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Parental experience with whole exome sequencing reanalysis and its impact on the diagnostic odyssey

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Abstract

Advances in genomic technology and an increase in the number of gene-disease associations have helped reduce the number of individuals living without a diagnosis. Whole exome sequencing (WES) analyzes the entire human exome in an attempt to determine if there is a molecular etiology for individuals who remain undiagnosed after other clinical or molecular investigations. Still, WES leaves most individuals undiagnosed, resulting in feelings of disappointment and uncertainty. Individuals who remain undiagnosed after WES can subsequently undergo WES reanalysis later due to improvements in bioinformatics, software updates, and an increase in known gene-disease associations. This is the first study, to the investigator's knowledge, which investigates parental perspective of those undergoing the most current genetic testing available. This study recruited parents of undiagnosed individuals who have completed WES and subsequent reanalysis through the Greenwood Genetic Center to investigate their response to and experience with WES reanalysis while on their diagnostic odyssey. Six semi-structured interviews were conducted, recorded, and transcribed verbatim. Transcripts were analyzed using grounded theory and assigned codes to meaningful segments of text. Results showed most participants had lower expectations of reanalysis compared to the initial WES and felt it would not lead to a diagnosis. Most participants responded to nondiagnostic reanalysis results with feelings of disappointment and worry about the future. However, some exhibited a difference in the degree to which they negatively responded. Most participants recognized that reanalysis has been unhelpful for

their child but expressed willingness to contribute to science if it will assist future individuals on a diagnostic odyssey. Despite feelings that reanalysis was unhelpful, most participants would consider reanalysis again for their child. Considering the apparent comprehensive nature of genomic testing, these results show there is a need to balance hope and realistic expectations during counseling and consent of WES reanalysis. In addition, parents desired ongoing medical support which can be offered through reanalysis.

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Chapter 1: Background

1.1 A diagnosis

The journey and search for a diagnosis is referred to in the clinic and literature as a "diagnostic odyssey." The process of a diagnostic odyssey has been defined as "the time between when a parent or provider first becomes concerned about a child's development and a diagnosis is eventually reached" (Carmichael, Tsipis, Windmueller, Mandel, & Estrella, 2015). All individuals who have received a diagnosis have been on varying lengths of a diagnostic odyssey. For some, their diagnostic odyssey may last for months or years while others may remain undiagnosed in their lifetime.

There are many different times during the lifespan when an individual can be diagnosed. The earliest time one might receive a diagnosis is prenatally. For example, a diagnosis of Down syndrome can be made during the first trimester of pregnancy through a procedure known as chorionic villus sampling (CVS). Using the tissue obtained through CVS, a chromosomal karyotype is completed to assess the baby's number of chromosomes.

Postnatally the pursuit of a diagnosis begins with clinical recognition and evaluation of an individual's symptoms. If a condition is not readily suspected or diagnosed, an individual may spend time as an in- or out-patient, undergoing various imaging, clinical or laboratory tests, and consults with experts. In general, individuals who undergo a diagnostic odyssey have unexplained, medically complex features.

In the new age of technology, many will undergo genetic testing as part of their diagnostic odyssey. Genetic testing can help clarify a clinical diagnosis or give an individual a molecular diagnosis when a clinical diagnosis is unclear. A molecular diagnosis means that the diagnosis has a known biological cause that can be tested. This is different from a clinical diagnosis which describes physical features but the diagnosis does not necessarily have a known biological cause that can be molecularly detected. Typically, to make a clinical diagnosis there are standardized criterion that must be met and published guidelines that are followed (Makela, Birch, Friedman, & Marra, 2009). When a molecular and clinical diagnosis has been thoroughly researched, the medical field has prognostic and anticipatory information to guide the family and dictate treatment. Although some molecular diagnoses may be well researched, many can be rare or newly discovered and, therefore, not have as much clinical information available.

A third type of diagnosis is known as a "working diagnosis." A working diagnosis is used when a clinical or molecular diagnosis has not been confirmed, but there may be suspicion of a condition (Lewis, Skirton, & Jones, 2010). Even though a condition has not been confirmed, a working diagnosis can be beneficial because the individual may have the ability to obtain services and access to support groups (Lenhard, Breitenbach, Ebert, Schindelhauer-Deutscher, & Henn, 2005).

