I, Grace Tran, hereby submit this original work as part of the requirements for the degree of Master of Science in Genetic Counseling.

It is entitled:
Parents' Perspectives: Child's Whole Exome Sequencing (WES) Research Results of Uncertain Significance

Student’s name: Grace Tran

This work and its defense approved by:

Committee chair: Cynthia Prows, R.II., M.S.N.

Committee member: Armand H. Matheny Antommaria, M.D., Ph.D

Committee member: Rita Pickler, Ph.D. R.N., P.N.P., F.A.A.

Committee member: Kristen Sund, Ph.D.
Parents’ Perspectives: Child’s Whole Exome Sequencing (WES)

Research Results of Uncertain Significance

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Committee Chair: Cynthia A. Prows, MSN, CNS, FAAN¹
Committee Members: Armand Antommaria, MD, PhD, FAAP¹
Rita Pickler, PhD, RN, PPCNP-BC, FAAN¹
Kristen Sund, PhD, MS, LGC¹

¹ Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, USA
Abstract:

Introduction: Whole exome sequencing (WES) is becoming a common tool used in both research and clinical settings. Stakeholders’ attitudes regarding disclosure of research results are variable and a better understanding of participants’ preferences is needed. Contradictory evidence exists regarding adult participants’ comprehension and perceived impact of disclosed results of uncertain significance. There is also limited literature discussing how parents make sense of WES research results of uncertain significance.

Purpose: To explore parents’ understanding of, reactions to, and ascribed meanings of WES research results of uncertain significance.

Methods: The investigators conducted semi-structured interviews with 8 parents of 5 children who received results of uncertain significance. The investigators used an interpretive phenomenology approach to analyze the data. They developed codes using deductive and inductive logic and two coders coded all transcripts.

Results: Parents fluctuated in their understanding of their child’s WES research results of uncertain significance. Parents expressed disappointment and lingering uncertainties with disclosed results. Parents still had positive views regarding the research results because they saw it as an opportunity to benefit other children/families. In general, parents were willing and wanting to share their child’s results with friends and family members. Parents had high hopes for continuing research on their child’s results.

Conclusion: Genetic providers will need to evaluate the efficacy of current strategies in explaining results of uncertain significance and may need to develop educational tools. Alternatively, genetic professionals have to better address underlying reasons for parents’ fluctuating interpretations of research results. Further studies are needed to assess how receipt of research results of uncertain
significance can impact parents’ psychosocial health. Future studies are needed to assess the potential impacts decreased funding of future research on participant recruitment.

**Keywords:** Whole exome sequencing (WES), return of research results, next generation sequencing (NGS), variants of uncertain significance
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Introduction

Overview of Whole Exome Sequencing

Whole exome sequencing (WES) is a next generation sequencing (NGS) technique used to scan all genetic coding sequences (exons) of an individual. The resulting sequence can then be compared to a reference exome, derived from a standardized genome maintained and updated by the National Center for Biotechnology Information (NCBI) that is a compilation of many different individuals’ DNA sequence [Bick and Dimmock, 2011]. This process can identify variants in the individual’s exome that are not identified in the reference exome. These variants may be of known or unknown significance. Variants of known significance are recognized as pathogenic or benign based on established scientific evidence. When current knowledge evidence cannot confirm whether a variant is pathogenic or not, it is called a variant of unknown significance. Variants of uncertain clinical significance (VUS) or a variant novel genes of uncertain clinical significance (VGUS), have never been reported to cause specific disease and are only predicted to have deleterious effects on the gene product [Wallis, et al., 2013].

More traditional methods used to identify the genetic etiology of a condition often require the suspicion of a specific genetic condition or group of genetic conditions, followed by targeted gene testing of known variants or mutations in the DNA sequence that is associated with the condition(s). With the development of WES, the majority of an individual’s exome can be sequenced yielding information about most genetic variants present in an individual’s exome without needing prior knowledge about candidate genes or a list of suspected genetic conditions [Bamshad, et al., 2011; Raffan and Semple 2011; Ku et al., 2012].

WES has been successfully used to identify new and previously described genes that contribute to Mendelian disorders, undiagnosed conditions with genetic and phenotypic
heterogeneity, and rare variants in complex diseases [Choi et al., 2009; Ng et al., 2010; Futema, Plagnol, Whittall, Neil, & Humphries, 2012; Gilissen, Hoischen et al., 2011; and Need et al., 2012]. Although many studies have demonstrated that WES has been successful in identifying a variety of genetic conditions in research settings, the question of whether or not research results from WES should be disclosed to research participants has been debated in the scientific community. However, little data exists regarding the logistics and effects of disclosing research exome results, such as proper protocols delineating when, by whom, how, and what research results should be returned. Additional considerations need to be taken into account when children, a vulnerable population, are involved, including how the process of undergoing WES and receiving research results can affect children and their parents [Avard, Senecal, et al. 2011; Caleshu, et al., 2010; Ravitsky & Wilfond, 2006, National Heart et al., 2010; and Levesque, Joly, & Simard, 2011].

The difficulties in interpreting and communicating individual research results are of a similar type but much greater magnitude in WES. Human WES yields about 200,000 to 400,000 single-nucleotide variants and small insertions and deletions compared to a reference genome [Yang, et al., 2013]. Thus, it is expected that many of the initial variants identified through WES have the potential to be returned to research participants after filtering and analysis. After filtering of all variants identified in a proband, an extensive process occurs to characterize the variant in relation to the presenting disease phenotype. However, the types and amount of analysis conducted by each research laboratories for variant-calling are vague and inconsistent. Even after a rigorous process of analysis, laboratories still have to decide on the criteria and standards for returning different types of results (i.e., pathogenic, benign, VUS/VGUS). It has been estimated that there are about 4,000-17,000 variants that meet criteria for disclosure with the use of WGS in research settings based on the National Heart, Lung, and Blood Institute (NHLBI) Working Group’s most
recent recommendations for the return of genetic research results to research participants [Cassa et al., 2012]. With the increasing amount of genetic information that can be generated from genomic research studies, there is a need to develop flexible as well as ethically and logistically sound frameworks to help guide the disclosure of WES research results [Cassa, et al., 2012; van El, et al., 2013, Brendenoord, et al., 2011a].

**Incidental Findings**

There is significant controversy regarding whether or not a particular type of results, called incidental findings should be disclosed [Jackson, Goldsmith, O’Connor, & Skirton, 2012; Johnston, Rubinstein et al., 2012; Wolf et al., 2008]. Incidental findings are additional findings accidentally revealed during the testing process that do not pertain to the primary indication for testing, and thus are considered unanticipated results. These results may however have health and/or reproductive implications [Jackson, Goldsmith, O’Connor, & Skirton, 2012; Johnston, Rubinstein et al., 2012]. Different types of incidental findings include genetic health information regarding medically actionable/preventable diseases, carrier status, pharmacogenetic risks, and/or treatable/untreatable adult-onset diseases. Some scholars differentiate incidental findings from a similar type of results called “non-incidental secondary findings” [Green, et al. 2013]. Secondary findings are also unrelated to the initial reason for testing, but in contrast to incidental findings, clinical or research laboratories actively search for these findings to report results to patients or participants. The reporting of unanticipated genetic information may have medical and psychosocial effects on research participants and possibly on other family members [Merrill, et al., 2012].

