrepancies discovered by those who order panel testing, syndrome-specific testing, or both about equally. This

shows that cancer genetic counselors do not avoid encountering variant discrepancies by ordering syndrome-specific panels.

Most respondents to this survey discovered an average of one discrepancy per year, which is less prevalent than the 16-26% frequency of discrepancies in genes associated with cancer cited in the literature (Balmaña et al., 2016, Maxwell et al., 2015). Some previous studies, however, were looking at results that included many more genes that the typical cancer panel, which today contains anywhere from 2 to 80 genes. Studies show the more genes tested, the more likely there will be a variant found (Lincoln et al., 2015, Maxwell et al., 2016). Therefore, it is not surprising that the previous studies using WES found more discrepancies. It is important to note that the research in cancer genetics is constantly being updated, so it is likely that more genes will be gradually added to cancer panels and could increase the rate at which counselors see discrepancies in the future. Another reason that this study may have found a lower rate of discrepancies is that counselors ordering hereditary cancer testing may not research every result they get. Though 96% of our study population indicated researching variant results independently, it is possible certain classifications are more likely to trigger further research, particularly if there is suspicion that the result may not be appropriate for a patient's clinical presentation or family history. If so, some discrepancies may not be discovered.

Researching a discrepant variant was more time-consuming than researching a non-discrepant variant. In fact, a third of counselors (33%) said they spend over 45 minutes researching a variant discrepancy. This is concerning because it consumes the genetic counselor's valuable clinic time. The time that counselors spent on a variant discrepancy was not dependent on the number of discrepancies seen, licensure status, or concern about legal liability. The fact that time spent was not dependent on how many times they had seen discrepancies demonstrates that prior experience did not impact the

amount of research time needed. None of these influenced the amount of time spent researching a discrepancy, so there must be some other factor that drove the differences between respondents that was not addressed in this survey.

Databases and Barriers to Counseling

The vast majority of respondents (96%) conducted their own independent assessment on variants using available resources, including the 87% of counselors who used disease databases. Disease databases were a widely utilized resource for counselors when researching variants, and our respondents reported this is the most frequently used line of evidence. This could be because many databases with information about variants are readily accessible online, such as the disease databases OMIM and ClinVar (Richards et al., 2015). Survey participants also reported that disease databases were the most common way to discover a variant discrepancy. However, those that refer to disease databases more often were not more likely to discover discrepancies than counselors who used them less often. This shows that counselors that did not use databases were not avoiding discovery of discrepancies. Other methods of discovery, in order from most commonly selected to least, were using different laboratories to test relatives, discovering the same variant in two patients tested at different laboratories, and testing one patient at two laboratories.

Our study demonstrates that very few counselors are satisfied with resources currently available to evaluate variant discrepancies given that a majority (99%) of respondents selected at least one barrier. The most commonly selected barrier was lack of data sharing followed by lack of a central database. Many obstacles would need to be addressed in order to overcome lack of data sharing and create a central database. One of these obstacles is curation. This includes the responsibility of updating the information and ensuring common standardized nomenclature. Furthermore, some may feel that sharing data means that others can make monetary gain from their work (Savage,

2017). In addition, current databases have limited amount of phenotypic information available about the patient with the variant, making it difficult to know if the variant is clinically significant (Johnston et al., 2013).

Despite these obstacles, there are efforts underway to help reconcile the deficit of a central database. One example is the Prospective Registry of Multiplex Testing (PROMPT), which allows patients to self-enroll by inputting their panel testing results. This registry allows data sharing through patient input and allows comparison of variant classifications (Balmaña et al., 2016). Another database, BRCAShare, gathers information about variants in the *BRCA1* and *BRCA2* genes. The data is obtained from the Universal Mutation Database owned by the French Unicancer Genetic Group, along with data from two commercial testing laboratories. This effort combines academic and commercial knowledge into a central database (Rehm et al., 2015). However, BRCAShare only gathers information for two of the many known cancer genes, so much more is needed in the ideal database. There are other available resources, including ClinVar, which is an initiative from the National Institute of Health that gathers information about variants associated with many diseases including cancer, and GENIE, which is an effort from the American Association for Cancer Research that gathers patient information and genomic results (Savage, 2017).