1.2 The impact of a diagnosis

Receiving a diagnosis can help provide families with emotional, medical, and educational benefits. Many studies have found that a diagnosis can give a family a sense of closure, help guide family planning, and provide the recurrence risk in future children (Carmichael et al., 2015; Lenhard et al., 2005; Graungaard & Skov, 2007). An additional

benefit of receiving a diagnosis includes improving the psychosocial outcomes for individuals and families affected by disabilities (Rosenbaum, 1988). Moeschler and Shevell (2014) explain the effect of receiving a diagnosis as a "healing touch" that bolsters well-being. Carmichael and colleagues (2015) found that receiving a diagnosis lifted some of the emotional burden associated with being undiagnosed. Emotional burden was lessened because a diagnosis validated parental concerns, justified their pursuit of a diagnosis, gave them access to certain support groups, and allowed them to properly plan for the future.

How an individual receives a diagnosis affects satisfaction with the medical field. A qualitative study interviewing parents of physically and mentally disabled children who recently received a diagnosis found that the process of receiving a diagnosis and the certainty of the stated diagnosis strongly influenced the parents' experiences and abilities to cope with a diagnosis. Themes that influenced satisfaction with the diagnostic process depends on the context. This includes the setting of where the information was given, the timing of the information, and level of information related to the parents' readiness to receive the information (Graungaard & Skov, 2007). These results showed that there are many variables that influence a diagnostic odyssey, making it a complex time for families.

The diagnosis of a rare condition may happen years after symptoms appear and many tests later. The natural history, prognosis or medical management of rare conditions may not be known. Some families prefer a diagnosis, even if it involves a poor prognosis, rather than remain uncertain (Makela et al., 2009; Stewart & Mishel, 2000). In addition to preferring a diagnosis rather than not, many parents understand that receiving a specific

diagnosis may not change medical management or have a known cure. Notably, these parents recognized the importance of their child's test results for future medical research (Rosenthal, Biesecker, & Biesecker, 2001).

1.3 Living without a diagnosis

Unfortunately, some may never have an end to their diagnostic odyssey. One study found between 30 and 50% of individuals with intellectual disability go without a known etiology (Daily, Ardinger, & Holmes, 2000). According to The Rare and Undiagnosed Network (2017), one in ten individuals are living with a rare or undiagnosed disease; half of these individuals are children.

Living without a diagnosis can be challenging and have various adverse effects for individuals and their families. Effects may include the inability to receive certain medical or educational services provided and covered by insurance or the state. In addition, living with an undiagnosed condition may involve a lack of direct treatment, anticipatory guidance, and information on prognosis (Carmichael et al., 2015; Lewis et al. 2010). One study found that families may feel emotionally isolated, unable to connect with others living with a similar diagnosis, and have difficulty in coping with an uncertain future (Graungaard & Skov, 2007). Overall, many studies have repeatedly reported time spent undiagnosed as stressful, overwhelming, and involving various negative emotions. This is a result of added medical care for their child and required medical appointments. Additionally, feelings of being out of control may result in emotional distress and burden (Lewis et al., 2010).

Those on a diagnostic odyssey spend much of their time wondering how to plan and manage medical concerns (Rosenthal et al., 2001). A recent study investigated

uncertainty and lack of control in parents of children living with various medical conditions, some of which were undiagnosed. Lower levels of optimism and higher levels of uncertainty were reported in individuals who perceived less control over their child's undiagnosed condition. Although parents felt they did not have control overall, they felt in control of some aspects of their child's condition. The aspects they felt they could control included information and decision making, advocacy, the child's comfort, and self-care (Madeo, O'Brien, Bernhardt, & Biesecker, 2012).

The emotional burden associated with being undiagnosed shows the importance of establishing a strong support system for these individuals and their families as they search for a diagnosis. Although they may not have direct access to certain medical or support services, there are a handful of online support groups created specifically for those who are undiagnosed or diagnosed with rare conditions. One online support group, Syndromes Without a Name (SWAN), is nationally available and officially became a nonprofit organization in the United States in 2006. The site allows families facing similar challenges to connect either through the SWAN website or Facebook group. A few goals of the group are to address the lack of information associated with being undiagnosed, offer emotional support, and help with psychosocial concerns such as isolation, guilt, or helplessness (Syndrome Without a Name, 2017).

