

**PSYCHOSOCIAL IMPACT OF TARGETED EXOME SEQUENCING OF
CHRONICALLY ILL CHILDREN**

by

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ABSTRACT

Whole exome sequencing (WES) is a relatively new testing option, currently offered primarily in the pediatric setting, that is open to more uncertainty than single gene or gene panel testing. This uncertainty makes pre-test counseling challenging yet critical to ensure informed consent. Adaptation of counseling strategies for this type of testing requires a better understanding of parents' experiences throughout the WES testing process. However, the experience of parents of chronically ill children who have actually gone through the entire WES testing process has not been widely reported in the literature. Currently, analysis of exome test results at Children's Hospital of Pittsburgh does not include the incidental findings that are typically reported in WES results (i.e. variants in genes that are not associated with the child's present condition) and is, therefore, referred to as "targeted exome sequencing" (TES). This study was designed to gain an understanding of the psychosocial impact on parents of the TES testing process for chronically ill children in order to improve test education, consent and results disclosure processes, and to better help parents cope with the results. Semi-structured interviews were conducted with 11 parents of children who received targeted exome sequencing results and thematic analysis was performed on transcripts generated from the interviews. The experiences and opinions of parents whose children received positive, likely negative or uncertain results were analyzed, in order to

develop a robust understanding of the full TES process. This study has Public Health significance because the results may contribute to the development of updated recommendations for optimizing informed consent and results disclosure for TES.

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PREFACE

This project would not have been possible without the support, guidance, and participation of a number of individuals whom I would like to take this opportunity to thank. First of all, to all the interviewees willing to share their stories, I am truly grateful for the time taken out of busy schedules and the unflinching glimpses they offered into their lives. I would also like to thank Dr. Jerry Vockley for allowing me to pursue this project in the Medical Genetics Department of Children's Hospital of Pittsburgh of UPMC and Marianne McGuire for helping to recruit the participants. Additionally, I am extremely grateful to my thesis advisor, Catherine Walsh Vockley, and Program Director, Dr. Robin Grubs, for helping to conceptualize this project, for their guidance through the world of qualitative research, and for their feedback through multiple drafts of this document. Finally, I would like to thank my family and friends for their love and support.

1.0 INTRODUCTION

Whole exome sequencing (WES) is a relatively new testing option currently used primarily in the pediatric setting and is open to more results uncertainty than single gene or gene panel testing. Counseling strategies must be adapted to adequately provide informed consent for this type of testing. Adaptation of counseling strategies requires a thorough understanding of parents' experiences throughout the WES testing process. A number of studies investigating individual experiences, opinions, and beliefs related to WES have been conducted in various populations. The subjects of these studies typically either involve parents of chronically ill children who have not undergone testing or relatively healthy adults who received testing by volunteering for a research study. Thus, these studies focused on hypothetical preferences. The majority of these studies focus on preferences for return of results and reasons for these preferences¹⁻¹⁰.

There remains, however, a dearth of literature exploring the experience, perceptions and beliefs of parents of chronically ill children who have gone through the entire WES testing process. It is unknown how parents' views might change following results disclosure and how to best prepare them for multiple, uncertain outcomes. In order to tailor counseling to the unique needs of patients undergoing WES, a deeper understanding of parents' experiences with WES is required. This understanding should include parents' perceptions of WES and how these perceptions evolve throughout the testing process as well as parents' opinions of the informed consent process and how prepared this process made them for the disclosure of results.

Currently, analysis of exome test results at Children’s Hospital of Pittsburgh (CHP) are “targeted” to the patient’s clinical presentation and does not include the incidental findings that are typically reported in WES results¹¹ (i.e. variants in genes that are not associated with the child’s present condition). The results from targeted exome sequencing (TES) typically fall into three broad categories: (1) known pathogenic variants in a gene associated with patient’s condition (“positive” result), (2) a variant of unknown significance that might be diagnostic pending further developments (“uncertain” result), and (3) a variant of unknown significance that will likely never be diagnostic and is thus considered a "negative" result. It is important to consider the perceptions of parents in all three categories in order to develop strategies to prepare parents for any possible outcome.

The present study used qualitative thematic analyses of semi-structured interviews to identify the perceptions, beliefs, and experiences of parents of chronically ill children who have undergone targeted exome sequencing. A deeper understanding of their experience may help direct medical genetic practice in the informed consent process and disclosure of results.

2.0 RESEARCH QUESTIONS AND SPECIFIC AIMS

2.1 RESEARCH QUESTIONS

Question 1: What is the psychosocial impact on parents of targeted exome sequencing of chronically ill children?

2.2 SPECIFIC AIMS

Aim 1: To conduct semi-structured interviews with parents of children who received targeted exome sequencing results.

Aim 2: To perform thematic analysis on transcribed interviews and develop a comprehensive understanding of the data.

Aim 3: To contribute to recommendations for optimizing informed consent and results disclosure for targeted exome sequencing at Children's Hospital of Pittsburgh of UPMC based on study findings.

3.0 BACKGROUND AND SIGNIFICANCE

3.1 EVOLUTION OF GENETIC TESTING

The etiological bases of at least 7000 genetic disorders are currently known. While each is relatively rare, collectively their incidence is estimated to be 1/50 live-born individuals. Furthermore, with roughly 20,000 genes in the human genome, the number of known genetic disorders is likely to increase as more data becomes available. In a recent analysis of 500 unselected consecutive patients, it was reported that 46% of patients with unknown suspected genetic disorders receive a genetic diagnosis through traditional genetic approaches, while the remaining 54% do not receive a diagnosis¹². Traditional clinical genetics refers to a comprehensive genetics evaluation (pregnancy, family, developmental and medical histories and detailed physical examination) followed by targeted testing based on the evaluation. Testing may include chromosomal studies, biochemical studies, single gene testing, gene panel testing, or microarray analysis. Of those patients who did receive a genetic diagnosis through traditional genetic approaches, 72% were diagnosed during the first diagnostic evaluation¹², suggesting WES or whole genome sequencing (WGS) as a second-line test might increase yield and decrease time to diagnosis thereby decreasing costs of subsequent testing.

3.2 WHOLE EXOME SEQUENCING (WES)

3.2.1 Utility

Whole exome sequencing (WES), first introduced in 2009, takes advantage of targeted exon capture and massively parallel sequencing capabilities to sequence almost all the coding regions of human DNA^{13,14}. While the exome covers only 1% of human DNA, it accounts for over 85% of known genetic diseases. Using Next Generation Sequencing technologies rather than relying on conventional sequencing methods, WES is able to sequence over 30Mb of DNA for a fraction of the cost and at greater speeds. Single gene or panel testing are still preferred clinical genetic testing methods when a specific disorder and/or a small subset of genes are suspected to cause the patient's phenotype. WES has been increasingly used clinically when the suspected condition is genetically heterogeneous or the underlying genetic cause is unclear¹⁵. Because WES is the most comprehensive and unbiased genetic test currently available clinically, it is particularly useful for diagnosing rare Mendelian disorders or uncovering complex mutation patterns by sequencing thousands of genes at once.

Most institutions report a 25-35% success rate in identifying pathogenic variants with WES.¹⁵⁻¹⁹ Patients tend to be referred for WES following extensive evaluations by geneticists and other specialists with testing that may include MRI/CT scans, muscle biopsies, metabolic panels, karyotype analysis, single gene testing, gene panels, chromosomal microarrays, and others depending on the indication. Utilizing WES as a first line test, would likely result in a higher yield. A recent analysis of the 153 exome cases that have been seen at Children's Hospital of Pittsburgh of UPMC (CHP) revealed a 39.2% diagnosis rate²⁰.

The utility of the unbiased approach afforded by WES has been highlighted in a number of recent retrospective studies by the major clinical laboratories offering WES testing. An assessment of 2000 and 3040 consecutive cases receiving WES through Baylor Miraca Genetics Laboratories (BMGL) or GeneDx, respectively, revealed that 37-41% of confirmed cases were due to *de novo* autosomal dominant variants, 29-34% were autosomal recessive, and 11-12% were X-linked^{15,19}. These confirmed cases corresponded to 25.2% of BMGL's total WES cases and 28.8% of GeneDx's WES cases. Importantly, 3-4.6% of cases were found to harbor two or more pathogenic variants and BMGL reported that 58% of the total pathogenic variants uncovered had not been previously reported^{15,19}. Moreover, a study of 362 Canadian individuals receiving WES testing at the end of their diagnostic odysseys found that 29% had mutations in genes known to cause disease²¹. The study reported that these mutations were not previously identified due either to genetic heterogeneity or an atypical presentation²¹. These numbers suggest that traditional genetics approaches of single gene and gene panel testing were unlikely to determine the genetic basis for most of the cases included in the studies.

The 5000 cases analyzed at BMGL and GeneDx revealed a high diagnostic yield for neurologic conditions (24.6%-36.1%) and multiple congenital anomalies (24%)^{15,19}. Other studies have shown the ability of WES to detect pathogenic variants related to neurodevelopmental disorders²², speech apraxia²³, atrial septal defects²⁴, skeletal dysplasias²⁵, limb-girdle muscular dystrophy²⁶, and retinal dystrophies²⁷, to name a few. In a post-hoc analysis of patients who received testing either through traditional Sanger sequencing or WES for similar indications, WES provided a significantly higher diagnostic yield for heterogeneous conditions such as blindness (52% vs 25%), deafness (44% vs 10%), and movement disorders (20% vs 5%)²⁸.

3.2.2 Sequencing Method

Currently, most WES protocols rely on solution-based methods for target enrichment^{18,29,30}. To begin, a standard pool of biotinylated oligonucleotide baits with sequencing-platform specific adaptors is made against the target regions of 180,000 exons in the human genome. The patient's DNA sample is fragmented and allowed to hybridize with the biotinylated baits. Streptavidin beads are used to pull down the biotinylated bait-DNA fragment complexes while the unbound, non-targeted DNA fragments are washed away. The pool of targeted fragments is next enriched through PCR amplification followed by high throughput sequencing. Because WES relies on massively parallel sequencing methods, it is important to have sufficient read depth for each target in order to build an accurate and reliable consensus sequence. All participants in this study received testing through BMGL. BMGL quotes a mean coverage of 100-120X with 95% of the exome covered at a depth of at least 20X³¹. BMGL also provides a WES coverage search tool for clinicians to determine whether specific genes included in their differential diagnoses are covered at sufficient depths to be meaningful for their particular patient. This method for WES does not reliably detect triplet repeat expansions, copy number variations, long insertions/deletions, chromosomal translocations, aneuploidy, or epigenetic alterations^{31,32}.

3.2.3 Variant Interpretation

An initial list of variants is identified by comparing the consensus sequence to a reference sequence. In order to produce a consensus sequence, multiple overlapping reads are aligned and compared, generating a sequence that represents the most likely true sequence of the patient.

This consensus sequence can then be compared to a reference sequence, or a sequence used as a ‘normal’ control. A read depth of 20 or more allows for interpretation of any reads that don’t match the reference sequence. For instance, if a small proportion of the reads show a T at a particular position while the remaining reads have an A, the discrepancy can be attributed to a replication error and an A will be called for that position. However, if 50% of the reads are T and 50% are A, the patient will be considered a heterozygote for that variant. By simply identifying the base pairs that differ between the consensus and reference sequence, the variant lists can contain on the order of 10,000 different variants³². Filters must be applied to reduce the list to those variants that have a higher likelihood of being pathogenic. Tools such as minor allele frequencies, mutation databases and, disease specific databases are used to filter out common variants that likely represent natural human variation²⁹. Still, most samples reveal 50-150 ‘personal’ rare variants. Board certified molecular lab directors, genetic counselors and medical directors then use clinical phenotype information to further narrow down the list to potential disease causing variants³¹. Data from parental samples, if available, can also aid in determining both pathogenicity and mode of inheritance. Once a list of reportable variants is assembled, the variants are verified by Sanger sequencing in order to rule out false positives before finalizing the report.

3.2.4 Reporting results

Generally, when ordering WES from BMGL, clinicians receive a focused report with the option to also order an expanded report. The focused report for WES includes results for pathogenic variants or variants of uncertain significance (VUS) in genes that are known to be related to the patient’s clinical phenotype. It also includes information on any incidental findings such as

variants determined to be medically actionable by American College of Medical Genetics and Genomics (ACMG) and BMGL, carrier status, and pharmacogenetics, depending on what information the patient/parent chooses to receive. In 2013, the ACMG issued a policy statement regarding the reporting of incidental findings in clinical exome and genome sequencing¹¹. Incidental findings typically mean variant discoveries that are not based on the patient's current phenotype. This statement recommends actively searching for specific kinds of mutations in genes associated with 24 different conditions on the 'minimum list' compiled by the Working Group on Incidental Findings in Clinical Exome and Genome Sequencing. Inclusion on this list was based on (1) the existence of standard methods to confirm a medical diagnosis, (2) long latency period in asymptomatic, pathogenic mutation carriers, and (3) availability of preventive measures and/or treatment. Furthermore, the recommendation included a restriction on the types of variants reported to those that were categorized as "Known Pathogenic" or "Expected Pathogenic". These terms would refer to "Pathogenic Variant" and "Likely Pathogenic Variant" under the updated ACMG Variant Classification Guidelines³³. Finally, the Consensus Statement recommended that reporting these incidental findings should not be limited by the patient's age, even though many conditions listed are adult-onset conditions. Generally, genetic testing for adult-onset conditions is not offered to individuals under the age of 18. However, the ACMG Working Group argued that since adults do not routinely have access to exome or genome sequencing technology at the current time, the potential harm to the future adult due to the loss of autonomy was outweighed by the potential benefit to the individual's future health¹¹.