A Working Group recently released recommendations endorsed by the American College of Medicine (ACMG) regarding the reporting of non-incidental secondary findings for patients undergoing clinical whole exome and genome sequencing. They recommend laboratories actively
identify disease-causing variants using a “minimum list” of 56 genes regardless of the patient’s age and preference. Variants in these genes are considered the more frequently diagnosed of the rare, single-gene conditions that may be identified in patients’ exomes/genomes. Most listed conditions have diagnoses that can be confirmed through an alternative method, and preventative actions or treatments are available [Green et al., 2013]. Burke, et al. (2013) raised concerns regarding ACMG recommendations including the lack of evidence proving clinical and personal utility of screening for these conditions in this patient population and the absence of patient voice in this process. They propose a broader stakeholder discussion to address the benefits/harms of the proposal. Initial studies have found that research participants prefer choosing which IFs they would like to receive, and changing their decision about receipt of such results. This supports a flexible process for return of research results [Lakes et al., 2013; Tabor et al., 2012].

Although many ethical issues have not been resolved regarding the disclosure of incidental findings, WES has been adopted as a diagnostic tool in many clinical settings. However, there is a lack of consensus regarding whether or not research results, including incidental findings identified through genomic research, should be disclosed to research participants. There is moreover, a lack of understanding about how disclosure of WES research results may affect research participants.

**Ethical and Social Considerations**

A significant component of this debate centers on the ethical and social implications of NGS. The debate regarding whether or not to disclose individual research results is not unique to WES [Knoppers, et al., 2006; Miller, et al., 2008]. Arguments in favor of returning individual research results have focused on upholding ethical principles such as autonomy and beneficence. Other arguments have underscored the importance of meeting the research participants’ expectations for reciprocity. Meeting these expectations may help maintain a trusting relationship between
researchers and the public, thereby promoting participation in future studies [Majewski, et al., 2011; McGuire and Lupski, 2010] A major concern of opponents of the disclosure of individual research results is the possibility of therapeutic misconception [Brendenoord, et al., 2011a; Brendenoord, et al., 2011b; Knoppers, et al., 2006; McGuire and Lupski, 2010]. Research participants may not understand the distinctions between research and clinical care and falsely believe that the goal of research is to provide them with therapeutic benefits. Returning results may also cause increased temporal and financial burdens for researchers. Returning genetic information would create even greater burdens given the demands of counseling [Brendenoord, et al., 2011a; Brendenoord, et al., 2011b; Knoppers, et al., 2006; McGuire and Lupski, 2010; Miller, et al., 2008].

Even though there are continuing ethical debates surrounding the return of research results, a developing national and international consensus favors the return of genetic research results, including uncertain or unexpected results [Baret & Godard, 2011; Bollinger, et al., 2011; Facio, et al., 2011; Knoppers, et al., 2006; Namey & Beskow, 2011]. Even if results do not have immediate medical significance, they may have personal significance for research participants such as having access to one’s own genetic health information, general desire for more information, or increased knowledge that can lead to possible treatments in the future and/or influence health behaviors, including future healthcare or reproductive decisions [Baret & Godard, 2011; Yu, et al., 2013; Murphy, et al., 2008; Wright, et al., 2012]. Although there seems to be a general consensus in favor of returning individual genetic research results, some research participants have acknowledged potential drawbacks to disclosure. Potential harms include feelings of anxiety when the variant has an unclear association to the risk of developing disease or concerns about the obligation to inform family members about their potential genetic health risks [Bollinger, et al., 2011; Murphy, et al., 2009; Wright, et al., 2013]
Results of Uncertain Clinical Significance

While much emphasis has been placed on return of IFs, returning research results of uncertain significance creates even greater dilemmas. The Clinical Molecular Genetics Society has issued practice guidelines on the interpretation and reporting of unclassified variants. The practice guidelines state the importance of reporting all VUSs [Wallis, et al. 2013] and currently, most laboratories offering clinical WES report results of uncertain significance pertaining to the proband’s primary indication for testing. Some laboratories are reporting VGUSs while others are only reporting VUSs in genes known to be associated with a disease. Several labs do not differentiate between VGUS and VUS but uses an umbrella term of “variant of uncertain significance.” ACMG guidelines indicate that the decision of whether or not to report VGUS is up to the lab’s discretions [Rehm, et al., 2013].

Various professional working groups, associations, and researchers have published recommendations for returning VUSs in clinical settings [Rehm, et al., 2013; Bookman, et al., 2006; Knoppers, et al., 2014; Sénécal, et al., 2013; Shalowitz and Miller, 2005]. However, recommendations for the return of uncertain results in research settings are inconsistent. For example, some authors recommend returning only certain results for diseases with clear clinical benefits to the participants (proven therapeutic/preventative interventions), while other scholars propose returning all research results that participants elect to receive, including primary research results of uncertain significance [Bookman, et al., 2006; Knoppers, et al., 2014; Sénécal, et al., 2013; Shalowitz and Miller, 2005]. The lack of consensus on the return of research results has raised concerns about the legal ramifications if researchers do/do not return results. Ethical guidelines proposed by working groups and published literature should be differentiated from enforceable laws with which researchers must abide [Wolf, 2013]. The ambiguity in defining what standards
need to be met in order to deem research results important enough to return makes establishing a standard protocol, and potentially enforceable laws, for disclosure of research results difficult.

Perceptions and effects of uncertainty surrounding a diagnosis have been recognized in previous research. On the one hand, parents perceive the lack of a clear diagnosis and prognosis for their child’s condition negatively. Uncertainty about their child’s diagnosis can have long-term adverse psychological impacts on parents, including posttraumatic stress disorder-associated symptoms and general distress [Lipinski, et al., 2006; Lenhard, et al., 2005; Santacroce, 2003]. On the other, many parents believe that a clear diagnosis can provide information regarding causes, prognosis, treatment and social support [Lenhard, et al., 2005, Rosenthal, et al., 2001, Makela, et al., 2009]. Giving a name to a child’s condition may help parents cope with and accept their child’s diagnosis [Lipinski, et al., 2006; Rosenthal, et al., 2001]. However, in cancer genetics, some patients have noted increased anxiety, anger, and frustration after receiving inconclusive or uncertain results [Vadaparampil, et al., 2004; Hallowell, et al., 2002], while others do not experience higher levels of distress [Culver, et al. 2013]. Some asymptomatic (cancer-free) patients undergoing testing for the hereditary breast and ovarian cancer syndrome (identification of mutations in the BRCA1/BRCA2 genes) have expressed a sense of relief with their inconclusive results, believing that negative test results indicates the absence of a genetic mutation running in the family [Hallowell, et al., 2002]. Patient/participant’s responses are therefore variable.

This variability in patient/participant response to disclosed results of uncertain significance may indicate a misunderstanding of such results [Frost, et al., 2004]. This is concerning when performing research genetic testing if research participants have incomplete comprehension of
research results and make healthcare or personal decisions based on inconclusive research results [Caleshu, et al., 2010].

Wright, et al. (2012) suggested that attitudes for the return of different types of individual genetic results are variable and more research and understanding of research participants’ preferences are needed. Because of the novelty of WES sequencing, there is limited discussion about, but a great need to understand the psychosocial effect of returning results of uncertain significance on research participants and their families [Tabor, et al., 2011; Culver, et al., 2013].

Significance of Study

Results of uncertain significance are not unique to WES. However, the use of NGS technology will likely yield a greater number of variant or variant genes of uncertain significance compared to traditional genetic testing technologies. There is contradictory evidence regarding adult participants’ comprehension and the effect of results of uncertain significance. Furthermore, there is limited literature discussing how parents make sense of the WES research results of uncertain significance for their children with unknown, rare genetic disorders. This study addresses the knowledge gap regarding parents’ understanding of, reactions to, and ascribed meanings of WES research results of uncertain significance. Findings from this study may provide valuable information for clinicians and researchers seeking to improve the informed consent and results disclosure processes for parents of pediatric patients and research participants.