A second online support group known as the Rare & Undiagnosed Network (RUN) aims to address similar issues. Their mission is to "empower rare and undiagnosed patients and their families with genomic information through community, advocacy, networking, and support" (Rare and Undiagnosed Network, 2017). Like SWAN, families can share their stories, and RUN helps give them a sense of community.

These two organizations are wonderful resources for families on a diagnostic odyssey and dealing with uncertainty surrounding their child's health.

Not only is a diagnostic odyssey emotionally exhausting, but it is financially costly as well. The cost of discovering a diagnosis may include more expensive, large-scale genomic sequencing that is recommended as second-tier testing completed after cheaper and targeted testing is negative. Genomic testing is broad, nonspecific testing that looks at a much larger part of the human genome than targeted genetic testing. A recent study found that patients who had previously completed basic and complex investigations searching for a diagnosis could be spending up to $21,000 (Stark et al., 2017). In this study "basic investigations" referred to standard clinical assessments including biochemical, imaging, and neurophysiological studies while "complex investigations" referred to non-standard testing that may have included complex biochemical or genetic testing.

1.4 New technologies and genomic sequencing

In recent years, major medical strides and technological advancements have worked towards decreasing the number of undiagnosed individuals and increasing knowledge of rare conditions. To aid in diagnosing medically complex cases, the Undiagnosed Diseases Network (UDN) was created in 2008. The UDN is a multisystem research study funded by the Nation Institute of Health (NIH) known as the NIH Undiagnosed Disease Program (UDP). The purpose of the UDP is to gather clinical and research specialists working in the U.S. with the common goal to solve medical mysteries using new technology (Gahl, Wise, & Ashley, 2015). Thirteen research and clinical sites contribute to the UDP including Duke Medicine, Harvard Teaching Hospital, Stanford

Medicine, and Vanderbilt University Medical Center (Undiagnosed Diseases Network, 2017). These centers collaborate with each other and their patients to understand better the origins of disease. By publishing their work, the UDN is making great efforts to improve the level of diagnostics and care in hopes to relieve some of the burden felt by individuals and parents of those living with undiagnosed conditions.

Since the publication of the completed human genome sequence in 2004, decrease in the cost of sequencing DNA has changed the landscape of clinical testing and is a driving force behind changes in genetic testing practice guidelines (International Human Genome Sequencing Consortium, 2004). The initial sequencing of the human genome utilized a technique known as Sanger sequencing. Although Sanger sequencing still is used, most laboratories now heavily rely on a more recently developed sequencing technique known as Next Generation Sequencing (NGS). NGS allows for rapid sequencing of single and multiple genes at a reduced cost and faster turnaround time (TAT) compared to traditional Sanger sequencing (Mardis, 2008). The first commercially available NGS sequencer, known as the 454 Life Sciences Next Generation Sequencing system, was launched in 2005 (Van Dijk, Auger, Jaszczyszyn, & Thermes, 2014). Since then, data output has more than doubled each year and the cost of genomic sequencing has decreased at a rate faster than anticipated by Moore's Law. Since the cost of genomic sequencing is decreasing more quickly than anticipated, its clinical use is becoming more accessible (Sarda & Hannenhalli, 2014).

When DNA sequencing first was offered, clinical testing was limited to a single gene or small collection of genes. A gene is a unit of genetic material that provides the instructions for our bodies. Genes are housed within the human genome. Available

testing has expanded to include multi-gene tests, known as panels. Panel testing targets specific genes that are indicated when clinical evaluation suggests a diagnosis. Panels can be thought of as "first-tier" testing because they are the most clinically efficient in terms of cost and diagnostic yield.

Genetic technology now has allowed the ability to clinically offer analysis of an individual's entire genome. The entire human genome consists of approximately 20,000 genes and therefore, encompasses a complete set of DNA (Ezkurdia et al., 2014). Genomic sequencing is currently recommended when clinical evaluation is unclear or uncertain, the genes involved are generally unknown, the patient has tested negative using other first-tier testing options, or a broader testing approach is warranted. These broader approaches can include whole genome sequencing (WGS) or whole exome sequencing (WES).