Since patients/parents undergoing WES must opt in to receive information on each category of incidental findings separately, the visit to discuss informed consent for the test can be quite extensive and time consuming. After the clinician receives the focused report, there is the

option to order an expanded report, if felt necessary. The expanded report includes results for pathogenic variants or VUSs in genes unrelated to the patient's clinical phenotype. Clinicians generally rely upon the expertise of the testing company to determine what results are clinically relevant and therefore, included on the focused report. However, in some cases, after receiving a negative focused report, a clinician may order the expanded report, rationalizing that he/she is more familiar with the patient's clinical phenotype and wants to verify nothing was overlooked.

Even with a focused WES report, most patients are typically found to have variants in multiple genes that could be related to some aspect of their clinical phenotype. *In silico* prediction software lends evidence about the possible pathogenicity of the variants. It is then up to the ordering clinician to research the conditions associated with those genes to determine whether each is more likely or less likely to explain the patient's phenotype. WES reports rarely show completely negative results; typically, reports either contain one verified pathogenic variant plus a few VUSs in genes that are less likely to be causative, one or two very suspicious VUSs with or without a few less likely to be pathogenic VUSs, or a number of VUSs in genes that all appear unlikely to explain the patient's phenotype. When disclosing results, the clinician must be able to clearly explain the difference among known pathogenic, suspicious variant, and variant not likely to be related to the phenotype. The distinction may seem quite ambiguous and be open to much interpretation on the part of the patient/parent.

While such a comprehensive genetic test can provide unexpected yet explanatory answers, very often parents and patients receive unexpected yet uninterpretable results. As opposed to single gene or even gene panel testing, WES is open to more ambiguity and uncertainty, making pre-test counseling challenging but also critical to ensure informed consent.

3.3 TARGETED WHOLE EXOME SEQUENCING (TES)

In collaboration with Baylor Miraca Genetics Laboratories (BMGL), the Medical Genetics Department of Children's Hospital of Pittsburgh (CHP) recently developed a Targeted Exome Sequencing (TES) test in order to limit the number of incidental and secondary findings. When submitting a test requisition form for whole exome sequencing, clinical notes including a full description of the patient's symptoms, characteristics and family medical history are sent along with the blood sample. As part of the data analysis after performing WES on the patient's DNA, an additional filter to exclude any genes unrelated to the patient's clinical presentation is applied. Therefore, the final report is 'targeted' to include only those variants in genes that overlap with the patient's phenotype. Additionally, variants in the ACMG Recommended List of Incidental Findings¹¹ are not included in TES reports at CHP and there is no option for an expanded report. Since the results are targeted based on the patient's clinical phenotype and there are no additional incidental finding options that require an opt in, the time spent in pre-test counseling is greatly reduced. Parents are informed of this limitation of TES testing during the consent process.

3.4 GENETIC COUNSELING PROCESS AT CHILDREN'S HOSPITAL OF PITTSBURGH OF UPMC

3.4.1 Genetics visits prior to targeted exome sequencing

In the majority of cases, patients who eventually receive TES have multiple genetics visits prior to receiving TES. Whole exome sequencing is not typically considered a first line genetic test,

therefore, the more readily testable genetic conditions often are ruled out first based on suspected diagnoses given the individual's phenotype before exome sequencing will be offered. Beginning in December 2013, CHP instituted an algorithm for molecular testing. Single gene tests are considered first if the patient's symptoms suggest a specific genetic condition. If a single gene test is not ordered or if no mutations are found, then a SNP microarray is pursued. Next, if the SNP microarray results do not explain the patient's clinical presentation, symptom specific gene panels are then considered. Finally, if the gene panel does not return any pathogenic mutations, WES/TES may then be pursued. Most parents of children who receive TES have some prior understanding of genes and chromosomes due to this exposure to genetic testing but are unfamiliar with more complex concepts such as exome sequencing. When TES is first offered to parents at CHP, either a genetic counselor or geneticist provides a brief overview of the process and the types of possible results, including positive, uncertain and uninformative/negative. Parents are told ahead of time that positive results are returned in 30-40% of cases and that it can take up to six months to receive results following insurance approval. The genetic counselor is available to answer any questions after the introduction to TES but there is no formal presentation introducing parents to the details of exome sequencing and no materials are provided at the time of testing.

3.4.2 Targeted exome sequencing results disclosure visit

CHP works with one counselor affiliated with BMGL whose role is solely dedicated to analyzing and disclosing exome results. For each results disclosure visit, she prepares a detailed handout describing genes, chromosomes, and TES followed by a meticulous description of each variant identified, an assessment of what those variants mean for the patient, and any follow-up

recommendations possible. The handout is then given to the parents to take home for their reference along with a copy of the original report. A letter summarizing the visit and the results is sent to the parents and any physicians as requested by the parents. The counselor is then available by phone and email for any future questions.

3.5 PUBLIC PERCEPTION OF WHOLE EXOME SEQUENCING

A number of studies investigating individual experiences, opinions, and beliefs related to WES have been conducted in various populations. The subjects of these studies typically either involve chronically ill children who have not undergone testing or relatively healthy adults who received testing by volunteering for a research study, thus they focused on hypothetical preferences.

3.5.1 Parents of affected children hypothetically considering whole exome sequencing

The majority of these studies focus on preferences for return of secondary findings and reasons for these preferences. For instance, a qualitative study of 25 parents of 13 probands with rare genetic conditions asked participants about their motivations for return of results preferences if they were offered WES testing³⁴. These participants did not go through the informed consent process for WES nor did their children receive WES testing. The majority of subjects provided either positive or neutral responses to questions about receipt of results indicating carrier status for recessive conditions as well as both treatable and untreatable childhood conditions. The only negative responses were generated regarding receipt of secondary variants for untreatable childhood conditions. A number of themes emerged from the analysis of semi-structured

interviews including, parental responsibility to find answers for their children, preference for knowledge, control over genetic information, and altruism³⁴.

In a focus group study of parents whose children have suspected genetic conditions and could potentially be offered WES, parents raised concerns about stigmatization, insurance and employment discrimination, loss of privacy and restrictions on their child's future reproductive decision making. However, parents would still pursue testing because results could have therapeutic benefit and could help elucidate disease course. Concerns about the limitations of WES were not mentioned. Parents wanting all results to be disclosed, including incidental findings, mentioned entitlement to their own genetic information and possible future utility as reasons. Having results for conditions unrelated to their child's current clinical presentation may be therapeutically beneficial if there are treatments, therapies or preventive methods available now or in the near future. These parents would also prefer to receive results for adult-onset untreatable conditions because they place value on the ability to prepare and to know what to expect³⁵.

Importantly, parents revealed that the strong desire for a diagnosis and prognosis for their child would likely lead them to pursue WES without fully contemplating the consequences of secondary findings³⁵. As mentioned above, parents raised a number of concerns regarding WES beyond that of secondary findings and yet all would pursue testing. The potential to end the diagnostic odyssey seems to override any other trepidation these parents may have about exome sequencing, which highlights a possible barrier to informed consent. Parents also conceded that the complex nature of exome sequencing could further challenge and complicate informed consent because parents may not sufficiently understand what the test can and cannot do. This lack of understanding could lead to misplaced expectations and/or misinterpretations of results.

It is unclear how well parents are able to adequately consider the potential impact of pursuing WES when a singular focus to find a diagnosis and a limited understanding of a complex test may prevent parents from appreciating both the pros and the cons of testing.

3.5.2 Adult volunteers in Whole Exome or Whole Genome Sequencing research projects

ClinSeq is a large pilot study of >1000 participants aimed at optimizing the use of clinical whole exome and genome sequencing to improve patient care³⁶. One of the study goals is to “establish approaches for informed consent and the return of genetic information to subjects”³⁶. The ClinSeq group has published a number of studies investigating the experience of participants with WES/WGS and their preferences for the return of results^{2,9,37}.

Recently, the perceived value of WGS results was surveyed among 320 ClinSeq participants². Subjects were questioned about the value of receiving results related to treatable conditions, untreatable conditions, carrier status and VUSs. Overall, the majority of participants valued all 4 types of results, even uninterpretable ones, although those providing information on treatable conditions and carrier status were valued more highly. In addition to medical utility, participants placed value on knowledge, the impact on increased health awareness, and the ability to inform family members².

A recent study of ClinSeq participants investigated the perceived utility of WES results for individuals who received urgent and actionable secondary results³⁸. Most of the 31 participants in this qualitative study received results pertaining to either cardiac or cancer risks. Responses to receiving results were largely positive or neutral with negative responses uncommonly expressed. Participants conveyed relief that the implications of the results were not more serious and that there were adequate surveillance options available. The experience with

genetic counseling and receipt of WES results also induced a general increased awareness of health in participants who then pursued more healthy living practices. Participants also found personal value in the results in that they provided an answer for certain health issues running in the family or in that they satisfied curiosity. Generally, the participants in this study were self-referred, early adopters of technology and over 60 years of age³⁸.

3.5.3 Parents of children undergoing clinical whole exome sequencing

Recently, two qualitative studies investigated the experience of parents whose children received whole exome sequencing in an attempt to diagnose their chronic conditions^{39,40}. In both cases, WES was offered as part of research study rather than in a clinical genetics setting. One study explored the expectations, understanding, utilization of results, and communication of findings of 19 parents of children who underwent WES⁴⁰. Parents' interest in WES was driven by a sense of duty to pursue any course of action that will lead to a diagnosis for their child. Even though they felt this duty to find answers, parents expressed fear that WES would uncover a lethal diagnosis. While parents hoped for a diagnosis, their expectations of WES were moderated by prior experiences. For those that did receive a diagnosis, some remained frustrated with sense of isolation due to limited information about their child's newly diagnosed rare disorder⁴⁰.

Another study sought to evaluate a novel counseling strategy designed to mimic a possible future in which WES is part of standard diagnostic routine and counseling is performed by physicians, not genetic counselors³⁹. This study involved children with undiagnosed neurological disorders and neurologists performed pre-test and post-test counseling. In their protocol, pre-test counseling was performed in clinic but test results were disclosed by telephone. Parents were then referred to a website with information specific to their child's diagnosis. An

appointment could then be made if parents wanted to discuss results further. Most parents in this study expressed a preference for an in-person results disclosure and prefer the hospital initiates the follow-up consult rather than leaving it up to the parents. Furthermore, parents expressed a desire to be kept informed about the progress of testing during the wait for results. Upon receiving the results, parents are most interested in discussing what the results mean for their child's care and they also articulated a need to share questions and concerns with health care providers to deal with practical and psychosocial problems. Finally, one requirement to participate in this research study was that participants had to accept the receipt of incidental findings. Some saw value in obtaining extra health information but others want to be able to decide for themselves whether or not to receive this information³⁹.

While these studies provide insight into the public perception of WES in hypothetical or research settings, the experience of parents whose children have undergone WES in clinical settings has not been adequately explored in the literature. It is unknown how parents' views might change following results disclosure and how to best prepare them for multiple, uncertain outcomes. In order to tailor counseling to the unique needs of patients undergoing WES, this study seeks to gain a deeper understanding of parents' experiences with WES. This understanding includes parents' perceptions of WES and how these perceptions evolve throughout the testing process as well as parents' opinions of the informed consent process and how prepared this process made them for the disclosure of results.

4.0 DESIGN AND METHODS

4.1 PARTICIPANT SCREENING AND RECRUITMENT

4.1.1 Ethical considerations

The Institutional Review Board of the University of Pittsburgh approved this study (PRO15030727 – APPENDIX A). Informed consent was obtained from all participants.

4.1.2 Inclusion criteria

Participants were recruited from parents of children who received targeted exome sequencing test results and who then consented to participate in interviews.

4.1.3 Exclusion criteria

Because this study requires in-depth interviews, any participants who met the inclusion criteria but were not fluent English-speakers were excluded from participation. Participants with intellectual disability were also excluded.

4.1.4 Screening Procedures

Screening of potential participants was based on results of targeted exome sequencing (TES). Marianne McGuire, a Baylor College of Medicine (BCM)-affiliated Genetic Counselor specializing in TES, compiled a list of eligible participants according to the stated inclusion and exclusion criteria. Since she was already familiar with the patients' medical records, she categorized the potential participants as parents of children who received either definitively positive, likely negative, or uncertain results from targeted exome sequencing. Ms. McGuire contacted the eligible participants to introduce the study to the potential participants by briefly describing the study goals, risks and requirements. The participant could then agree or decline to either contact the PI or allow the PI to contact the participant. The goal was to recruit participants until we had 5 participants in each category. The members of the study team did not access the medical records of the potential research participants or their children and were not informed of any specific test results.