Study Purpose

The purpose of this study is to explore parents’ understanding of, reactions to, and ascribed meanings of their child’s WES research results of uncertain significance. Institutional Review Board approval was obtained from Cincinnati Children’s Hospital Medical Center (CCHMC) and the
University of Cincinnati for the larger primary longitudinal study titled Performance of Exome Sequencing for Rare Disorder and Parents’ Experiences Expectations Regarding Results, of which this study was a part.

Methods

Study Design

The primary study from which these data were drawn is a longitudinal study with two aims:

1. Estimate the success rate of WES for finding predicted functional gene variants that co-occur with rare disorders in children.

2. Explore parents’ expectations and experiences following obtaining informed consent for WES and disclosing results and the meaning parents’ assign to the results.

This sub-study analyzes parent interviews following disclosure of the child’s WES research results of uncertain significance. Semi-structured qualitative interviews were conducted using an IRB-approved list of topical questions and related interview probes. An interpretive phenomenology approach was used for data collection and analysis.

Parent Study

Selection of Research Participants for the Parent Study

An expert panel, consisting of up to seven professionals from the Division of Human Genetics at CCHMC, was responsible for selecting appropriate participants for the primary study, evaluating and interpreting WES results, and confirming clinically actionable incidental findings. The professionals had expertise in phenotyping rare disorders, analyzing genomic data, determining significant variants, and evaluating the ethical and social implications of genetic/genomic testing.
Initial recruitment for the primary study was accomplished through purposive sampling. Practicing clinicians at CCHMC identified children, the pediatric probands, whom they believed might benefit from the use of WES to identify a genetic cause to a rare disorder. Treating physicians discussed the possibility of participating in the study with the biologic parents and/or legal guardians of these patients. During the initial assessment of parents’ interest in participating in this research study, the treating clinicians provided a general description of the aims and purpose of the primary study to each prospective participant. If the parents expressed interest, the treating physician submitted a one-page application for consideration by the study’s expert panel. The submitting clinician also suggested a method of analysis based on the family’s medical and family history information. For example, a trio analysis would be suggested to compare the unaffected biological parents’ and affected child’s exomes. Each type of analysis method is used as a pilot test to assess the utility of WES in different clinical situations. Therefore, the application was reviewed by the expert panel and accepted if the panel believed that the method of analysis could contribute to evaluating the clinical application of WES. Once an application was accepted, the clinician confirmed with the parents their continued interest in participation.

In addition to the approval by the expert panel, families invited to participate in the primary study were required to meet the study’s inclusion criteria: 1) the proband had been evaluated by a specialist with expertise in genetics and had previous genetic testing with inconclusive results; or 2) the child had a clinical diagnosis of a condition with locus heterogeneity (condition that can be caused by mutations in genes located at different chromosomal loci). Additionally, at least one biologic parent and other informative biologic family members were willing and able to provide DNA samples. The parent(s) and/or legal guardians of the proband must agree to be informed about the WES results related to both the child’s primary disorder and IFs the expert panel and the
CCHMC IRB classified as “clinically actionable.” Finally, the biologic parent(s) also had to choose whether or not to learn the WES research results pertaining to their own exomes if results were available.

Invitation for primary study participation was extended in-person, through e-mail, via standard mail, or by telephone by a treating physician or healthcare professional at CCHMC. Each family confirmed their interest in participating in the primary study and gave written informed consent.

**Parent Study Disclosure Procedures**

Parents were contacted to schedule their child’s disclosure visit no more than one year after their DNA was submitted for WES. They were encouraged to return to CCHMC for results disclosure and the study interview. During the disclosure visit, the study genetic counselor disclosed the primary research results to the parent(s). There were occasional deviations from the disclosure process. A geneticist was present during the disclosure of results for one family (Family 008) due to the progressive nature and poor prognosis of the child’s condition. The treating geneticist for another family (Family 015) requested permission from the investigators to conduct disclosure.

During the disclosure visit, the genetic counselor or geneticist provided each family a disclosure handout tailored to the child’s WES research results. She/he used the handout to review the aims of the research study, the general technique and limitations of WES, the difference between primary and incidental WES research results, and the different categories of possible test results. The general template for the disclosure handout can be found in the Appendix A. The individual performing the disclosure used simple terminology, metaphors, and applicable figures to help explain the child’s results. The counselor or geneticist provided the parents an explanation of
the different lines of evidence used in the analysis and interpretation of the results. Moreover, she/he identified additional evidence or further research that would be needed. Finally, the counselor or geneticist provided a concluding summary about the child’s WES research results as well as recommendations for the family regarding follow-up. It was explained that the child’s WES research results would be documented in the child’s electronic medical records. Additionally, parents were informed about follow-up clinical visits, imaging, referrals, and further testing.

Sub-study Methods

Selection of Sub-study Participants

Participants in this sub-study were parents of children whose results were of unknown significance. Although parents were able to choose whether or not they wanted to receive their own WES results, all parents in this sub-study were informed that their genomes were only used as a comparison and thus not analyzed sufficiently to identify IFs.

Primary Interview Procedures

The study genetic clinician/genetic counselor who conducted disclosure was not involved in the interviews. Parents were interviewed individually, either in person immediately after results disclosure or by phone soon after results disclosure (within 2 weeks). Two interviewers (G.T. and C.P.) conducted the qualitative interviews for all parents participating in the larger study and alternated mother/father interviews to reduce interviewer bias. For the sub-study, G.T. interviewed one mother and two fathers and C.P. interviewed four mothers and one father. A topical interview guide (Appendix B) was used to initiate and guide the interview to explore parents’ perspectives regarding the disclosed WES research results. The interviewers also explored unanticipated and unexpected responses to better understand research participants’ experience of receiving a specific type of disclosed WES research results. Parent interviews typically lasted 30-60 minutes. Interviews
were audio-recorded and transcribed verbatim by a transcriptionist who had received human subjects research training.

**Data Analysis**

This sub-study used an interpretive phenomenology approach to explore parents’ understanding of, reactions to, and ascribed meanings of disclosed WES research results. A qualitative data analysis software, Atlas.ti 7.1.5, was used to store, organize, and manage qualitative data and investigator comments. The primary coder (G.T.) listened to the audio-recorded interviews while reading the transcripts and taking notes. This allowed the coder to verify the accuracy of the transcripts, and record initial impressions, thoughts, and/or observations. The notes provided documentation of the coder’s initial perceptions of the parents’ responses for more in-depth analysis during the coding process. This process allowed G.T. to build familiarity with the interview transcripts and understand the experience of receiving WES research results of uncertain significance from the research participants’ perspectives (Smith, 2009).