This study clarified the ideal source for inputting data into databases and the ideal owner of a centralized database. The majority of counselors (95%) indicated that the laboratory alone or the laboratory combined with another source should input variant data into a centralized database. Most survey participants (59%) indicated that a non-profit should own such a database and about a quarter (27%) of respondents selected government as the ideal owner. Based on these responses, counselors desire that laboratories input information into a central database that is owned by a non-profit organization without a potential conflict of interest.

Approaching the Counseling Session

When it comes to patient care involving variant discrepancies, counselors were consistent in their approach regardless of the hereditary cancer syndrome involved. Additionally, counselors were statistically likely to choose the same approaches to the session whether or not there was a difference in clinical actionability of the classifications. When there was a discrepancy in actionability, counselors were more likely by percentage to emphasize that the classifications were not the same and to discuss management based on each classification.

The free response field after each scenario indicated that many counselors tailored the session to the individual patient based on the personal and/or family history of cancer, rather than tailoring the session to the syndrome involved or the clinical actionability of the result. Genetic counselors are able to tailor recommendations by synthesizing information from test results, personal history, and family history, which allows them to provide patients a personalized risk assessment. When treatment and management options are discussed in the context of the individual, there is an increase in the quality of care. Other free responses focused on psychosocial support. This type of support is unique to genetic counselors in the context of genetic testing. The fact that respondents were able to tailor their session to the individual patient may be useful for informing counselors who are deciding how to approach a discrepancy and for informing future practice guidelines on variant discrepancies. Specifically that patient care requires individualization based on test results, personal history, family history, and psychosocial situation.

Laboratories and Other Resources

Most counselors (83%) indicated that their confidence in a classification depends on the laboratory providing it. Furthermore, additional support from the laboratories was the most commonly selected resource that would help with addressing a variant

discrepancy. These results indicated that interaction with the laboratory and which specific laboratory is involved were important factors when dealing with variant discrepancies. It also demonstrates the need for laboratories to provide support and resources to providers regarding hereditary cancer testing as well as the responsibilities laboratories have for communicating effectively with providers.

Following additional support from the laboratories involved, practice guidelines from a major society/organization and continuing education opportunities were the second and third most commonly selected resources, respectively. Currently, no guidelines address discrepancies in variant classification, including those from the National Comprehensive Cancer Network, American Society of Clinical Oncology, National Society of Genetic Counselors, or the guidelines on interpretation of sequence variants from the American College of Medical Genetics.

Furthermore, more than half (58%) of counselors chose functional studies as a resource that could help with addressing a variant discrepancy. Functional studies allow creation of the gene product from the DNA sequence. By creating the product from the sequence with the variant, researchers can ascertain if the DNA change maintains functionality of the gene. Functional studies were not widely utilized as a line of evidence for researching variants, which could be because this type of data was not available for many variants. This lack of availability could be due to the fact that they require a high monetary and temporal investment (Simpson et al., 2017). Despite these challenges, functional studies are known to be valuable for interpretation of variants, and performing these studies is of importance to advance knowledge of hereditary cancer (Amendola et al., 2015, Imyanitov et al., 2004, Richards et al., 2015). Fortunately, some genetic testing laboratories have started performing functional studies ("Myriad myChoiceTM HRD Technical Specifications.").

Limitations

The respondents to this survey may represent a skewed sample due to selection bias. Genetic counselors who have seen a variant discrepancy in practice or who conduct their own research on variants may have been more likely to take the survey.

The survey itself was created by the investigators and was not validated. Additionally, some responses had a small sample size, which limited the ability for statistical comparisons between groups. For example, there were a small number of respondents that order syndrome-specific testing most often (n=4, 2%). This made comparisons between that group and the group that ordered panel testing most often (n=186, 87%) less robust.

Future Directions

These results call for additional resources to aid counselors in addressing variant discrepancies, including creation of a centralized database, additional support from laboratories, practice guidelines from a professional organization, continuing education on discrepancies, and functional studies. While respondents noted that having a central database owned by a non-profit with variant information from testing laboratories was desirable, additional research could help further define the ideal disease database and inform the creation of such a resource. A similar study could be conducted in the future to learn if counselors indeed see an increase in the number of variant discrepancies in practice over time.