4.1.5 Participant Recruitment

Eligible participants who agreed to be contacted were consented by telephone. Verbal informed consent was obtained following an explanation of the rationale, goals and design of the proposed research and consent for one-on-one semi-structured interviews. Individuals could choose to consent to research immediately, to consider it further and consent at a later date, or decline to participate. If participants chose to think about participating, the participant and contact person discussed a mutually agreeable date and time for re-contact. Following the informed consent process, a mutually convenient time to conduct the interview either in a private room at

Children's Hospital of Pittsburgh or by telephone was chosen. Recruitment was based on whether the children received positive, likely negative or uncertain results. We continued to recruit participants until we had 5 participants in each category. Refusal to participate in this study did not affect their child's continued medical care. Participants were not compensated for participation. Seventeen participants were contacted for participation in this study. Thirteen provided informed consent for participation and 11 completed interviews with the researcher. Two participants withdrew after providing informed consent due to scheduling conflicts and initial contact was not made with 5 potential participants despite two attempts to contact them by telephone per the Institutional Review Board-approved protocol. Two of the interviews were performed in person and the remaining nine were conducted by telephone. One of the in-person participants was accompanied by her husband and child. The husband was consented on site so he could offer comments as well but he did not undergo a separate interview and their joint interview was analyzed as one participant.

4.2 PARTICIPANT INTERVIEWS

Participants were interviewed by telephone or in person between September 2015 and December 2015 by Bess Wayburn, a Genetic Counseling Intern with a PhD in Molecular Genetics, and supervised by a licensed, board-certified Genetic Counselor. Consent to participate included permission to record and transcribe interviews to maintain accuracy. Both the recordings and transcriptions were coded to protect the participants' identities and these were maintained on password-protected computers in card-access only locations. A semi-structured interview guide was developed to gauge parental perception, expectations and experience with the entire targeted

exome sequencing testing process, including test education, informed consent, and results disclosure (See APPENDIX D for interview guide). Initial drafts of the interview guide were reviewed by Ms. McGuire as well as by two members of the thesis committee with experience in qualitative research (RG and CWV). Their input was incorporated into the final guide used in this study. Each interview lasted approximately one hour. Interviews are reviewed, transcribed and coded as each was completed. Consistent with a qualitative approach, analysis began before all the interviews were conducted and later interviews were informed by earlier interviews. This means that new lines of questioning could be developed based on early responses that led to a deeper exploration of parents' experiences and perspectives. Upon completion of each primary interview, participants were requested for permission to re-contact by the interviewer in the event that early analysis uncovered topics that necessitated further exploration. All participants agreed to this request, however, based on their responses, it was not deemed necessary to re-contact any participants.

4.3 THEMATIC ANALYSIS

A wide variety of methods have been developed and employed in the field of qualitative research. Thematic analysis, one widely used qualitative method, is a flexible and easily accessible method that is used to describe patterns (themes) across a qualitative data set. Braun and Clarke further define thematic analysis by three different parameters: (1) the method can be realist, constructionist, or contextualist, (2) analysis can be inductive or theoretical/deductive, and (3) themes can be semantic or latent. The realist method focuses on a direct reporting of participants' experiences, perceptions and beliefs while the constructionist method examines

how the wider social context constructs those experiences. The contextualist method considers the reality of participants' experiences, the meanings they attach to them, and the broader social context. When performing inductive analysis, themes are developed from the content of the data. Theoretical data analysis, on the other hand, develops themes from existing concepts. Finally, semantic themes explicitly reflect the data whereas latent themes describe underlying concepts. Realist methods tend to cluster with inductive analysis and semantic theme development while constructionist methods cluster with theoretical/deductive analysis and latent theme development.

This study was developed to obtain a deeper understanding of parents' beliefs, perceptions and experiences with targeted exome sequencing. Therefore, our approach followed the constructionist method with inductive analysis and development of latent themes. Using this approach, we considered the reality of TES testing as well as the broader context in which parents' experiences and the meanings attached to them were formed. Using this approach, we intended to fill gaps in the literature and direct further research in this field.

The thematic analysis approach used follows six steps. While listed sequentially, the steps are performed through an iterative process where codes and themes are developed following initial familiarization with the data, the development of codes and themes leads to greater familiarization with the data and further refinement of the codes and themes. In addition, analysis begins after the first interview is completed and this early analysis is used to inform the remaining interviews. In this way, experiences that are revealed can be explored more deeply throughout the interview process. The six steps to thematic analysis as proposed by Braun and Clarke are as follows:

1. Familiarization with the data
2. Generation of initial codes
3. Searching for themes
4. Reviewing themes
5. Defining and naming themes
6. Writing the report

4.3.2 Familiarization with the Data

Each interview was reviewed in full at least once soon after recording and before transcribing in order to develop a deeper understanding of the experience and emotion expressed by the participants. The majority of the interviews were transcribed by the investigator, which also increased familiarity with the data. A medical transcriptionist was contracted to transcribe a subset of the interviews. Whether transcriptions were transcribed by the researcher or by a transcriptionist, the documents were reviewed thoroughly by the researcher while listening to the original audio recording, prior to starting the coding process. Any incorrect or missing parts of the interview were then resolved. Finally each transcript was read completely at least once more before coding began in order to develop a holistic understanding of each participant's perspective.

4.3.3 Coding the Data

Transcripts were coded by the researcher in Microsoft Word® using Comments to highlight each code. Use of comments allowed for the codes to remain linked to the parts of the transcript to which they refer for reanalysis and for extracting relevant quotes. Initial codes remained true to

the participant's language in order to preserve nuance. A codebook was assembled in Microsoft Excel® based on the interview guide and iteratively revised following review and input from two co-authors experienced in thematic analysis. An initial set of 1273 codes were identified from 11 hour long interviews.

4.3.4 Theme Identification

Boyatzis (1998) defined a theme as “a pattern in the information that at minimum describes and organizes the possible observations and at maximum interprets aspects of the phenomenon” (p. 161). The process of theme identification began by condensing the 1273 codes into broader categories. Categories were assigned a separate column in the Excel® codebook in order to maintain the connections between the codes and the corresponding text. Using Excel®, the data could be re-sorted by category and the codes assigned to each category could be easily re-analyzed in this context. Categories were reviewed to ensure the codes assigned complement each other and that each category represented a distinct idea⁴¹. Codes were reassigned to other categories, categories were condensed, and new categories were created based on recursive review. Themes began to emerge when considering the coherence of these categories across the full data set⁴¹. When sorting by category in Excel®, it is also possible to quickly identify how many participants were coded for within that category and to which group they belonged. If a category appeared highly representative of a shared experience across interviewees, it became a strong candidate for further theme development. If a category did not demonstrate a common experience, the codes and data source were re-explored to determine if there is meaning in the dissonance or if the category is simply not relevant for the entire data set⁴¹. A category that represented a compelling experience relevant to the research question or that held implications

for genetic counseling practice was also a strong candidate for theme development regardless of its frequency among the participants. Additionally, as potential themes were developed, the data set was reviewed again to be certain codes were not missed during the early stages of analysis. Finally, themes are refined and defined by determining their scope, focus and narrative⁴¹.

5.0 RESULTS

5.1 DEMOGRAPHICS

All participants in this study were female and all but two are currently married. Out of the 11 participants, five had a child with a positive TES result, three had a child with an uncertain result, and 3 had a child with a likely negative result. There was a wide range in years from onset of symptoms prior to TES spanning from 3 to 24 years with an average of 10 years. Participants were generally well-educated with five participants completing Master's Degrees and all who reported had completed at least a high school education.

Table 1: Demographics

Participant ID	Result	Years from Symptoms to TES	# of other children	Education Level of participant	Marital Status of participant	Gender of participant
TES01	Positive	24	1	Masters	Married	Female
TES02	Positive	4	2	Masters	Divorced	Female
TES03	Positive	3	1	Unknown	Married	Couple
TES04	Positive	13	1	Masters	Married	Female
TES05	Positive	9	2	High School	Married	Female
TES07	Negative	8	2	High School	Married	Female
TES08	Uncertain	12	1	Unknown	Married	Female
TES09	Negative	13	0	Unknown	Married	Female
TES10	Negative	8	1	Masters	Divorced	Female
TES12	Uncertain	8	1	Bachelors	Married	Female
TES13	Uncertain	8	2	Masters	Married	Female

5.2 THEMES IDENTIFIED IN THE ANALYSIS

5.2.1 Managing expectations

All participants voiced understanding that the chance of getting an answer from TES was low and were appreciative that their expectations were managed prior to performing the test. Still, even with this understanding, all hoped for an answer and there were wide differences in what participants actually expected. For example, one participant expected an answer because the test was new and because of the extent of her child's symptoms:

“I was expecting them to find something, because of how just, they seem to test everything. And it was new; I expected them to find something to answer what was going on with her. But I was scared to know what it was. But I did expect, with all the symptoms she has, and you know, I was expecting to find out something new.” (Negative)

Others expected no answer based on their prior experience with inconclusive genetic testing.

“Honestly, I wasn't hopeful because she had not been diagnosed before. I wasn't thinking that there would be an answer. I thought that it might be an inconclusive again but I was, I would like to know. I was hoping that it would turn out to give us some answers but I wasn't sure that it would. I wasn't convinced that we definitely would get an answer.” (Positive)

“I went in with no expectations. ... Just because nothing, to my knowledge, had shown up before. Even though I know what I know about the exome, I just.... You know, when you've been told for, geez it was 7 years at that point... 8 years – what you've been told for 8 years: this is the way your daughter is and we don't know why she is the way she is.

You know, you just get used to hearing that. So I just expected to hear again, we still don't know why she is the way she is and maybe down the road with the advancement of medical science we can find something out – that's kind of what I expected to hear.”

(Uncertain)

Due to the amount of analysis involved, exome results typically take up to 6 months to be returned. Managing expectations regarding the wait time, therefore, was another crucial part of anticipatory guidance. All parents understood how long the return of results was expected to take, however, the actual experience of waiting varied widely among participants from surprise the results came back as quickly as they did to them taking a seemingly interminable expanse of time:

“I was actually surprised. You know, I don't know if I blocked that out for a while and didn't think about it. I just thought with how amazing that test was that I figured it wouldn't be really quickly for us to get it back anyway.” (Negative)

For one parent in particular, who eventually received a positive result, the wait “*seemed like it was forever. It probably wasn't. ... It was six months. Just under six months.*” Part of the struggle with waiting was due to this parent's constant worry over the different possible diagnoses that could be made. Other parents were better able to put these thoughts out of their mind, making the wait more bearable:

“They're pretty punctual with their timing. So it was ok because I just figured, you can't, stressing over this stuff isn't going to change it. You can't do anything about it so just deal with it as it comes. So we were all right.” (Positive)

“Honestly as soon as we had the blood work done, I knew it was going to take a few months so I put it out of my mind other than just making the mental note that by June or July I had thought we should have the results back. So at that time, I was like oh hey, we haven’t heard anything yet, I wonder what’s going on. So, you know, I didn’t really think much about it until I knew it was time to be getting results.” (Negative)

Even for those parents waiting furtively for results, participants seemed to understand that the complexity of exome requires time-consuming analysis. Parents were also buoyed by their prior experiences with waiting for genetic test results and by the willingness to wait for answers if it means the analysis is thorough and correct:

“The wait, when they finally did it that was hard. It took a long time for the results and I can imagine what they would have to do to interpret those results. I can’t imagine how many hours were spent just on his one test. But waiting, it’s like forever. That is like a trip you would like to make just to hug someone. [Laughter]. For all of those hours invested.” (Positive)

“Well, you know what, we’ve done this so many times that it really wasn’t. I mean this isn’t the first test; our last one wasn’t quite that long, but it’s not the first test that we’ve had to wait for. So as much testing and stuff that we’ve been through, for me it really wasn’t... I mean it kind of stinks because you want to know, but I understand that it’s a necessary time period to go through all that, I mean they’re going through a lot of data. And I’d rather them take their time and do it right, than rush it to get me back an answer. So I’m OK with the waiting. I mean it sucks, but I’m OK with it. Because I want it to be right.” (Uncertain)

Interestingly, all participants felt exome met or exceeded their expectations and would do it again – even those who did not get answers and were previously frustrated by or found no meaning in prior experiences with genetic testing. One participant whose child received a positive result from TES, for example, described prior genetics visits as *“Empty. Not very helpful. They didn’t offer [anything] useful, which is why I don’t think that there was any...any need for us to continue to think about going back to them. There was nothing useful coming out of that.”* Another parent expressed frustration over the repeated testing for single genes to rule out conditions that are more common but that the physicians didn’t really think explained her child’s clinical phenotype.