Both deductive and inductive content analysis methods were used to explore parents’ perspectives. G.T. and C.P. identified quotes from all parent transcripts consistent with pre-determined, deductive codes based on interview questions and probes. The primary and secondary coder reviewed areas of disagreement until they reached consensus on the best code to apply. G.T. then coded all remaining transcripts and C.P. reviewed all coding, noting areas of disagreement for discussion with G.T. Coding of parent transcripts helped identify emergent themes prevalent across multiple codes. A research advisory committee served as a validation source to ensure that interpretation, analyses, and conclusions were credible. Overall assertions, interpretations, and analyses of the collective cases will be discussed in this paper.
Results

Study Sample/Sample characteristics

Overall, 44 parents chose to participate in the primary study; as of 01/30/2014, 13 parents had received their WES research results. This thesis focuses on interviews conducted with the parents of five children. The families’ backgrounds can be found in Table 3 in the Supplemental Materials. These parents received results of variant gene of unknown significance (n=5 parents and n=3 children) and variant of unknown significance (n=3 parents and n=2 children). A total of 8 parent interviews were conducted for this sub-study, 3 in-person interviews and 5 telephone-interviews. Five of the parent participants were female and 3 were male. All participants were White/European. All participants identified as biological parents of the pediatric proband. The majority of participants were married (n = 6), 1 was single and 1 was not married but living with a partner. Broad socioeconomic status was indicated by varying education levels including 1 parent with less than a high school education, 2 with vocational/technical school (2 years) training, and the majority (n=5) with some college education. Additional demographic information is presented in Table 1.

Themes are grouped and discussed under the following five major categories: 1) evidence of fluctuating interpretation 2) degree of resolution 3) future benefits to others 4) keeping support system informed and 5) somewhere to start. All of the codes, except for one code (i.e., evidence of fluctuating interpretation) were based on deductive coding.

Evidence of fluctuating interpretation

The majority of the parents (n=5) demonstrated a fluctuating interpretation of the certainty of their child’s WES research results ranging from uncertain to certain. When a research participant was asked what he/she learned during the disclosure visit, the parent’s answer often reflected
understanding of the uncertainty of the research results, but throughout the remainder of the interview parents also reported that or responded as though the results were certain. The following paired quotes illustrate this spectrum of understanding for several parents:

Mother, Family 001:

Understanding - “We know that she has a disorder with her brain. We don’t know 100% what caused it.”

Misunderstanding - [Mother’s response to the interviewer asking her how she feels that her unaffected child included in the WES analysis is a carrier of the VGUS] – “It’s just nice to know [...] It’s just comforting to know, that I do know, that I’m not wondering, “Does he have either, does he have one? And I do know, I know that he’s got one copy of this, and I do know that it’s a possibility that if he met up with somebody else that had one copy, that his children could be like this as well.”

Father, Family 005:

Understanding – “I think it did give us the answer we were lookin’ for. It’s not a very well-understood answer, rather than the technical part of it. Technically we can say that it’s this gene and this sequence and whatever, and it overexpresses in these parts of the body. I guess we understand the technicalities of it, but we may not understand the full effects of it and what it means for people like her as a whole.”

Misunderstanding – “We learned what gene, what part of the gene was causing most of H’s—or what they attributed to most of H’s issues as far as how she presents in clinic.”
Affected mother, Family 002:

Understanding – “I guess we were kind of expecting maybe to not get a definite answer, but kind of like what she told me about this could possibly be the cause of it. They don’t know if it’s something that they can maybe try to do anything about later on in the future, but hopefully maybe they can.”

Misunderstanding – “In the past, they’ve asked me questions about it, when I was younger and stuff. “I don’t know, it’s just the way I was born. I don’t know nothin’ about it.” Now I can tell ‘em it’s because of a protein. [laughs]”

Although most parents had fluctuating interpretations of the certainty of the research results, at some point during the interview, all parents (n=8) seemed to attribute it with a greater degree of certainty than was correct for this type of research results.

Moreover, the three parents who received results with a geneticist present or returned by a geneticist directly, did not have fluctuating interpretations of the results, and saw their child’s research results as conclusive. For example, a mother shared, “Never give up. Other than that, nothin’. Just never give up, regardless. My goal was to find answers, regardless good or bad. I did accomplish that” (mother, Family 008). These participants explained that the results helped resolve questions they had regarding their child’s condition. Thus, some parents believed that the disclosed WES research results confirmed a diagnosis for their child:

“If they would have came back and said they didn’t know anything, they couldn’t find anything, it would have left the idea I think in my head for the rest of our lives of what went wrong? What happened? And especially with us having another child, my wife being pregnant now, the whole time, they’re sittin’ there goin’, ‘Well, we don’t know what
happened to this one, let’s see what happens to this one.” And there’s no way we can figure it out until the end, if they’re gonna whatever, suddenly just pass. It makes it better I guess to know that you’re not sittin’ there thinkin’, “Was it this? Was it that?” Everything under the sun. Now you have an idea that it was this. It kind of just solves that question of never having the answer” (father, Family 015, deceased proband).

Based on these findings, the primary coder (G.T.) reviewed the transcripts from the disclosure visits for the 3 parents in which a geneticist participated. The treating geneticists presented information in a manner suggesting certainty regarding the results of uncertain significance. For example, when discussing the need to confirm the VUSs through a clinical laboratory, the child’s treating geneticist for Family 008 stated, “I’m going to tell you, it’s possible that [the lab] could also say that one or both is a variant of unknown significance. If they do that, it’s up to us to make a formal clinical opinion on that. I will be completely frank with you. If they tell me that’s a variant of unknown significance and they’re not sure, I’m very likely to say, and I probably will say that I still think this is probably what is.” The parents, therefore, may not have misunderstood the results that they received.

Degree of resolution

After receiving results of uncertain significance, a majority of the parents (n=5) expressed additional unanswered questions or lingering uncertainty. One participant stated, “We still kind of have that bit of wondering, is this everything that caused this? Is it even related at all? Versus being told, “Hey, we found it. This is it. This is where it’s at. This is what it does and will do.” [...] but I still question in my mind, is this for sure what the cause is?” (mother, Family 001). These participants hoped to have more information on other children with the same condition as their child so that they could learn about prognosis or projected symptoms associated with the condition. As one
parent shared, “We were just kind of hoping to get a little more on their health issues as far as if there was anything, immediate danger, comin’ up that maybe we could prepare ourself for, anything” (father, Family 001). One mother explained the difficulty of not having a diagnosis for her child, demonstrating how a definitive answer can provide closure for this parent:

“When you don’t have a diagnosis but just a list of symptoms, it’s very difficult to advocate. Nobody, when you’re applying for a government aid program or anything, wants to hear a list of symptoms. They want to hear a word, like Down syndrome. Because that encompasses a whole list of symptoms that are commonly known, to at least a group of physicians. So when you lack something like that, it’s sort of like you’re out there hoping people will believe what you’re saying and that there’s weight to what you’re saying” (mother, Family 005).

As a consequence of the child’s WES results not providing the parents with a sense of resolution, three parents also expressed feelings of disappointment after the receipt of their child’s research results of uncertain significance. Interviewees used modifiers in their speech when describing their feelings about the disclosed results, suggesting the parents’ method of managing their disappointment. As described by one parent, “I guess there’s really nothin’ we can do to change anything. No use in bein’ upset or depressed or down about it. Just kind of let nature take its course” (mother, Family 002).

In contrast to the majority of the parents, three participants indicated that their child’s WES research results provided them with a sense of closure. As described by one participant, “It helps put things in perspective. It helps the closure” (mother, Family 015). Of note, a geneticist was present or had directly returned results during the disclosure visit in each of these cases. Furthermore, two participants expressed positive emotions after the receipt of WES research
results rather than disappointment. For example, a father explained that “after finding out that [the researchers] found out what it was, we felt pretty good” (father, Family 015).