Whole genome sequencing and WES are sequencing techniques that use NGS technologies. Rare or unexpected diagnoses often are revealed by WGS and WES, which sequence the entire human genome and exome, respectively. The Human Genome Project found that the human genome contains a total of about three billion base pairs. The human exome is the portion of the human genome which codes for proteins made within the body and accounts for less than one percent of the genome (International Human Genome Sequencing Consortium, 2004). This estimates the human exome to approximately 60 million base pairs split across about 180,000 exons (Ng et al., 2009).

1.5 Whole exome sequencing

Laboratory procedure involved in genomic sequencing is complex. In simplified steps, WES involves the lab's receipt of the patient's specimen, usually a blood sample,

followed by DNA extraction. The exome then is targeted, enriched, and sequenced by NGS (White et al., 2017). One of the more complex, and last steps is variant classification and annotation. In previous years, variant classification and annotation encompassed a significant amount of WES result analysis. In 2014, it was estimated that 20 to 40 hours of expert time was needed to analyze a clinical exome (Dewey et al., 2014). Recent improvements in bioinformatics tools, updated analysis software, and new public variant databases have drastically reduced the time spent analyzing genomic data (Stenson et al., 2017; Wright et al., 2018).

Variant analysis involves filtering through and deciphering which detected variant(s) best matches with the patient's clinical features, or phenotype. In this way, WES and WGS results are phenotypic-driven. This means that labs will report variants that potentially explain what is clinically indicated. For example, if a patient presents with many unexplained features such as seizures, low muscle tone, strabismus, and a congenital heart defect, only variants associated with any of those features are reported.

To help standardize variant classification, the American College of Medical Genetics and Genomics (ACMG) in collaboration with the Association for Molecular Pathology (AMP) has published standards and guidelines on variant classification and interpretation that laboratories can use when analyzing genomic data. A variant must meet certain criterion to be correctly classified. Included in the criterion are specific variant evidence such as population data, computational (*in silico)* data, functional data, symptomatic data, etc. The five standard categories of variants detected by WES include 'pathogenic', 'likely pathogenic', 'uncertain significance', 'likely benign', and 'benign'. A variant is classified as pathogenic or likely pathogenic when evidence suggests the

change is causative of the patient's features. A variant of uncertain significance is classified as a change for which there is not enough data to support its classification as likely pathogenic or likely benign. Finally, the classification of likely benign or benign is justified when evidence indicates the change is not disease-causing (Richards, Aziz, Bale, Das, & Gastier-Foster, 2015).

Based on the laboratories' classification of variants detected, laboratories will then classify genomic test results into four categories. Result classification is separate from variant classification but is influenced by the type of variant detected. The first type of result is a positive, or definitive result, meaning the lab found a pathogenic or likely pathogenic variant in the patient resulting in a molecular diagnosis. The second result is a possible, or probable diagnosis. This means the lab detected a variant that is located in a known disease-causing gene possibly associated with the patient's clinical features. A third result is a variant in a new 'candidate gene' not previously associated with human disease but suspected to be disease-causing based on the nature of the variant and the known function of the gene product. The final type of result is a negative result meaning the lab found no variant associated with disease or the phenotype of the patient (Williams, Retterer, Cho, Richard, & Juusola, 2016). This final result leaves the patient undiagnosed.

1.6 Clinical implementation of whole exome sequencing

Genetic testing is currently at a turning point with the advent of genomic sequencing. Although the cost of DNA sequencing is decreasing, interpretation of genomic sequencing data continues to become more complex and time-consuming due to the large amount of data generated. Whole genome sequencing of a single sample

generates about 3 million variants that are different from the human reference genome while WES generates a range of 30,000 to 70,000 variants per sample (Hedge et al., 2017). Although WGS can be ordered clinically, it is not utilized as frequently as WES because it is more expensive and results in a greater amount of data that requires much more analysis and interpretation than WES.