“What I think was always so frustrating about that was ‘let’s put her through a blood test for something that we don’t think it is’. And we really felt like she was being treated as a test case more than really trying to find what’s going on with her. ... I think what bothered us so much is that a lot of the time, the doctor would say ‘we’re gonna test for this but we don’t really think that’s what it is – we just want to rule it out’. And it was like they didn’t understand what an effort it was just to get the testing done in the first place. ... It’s hard to have to restrain her and it’s physically difficult to restrain her so it’s been... it’s been emotional to say the least.” (Uncertain)

Parents, including those who were previously frustrated by the genetic testing process, expressed positive reactions to TES partially because this is the most extensive test currently available. With or without an answer, parents felt they have done everything they can at this point to help their child.

“I think that it’s definitely something where if you have a child up there with all these question marks, I would tell any parent to do it just because whether or not you still come

back with question marks, you still know that you've exhausted all these resources that are out there for your child and not just sitting back and not trying to help.” (Negative)

Parent satisfaction with TES also extends from the counseling experience itself. Parents voiced appreciation for delivery of information in a clear, concise, and thorough manner, attending to parents' questions and concerns without feeling rushed, and providing materials to review at home and share with others. One issue that many participants raised, though, was the concern for information overload during the results disclosure visit. Results disclosure typically includes a review of genes, chromosomes, and the TES process in addition to discussing the relevance of each variant identified. For some participants, trying to understand genetics while processing the meaning of their child's test result was overwhelming. These parents would like more information about basic genetics and the TES testing process prior to results disclosure either in the form of a brochure or links to trusted websites so they can study the background while waiting for results and feel better prepared:

“Very overwhelming! To get all that information at one time. So having the ability to have that information ahead of time may have made that a little bit easier 'cause then we could have concentrated on just that [variant].” (Uncertain)

“To have to sit and kind of process everything and luckily she makes a really nice packet because there is just...me anyways, I was just kind of emotional and then to have to actually focus and pay attention to everything that she's telling us because she walked through the whole process, broke down things in diagrams, so thankfully we had those things to take home with us to kind of work through even in the days after to look over. We emailed her a few times after we got home about different questions that kind of came after the fact. It was a little overwhelming to say the least.” (Positive)

Another parent, however, did not see the utility of receiving more information on TES upfront because the complexity of exome sequencing precludes non-genetics professionals from truly understanding its fundamentals. This parent preferred to place her faith in health professionals to determine an appropriate testing strategy:

“We were just glad. Do what you need to do. That is really what it comes down to. I think they do a nice job of explaining, but again, you are talking about such specific knowledge set. No matter how hard they try to explain it or go into detail, I don’t think the average person is going to get it. I think that we just have to have faith that based on our awareness as a parent and conversations that you’re having, that the correct tests are being selected.” (Positive)

Others were not overwhelmed at all and expressed appreciation for the in depth results disclosure. For instance, one parent in the positive category when asked if she felt overwhelmed during the results disclosure remarked, *“No, no. I was just intrigued.”* While another parent in the negative category conceded that the information presented during the results disclosure was *“a little over our heads”*, she still did not see the need for more information prior to that visit.

5.2.2 Living with uncertainty

One of the major themes to emerge from this research was that of living with uncertainty. Feelings of uncertainty were pervasive both prior to and following TES regardless of whether patients received a definitive or uncertain answer. Prior to TES testing, participants in all groups expressed uncertainty regarding a diagnosis and all participants were most concerned with the uncertainty around life span. Regardless of the outcome, participants described complex

relationships with uncertainty throughout their diagnostic odyssey, often finding a mixture of frustration, fear, meaning and comfort in the ambiguity.

For instance, as one mother whose child eventually received a diagnosis following TES described, it was clear early on that there was something not quite right with her son but doctors could provide neither a diagnosis nor a prognosis:

“[Our doctor] felt that there was a genetic condition, he just didn't know what it was and felt that all of these things including the seizures, the tremors, the failure to thrive, being small, his characteristics... he felt that...the way he explained it was that just all fell under this umbrella. He just wasn't certain as to what the umbrella was.”

Without an answer, various conditions are continually added to the differential diagnosis leading the parents to continually investigate each in an attempt to reconcile the uncertainty. Additionally, TES results often take up to 6 months to be returned. An extended wait for answers can leave parents trying to prepare themselves in advance by researching all possible outcomes online. As this mother continues to relate, having access to information without knowledge can be detrimental to a parent's psychological wellbeing:

“A hundred different things go through your mind. You get on the internet and research all of these characteristics. Some of the syndromes are pretty awful...pretty awful that they mention. So along the way you still have all of your regular doctor's appointments and check-ups and follow-ups with everybody. They all mention something different, so of course, you are looking these things up, which does more harm than good. It was just the unknown, the uncertainty. You didn't want to be disappointed by no answer, but then on the other hand, you were fearful of getting an answer. What happens if it's something

that is terrible, that's just devastating, that's awful? You kind of psych yourself out and probably scare yourself more than what you need to."

This mother's child, similar to all the cases who received positive results in this study, was found to have a rare, *de novo* pathogenic mutation. Most of the conditions associated with mutations in these genes were newly characterized without much research. While receiving a diagnosis alleviated some pre-existing uncertainty, the rarity of the associated condition preserved that which remained. Parents now had an answer but most did not know what it meant for their child. The incomplete removal of uncertainty generated complex emotional responses to receiving a positive result in this participant:

"I was devastated and relieved. ... Now I know what it is and what's entailed. How do I treat it? How do I fix this? But then your heart, this is your little guy that you love more than anything and you are telling me that he has this syndrome that you don't know anything about. So that was hard too. Just that immediate uncertainty. I want to know exactly what it is. The question that just even today is just the longevity. I don't like ... not knowing the life expectancy or longevity. What can we predict? So it was just emotionally overwhelming. ... that uncertainty of longevity is probably the hardest part for me...not knowing. What is the life expectancy for these children? There are so many different variations of them that it is hard for them to tell. They can't really give you an answer. ... That part is probably the worst part for me. He is happy and fairly healthy otherwise and it's just that longevity aspect that I am having a hard time with."

Nearly all participants in this study similarly placed life expectancy as their primary concern when faced with an uncertain prognosis, as echoed by another parent who said, *"I would take anything as long as you tell me for whatever reason it is that my son is not going to have*

short lifespan. We can deal with it. We will make it work.” However, once her main fear of a shortened lifespan was alleviated upon learning of her child’s positive result, the remaining symptoms and issues that accompany this diagnosis predominated. No longer paling in comparison to pre-mature death, the newfound weight of these concerns created an emotionally complex reaction.

“My first question was: is there a short life span? She said no. I was so happy, so obviously there were more emotions. Then you realize like the end result is I wanted to hear that my son will live a fairly healthy life and not have a short life span, but now we have to face all this. You go from excited, to happy to sad all in a matter of one hour.”

Conversely, parents whose children did not receive a definitive diagnosis from TES cited continued uncertainty regarding life expectancy as a positive outcome. As explained by one participant:

“This way we just have our normal life even if it’s not normal for others. It’s our life and it’s scheduled and organized and we view things importantly in that way and I think in that respect, I think it’s kind of good not to know. I know our lives would be radically different if we knew, especially if it was something that we only had a few more years. I think that would plague you and I think that would make you miserable. And I would think every time they would cough or they would sneeze you’d be freaking out thinking ‘is this it??’ You know, I don’t think I’d want that. So in some ways, maybe not having answers is better too. Even though it seems contradictory but...”

Likewise, participants who did receive a positive result from TES recognized the psychological benefits uncertainty can provide and somewhat lamented its loss:

“We were much better off getting an answer, but it was hard getting an answer. If we wouldn't have gotten an answer we could have said oh, you know, maybe it's nothing. Maybe he is going to outgrow whatever it is, but when you get an answer it's just like ok it's in black and white.”

Another parent whose child received a positive result from TES similarly described potential limitations that may have been placed on her daughter had there been a firm diagnosis earlier:

“I think one of the benefits that I had in not having a firm diagnosis was that I had no reason to not think that she could not develop further, grow more, become over time, overcome some of her challenges, and just gave it a lot of space to work its way out. Now I can be comfortable with, I think I know who [my daughter] is, I know what [my daughter]'s going to need in the future and I can be ok with that.”

This same parent, however, also recognized the potential benefits that may have been possible with more targeted therapies and interventions had a specific diagnosis been made earlier:

“I think if we had the genetic testing and we knew this when [our daughter] was just a baby we wouldn't have had sort of this wait and see how things go - what is the label going to be. And I don't really care about the label just accessing the resources, understanding what the needs are and having her try and reach her potential.”

Furthermore, parents whose children did not receive a definitive diagnosis from TES expressed frustration with the continuing uncertainty largely because they want to know how best to help their child, as one parent in the uncertain category said: *“I just want to know what it is so we can deal with it. And you learn about it, figure it out, help her the best we can, it'll be better to help her.”* Another parent whose child received a negative result similarly related:

“I just would love an answer. It doesn’t seem like you get them. I have to, you know, be ok with that. I get ok with it and then when she doesn’t feel good, I’m not ok with it. But I don’t know, there are so many emotions with all of this. I do wish, I just wish I knew like exactly the right way to help her and what to do and what not to do. When to push and when not to push with her. But, I don’t know, I just do the best that we can. With the information we have.”

This mother also voiced a common fear of the unexpected that accompanies living with an uncertain diagnosis, saying, *“Still like uncertainty that something could be wrong and we just don’t know it yet. ... I usually handle it pretty well but every couple of months I cry about it and then I get on with life.”* Interestingly, this parent concluded her daughter was the source of uncertainty, not the test: *“I was hoping to get an answer, but that’s nothing the testing does, that’s just [my daughter], like there’s just nothing good to find out I guess. As of right now.”* Another parent similarly absolved any limitations of the test by blaming bad luck: *“we didn’t get more of a clear cut answer that we were looking for, hoping for but, I mean, that’s nothing against the test. That’s just our luck.”*

The ambiguity and complexity of exome results left openings for parents to find meaning in the uncertainty. Participants in the negative and uncertain categories, while frustrated by the lack a diagnosis, often interpreted non-answers as evidence their child does not have any of the more severe conditions listed on their doctors’ differential diagnoses. They, therefore, found significant meaning in their child’s TES result even though it did not provide a causative answer. For example, one parent whose child received an uncertain result said: *“In a way we were really looking forward to finding out something, but then on the other hand, when there are so many things out there, and some of the things that they were pointing us in the direction of I was really*

relieved we didn't have." Similarly, following a negative TES result, another parent said: *"And what's unfortunate is that it hasn't led us to anything. It's ruled out a lot but it's not confirmed anything. At least we know what he doesn't have, which is a good thing."* Participants whose children did not receive a definitive diagnosis from TES perhaps searched for meaning as a way to cope with the uncertainty. As one mother vividly described the struggle to resolve uncertainty: *"...the entire process whether it is the full exome sequencing or just a regular genetic test it is extremely emotional draining -the unknown, you can't control it and the waiting time. ... At a point, I can tell it is like you are playing a game and at a point you just want to give up, but you have to put your shoes on and keep going."*

Furthermore, since TES only reports back variants in genes that could explain at least some of the patient's symptoms, searching for meaning in uncertain or negative results can lead to confusion and false hope. For instance, one parent whose child received VUS results in genes that were not thought to explain his condition (meaning, she was in the negative category) seemed convinced that the test proved her child was different because of genetics, which brought her comfort. Moreover, she seemed to interpret 'variant' as meaning a variant of the condition rather than a genetic variant.

"I think it definitely helps you explain your child better even if it's not a definitive but you can still say there are these things genetically wrong with my child and even though they don't know exactly definitively this is the syndrome he has or whatever that I can say with certainty because of this test that he has these variants in his DNA. ... You can't say yes, this is exactly what it is but it still gives you that idea because he does technically have these things but they're not 100%. It may be 30% of this and 50% of this and you know what I mean. So like I said, that's just what makes my kid so unique and amazing."

“It helps me to, you know... I think even with family and friends, they just always looked at [my son] as this bad, hyper kid when he’s not bad at all. Yes, he used to be hyper. Yes, he’s a little different than other children. But I can tell you because we did this certain test, even though it’s not definitive, that this is why my son is the way he is. So I think it just helps me explain him better to people and not get angry for people not understanding or make me upset when people make comments because they just...they can’t judge him based on what they see on the outside. They need to realize that genetically, that’s why [my son] is the way he is. So I think it almost gave me some fuel, you know, like, it really did. It gave me something to just kind of defend [my son] in a way. I mean, definitely. Even though it’s not definitive.”

Another parent whose child did receive a pathogenic variant from TES, also interpreted the other VUSs as meaningful, believing them to indicate that certain conditions were being passed on in the family even though these conditions did not match her daughter’s presentation.