Conclusions

This investigation was unique because it was the first to address variant discrepancies from a clinical point of view. Cancer genetic counselors discovered fewer variant discrepancies in practice than suggested in previous literature, but addressing this issue is a concern for the genetic counseling profession since the vast majority of counselors have seen discrepancies in practice. Genetic counselors desire

further resources to aid in addressing variant discrepancies, including a centralized database, support from the genetic testing laboratories, practice guidelines from a major organization, continuing education opportunities, and functional studies.

BIBLIOGRAPHY

Amendola, L.M., M.O. Dorschner, P.D. Robertson, J.S. Salama, R. Hart, B.H. Shirts, M.L. Murray, M.J. Tokita, C.J. Gallego, D.S. Kim, J.T. Bennett, D.R. Crosslin, J. Ranchalis, K.L. Jones, E.A. Rosenthal, E.R. Jarvik, A. Itsara, E.H. Turner, D.S. Herman, J. Schleit, A. Burt, S.M. Jamal, J.L. Abrudan, A.D. Johnson, L.K. Conlin, M.C. Dulik, A. Santani, D.R. Metterville, M. Kelly, A.K.M. Foreman, K. Lee, K.D. Taylor, X. Guo, K. Crooks, L.A. Kiedrowski, L.J. Raffel, O. Gordon, K. Machini, R.J. Desnick, L.G. Biesecker, S.A. Lubitz, S. Mulchandani, G.M. Cooper, S. Joffe, C.S. Richards, Y. Yang, J.I. Rotter, S.S. Rich, C.J. O'Donnell, J.S. Berg, N.B. Spinner, J.P. Evans, S.M. Fullerton, K.A. Leppig, R.L. Bennett, T. Bird, V.P. Sybert, W.M. Grady, H.K. Tabor, J.H. Kim, M.J. Bamshad, B. Wilfond, A.G. Motulsky, C.R. Scott, C.C. Pritchard, T.D. Walsh, W. Burke, W.H. Raskind, P. Byers, F.M. Hisama, H. Rehm, D.A. Nickerson, and G.P. Jarvik. 2015. Actionable exomic incidental findings in 6503 participants: challenges of variant classification. *Genome Research Genome Res*. 25: 305–315.

Amendola, L. C. A. M., G. P. Jarvik, M. C. Leo, H. M. Mclaughlin, Y. Akkari, M. D. Amaral, J. S. Berg, S. Biswas, K. M. Bowling, L. K. Conlin, G. M. Cooper, M. O. Dorschner, M. C. Dulik, A. A. Ghazani, R. Ghosh, R. C. Green, R. Hart, C. Horton, J. J. Johnston, M. S. Lebo, A. Milosavljevic, J. Ou, C. M. Pak, R. Y. Patel, S. Punj, C. S. Richards, J. Salama, N. T. Strande, Y. Yang, S. E. Plon, L. G. Biesecker, and H. L. Rehm. 2016. Performance of ACMG-AMP Variant-Interpretation Guidelines among Nine Laboratories in the Clinical Sequencing Exploratory Research Consortium. *The American Journal of Human Genetics* 99: 247.

Balmaña, J., L. Digiovanni, P. Gaddam, M. F. Walsh, V. Joseph, Z. K. Stadler, K. L.

Nathanson, J. E. Garber, F. J. Couch, K. Offit, M. E. Robson, and S. M. Domchek. 2016. Conflicting Interpretation of Genetic Variants and Cancer Risk by Commercial Laboratories as Assessed by the Prospective Registry of Multiplex Testing. *Journal of Clinical Oncology* 34: 4071–4078.

Gallego, C. J., B. H. Shirts, C. S. Bennette, G. Guzauskas, L. M. Amendola, M. Horike-Pyne, F. M. Hisama, C. C. Pritchard, W. M. Grady, W. Burke, G. P. Jarvik, and D. L. Veenstra. 2015. Next-Generation Sequencing Panels for the Diagnosis of Colorectal Cancer and Polyposis Syndromes: A Cost-Effectiveness Analysis. *Journal of Clinical Oncology* 33: 2084–2091.