**Future benefits to others**

Although the results did not provide these parents with the exact information they were looking for, many parents (n=6) had a positive reaction to the disclosed results because they believed that their child’s results of uncertain significance might be helpful or useful for future children/families. As described by a parent:

“I kind of think that her results kind of leave behind a H___ legacy [...] even if it doesn’t help her, I hope that it will be helpful to future H___-like patients or patients similar to her and her conditions. I hope that it can be used for—even if it’s not for her good, but for the good of others, almost as a bit of a sacrifice on her part to help other folks. I don’t know necessarily that there will be any quick cures or fixes or changes in management of her, but maybe over time there will be, at least for other people. In that way I feel good. I feel like she’s contributed in a huge way to what doctors and research facilities can understand about the human body” (father, Family 005).

These parents believed that the knowledge and information gained from their child’s results might contribute to furthering science and helping other children/families who might have a similar condition/experience in the future. As one parent shared, “Maybe our research will help someone else. It may be a factor in the future that they look for before people decide to have kids. They know it exists, but she was saying the testing’s not that available yet for this particular gene, so maybe it’s somethin’ one in a million. Maybe it’s somethin’ that they start lookin’ for more often” (mother, Family 001). One father explained his traumatic experience of watching his child die without
knowing the cause. This father believed that he would want his child’s WES research results to benefit other parents by providing other parents with an answer even if there was no cure or treatment to the disorder:

“It doesn’t matter who they are, if you can catch this sooner or get an idea of what’s wrong with their child, or worst case, even if their child comes out and is having problems and you figure out that it’s this, even though there’s nothing you can do, the child’s gonna pass no matter what, at least it gives them an answer. [...] There’s nothin’ worse than just sitting there and watching your child die and nobody know what it is or what to do, but at least they could maybe go, “Hey, we found out what it is. We can’t stop it from happening. Even though your child’s been born, there’s no medicine that can stop it.” At least it answers that question, makes it a little easier than just sittin’ there and watchin’ your child die and not havin’ a clue why” (father, Family 015).

Keeping support system informed

Nearly all parents (n=7) voiced their willingness to share their child’s results to other friends and family members. One mother stated, “Yeah, [I’ll share] with my family. I mean, because that’s been my whole support group through all of this” (mother, Family 008). Participants clarified that they will share their child’s results because they are not “ashamed” of their child’s disease and would share the information with people who have been with them through their child’s diagnostic journey. For example, a father said, “We want the word out. My wife does a blog. Even as soon as we leave here, I’m sure she’ll update the blog. There’s no need to hide nothin’. We’re not ashamed of ’em. We want people to know” (father, Family 001). Similarly, another parent expressed:
“Yeah, I think we’ve probably told every family member we know. There’s a lot of people that are aware, maybe not to the detail, that it’s gene number this and sequence number that, but anyhow, yeah, we have shared and been happy to share. We have a lot of people who have prayed for her and prayed for us and taken her health into high regard in their own thoughts and prayer life. So yeah, we have told—kind of shouted it from the rooftop by sharin’ it with friends and family and the acquaintances we know who have followed us through our ongoing journey” (father, Family 005).

A mother shared that she was “glad that [the results are] in EPIC [the electronic medical record] so that... [healthcare providers] have her information, [and] felt good about [the results] being in her record, that it’ll be there for them to access in case they needed it” (mother, Family 005). This mother’s willingness to share her child’s results extended beyond that of her friends and families, expressing contentment in knowing other healthcare providers will also have access to her child’s disclosed WES research results. This mother was unique in that she also indicated the importance of sharing results because of the reproductive health implications the information can have for other family members. The mother stated, “I think it’s kind of an important piece of information for them to have, to be aware of. Like [the genetic counselor] said, it was one in a million for my husband and me to meet up, but we did. So they’re gettin’ married and they’re having kids and their kids is gonna grow up to have kids, and it’s a possibility that they will carry this mutation as well” (mother, Family 005).

One parent stated that she did not plan to share her child’s WES research results. This mother explained, “Me personally, I’m pretty private about the whole thing, and I’m kind of upset
‘cause my husband told his mother about it.” She elaborated that she is “a private person [...and...] internalize things differently, maybe, than other people” (mother, Family 015).

**Somewhere to start**

Many participants (n=5) stated that the disclosed result provided the researchers a starting point to better understand their child’s condition. A mother described the result as “a step in the right direction” (mother, Family 005). Research participants explained that the disclosed results of uncertain significance can guide future research, which ultimately might help discover the answer to their child’s condition. As explained by one research participant, the disclosed uncertain result is “somewhere to start at now, somewhere [the researchers] can look” (father, Family 001). Other participant responses mirrored this idea, “the more this [research] goes on, they said they was still doin’ some studies and things, we may find out even more in the future” (mother, Family 001).

Furthermore, a majority of the parents conveyed positive reactions to and high hopes of researchers continuing research to gain more understanding/information about their child’s results. Parents were “glad” and “encouraged” in knowing that the researchers will be progressing with the information they gained from their child’s research results, further suggesting the belief that the disclosed WES research results is the initial step in finding the answer to their child’s condition. For example, one parent explained her understanding was that the researchers “were gonna keep [...] going through it and try to find other candidates to compare it to and everything, which is good” (mother, Family 002).

Three participants who believed their child’s results had provided the parents with an answer to their child’s rare condition did not see their child’s results as “a step in the right direction” or closer to finding an answer to their child’s disorder.
Discussion

This sub-study explored parents’ perspectives in receiving WES research results for their child diagnosed with an unknown, rare condition suspected to have a genetic etiology. Specifically, this study adds to the literature by focusing on parents’ understanding of, reactions to, and ascribed meanings of the WES research results of uncertain significance. The following section will be a discussion of the themes identified, raising pertinent issues and concerns in the context of current literature related to each theme and providing relevant recommendations when possible.

Fluctuating Understanding

Previous studies have demonstrated patient/participant misunderstandings of genetic test results [Cadigan, et al., 2011; Klitzman, 2010; Namey & Beskow, 2011; Reiff, et al., 2013; Richter, et al. 2013]. This finding is consistent across diverse research participant populations in cancer, pediatric, and adult genetics specialties. Similar to other studies, the 8 parents in our study consistently misinterpreted the results of uncertain significance and conveyed a greater degree of certainty to the disclosed results. Parents seemed to perceive the results as a conclusive answer at some point during the interview, indicating that the identified VUS/VGUS provided a causal explanation for their child’s clinical condition. However, unlike previous studies, the 5 parents whose results were disclosed by a genetic counselor also accurately provided responses that demonstrated their understanding of the inconclusive results. For example, several participants recounted the uncertainty of the results returned and expressed additional unanswered questions regarding their child’s research results indicating their ability to process the meaning of a VUS/VGUS. Parents’ belief that the VUS/VGUS provided an answer to their child’s condition and communication of lingering uncertainties after receipt of results illustrates their vacillating interpretation of their child’s research results of uncertain significance.
Patients, influenced by various external factors and/or emotional needs, may uphold their misunderstandings even if they are cognitively capable of understanding the genetic test results [Klitzman, 2010]. Cadigan, *et al.* (2011) discussed how research participants with a diagnosis of cystic fibrosis are more likely to assume clinical validity and utility in genetic research results compared to healthy research participants. Similarly, Namey & Beskow (2010) demonstrated that different research participant populations may have unique expectations and preconceptions prior to results disclosure that influence the meanings and understandings of the research results. Thus, parents’ desire and strong motivation in obtaining a conclusive answer may influence the certainty they attribute to their child’s VUS/VUGS.