“Well we passed on a lot of muscular skeletal disease in our family. But there’s no real evidence of it. She has kyphosis and scoliosis, so there’s probably, it’s certainly not a surprise if you look at the number of muscular skeletal variants that she has, what variants could be a combination of certain things that she ends up having that more dramatically. It could not be related at all, who knows? But interesting that it’s coming through the family and yet nobody’s had Duchene’s muscular dystrophy, I mean not that that’s one of them, but there’s really, really strange rare conditions.”

5.2.3 Valuing the Intangible

Participants in all categories described intangible yet profoundly meaningful benefits to TES. In addition to clinical utility for some, TES has the potential to provide personal utility to many more families. Subthemes include relief, validation, inspiring altruism, hope for the future, and obtaining support.

5.2.3.1 Relief

Relief was a major sub-theme expressed by the participants consistently across the three different groups. Relief took on many forms including peace of mind, reducing anxiety and stress, clarifying risks to other relatives, and absolving blame and guilt. Importantly, relief not only ameliorated one's own psychological distress but also led to positive impacts on family relationships by helping to ease marital stress and opening up a dialogue with their children and other relatives, as described further below.

Without a diagnosis, many parents automatically think they are somehow to blame for their child's condition and must find a way to cope with that thought, as exemplified by one parent who shared:

“My husband and I were grasping at straws. Was it because I drank too much chocolate milk and I found out later that chocolate milk ... had caffeine in it. I also was on a gallbladder diet and ate tuna fish all the time and I thought was it the mercury. Was it the vaccinations? Um, I had a fall when I was pregnant and had physical therapy and they put some heat on my back. Just, you know, we just were searching, you know, for answers and causes. But finally we came to a peace that, ... we're probably never going to find out.” (Positive)

When TES was offered after years had passed since they stopped searching for answers, this peace was momentarily disturbed by bringing questions of cause back to the surface: *“I guess that it just brought to mind the whole questioning again and we had kind of accepted everything and not thought about the why of her condition so it just brought that to mind.”* Yet upon receiving an answer from TES, this parent expressed relief as they were absolved of their perceived guilt:

“When we found out the cause of her, after the exome sequencing, we found out the cause of her disability, I thought I had accepted everything but I just started to cry. It was finally an answer to, you know, what had happened. I think that I had accepted that I wouldn’t get an answer but it was a gift that I did get an answer.”

Moreover, feelings of guilt and blame for causing their child’s condition had contributed to increased marital stress. Receiving an answer from TES helped relieve this stress in this relationship:

“It has kind of given us a peace of mind and I think it has taken that stress, you know, this whole disability that she has has been very stressful on our marriage. We, you know, we’re doing fine now but we were told that 85% of parents that have special needs children end up getting divorced and um, we never considered getting divorced but it definitely put a strain on our relationship because of all the caretaking responsibilities. So I think this diagnosis since we’ve know, it’s just given a little bit more peace about it and we’re able to, you know, that aspect is just one less stressor in the relationship. It took a little bit of that away.”

Even those with uncertain results found comfort in finding a potential genetic cause for their child’s condition. As one parent related:

“Did I eat something wrong? I was working full time – should I not have worked? Should I have rested more? Should I have drank more? Yeah, yeah, you have no idea the things... oh that one time that I kind of slipped and fell – did I twist wrong? One time, you know, [my husband] knocked me in the stomach because he rolled over too fast in bed. You know, it’s everything under the sun that goes through your head that you did something. So that was really huge for us. Or at least for me. That alone was well worth taking the time and the test. That’s a whole huge peace of mind. That’s a whole load. Which gives you more time to focus on things you should be focused on, than worrying about something that doesn’t matter anyway because you can’t change it anyhow.”

Upon receiving a potential genetic cause for their child’s condition, some parents shifted the narrative from one of self-blame and guilt to one of Divine purpose. Parents who interpreted their child’s TES results as providing some kind of answer, also concluded that their child was made this way by G-d for a purpose and a reason. Parents found strength in this realization and were less anxious about their child’s future:

“But it just confirmed that, this was, you know, was meant to be and that she was designed that way.” (Positive)

“We know we didn’t cause it and even though we don’t know what’s to come, I think it’s just made it easier to just accept that she is who she is because G-d wanted her to be this way and for us that’s enough to say ‘ok, we give up’ [laughter] . Now this is what she’s meant to be. G-d gave her to us specifically. He chose us to raiser her, so we’re going to do the best we can and I think that has really impacted both of our lives with more an urgent responsibility to do our best for her. And to kind of get that divine intervention

thing going where you feel, ok she's here for a reason and He gave her to us for a reason. And that's even helped with my younger daughter to be able to say, look G-d made her this way for a reason, and He put you here in your situation for a reason. So it's definitely alleviated some stress and opened up a dialogue and, you know, maybe impacted all of us in, I think, a positive way.” (Uncertain)

Another source of relief identified in this study was that TES results helped clarify risks to other family members. All participants in this study who received results with pathogenic variants had *de novo* mutations. Therefore, the test result alleviated the fear of another child in the family being born with the same condition in the future:

“The main thing was having the knowledge that my daughter, my other daughter wouldn't have to worry about what she might be passing along to children that she would want to bear in her life . So the fact that I could spare her, that was probably the best outcome.”

5.2.3.2 Validation

Without a diagnosis, parents often had lingering concerns that they were being hypochondriacs, that they were bad parents, or that others were judging them as bad parents because of their child's condition. Participants who received a diagnosis from TES cited that having their concerns and struggles validated was an important outcome of testing:

“I think that that's more validating that there something really definitive, that there's a reason why she is built, that there is a reason why she looks the way she does. Just her genetics.”

“I have to tell you quite frankly, I am happy with the results, not that I have an option to be happy. I am quite satisfied with them because it just really affirms every concern I have shared with the doctors at each of the clinics.”

“It felt like we were validated. Like our struggles had been validated. ... I kind of felt validated you know, that I was able to tell like close family members, past teachers and friends that there really was something unique about her.”

The positive impact of having a child with special needs was well reported among this group of participants. Siblings in particular were noted to develop a strong sense of compassion and universal inclusion. Families were also noted to have gained strength through adversity. Parents did, however, also note the difficulties of growing up with a sibling with special needs who is given more attention and treated differently. Participants were able to use TES results to discuss the differences between their children in order to validate the struggles they endured and to alleviate feelings of worry and resentment:

“And that’s even helped with my younger daughter to be able to say, look G-d made her this way for a reason, and He put you here in your situation for a reason. So it’s definitely alleviated some stress and opened up a dialogue and, you know, maybe impacted all of us in, I think, a positive way.” (Uncertain)

“... the older daughter did have to sacrifice a lot and I’m sure she thinks that, when she was younger, [my affected daughter] got a lot more attention than she did, which she did. But it wasn’t our choice. So I think that her knowing that there was a definite cause for this may help her accept all the requirements of her special needs a little bit easier. I

don't want to say anything negative about my older daughter. She's just wonderful and she's now a Special Ed teacher and she's just so compassionate. I think that this whole process of having [a daughter] with special needs has only made our family stronger and has only made our family better. So you know I don't want to say anything negative about her but I do think that having the diagnosis probably helps her understand that you know, that everything was justified.” (Positive)

5.2.3.3 Altruism

Parents in all groups also expressed motivations of altruism before and after TES testing. Those who received a diagnosis felt inspired to participate in research to further advance knowledge about the condition and to join support groups in order to serve as mentors to others who may be diagnosed currently or in the future. As one participant expressed:

“I connected with some other parents that have a child with the same genetic disorder and we set up a Facebook support page and that's been a positive since most of their kids are age 5 and under, I can be a support to them and they ask me questions and I can feel like I can be a mentor so that's been a positive outcome of knowing.”

Additionally, those who received uncertain results were still encouraged by the idea that just getting their child's genetic information into databases will help advance the field and may lead to answers for others, if not for themselves. As one parent stated:

“Knowing that I'm not only doing something for myself and my child but that it's potentially helping another parent find out something about their child in the process – that was the biggest reason we decided to do it because it's not just for us but it's for parents that might not have any answers about their kids. There's not much that we can do but one blood draw and that's it, you know, and her map is in the system and my

husband's all about the maps. [Laughter]. You know the more information that is available, the further we can advance."

Even those without an answer found positives in the option to enter into the research phase because of the potential to help others in the future. For instance, one parent shared:

"I also like that they said that if this doesn't help, all this testing can further... like they were going to put us in this study group too, that they could further help other people even if it wasn't helping [our daughter] directly, we weren't getting answers directly, it would help out with the research."

One parent who described a particularly frustrating and lengthy fight to get insurance approval after multiple rejection letters discussed a strong desire to use her experience to help other families through the process:

"I think that for us if there is something we can do to advocate for another family, to write letters to insurance panels. Whatever we can do I think for us is what we want to do. We have our answers. We have our plan, but there are so many other families that are in the boat now that we were in three years ago and that are just waiting. I think for us it would be just if there is something else we can do just to make it easier for somebody else. Make it fast or more accessible. That's the big thing for us."

5.2.3.4 Hope for future

Parents in all categories also had a well-developed hope for the future following TES testing. Some who did not receive answers were amazed by the advances in technology that made TES a possibility and looked forward to future advances that may lead to an answer for their family. Others who had a diagnosis hoped that this answer would lead to increased knowledge about the

condition and better treatment or management strategies. This was exemplified in the following statements:

“And who knows, maybe they’ll end up identifying some sort of drug down the line that plugs in that broken X and things change.”

“The negative is there’s just so much unknown still. You know, in [my son]’s case, he has these variances but we don’t know what they mean. And that’s just...that’ll come I think as technology advances and new discoveries are made in science. It’s disappointing but it is what it is [laughter]. That we’ve come this far is pretty cool though.”

5.2.3.5 Obtaining support

The importance of finding support groups has been well documented in the literature. Similarly, participants in this study spoke about the positive impact of sharing ideas and connecting with parents going through similar experiences has had on their lives. As one parent voiced, finding a support group *“has been life-changing”* ... to be able to *“network and find answers to questions you may be having. Then to be able to talk to a mom who gets you, who understands your frustration and your stress. You know because our lives as a disabled parent is not like a normal parent. ‘cause you know, their biggest issues to us – we would love to have that as our biggest issue.”* Support doesn’t have to be disease or condition specific as this participant continues to relate: *“Even just the fact that they have a disability – sometimes that’s just all it takes. Just to get the understanding where you can say ugh, I had 6 doctor’s appointments this month, you know, and have somebody understand what that means. Or you know, we did this test or we did that test, you know, just somebody who gets it, is nice.”*

As one parent described, however, for those parents who received a diagnosis, seeking support from other parents whose kids have the same condition provides the opportunity to share information about therapies or strategies that have worked, insight into what to expect at different developmental stages, and the knowledge that they are not alone:

“So that was the good thing too with the social media that got us hooked up with these families. To see what works and what isn't working for them. What is available in different parts of the world. ... I think it has bridged us to a lot of other people with the same diagnosis. ... When we started connecting with those folks it just didn't feel so bad. We kind of had the overwhelming bad feeling at first and then once we started connecting with these folks it didn't seem so bad. ... We can kind of relate. We have some friends with little ones, but not many. I think it's just opened us up to more people.”

While physicians can serve as a source for some of this information, when coming from those who are living it daily it is much more comforting and meaningful, as exemplified by one participant:

“And just thinking and feeling – you know, we knew that it was rare – but thinking that we were... feeling like we were the only ones dealing with these particular issues is very, very lonely and to not have anybody but each other to bounce ideas off of. ... Because of them we get more information about the condition and we can talk to other parents who are dealing with the exact same stuff that we're going through. It's just so helpful to have that kind of peer support. ... It's coming from the people who are affected, it's not coming from a physician's office who says well this is how you should be feeling or this is what we expect that you ... this is the parents that have gone through this and they've made into a, literally, worldwide organization of support and it's fantastic.”

6.0 DISCUSSION

This exploratory qualitative study was conducted in order to understand the attitudes, perceptions and beliefs of parents towards targeted exome sequencing of their chronically ill children. Through thematic analysis of transcribed semi-structured interviews, a number of central themes emerged related to managing expectations, living with uncertainty and valuing the intangible. By developing a deeper understanding of the complexities and nuances of the parental experience with TES, alternative strategies for counseling this population of patients may be considered.

6.1 DISCUSSION OF THEMES

6.1.1 Managing Expectations

According to the Orphan Product Act of 1983, a rare condition is defined in the United States as one that affects less than 200,000 people in the US⁴². Among populations in the US and Europe, there are approximately 5000-8000 conditions that can be defined as rare. Likewise, there are 6800 conditions on the National Institutes of Health's list of rare diseases⁴³. Over 30 million individuals in the US are thought to currently be diagnosed with a rare condition^{42,43}. Genetic

conditions make up approximately 80% of rare conditions, which also includes infections, toxic exposures, and nutritional deficiencies.