Gradishar, W., K. Johnson, K. Brown, E. Mundt, and S. Manley. 2017. Clinical Variant Classification: A Comparison of Public Databases and a Commercial Testing Laboratory. *The Oncologist.*

Harrison, S. M., J. S. Dolinsky, A. E. K. Johnson, T. Pesaran, D. R. Azzariti, S. Bale, E. C. Chao, S. Das, L. Vincent, and H. L. Rehm. 2017. Clinical laboratories collaborate to resolve differences in variant interpretations submitted to ClinVar. *Genetics in Medicine.*

Imyanitov, E. N., A. V. Togo, and K. P. Hanson. 2004. Searching for cancer-associated gene polymorphisms: promises and obstacles. *Cancer Letters* 204: 3–14.

Johnston, J. J., and L. G. Biesecker. 2013. Databases of genomic variation and phenotypes: existing resources and future needs. *Human Molecular Genetics* 22.

Karbassi, I., G. A. Maston, A. Love, C. Divincenzo, C. D. Braastad, C. D. Elzinga, A. R. Bright, D. Previte, K. Zhang, C. M. Rowland, M. Mccarthy, J. L. Lapierre, F. Dubois, K. A. Medeiros, S. D. Batish, J. Jones, K. Liaquat, C. A. Hoffman, M. Jaremko, Z. Wang, W. Sun, A. Buller-Burckle, C. M. Strom, S. B. Keiles, and J. J. Higgins. 2015. A Standardized DNA Variant Scoring System for Pathogenicity Assessments in Mendelian Disorders. *Human Mutation* 37: 127–134.

Kurian, A. W., E. E. Hare, M. A. Mills, K. E. Kingham, L. Mcpherson, A. S. Whittemore, V. Mcguire, U. Ladabaum, Y. Kobayashi, S. E. Lincoln, M. Cargill, and J. M. Ford. 2014. Clinical Evaluation of a Multiple-Gene Sequencing Panel for Hereditary Cancer Risk Assessment. *Journal of Clinical Oncology* 32: 2001–2009.

Lincoln, S.E., Y. Kobayashi, M.J. Anderson, S. Yang, A.J. Desmond, M.A. Mills, G.B., Nilsen, K.B. Jacobs, F.A. Monzon, A.W. Kurian, J.M. Ford, and L.W. Ellisen. 2015. A Systematic Comparison of Traditional and Multigene Panel Testing for Hereditary Breast and Ovarian Cancer Genes in More Than 1000 Patients. The *Journal of Molecular Diagnostics*. 17: 533–544.

Maxwell, K. C. A. N., S. N. Hart, J. Vijai, K. A. Schrader, T. P. Slavin, T. Thomas, B. Wubbenhorst, V. Ravichandran, R. M. Moore, C. Hu, L. Guidugli, B. Wenz, S. M. Domchek, M. E. Robson, C. Szabo, S. L. Neuhausen, J. N. Weitzel, K. Offit, F. J. Couch, and K. L. Nathanson. 2016. Evaluation of ACMG-Guideline-Based Variant Classification of Cancer Susceptibility and Non-Cancer-Associated Genes in Families Affected by Breast Cancer. *The American Journal of Human Genetics* 98: 801–817.

"Myriad myChoiceTM HRD Technical Specifications." N.p., Feb. 2016. Web. <https://s3.amazonaws.com/myriad-library/technical-specifications/myChoice HRD Tech Specs_012216_FINAL PMRC APPROVED.pdf>.

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Rehm, H. L., J. S. Berg, L. D. Brooks, C. D. Bustamante, J. P. Evans, M. J. Landrum, D.

H. Ledbetter, D. R. Maglott, C. L. Martin, R. L. Nussbaum, S. E. Plon, E. M. Ramos, S. T. Sherry, and M. S. Watson. 2015. ClinGen — The Clinical Genome Resource. *New England Journal of Medicine* 372: 2235–2242.

Richards, S., N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W. W. Grody, M. Hegde, E. Lyon, E. Spector, K. Voelkerding, and H. L. Rehm. 2015. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine* 17: 405–423.

Savage, N. 2017. Getting Data Sharing Right to Help Fulfill the Promise of Cancer Genomics. *Cell* 168: 551–554.

Simpson, K. J., and J. A. Smith. 2017. Knocking down the obstacles to functional genomics data sharing. *Scientific Data* 4: 170019.

StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.

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