Alternatively, parents may be choosing to interpret uncertain results as a conclusive explanation of their child’s disorder as a mean of constructing order and concrete meaning in an uncertain situation regarding their child’s diagnostic odyssey. For example, parents may want to view the VUS/VUGS as the cause to their affected child’s condition in hopes of knowing that an unaffected child or family member may be able to undergo testing to better quantify their risk or risk for future generations to develop the same disease. This fluctuating understanding within most parent transcripts seems to reflect how parents perceive and make sense of their child’s results of uncertain significance.

Geneticists were present during the disclosure of results to 3 parents. Several authors have discussed the risk of conflating the roles of researchers and clinicians with the return of individual research results, thus allowing for the “diagnostic” misconception to occur [Clayton and Ross, 2006; Meltzer, 2006]. Parents may be interpreting results of uncertain significance as a conclusive answer when the child’s treating clinician (i.e. geneticist) is involved in the disclosure process.
A review of the disclosure conversations, however, suggests that these parents correctly interpreted what was told to them. The parents’ erroneous interpretations may be a direct reflection of the geneticists’ attitude and language used to describe the results of uncertain significance. Geneticists are trained to interpret genetic test results in the context of the patient’s clinical presentation. In contrast, a clinical or research genetic counselor may take a different approach in explaining the definition of a VUS/VGUS and limitations of research results. As a consequence, the discloser’s clinical and/or research experience may influence the degree of certainty they feel regarding the VUSs and the manner with which they disclose research results to the participants. Therefore, in order to separate the goals of research and clinical care, it may be important for research results to be disclosed by a trained healthcare professional not involved in the medical care of the participant and his or her child.

Concerns arise when parents have misinterpretations or a fluctuating understanding of genetic research results of uncertain significance. Research participants are advised that clinical decisions should not be made based on research results due to the experimental nature of research testing [Fabsitz, et al., 2011]. However, research participants may make personal and/or clinical decisions despite cautions against doing so by researchers [Caleshu, et al., 2010]. Discourse between research, medical, and public communities is warranted to reach consensus on whether or not research results of uncertain significance should be returned to research participants if harmful consequences are caused as a result of participant fluctuating interpretation of research results. Researchers may therefore have a duty to not disclose research results of uncertain significance even though there is a trend favoring the return of research results [Baret & Godard 2011; Bollinger, et al., 2011; Facio, et al., 2011; Knoppers, et al., 2006; Namey & Beskow, 2011].
Attributing results a greater degree of certainty is evident in all participant responses in our sub-study even though the genetic clinician used various modalities to help participants understand their child’s results. Genetic healthcare providers will need to evaluate the efficacy of current strategies in explaining results of uncertain significance and develop educational tools accordingly to help better explain this type of results. Alternatively, if parents are choosing to interpret results as means of coping with the uncertainty of the research results, there may be a need for genetic and non-genetic healthcare professionals to better address the underlying reason for why parents are viewing uncertain results as conclusive. For example, Klitzman (2010) suggested using exploratory strategies such as confronting patients’ inconsistent responses to their understanding of genetic results. Addressing any underlying psychological/emotional influences may help resolve the root cause for their attribution of certainty to the disclosed results. Ultimately, this probing technique may help improve their understanding of the results of uncertain significance. However, such thorough counseling would require specific expertise and additional time and funding allotted to the results disclosure process.

Degrees of Resolution

The majority of parents in our sub-study conveyed feelings of disappointment and lingering uncertainties upon receiving their child’s WES research results of uncertain significance. Parents had many unanswered questions regarding their child’s long-term prognosis or how the VUS/VGUS might affect their child. This emotional reaction further illustrates parents’ understanding of the uncertainty of their child’s disclosed VUS/VGUS as the research results did not provide these parents with a definitive answer to the cause of their child’s condition or the clinical impact of the VUS/VGUS on the child.
Previous studies have discussed how some parents receiving a diagnosis of well-known conditions such as fragile X, Klinefelter, or Turner syndrome [Whitmarsh, Davis, & Skinner, 2007] or an atypical presentation of cystic fibrosis [Tluczek, et al., 2010] welcome the uncertainty surrounding a diagnosis. They hope their child’s future will not be consistent with the disease’s typical natural history. Thus, some parents have favored a sense of uncertainty regarding a diagnosis, hoping their child would not be constrained by the limitations projected by the diagnosis of a specific condition [Whitmarsh, Davis & Skinner, 2007; Tluczek, et al., 2010]. In contrast, the majority of the parents in our study experience additional uncertainties, sentiments of disappointment, and lack of closure after receiving results of uncertain significance. Of the parents who did not fluctuate in their understanding of the results and saw the results as a conclusive answer to their child’s condition, these parents expressed relief and satisfaction, akin to a sense of closure, upon receiving results.

Parental uncertainty surrounding a child’s undiagnosed condition has been reported to have psychological impacts, such as general distress and helplessness [Lipinski, et al., 2006; Lenhard, et al., 2005; Santacroce, 2003; Graunagaard and Skov, 2006]. Receiving results of uncertain significance for a child with an unknown rare condition indicates the absence of prognostic information, which makes it difficult for parents to plan for the future. Similarly, parents in our study felt disappointed by the uncertainty of the results received and expressed wanting more prognostic information regarding their child’s condition. This finding is consistent with the idea that parents seek a definitive answer for their child’s undiagnosed condition in hopes of having knowledge to help prepare for the future and gain control in an uncertain situation [Lenhard, et al., 2005; Rosenthal, et al., 2001; Makela, et al., 2009; Graunagaard and Skov, 2006; Macleod, Craufurd, & Booth, 2002].
Further quantitative studies may be needed to assess how receipt of a child’s WES research results of uncertain significance can impact parents’ psychosocial well-being.

**Future benefits to others**

Although parents had reactions of disappointment from their child’s results, many parents still held positive opinions of the results because they believed their child’s results could have an impact by helping future children and families affected with a similar condition. Nearly all the parents believed their child’s results could contribute to scientific research and might help researchers develop treatments or a cure for other children in the future regardless of whether or not their child would benefit from such advances. Participation in genetic research study based on altruism has been reported in many participant populations [Rosenthal, *et al.*, 2001; Trottier, *et al.*, 2012]. Findings from this study show that research participants had mixed beliefs including altruistic ones. Parents’ primary intention was to find an answer to their child’s condition. However, parents still expressed altruistic thoughts after receipt of research results of uncertain significance, hoping the results would benefit children/families in the future regardless of whether or not future innovations would benefit their child. Ultimately, parent responses from this study support the idea that parents of children diagnosed with a rare, unknown disease see their child’s results as a way of benefiting other children/families by adding to scientific knowledge.

**Keeping Support System Informed**

Genetic testing can have important implications for not only the proband that underwent testing, but also for other family members as well, presumably because they share similar genetic make-up with the proband. Much of the literature focuses on practices and issues of sharing
genetic test results with other individuals (first degree, second-degree, and/or third-degree relatives) due to the genetic health implications it can have for at-risk family members [Aktan-Collan, et al., 2010; Stoffel, et al.; 2008, Peterson, 2005]. However, unlike previous studies, findings from our sub-study showed that parents were not sharing results for the reason that other family members may benefit from knowing genetic health information. Parents were willing and wanting to share research results to provide updates and keep support system informed about their child’s progress throughout their child’s diagnostic odyssey. Furthermore, parents in our sub-study chose to share results with not only other family members, but also friends who have been involved in the care of their child.