Parents of children with chronic conditions often express simultaneous feelings of joy and sorrow, hope and hopelessness, and defiance and despair⁴⁴. The demands of caring for a child with a chronic condition can be overwhelming and contribute to feelings of depression, anxiety, uncertainty over child's future, and restrictions on social life⁴⁵. While parents of children with rare diseases face issues related generally to caring for a child with chronic health needs they must also cope with issues related to lack of diagnosis, limited availability of support and limited knowledge among healthcare providers. The prolonged search for a diagnosis can substantially impact parents' hopes and expectations for their child and for genetic testing. Understanding how these hopes and expectations evolve can help tailor counseling strategies in the setting of targeted exome sequencing.

Hopes and expectations are related but independent constructs⁴⁶. A recent integrative literature review was conducted to devise a conceptual model of how hopes and expectations each develop and then either diverge or converge⁴⁷. This model described hopes as determined by preferences and expectations as determined by probabilities. The perceived likelihood of an event is influenced by past experiences and current knowledge as well as optimistic bias, or the tendency to overestimate the likelihood of positive outcomes and underestimate that of negative ones. Because hopes and expectations are derived from distinct constructs, both can be held concurrently even when they are divergent⁴⁷. Taking this concept into practice, a study found that while health professionals view a tension between parental hope and medical prognosis, parents did not see these two states in competition with each other. Health professionals feared

that seemingly unrealistic hope meant parents are not fully informed while parents placed value on hope in helping to cope with illness⁴⁸.

Similarly, in this current study, parents' hopes and expectations often diverged without creating tension. Additionally, while parents all had similar hopes of finding answers for their children, their expectations diverged from one another based on their individual past experiences and personally held optimistic biases. For instance, parents cited multiple rounds of negative genetic tests in the past as informing their expectation of more of the same from TES. However, other parents with an optimistic view of technology expected answers from this scientifically advanced test. Interestingly, parental satisfaction with TES among these participants did not depend on whether either their hopes or expectations for results were met. Rather, anticipatory guidance of the test's limitations along with the knowledge that they've pursued every avenue open to them at this point seemed to be the most significant components of parental satisfaction with TES.

Furthermore, while all parents expected up to a six month wait for results, their experiences during this wait period diverged as well. Some parents were able to put the test out of their minds after circling the six month mark on their calendar expressing surprise it passed so quickly, while others waited furtively for what seemed like an interminable length of time. Those participants who were not troubled by the wait time tended to have older children whose condition reached somewhat of an equilibrium that did not suggest a shortened life expectancy. These parents also acknowledged the futility of agonizing over issues out of their control. On the other hand, those that were more anxious tended to have younger children where the question of life expectancy was more uncertain. Further studies exploring the factors determining how parents experience the wait for TES results as well as strategies parents employed to reduce

anxiety during this time could help illuminate ways clinicians may help parents manage the long wait.

6.1.1.1 Uncertainty in chronic illness

Mishel first developed the theory of uncertainty in illness defining uncertainty as “the inability to determine the meaning of illness-related events, occurring when the decision maker is unable to assign definite value to objects or events, or is unable to predict outcomes accurately⁴⁹.” Illness uncertainty has been described as falling into four broad categories: ambiguity regarding the state of the illness, complexity of medical care, lack of information, and disease unpredictability⁵⁰. Mishel’s theory was later re-conceptualized to incorporate the temporality of chronic illness where uncertainty is continually reevaluated as the relationship with illness continually evolves^{51,52}.

Mishel’s theory of uncertainty in chronic illness is composed of three main components: Antecedents, Appraisal, and Outcomes. Antecedents are any factors that form the foundation for the individual’s worldview, which may include medical, psychological and social factors. Appraisal refers to the individual’s assessment of uncertainty as a danger or an opportunity based on the incorporation of their antecedents. Finally, viewing uncertainty as a danger or an opportunity will determine whether the outcome is psychological distress, coping, or maintenance of the status quo^{50,51}. Individuals who perceive uncertainty to be a danger tend to attempt to bring clarity by seeking information⁵³. Alternatively, when uncertainty is viewed as an opportunity, individuals prefer to maintain uncertainty rather than learn troubling information. Therefore, these individuals tend to avoid information⁵³. Interestingly, while all participants in this study initially proceeded with TES seeking information, some who remained with uncertainty ultimately recognized opportunity in the lack of information. These individuals found

solace in not receiving a diagnosis that was life limiting or with a deteriorating prognosis and did not have to face limiting their child's potential to the box created by a definitive diagnosis. The impetus to retrospectively interpret uncertainty as opportunity may have different psychological origins when uncertainty is the only option rather than when it is a preconceived choice. Parents who hoped for clarity may cope with continued uncertainty by searching for its benefits. However, given the choice, these parents still want answers.

Whitmarsh highlighted an additional view of uncertainty as opportunity when specifically addressing individuals diagnosed with genetic conditions⁵⁴. Because genes are described as unchangeable determinants of human biology, genetic diagnoses are often expected to be definite with a specific, inescapable trajectory. Introducing uncertainty in the severity or prognosis of a genetic condition can free individuals from being defined by a narrow label and allow hope for the potential to exceed the narrow limitations of their diagnoses⁵⁴.

In the midst of chronic uncertainty regarding a child's condition, parents' desire for a diagnosis may change in intensity and be motivated by different needs over time. A number of studies have described different issues that parents of children with unexplained chronic conditions may consider in their need for a diagnosis: acquiring a label, validation, determining etiology, clarifying a prognosis, obtaining proper treatment, reaching acceptance, and seeking social support⁵⁵⁻⁵⁷. Determining an etiology was cited as important for absolving blame and clarifying recurrence risks^{55,57}. Parents' complex attitudes toward uncertainty run through each of these dimensions, preferring to maintain uncertainty in some situations while seeking clarity in others and sometimes desiring both simultaneously. For instance, while some want a label in order to give a name to their child's condition and to obtain appropriate services, they also fear limiting their child's potential by establishing the boundaries of a definite diagnosis⁵⁵. Similarly,

many parents want a clearer prognosis so they can plan and anticipate for the future yet there is also the fear of possibly confronting a shortened life expectancy⁵⁵. Furthermore, in the absence of a definitive diagnosis, parents had greater difficulty reaching acceptance of their child's condition and also struggled with issues of self-blame for causing their child's condition⁵⁵. In one study, validation was cited as the most common reason parents sought a diagnosis⁵⁷. A genetic diagnosis would validate that there is a real problem with their child and that the parents are not to blame, relieving a major source of psychological stress.

By the time they were offered TES, some parents in this study had reached a point of acceptance concerning their child's condition. While some expressed lingering thoughts of self-blame and worries about life expectancy, other parents discussed eventually becoming accustomed to their new normal. Those parents whose children received an answer through TES expressed feelings of relief, possibly not acknowledging the remaining stress caused by guilt and/or concern over an uncertain life span until that stress was removed. Those that did not get an answer, while frustrated that their diagnostic odyssey continues, also felt a sense of relief in knowing they have done everything they can for their child by pursuing TES. These parents have become accustomed not only to their child's condition but also to not finding answers in genetic testing. While previous studies reported multiple factors driving parents' needs for a diagnosis for their child, parents in this study were primarily driven by a need to clarify life expectancy and a desire for information that will best help their child either obtain resources or find appropriate treatment. Although parents in the positive category remarked on the relief felt upon realizing they were not to blame for their child's condition, participants declared that they are not interested in knowing why their child is affected but rather how they can give him or her the best life possible. Similarly, parents who received an answer were happy to have it yet said they are

not interested in labels unless they can be used to obtain services, resources, treatments, or therapies that are otherwise unavailable.

6.1.1.2 Uncertainty in genetic testing

The need for interpretation of results in genetic testing can add another layer of uncertainty in the results themselves. Like typical lab tests, genetic tests can produce either positive or negative results, either confirming or ruling out the condition tested. However, unlike most typical lab tests, genetic tests can also produce an indeterminate result termed “variant of uncertain significance” (VUS). A VUS cannot officially be declared pathogenic or benign due to inadequate data, leaving parents with a result that is not an answer. Similar to WES, chromosomal microarray (CMA) is an unbiased approach to interrogating large sections of the genome simultaneously and can uncover rare, complex genetic anomalies that are not detectable by traditional genetic testing and also often results in VUSs. Parental perceptions, understanding and adaption to uncertain results following clinical CMA testing has been reported⁵⁸⁻⁶⁰ but similar studies in the setting of WES results have not been found.

Studies exploring the parental interpretation of VUS results from CMA revealed a conflict between the interpretation of a VUS providing a cause but not a meaning for their child’s condition^{58,59}. Many parents interpret this genetic difference as proof that their child’s condition is genetic, which they can use to alleviate guilt, fight for services, and hope for better information as more data is accumulated in the future. Again, even in the context of a VUS, parents described the result as delivering relief, providing validation, and helping reach acceptance⁵⁹. However, VUSs still leave parents unsure about the health implications for their child⁶⁰. The importance of support groups and connecting with other parents was further highlighted in the context of rare diagnoses that lack information being described as

“isolating⁵⁹.” Moreover, due to the rarity and novelty of many CMA results returned to patients, parents are often faced with uncertainty regardless of whether the genetic diagnosis is definite or uncertain, which engenders both frustration and hope⁶¹.

The current study reveals a number of parallels between the impact of uncertainty in CMA and TES. Similar to prior studies, participants in this research viewed VUSs as proof that their child is genetically different even if the genetic differences uncovered by the test are not themselves the definite cause of their child’s condition. Having evidence that there are differences in their child’s genes gives parents reassurance that the answer lies somewhere in their child’s genetic makeup but technology is not advanced enough yet to detect it. Based on this interpretation of VUSs, parents without answers still expressed feelings of relief, absolution of guilt and validation, used the information to defend their child, and had hope for better answers in the future. It is unclear if these misinterpretations or over-interpretations of the meaning of VUSs is due to a lack of understanding or is an attempt to cope with the reality of caring for a child with an undiagnosed chronic illness. It is important to understand the impetus for this response because each would require a different counseling strategy. If parents do not understand that genetic variability exists among all individuals or how to distinguish between variants of uncertain significance and pathogenic variants, then more in depth education on these topics during or prior to results disclosure is necessary. However, if parents are using VUSs like a life raft in a sea of uncertainty, then alternative strategies to seek relief from stress, absolution of guilt, and to maintain hope might be explored.

Similar to CMA, TES interrogates large, previously uncharted genomic territory and even results that provide certain diagnoses are not always able to provide certain prognoses. The children of participants in this study that did receive positive results from TES were all

diagnosed with rare conditions. As prior studies found subsequent to diagnoses with rare copy number variants, parents in this study expressed dichotomous reactions of both gratitude and frustration. While grateful for an end to the diagnostic odyssey, parents were also frustrated by the lack of clarity in the meaning of their child's result. The ability to participate in research related to their child's condition or the opportunity to serve as mentors to other parents of children with the same rare diagnosis may be avenues to channel some of their frustrations.

6.1.2 Valuing the Intangible

While the diagnosis or presentation of a chronic condition can initially spark feelings of shock, grief, loss, anger, and guilt, many parents are able to adapt over time⁶². Adaptation can occur through meeting the social, informational, and emotional needs of parents of children with rare, chronic conditions⁶³. Participants in this study disclosed a number of adaptations used to cope with their child's chronic condition, highlighting the potential for personal utility of TES. Adaptations included participation in support organizations and research, acquiring knowledge, maintaining hope for the future, and reframing their world views. In other studies, parents of children with chronic conditions have been shown to display positive adaptation through altering their perceptions and prior beliefs by focusing on their child's strengths, on the positive impact their children have on those around them and on their hope for the future, finding meaning, creating new dreams, and framing their situation as a 'new normal'^{62,64}. By actively altering their world view, parents are able to regain a sense of control⁶².

Social needs of parents of children with rare, chronic conditions include access to support groups, support of family and friends, and the ability to communicate with parents in similar circumstances. When these social needs are not met, parents display feelings of isolation and

loneliness⁶³. Likewise, study participants spoke about the life changing importance of peer support and support groups. Support groups did not need to be disease or condition specific to be beneficial. Even in the absence of a definitive diagnosis, therefore, referral to a group of parents that can understand the daily life of a parent of a child with special needs is critical.

Informational needs refer to a parental desire to be personally informed as well as frustration over lack of knowledge of rare diseases among healthcare providers. Because it is often difficult for healthcare providers to be knowledgeable about every rare disease, the parents may become the experts and many parents must gain knowledge through doing their own research, going to conferences, searching the internet and talking to others with the same condition⁶³. Similarly, participants in this study wanted to know disease course, life expectancy, and availability of services, treatments and management so they can plan for the future and so they can provide the best life possible for their child today. TES results disclosures address these topics and should include information that can be easily shared with other healthcare providers who may not be familiar with rare conditions.