There have been several publications addressing the clinical utility and implementation of WES. Challenges identified in clinical implementation include cost, TAT, lack of clinical guidance, variant interpretation, and potential incidental findings (Bertier, Hetu & Joly, 2016; Iglesias et al., 2014; Williams, Cashion, & Veenstra, 2015). There has only been one publication suggesting comprehensive guidelines for the implementation of exome sequencing in the clinic (Matthijs et al., 2015).

Recently, studies have investigated the utility of clinically offering WES as first-tier testing (Krabbenborg et al., 2016; Stark et al., 2016; Tan et al., 2017). A 2015 study compared three different tests which utilized Next Generation Sequencing (NGS). Sun and colleagues (2015) used nine samples to investigate the differences between gene panels, WES, and WGS data from patients with intellectual disability. The study looked at 537 gene panels targeted toward intellectual disability. The largest limitation of panel testing is that they are targeted and only successful if the causative gene is on the panel. Interestingly, WES did not miss any of the variants detected by the more comprehensive WGS. Although they recognized that panel testing is the cheapest and WGS is technically the most inclusive test, they concluded that WES was the best test option when clinical indication involves intellectual disability. WES analyzes more genomic material and will

find variants in genes not included on targeted panels. WES is a reasonable alternative to WGS due to cost, TAT, and ability to clinically implement the tests.

The economic cost of WES has been a large challenge for clinical implementation. When WES first was offered in 2011 the cost ranged between $4,500 and $9,000 (Atwal et al., 2014). Since then, the cost of WES has decreased. Stark and colleagues (2017) investigated the cost effectiveness of WES and quoted the cost of a clinical WES as approximately $2,412. A review of articles published between 2014 and 2015 found that the cost of WES was thought to be too expensive for use as standard testing (Bertier et al., 2016).

In addition to cost, the length of time waiting for results is an important aspect of WES. In 2014, the TAT for WES ranged from 11 to 21 weeks with an average of 18 weeks (Atwal et al., 2014). Since 2014, TAT has substantially decreased in 2017 to an average of 40 days in cases where individuals needed results quickly (Bourchany et al., 2017). The high cost and lengthy TAT is due partly to the process and analysis of WES variants detected by the laboratory.

Practical concerns regarding patient education and consent in pretest counseling are a barrier to clinically offering WES (Iglesias et al., 2014). Due to the vast amount of data analyzed in WES, pre-test counseling can be extensive. In many clinics, a genetic counselor is the medical professional working with these families. Patients and families pursuing WES should be counseled on many aspects including TAT, possible results, yield of testing, insurance coverage, cost, updates of test results or reanalysis, and impact on clinical care. A recent study found that parents were able to accurately describe their child's WES results and communicate the implications (Tolusso et al., 2016). This shows

that genetic counseling in that study had provided appropriate informed consent and follow-up for WES despite its complexity.

Another important aspect lending to the challenges of offering clinical genomic testing is the possibility of incidental or secondary findings. Guidelines introduced by the ACMG in response to challenges associated with incidental findings (Green et al., 2013 & Kalia et al., 2017). Incidental findings pertain to results discovered after completion of filtering and segregation analysis but are not related to the primary indication for testing. Secondary findings pertain to results not related to the primary indication but that are sought purposely during the analysis of the test results (Weiner, 2014). Importantly, both incidental and secondary findings may have health, reproductive, or personal importance for the patient or the family. Different from incidental findings, secondary findings are sought because they are medically actionable and have published health management guidelines. More time may be spent informing patients about the possibility of secondary findings since these results may be more medically actionable than other variants detected (Tolusso et al., 2016).

The ACMG Working Group published a secondary findings list of 59 genes and 26 conditions that clinical laboratories have an obligation to test for during the course of WES and WGS (Green et al., 2013; Kalia et al., 2017). The list includes childhood- and adult-onset conditions such as Li-Fraumeni syndrome, *PTEN* hamartoma tumor syndrome, Hereditary Breast and Ovarian Cancer syndrome, and Marfan syndrome. Certain variants found in any of these conditions should be reported by the laboratory, regardless of the indication for testing. This is grounded in the duty to prevent harm by warning patients and their families about medically actionable information. There is

controversy surrounding this aspect of genomic testing and if the duty to report these findings supersedes patient or parent autonomy. On the other hand, health providers may be liable if they fail to report secondary or incidental findings that could have prevented disease or changed medical management. In 2014, ACMG revised recommendations to state that patients should be given the option to opt-out before the testing takes place. That way the patients do not receive results that they did not desire (Clayton et al., 2013).