Parents’ willingness to share their child’s WES research results underscores the importance of a support system in aiding parents through their child’s diagnostic odyssey. The utility of a support system in helping parents cope with a child’s diagnosis has been discussed extensively in the literature [Barnett, et al., 2003; Canam, 1993; Hodapp, Fidler, & Smith, 1998; Yu, J.H., et al., 2013]. Nearly all parents in this study indicated their firm preference for sharing their child’s results with other family members and friends. Specifically, parents emphasized that they are excited to share this information because people are interested in learning about their child’s results. This finding suggests that parents make active efforts in maintaining connection and communication with their support system throughout their child’s diagnostic journey, and the research results is another piece of information about their child that parents can share with people.

Somewhere to start

Parents in our sub-study appeared to construct meanings from their child’s disclosed WES research results by having a positive attitude and high hopes for further research and gaining more
understanding/information about their child’s results. Previous studies have suggested that hope and positive expectations may protect parents from negative emotions and help parents cope with an uncertain situation [Grootenhuis and Last, 1997; Tluczek, McKechnie, & Lynam, 2010]. Similar findings are noted in parent responses from this study. Parents were optimistic and “encouraged” in knowing that researchers are invested in learning more about their child’s WES research results. In a study exploring parents’ perspectives on participating in genetic research of autism, Trottier, et al. (2012) found that parents had similar expectations for investigators to continue ongoing research to help better understand the etiology of autism.

Participants’ high expectations for continual research brings forth issues of duty to re-contact with updated research information, pressure on investigators to have ongoing research, and the potential impact on participant recruitment of future studies if participants lose trust in the research community. In our study, the genetic clinician disclosing results explained to parent participants that investigators have plans to publish and continue research in hopes of learning and understanding more about their child’s results of uncertain significance. By planting the seed of this possibility, are researchers promoting participants’ overly high hopes for ongoing research? Recent national budget cuts and sequestration on biomedical research in the United States will likely increase competition for genetic/genomic research grant funding [“Budget cuts,” 2013]. Although researchers may be motivated to continue research projects, diminished or absent funding can affect their ability to continue research in this area. Research participants have stated their desires for researchers to provide continual updates on research pertaining to their own/child’s genetic research results [Lakes, et al., 2012; Miller, et al., 2007; Trottier, et al., 2012; Wright, et al., 2013]. The inability of investigators to continue research studies or re-contact participants with updated
genetic information may disrupt the community’s willingness to participate in future research studies.

**Limitations**

There are several limitations to this sub-study. First, we used a small purposive sample and thus results have limited generalizability. Further, all research participants self-identified were white and thus, this research has limited focus in terms of ethnic/racial diversity. As this qualitative study did not reach saturation in terms of the themes identified, additional qualitative studies with larger samples will need to confirm our findings. Finally, parents in this study have undergone a diagnostic odyssey to search for an answer for their child’s rare condition with a suspected genetic etiology. Based on the inherent nature of parents who agreed to have their child undergo WES, this study may be subject to selection bias. Interviewed participants are parents of children who have gone through the testing diagnostic odyssey and are likely to be highly motivated in seeking the underlying genetic cause to their child’s disease. Therefore, responses may not be representative of all parents who have children with a rare, unknown genetic condition.

**Summary**

Parents fluctuated in their understanding of their child’s WES research results of uncertain significance, which may include both understanding and misinterpreting their child’s research results. Researchers may need to utilize different counseling or teaching strategies to effectively explore the underlying reason for why parents are attributing a greater degree of certainty to the research results of uncertain significance. Alternatively, if research participants are misinterpreting results when treating geneticists are involved in the disclosure process, it will be important for results to be returned by a qualified professional who does not have a clinical relationship with the
research participant. Further research is needed to better understand how research participants and genetic and non-genetic clinicians are using research results of uncertain significance when making personal and/or healthcare decisions to better assess the consequences of parents vacillating in their interpretations of the certainty of their child’s results of uncertain significance. Parents’ initial reaction upon receiving results of uncertain significance was a sense of disappointment and lack of resolution, further indicating parents’ spectrum of understanding for their child’s results. Nonetheless, many parents expressed positive views regarding the disclosed results because they saw their child’s results of uncertain significance as a way for their child to benefit other children/families in the future. Although parents expressed negative emotions upon receiving their child’s WES research results of uncertain significance, parents were willing and wanting to share their child’s results with friends and family members. Sharing research results of uncertain significance emphasize the importance of a support network and introduce the possibility of familial testing in some cases for this participant population and the personal utility the research results may have for parents included in this study. Overall, participants expressed appreciation and optimism for the researchers’ interest and plans for continuing the longitudinal study. Investigators may be unintentionally contributing to participants’ overly high expectations for continued research. Research is needed to further study the potential impacts on participant recruitment if ongoing research cannot be sustained with limited funding.
References


Ravitsky, V., & Wilfond, B. S. (2006). Disclosing individual genetic results to research participants. Am J Bioeth, 6(6), 8-17. doi: 10.1080/1526516060934772


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**Tables**

*Table 1 – Glossary*

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>WES</td>
<td>Whole exome sequencing</td>
</tr>
<tr>
<td>WGS</td>
<td>Whole genome sequencing</td>
</tr>
<tr>
<td>Incidental Findings (IFs)</td>
<td>Findings accidentally revealed using WES but do not pertain to the primary indication of testing and thus are considered unanticipated results. May have clinically significant and potential health or reproductive implications</td>
</tr>
<tr>
<td>Non-incidental secondary findings</td>
<td>Findings that are unrelated to the proband’s initial reason for testing, but clinical or research laboratories actively search for these findings</td>
</tr>
<tr>
<td>Variant of uncertain significance (VUS)</td>
<td>Variant identified in a gene that previously has been associated with a human disease. Specific variant has never been reported to cause the disease and is only predicted to have deleterious effects</td>
</tr>
<tr>
<td>Variant gene of uncertain significance (VGUS)</td>
<td>The gene has never been reported in association with a human disease, and the identified variant in this new gene is only predicted to have deleterious effects</td>
</tr>
<tr>
<td>Results of uncertain significance</td>
<td>Includes both VGUS and VUS</td>
</tr>
<tr>
<td>Uninformative results</td>
<td>Negative testing results for a hereditary cancer syndrome in an unaffected individual when no mutation has been identified in the family</td>
</tr>
</tbody>
</table>
### Table 2 – Demographic Information