Emotional needs vary with the level of disease severity, needs, and caregiving responsibility. While the initial diagnosis can induce feelings of shock, even with the passage of time, parents face chronic stress, guilt, frustration, anxiety, uncertainty, and fear. Receiving a diagnosis is important in providing some relief of these emotional stressors⁶³. For many couples, the stress of raising children with chronic conditions negatively impacts their relationships⁶⁵. Others, though, have been shown to benefit from parenting a child with special needs by strengthening their bond and becoming more unified as a family⁶⁶. Siblings and parents have benefited through the development of a positive world view to include greater acceptance, tolerance, and patience. Through participation in support groups and advocacy, parents and

siblings also develop new, meaningful relationships and become part of communities they would not have experienced otherwise^{65,66}. Likewise, parents in this study spoke of the stress caring for a child with special needs has had on relationships with their spouses and the struggles endured by their other children. However, families have also discovered strength in overcoming adversity and siblings tended to develop warmth, compassion and patience beyond their years. TES aided in adaptation to living with a child with a chronic condition by providing validation for their struggles and removing thoughts of blame. Having a reason or even the suggestion of a reason contributed to reduced parental stress and opened up a discussion with their other children concerning the seeming disparities in attention given to them compared to their affected siblings.

The personal utility found in meeting their social, information and emotional needs is also commonly cited in the literature as a benefit of receiving genetic testing results^{2,5,7,8,35,38,61}. Genetic testing has resulted in relief⁶¹, reduced uncertainty and anxiety⁶¹, improved ability to prepare for the future³⁵, reinforcement of compliance with recommendations³⁸, increased awareness of health risks³⁸, and empowerment and control⁵. Additionally knowledge is viewed as being intrinsically valuable and parents have described a sense of personal responsibility to know all they can about their child^{61,67}. For parents who feel this responsibility, any knowledge – even uninterpretable knowledge – is inherently valuable⁶¹.

6.2 STUDY LIMITATIONS AND FUTURE DIRECTIONS

This study had several limitations. The sample size was small and limited to one genetics center where all participants were seen by the same genetic counselor for post-TES counseling. Results, therefore, may not be generalizable. Small sample size also limited ability to compare

experiences among groups. Additionally, all participants were Caucasian females and most had at least some post-secondary education, limiting the diversity of the study population. Views that appeared common may reflect culture-specific norms and a richer description of the parental experience could be gained by similar studies with a more diverse population. Participation in this study was voluntary and results could be a reflection of selection bias whereby those agreeing to participate in research may have a particular perspective that is not necessarily representative of the general population. The index patients in this study had diagnoses that ranged in etiology and severity. Parental perceptions may vary as a result of specific diagnosis. This was a retrospective study. Stated expectations, therefore, were likely influenced by outcome and dependent upon the participants' recollection. Future longitudinal studies encompassing pre- and post-test data collection may provide a more accurate reflection of parents' thoughts, expectations, and perceptions in real time. Since the experience of parents whose children underwent TES testing has not been previously reported in the literature, this study intended to investigate the full experience of parents throughout this process. Therefore, the questions covered a broad number of topics and did not dig deeply into any one topic. Further research could focus more deeply on a specific aspect of testing. One such aspect that may benefit from additional investigation is how, when, and how much information on basic genetics and exome sequencing should be introduced to the parents. In this study of 11 parents, some were overwhelmed by receiving too much information in one sitting while others expressed fascination and appreciation for detail. Still others balked at the complexity of exome, concluding that no amount of information would bring parents to a full understanding of the test. A larger, more focused survey of parents whose children have undergone TES could help determine their preferences, needs and expectations for receiving this information. Based on

these results, alternative strategies could be developed for educating parents such as through the development of brochures or providing links to appropriate online resources.

6.3 CONCLUSIONS

This is one of the first studies to explore the parental experience with clinical targeted exome sequencing in a genetic counseling setting. The results describe the complexities and nuances of parents' attitudes and perceptions throughout the TES testing process. Satisfaction with TES was not dependent upon outcome but rather is a function of managed expectations. Awareness of the test's limitations, an accurate appraisal of the wait time, and a feeling that TES is the most comprehensive test currently available all contributed to parental satisfaction regardless of whether their child had a positive, uncertain or likely negative result. Furthermore, parents in all categories faced continued uncertainty in the face of their child's TES results resulting in feelings of both relief and frustration. For those who received an answer, there was relief in finally having a diagnosis yet frustration when that diagnosis was so rare it came with limited information. For those who did not receive an answer, the lack of a more serious and life threatening finding brought relief, yet parents were still frustrated by the unending diagnostic odyssey. Finally, in addition to feelings of relief, parents in all categories were able to find personal utility in undergoing the TES process such as validation of their concerns and struggles, the opportunity to participate in research, finding peer support and maintaining hope for the future. Developing a deeper understanding of parents' motivations, attitudes, and perceptions regarding the TES process has significant public health implications in that it may allow genetic counselors and other health professionals to find alternative strategies for counseling this

population of patients. Future research should focus on determining the generalizability of these results with larger, more diverse cohorts, on elucidating how these perceptions and attitudes evolve temporally through longitudinal studies, and on assessing the adequacy of counseling strategies aimed at addressing the nuances of the parental experience with pediatric TES.

7.0 PUBLIC HEALTH SIGNIFICANCE

In this study, parents expressed a desire for information on the basics of genetics and exome sequencing prior to receiving their child's result. Currently, parents receive most of this information during the results disclosure visit and some participants in this study were overwhelmed by the amount of information received at once. In order to reduce parents' stress and better prepare them for the results disclosure visit, the following is a proposed method for the development of educational materials for those undergoing exome sequencing. These materials could be sent to parents to study prior to the results disclosure visit without creating a strain on resources by requiring an additional clinic visit. This proposal includes a needs assessment, a method of material development and an evaluation of efficacy and parent satisfaction.

7.1 BACKGROUND

In the United States, a rare condition is defined as one that affects less than 200,000 people in the US⁴². According to the Genetic and Rare Diseases Information Center of the National Institutes of Health, there are 6800 conditions rare conditions known⁴³ affecting 25-30 million individuals in the US^{42,43}. Genetic conditions make up ~80% of rare conditions, which also includes infections, toxic exposures, and nutritional deficiencies. Almost half of all patients with

unknown suspected genetic disorders receive a genetic diagnosis through traditional genetic approaches¹². Traditional clinical genetics refers to a comprehensive physical evaluation, chromosomal studies, biochemical studies, single gene testing, gene panel testing, or microarray analysis. Of those patients who did receive a genetic diagnosis through traditional genetic approaches, 72% were diagnosed during the first diagnostic evaluation¹². Those without a diagnosis following their initial evaluation embark on a ‘diagnostic odyssey’ that can include multiple rounds of genetic testing, consultations with various specialists, radiological assessments (CT scans, MRIs, skeletal surveys), cardiology assessments (Echo, EKG), and neurological exams (EEG, sleep study) in an attempt to obtain a clinical diagnosis. This diagnostic odyssey can become emotionally and financially draining. WES or whole genome sequencing (WGS) as a second-line test might increase yield and decrease time to diagnosis thereby decreasing costs of subsequent testing.

7.1.1 Cost effectiveness of whole exome sequencing

Exome sequencing is a relatively new genetic test offered clinically since 2012. Some preliminary reports analyzing the cost effectiveness of WES are available, however, more comprehensive analyses are still needed. Recently, an economic analysis of WES/TES at CHP was performed (Madan-Khetarpal et al 2015 ACMG Abstract)²⁰. The results suggest that when a patient’s clinical presentation is indicative of a specific genetic condition, single gene or gene panel testing is appropriate. However, when the genetic etiology of a patient’s condition is not obvious, WES/TES as a first-line test might be the most cost effective option. This study compared patients whose initial genetics evaluation occurred before 2012 to those whose initial genetics evaluation occurred after 2012. WES/TES was not generally offered clinically prior to

2012, therefore, those patients tended to have a longer diagnostic odyssey before being offered exome sequencing. Diagnoses were made in 39.2% of cases overall and there was no difference in the diagnosis rate among both groups. The cost of diagnostic testing, however, was significantly different, with the group before 2012 spending an average of \$15000 prior to receiving WES and those after 2012 spending an average of \$7000 (Madan-Khetarpal et al 2015 ACMG Abstract)²⁰.

Since the patients in this study were receiving services over different time periods, it is difficult to directly compare costs as technological improvements alone can lead to decreased costs. Of note, however, the increased availability of WES/TES led to a reduction in the number of genetic tests ordered. Those patients with an initial genetic evaluation before 2012 received an average of 4.1 single gene tests and 1.2 gene panels whereas, those with an initial genetic evaluation in 2012 or later received an average of 1.5 single gene tests and 0.64 gene panels. This analysis does not account for possible reductions in specialist consultations, biochemical testing, radiological assessments, cardiology assessments or neurology assessments, which can significantly impact costs as well. The cost-benefit analysis, therefore, is likely an underestimate (Madan-Khetarpal et al 2015 ACMG Abstract)²⁰.

Similarly, another recent study found that 48% of patients who received WES had at least four prior genetic tests. In 3 out of their 40 cases, more than 10 genetic tests were performed, with a combined cost greater than that of WES¹⁷. In a 2014 study of children with undiagnosed neurodevelopmental disorders, the diagnostic rate for WES was 40% and the average cost of negative testing for all patients prior to receiving WES was ~\$19,000. The investigators determined that for WES to be cost effective as a first-line test for these patients, the cost of testing should be under \$7600 per family²². However, the cost of delaying WES testing does not

account for any non-genetic testing related costs such as CT/MRI scans, biochemical tests, or skin/muscle biopsies. It also does not account for the potentially increased detection rate that is likely to result from using WES as a first line test.

7.1.2 Utility of whole exome sequencing

WES has been increasingly used clinically when the suspected condition is genetically heterogeneous or the underlying genetic cause is unclear¹⁵. Because WES is the most comprehensive and unbiased genetic test currently available clinically, it is particularly useful for diagnosing rare Mendelian disorders or uncovering complex mutation patterns by sequencing thousands of genes at once. The utility of the unbiased approach afforded by WES has been highlighted in a number of recent retrospective studies. These studies revealed a diagnostic rate of 25-35%¹⁵⁻¹⁹ in which 37-41% of confirmed cases uncovered by WES were due to *de novo* autosomal dominant variants, meaning family history would not have provided clues to a diagnosis^{15,19}. Importantly, 3-4.6% of confirmed cases were found to harbor pathogenic variants in two or more different genes and 29-58% of the total pathogenic variants uncovered had not been previously reported^{15,19,21}. Furthermore, WES identified mutations in genes known to be associated with genetic conditions but had not been suspected due either to genetic heterogeneity or an atypical presentation²¹. These numbers suggest that traditional genetics approaches of single gene and gene panel testing were unlikely to determine the genetic basis for most of the cases included in these studies. A recent prospective study found that in a cohort of infants whose clinical presentation suggests a monogenic disorder of non-obvious etiology, 57% were diagnosed by WES as a first-line test compared to 13.8% who received standard testing⁶⁸,

suggesting marked utility for WES in this population as a first line test rather than test of last resort.

Other studies have shown the ability of WES to detect pathogenic variants in a wide range of conditions including but not limited to neurodevelopmental disorders^{22,69}, intellectual disability⁷⁰, deafness¹⁵, skeletal dysplasias²⁵, limb-girdle muscular dystrophy²⁶, and retinal dystrophies²⁷. As more studies emerge detailing the utility and cost effectiveness of WES, insurance coverage may become less of a barrier to testing. As of February 2016, 75 CHP patients received WES and 319 have received TES with an average of 8.4 WES/TES patients counseled per month (Internal Communication). As access to WES increases, extensive results curation and lengthy counseling sessions of 2-3 hours will become untenable. By developing targeted exome sequencing, which only focuses on variants in genes that could be related to the patient's clinical presentation, CHP has drastically reduced the amount time required to counsel patients receiving TES. However, with the bulk of the counseling provided during results disclosure, parents often come in to the session with a basic understanding of genetics, but with limited to absent knowledge about more complex genetics concepts such as exome sequencing. Trying to understand both new, complex genetics concepts along with their child's specific results can be overwhelming. By providing basic materials when TES testing is initially offered, parents can learn about genetics and the test itself while waiting for the results, which may help lower the anxiety level of parents and reduce the time needed for results disclosure.

7.1.3 Cultural perceptions of exome sequencing

Attitudes towards genetic testing in general have been shown to vary among different cultures^{7,8,71-75}, which can alter an individual's perception of the risks and benefits of pursuing

testing. One of the limitations of this current study is the lack of cultural diversity represented since effective genetic counseling requires an understanding of how risks and benefits are framed. Additionally, there is limited research available on the manner in which cultural context specifically alters the perception of whole exome or whole genome sequencing. One recent study, however, did compare the perceptions of whole genome sequencing for research among African Americans and non-African Americans through the use of focus groups^{7,8}.

The majority of participants in both groups voiced interest in receiving at least some exome sequencing results and saw the benefit in the ability to be proactive upon finding actionable results^{7,8}. Interestingly, the two groups displayed different temporal orientations when describing where this benefit is expected. The African American group was oriented more towards the present, focusing on potential short term health benefits as well as potential immediate psychosocial impacts. The non-African American group was instead oriented towards the future, focusing on the ability for results to help with long term planning. Furthermore, African American participants were also more community-focused than individualistic in decision making and considering the potential impact of their results compared to non-African Americans. When contemplating hypothetical actionable results, however, African American individuals raised concerns about a lack of health care access impacting their ability to follow-up and about the potential to overwhelm an already stressful life whereas non-African Americans did not display the same hesitation. Additionally, non-African Americans expected their health care providers to be fully aware of their results and actively involved in their continued care. While some African American participants, on the other hand, voiced concerns for law enforcement accessing their genetic information or misuse of this information by the health care system, preferring the ability to pursue this type of testing privately, outside of the system^{7,8}.