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3 (37.5%)</td>
</tr>
<tr>
<td>Female</td>
<td>5 (62.5%)</td>
</tr>
<tr>
<td><strong>Self-Identified Race/Ethnicity</strong></td>
<td></td>
</tr>
<tr>
<td>White/European</td>
<td>8 (100%)</td>
</tr>
<tr>
<td><strong>Relationship to Proband</strong></td>
<td></td>
</tr>
<tr>
<td>Biologic Father</td>
<td>3 (37.5%)</td>
</tr>
<tr>
<td>Biologic Mother</td>
<td>5 (62.5%)</td>
</tr>
<tr>
<td><strong>Highest Level of Education</strong></td>
<td></td>
</tr>
<tr>
<td>Less than high school</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Some College</td>
<td>5 (62.5%)</td>
</tr>
<tr>
<td>Vocational/technical school</td>
<td>2 (25%)</td>
</tr>
<tr>
<td><strong>Marital Status</strong></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>6 (75%)</td>
</tr>
<tr>
<td>Single</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Not Married but living with partner</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td><strong>Residential Region</strong></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>3 (37.5%)</td>
</tr>
<tr>
<td>Urban</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Suburban</td>
<td>3 (37.5%)</td>
</tr>
<tr>
<td><strong>Child's Parental Marital Status</strong></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>6 (75%)</td>
</tr>
<tr>
<td>Separated</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Participant ID</td>
<td>Exome Analysis</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>DHG_001_II01</td>
<td>ROH then trio if variant identified</td>
</tr>
<tr>
<td>DHG_001_II02</td>
<td>ROH then trio if variant identified</td>
</tr>
<tr>
<td>DHG_002_II02</td>
<td>Trio analysis for de novo mutation</td>
</tr>
<tr>
<td>DHG_005_II01</td>
<td>Trio analysis for de novo mutation</td>
</tr>
<tr>
<td>DHG_005_II02</td>
<td>Trio analysis for de novo mutation</td>
</tr>
</tbody>
</table>
| **DHG_008_II02** | Trio analysis for de novo mutation | VUS | Muscle biopsy, ETC I-IV studies; Microarray; Gene DX comprehensive epilepsy panel; Athena myoclonic epilepsy panel; Friedreich's ataxia testing; Phytanic acid; Acylcarnitine profile; CSF neurotransmitters; Organic acids (urine); Mitochondrial deletion panel (SUCL, TK2, POLG1, DGUOK)- Negative  
**Brain MRI/Spine MRI:** Normal | Progressive generalized epilepsy  
Complex gait abnormality, failure to thrive, and global developmental delay  
Clinically, epilepsy is reportedly similar to Doose syndrome (myoclonic atatic epilepsy) but additional features of ataxia and episodic loss of ambulation are not typical |
| --- | --- | --- | --- |
| **DHG_015_II01** | Autosomal recessive | VUS | Acylcarnitine profile: normal | Rapidly progressive lethal lactic acidosis  
Metabolic acidosis, liver failure, renal failure, respiratory failure, coagulopathy, fatty metamorphosis of the liver (mild), single cell hepatocyte necrosis (mild), extramedullary hematopoiesis of the liver, pulmonary congestion and focal hemorrhage (mild), bilateral pleural effusions, ascites, cardiomegaly with mild left ventricular hypertrophy |
| **DHG_015_II02** | Autosomal recessive | VUS | |  |
Table 4 – *Family background (Families receiving results of uncertain significance)*

<table>
<thead>
<tr>
<th>Family</th>
<th>Parents</th>
<th>Child</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>Mother</td>
<td>Affected daughter and son</td>
</tr>
<tr>
<td></td>
<td>Father</td>
<td></td>
</tr>
<tr>
<td>002</td>
<td>Affected Mother</td>
<td>Affected daughter</td>
</tr>
<tr>
<td>005</td>
<td>Mother</td>
<td>Affected daughter</td>
</tr>
<tr>
<td></td>
<td>Father</td>
<td></td>
</tr>
<tr>
<td>008</td>
<td>Mother</td>
<td>Affected daughter</td>
</tr>
<tr>
<td>015</td>
<td>Mother</td>
<td>Affected son who passed away within 24 hours of life</td>
</tr>
<tr>
<td></td>
<td>Father</td>
<td></td>
</tr>
</tbody>
</table>
Appendices

Appendix A – Disclosure Handout (General Template)

Slide 1:
Exome Sequencing Research

• **Goal:** Use new technology to find the genetic reason for your child’s health concerns
  – Diagnosis, prognosis, treatment, screening, recurrence risks
• **Goal:** Find out parents expectations of testing/ reactions to results
  – Parent interviews follow discussion of results
• **Limitations:**
  – Cannot guarantee we will find a result
  – Not sequencing the entire genome
    • The study is not designed to find ALL DNA changes
    • Sequencing does not cover 100% of exome
  – May find results of uncertain significance
    • We may not be able to understand the meaning of every DNA change

Slide 2:
Gene structure

Slide 3:
Sequencing/Exome Analysis

DNA Alphabet: A,C,T, & G

**SEQUENCING**

**ANALYSIS**

Mutations are like misspelled words
Slide 4:
Primary vs Secondary findings
• Primary: reason for study, gene change we think is causing your child’s condition
  – Results will be returned
• Secondary: gene changes that are found by chance because of the way we do the study
  – Child: Share if IRB agrees it is clinically actionable before 18 years
  – Parents: Share if IRB approved by IRB and parents want information

Slide 5:
Possible Primary Results of Exome Sequencing
• Negative
• Positive
  – Known mutation in known disease causing gene
• Result of Uncertain Clinical Significance
  – Variant of Unknown Significance: Effect of variant on known disease causing gene not known at this time
  – Gene of Uncertain Significance: Mutated gene has not been associated with human disease at this time
  – Collect evidence

Slide 6:
Family Specific Analysis

Slide 7:
### Slide 8:

**Your result: Uncertain Significance**
- Gene Name
- Change in DNA instructions: *gene change*
- Change in protein: *protein change*
- Changes part of protein
- Protein may or may not be able to do job correctly

### Slide 9:

**Evidence Collected**

- **Collect evidence**
  - Test other family members ✓
  - Test healthy people ✓
  - Test other individuals with same clinical condition
  - Important part of protein ✓
  - Computer programs ✓
    - 2/3 call this disease causing
  - Further lab studies to find out how DNA change affects the ability of the protein to do its job: **in progress**

### Slide 10:

**Inheritance of Mutation Autosomal Recessive**

- **Recurrence Risk for parents:** 25%
- **Recurrence Risk for son (carrier):** Depends on genetic status of partner
Slide 11:

Secondary findings

- Not the goal of this study
  - Did not specifically look for secondary mutations
  - Compare exomes then look at candidate list to see if a gene on that list is known to be important
- No secondary findings found by chance in your family. There could still be secondary mutations but we did not look for/find any in this study.
- Clinical exome is available and some labs may offer additional information about secondary findings
- If you are concerned about other risks, please talk to a genetics professional

Slide 12:

Meaning for Patient, future plans

- **Research result** to go in patient medical record
- Follow up with physician
  - Clinical visits
  - Updates on research
    - Find other families with [child’s] who do not have mutation in the known genes
    - Functional studies
    - Permission to publish requested
- No secondary results
- If testing becomes clinically available, confirm mutation and consider use for reproductive risk assessment in carrier son
- Interviews for study
Appendix B - Qualitative Interview Guide

Disclosure Visit

1. What did you learn at today’s visit?

2. What information were you expecting to learn at today’s visit?
   2.1 Results for your child?
   2.2 Results that were possible for you?

3. How are you feeling after learning about your child’s results?

4. From your perspective, what do your child’s results mean for your child and family?

5. Do you plan to share your child’s results with anyone?
   “No” – Why?
   “Yes” – Who and why?

6. What are your thoughts about how your samples were used for this study?
   --If parents were given options for their own exome results--

7. I understand today you were told that your genes were also looked at. During your first study visit, you were given a list of the types of diseases we might be able to find when looking at your genes. What did you do with that list?

8. What choices did you make today about learning genetic information about yourself?

9. Please tell me about why you made the choice(s)...
   (If chose to receive 1 or more types of results) How do you expect to use your own exome results?

10. (If chose to receive 1 or more types of results) How do you expect to use your gene results?

11. What does learning your results mean for you? Your family?
   --If exome results were not available for parents--

12. At your first study visit, you were given a list of the types of diseases we might be able to find when looking at your genes. What did you do with that list?
13  How do you feel about the fact that the investigators only used your DNA to compare with your child’s exome to find the cause of your child’s disorder but did not take extra measures to look for disease genes in your DNA?

--All Parents--

14  If you could change anything about today’s visit, what would that be?

15  What else would you like to tell me about your child’s participation or your participation in this study so far?