Distrust of the health care system and lack of health care access can be major barriers in the decision to pursue genetic testing.

It is not clear how generalizable these study results are since the sample size was small and there may have been significant differences between the two groups other than ethnicity, such as education level and health status. Additionally, the study focused on exome sequencing in a research setting rather than a clinical setting. However, the responses highlight the potential for an individual's background to frame their perception of genetic testing and the importance for genetic counselors to be aware of these differences. While these studies focused on the differences among African American and predominantly Caucasian individuals, members of other ethnic backgrounds are likely to display their own unique perceptions of genetic testing and exome sequencing. Therefore, any counseling strategy aimed at the general population should take into account the various cultural contexts present in the population.

7.2 SURVEY WIDER POPULATION OF INDIVIDUALS WHO RECEIVED EXOME SEQUENCING RESULTS

While many parents in this present study described feeling overwhelmed when trying to learn about general genetics concepts and their child's specific results during the same consultation, some parents disclosed that they were not overwhelmed and, rather, appreciated receiving all the information at once. Since the population size was not powered for statistical significance, a larger survey of parents' preferences should be performed prior to developing materials or altering counseling strategies. A survey of parents whose children have completed TES testing should address parents' desire for information on general genetics prior to the results disclosure

and what information, if any, should be included. Furthermore, as described above, perceptions and concerns regarding genetic testing vary across cultures. Therefore, the study population should encompass diverse backgrounds in terms of ethnicity, gender, educational and socioeconomic backgrounds.

7.3 DEVELOP TES EDUCATIONAL MATERIALS

If the survey demonstrates that a majority of parents prefer more information about TES before the results disclosure, educational materials would be developed in collaboration with geneticists, genetic counselors, patients/parents, and community partners to ensure cultural sensitivity as well as accurate and understandable explanations of complex concepts, such as variants of uncertain significance (VUS). The process would follow a similar overall approach taken by Sanderson et al when developing an online educational video for WGS⁷⁶ and begin with a review of existing publically available educational resources, determining their strengths and weakness and where improvements can be made.

The initial development would focus on written materials since, compared to videos, they are more economical, easier to update, and do not limit access to those with specific equipment. Moreover, prior studies of health related educational resources have not demonstrated any benefit to video over written materials⁷⁶. However, this approach would potentially limit access to illiterate and non-English speakers. The content would be directed by input from the stakeholders mentioned above as well as by results from the preliminary survey. Likely topics to incorporate include

1. An introduction to genes, chromosomes, proteins with a definition of the exome
2. An overview of WES/TES including method, purpose, benefits, and limitations (diagnosis rate, types of conditions not covered, wait time, insurance barriers).
3. A discussion of the different types of possible results, their interpretations, and potential psychosocial reactions to each type of result

Focus groups should be conducted with members of the aforementioned stakeholder groups following the initial development of the written educational resource to evaluate content, design, readability, and accuracy. The materials would be amended as directed by the results of the focus groups. Once a final product is created, these materials would be distributed to patients undergoing TES and/or their parents following the brief introduction to TES that is currently provided by geneticists or genetic counselors at CHP. The quality of the materials would then be evaluated based on patient/parent satisfaction and knowledge.

7.4 EVALUATE SATISFACTION WITH AND EFFICACY OF EDUCATIONAL MATERIALS

A survey would be conducted to determine participants' overall satisfaction with the educational materials, assessing areas such as language/word choice, relevance of content, organization, amount of information, and clarity of information. Participants would also complete a pre-test prior to receiving the educational materials and a post-test prior to results disclosure in order to assess how much knowledge was gained and retained. Since participants in this current study seemed to misunderstand the interpretation of VUSs and the meaning of the different types of

possible results, questions should be particularly focused in these areas in addition to areas of more basic genetics.

As exome sequencing technology continues to develop making testing more affordable and as insurance companies become more accepting, WES/WGS will likely not be limited to a small number of rare, esoteric cases. Rather, wider applicability of exome sequencing has been proposed in the prenatal setting^{77,78}, in newborn screening^{79,80}, to diagnose complex conditions, or to provide pre-symptomatic screening in healthy individuals. As the applicability of WES/WGS expands, so too will the types of providers offering this testing. If WES/WGS begins to be offered by non-genetics professionals, patients will increasingly benefit from the availability of educational materials describing exome sequencing along with its benefits and limitations clearly and accurately.

APPENDIX A: IRB APPROVAL LETTER



University of Pittsburgh *Institutional Review Board*

3500 Fifth Avenue
Pittsburgh, PA 15213
(412) 383-1480
(412) 383-1508 (fax)
<http://www.irb.pitt.edu>

Memorandum

To: Bess Wayburn

From: Sue Beers

Date: 8/11/2015

IRB#: [PRO15030727](#)

Subject: Psychosocial Impact of Targeted Exome Sequencing of Chronically Ill Children

The University of Pittsburgh Institutional Review Board reviewed and approved the above referenced study by the expedited review procedure authorized under 45 CFR 46.110 and 21 CFR 56.110. Your research study was approved under:

45 CFR 46.110.(7)

The IRB has approved the waiver for the requirement to obtain a written informed consent.

The IRB has approved the waiver for the requirement to obtain informed consent to use protected health information to identify potential research subjects.

The risk level designation is Minimal Risk.

Approval Date: 8/11/2015

Expiration Date: 8/10/2016

APPENDIX B: PARTICIPANT RECRUITMENT SCRIPT (TELEPHONE)

Hello,

(Marianne McGuire introduces herself)

I am contacting you to let you know about an opportunity to participate in research here at Children's. *(ask if now is a good time to talk or if they prefer that you call them at a later time. If later, arrange a time.)* The purpose of this research study is to better understand the experiences of parents whose child has been tested using targeted exome sequencing. We want to learn more about these experiences so we can improve our approach to offering this testing. To accomplish this, Bess Wayburn, a genetic counseling student, will be interviewing parents whose children have received results from targeted exome testing through the Medical Genetics Department at Children's Hospital of Pittsburgh. The researchers only be told of your child's results status (i.e. positive, negative, or uncertain), not the specific results of his/her testing. Those parents who choose to participate will be asked to complete an approximately 1 hour telephone or in person interview with Bess as the main investigator for the study. During the interview, those who choose to do the study will be asked questions about their thoughts and opinions on the information they were given before deciding to have the testing done, how well they thought they understood that information and whether they could ask questions freely before making a decision. They will also be asked about how things went when the results were given to them. Would you be interested in learning more about this research study?

If yes: May I provide Bess with your contact information so she can discuss the details of this study with you further?

APPENDIX C: PARTICIPANT VERBAL CONSENT SCRIPT (TELEPHONE)

Hello, my name is Bess Wayburn, a Genetic Counseling student working on the study entitled “Psychosocial impact of targeted exome sequencing of chronically ill children”. Marianne McGuire, a genetic counselor from Children’s Hospital of Pittsburgh, referred you to my study. Are you interested in hearing more about it?

You are being asked to participate in this research study because your child received results from a targeted exome sequencing test. The purpose of this research study is to better understand the experiences of parents with the entire testing process so we may improve the way we offer this type of testing. To accomplish this, we will be interviewing parents whose children have received positive, negative, or uncertain results from targeted exome testing through the Medical Genetics Department at Children’s Hospital of Pittsburgh.

Participants are asked to complete an approximately 1 hour telephone or in person interview with the main investigator in the study. The interview will ask about thoughts and opinions on the information parents were given before deciding to have the testing done, how well they thought they understood that information and whether they could ask questions freely before making a decision. They will also be asked about how things went when the results were given to them. Interviews will be audio recorded for transcription. Identifying information such as your name will be removed from the transcript.

All responses to the interview are confidential and responses will be stored in a secure manner. Personal password-protected computers and a locked file cabinet will be used to store records. Research records for this study will be stored indefinitely without information that can be linked to participants' identities. It is possible that in the future, other investigators interested in performing similar research will request access to data or materials from this study. If data is shared with other investigators, they will not be able to link the data to the participants' identities in any way.

While your participation may not directly benefit you, information gathered through the study will be used for improving our counseling process for targeted exome sequencing testing. This knowledge may benefit patients who undergo this testing in the future by allowing health professionals to design a more patient-focused testing process. You will not receive any payment for your participation and there is no cost to participate.

In unusual cases, in response to a court order, the investigators may be required to release identifiable information (which may include your identifiable medical information) related to your participation in this research study. If investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies. No medical procedures will be performed in this study but if you believe that the research procedures have resulted in injury to you, contact the Principle Investigator immediately. Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research-related injury requires

medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation.

Your participation is voluntary and you may withdraw from this project at any time. Any answers recorded in the interview prior to withdrawal will remain part of the study. Your decision to participate or not participate will not affect your current or future relationship with the University of Pittsburgh Medical Center or the University of Pittsburgh.

You are encouraged to ask questions, voice concerns or complaints about any aspect of this research study during the course of this study, and future questions, concerns or complaints will be answered by a qualified individual, by the Principal Investigator, Bess Wayburn at ____, or co-investigator Catherine Walsh Vockley at 412-692-7349. You may contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain information; offer input; or discuss situations in the event that the research team is unavailable.

Do you have any questions about the study or the information I just went over?

Are you interested in participating in this study?

APPENDIX D: PARTICIPANT INTERVIEW GUIDE

Date: ___/___/___

Study participant's ID: _____

Interview method: ___ Phone ___ In-person

Parent: ___ Mom ___ Dad

Parental age: _____

Education level of parent: High school degree ___ Associate's degree ___ Bachelor's degree ___ Master's degree ___ Doctoral degree ___ Other _____

Child's symptoms: _____

What testing did [child] receive prior to TES?:

How old is [child]? _____

Was [child] your first-born child? ___ If other children: What are the ages of the other children

Are other children in the family affected?

Prior to testing

Tell me about yourself (and your family)

Tell me about how you first found out about your child's condition.

People have many ideas about what causes birth defects or health problems. What were your initial thoughts about what may have caused your child's condition? Have those thoughts changed and if so, how?

Tell me about your experience since the onset of your child's condition.

- Prompts (if necessary)
 - How many other specialists had you seen prior to or along with Genetics?
 - What sorts of other tests/treatments had already been performed prior to TES?

Please describe your experience meeting with the geneticist or genetic counselor to discuss your child's condition prior to first being offered TES

- Prompts (if necessary)
 - What did you learn from the geneticist, if applicable?
 - What did you learn from the genetic counselor, if applicable?
 - Is there anything else that you would have liked to have learned from them?

Informed consent process

When you were first told about TES, what was the explanation given for providing your child with this test?

How was TES described to you?

What was your understanding of the goals of this test?

What information, in hindsight, was missing that you wish you had been given?

Did you have any questions that you do not think were answered, or do you have more questions now (address these)

What advice would you give to the person who discussed the test with you prior to testing in order to help other families who are considering the same test?

How would you describe this test to other families?

Thoughts/assumptions prior to results disclosure

After the testing process began, what were your expectations were for the results?

- Prompts (if necessary)
 - How definitive the results would be
 - How long test would take
 - How paying for the test would work

What questions or concerns, if any, did you have after the testing process began?

What were your thoughts about the possibility of not getting an answer from this test?

What were your thoughts about the possibility of getting an uncertain result from this test?

Did you and others discuss the testing while it was being done? If so, please describe for me the conversations about the test that took place between you and your partner and between you and other family members or any other important people in your life?

How was the waiting time for you to get the results once the test was started? Is there anything that the geneticist/genetic counselor could have done to assist you during that time?

Experience with results disclosure

When you think of your child's results, what is the first thing that comes to your mind?

What is your interpretation of what the test results mean?

Describe your experience with receiving your results.

- Prompts (if necessary)
 - Did you get information you hoped to get?
 - How did you react to the information?
 - How did it make you feel?
 - Were you given enough information to understand their meaning? Were you given too much information?

How did the experience match your understanding and expectations?

How did the experience differ from your understanding and expectations?

What questions, if any, did you ask at the time?

Post results reflections

What are some of the positives, if any, of the genetic testing process you went through?

What were some of the negatives, if any?

How has the test result impacted your perception of your child's future wellbeing?

How have the results impacted your child's ongoing medical care?

How has the test result impacted the important relationships in your life?

- Prompts (if necessary)
 - Will you share/have you shared your results with extended family members or other important people in your life? What are the reasons for your decision?
 - What is your understanding of the implications for other family members?

How has this experience affected your thoughts on future family planning?

When it comes to the experience of genetic testing, what I have missed that you would like to share?

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