1.7 Diagnostic yield of whole exome sequencing

Large scale studies and laboratory data show that WES can find a disease-causing pathogenic variation in approximately 25-40% of individuals, leaving up to 75% of individuals pursuing WES undiagnosed after completion (Baldridge et al., 2017; Farwell et al., 2014; Lee et al., 2014; Yang et al., 2014). Farwell and colleagues (2014) found the highest diagnostic rates were observed among patients with ataxia (44%), multiple congenital anomalies (36%), and epilepsy (35%).

Because WES does not yield a diagnosis for patients in up to 75% of cases, the limitations of WES and its inability to detect causative genetic variants in severely affected individuals is important to understand. In general, WES sequences exons and short exon-flanking regions, including consensus splice-site sequences (Hedge et al., 2017). The test will not detect genetic changes located outside of these regions. This includes non-protein coding regions such as introns, variants located in regulatory units, transcriptional units or mitochondrial DNA. Alterations that do not affect the sequence of the DNA, such as chromosomal rearrangements, inversions, trinucleotide repeats, or epigenetic changes will not be detected as well (Need & Goldstein, 2016).

Labs often analyze WES as “trios”, which includes sequencing three samples; the patient’s and both biological parents’ samples, or the patient’s and two other closely related relatives’ samples. The lab can then compare the patient’s findings to their biological parents or other relative. Although not essential, a trio allows for the lab to determine if the variant is *de novo*, or not inherited from either parent. The diagnostic rate of WES when run as a trio has been reported as 37%, specifically done on the patient and two first-degree relatives. This was compared with a singleton WES diagnostic rate of 21% (Farwell et al., 2014).

Importantly, having both parental samples may allow for the detection of certain genetic alterations such as uniparental disomy (UPD). Uniparental disomy is an atypical situation where a child has two copies of the same chromosome from one parent, as opposed to the expected one from each parent (Bis et al., 2017). When both parental samples are not available, WES would not be able to detect UPD.

Another reason WES may not detect a causative variant involves lab processes. Differences in laboratory bioinformatics, variant filtration techniques and, despite standardized guidelines, the definition of a pathogenic variant may all impact detection and yield. The laboratory will analyze all variants found in a patient through its own filtration system to determine which variant best matches the patient’s phenotype. If a variant is unassociated with the clinical indications, the variant may not be reported. It is possible some novel, yet causative, variants go unreported since WES results are phenotypic-driven. In addition, depending on the variant’s classification (i.e. pathogenic, benign, etc.) the result may or may not give the patient a straight forward diagnosis or answer. For example, a variant found in a potential candidate gene, for a new genetic

condition, will likely have little known data and have little impact on clinical care (Lee et al., 2014).

Additional components that affect WES diagnostic yield include data mining, gene discovery, newly available clinical information, and increasing collaborations between laboratories, clinicians and researchers (Wenger, Guturu, Bernstein, & Bejerano, 2017; Wright et al., 2018). Data mining refers to the process whereby laboratories sift through literature and research any new or helpful information on the detected variant. Data mining may also include searching for previously reported genetic variants using databases such as ClinVar (ncbi.nlm.nih.gov/clinvar), GeneMatcher (genematcher.org) and PhenomeCentral (phenomecentral.org). These sites match laboratories and institutes with one another when they both have identified individuals with a variant in the same gene, and with matching clinical features. Knowledge of other labs and individuals with the same rare variant can help the healthcare providers to properly explain the variant to the patient. Furthermore, finding others who have seen the same variants can aid in classifying a variant.

Genomic sequencing has lent itself to the revelation of new disease phenotypes but has also resulted in producing diagnostic dilemmas caused by genes previously unknown to cause human disease. Resources such as GeneMatcher and PhenomeCentral have likely helped establish some of these gene-disease associations in combination with new technology, such as WGS and WES. As White and colleagues (2017) conclude, the field must "...share data, clinical findings, and experiences..." to successfully implement an influential tool such as genomic sequencing.

It is evident that such rapidly evolving genetic research and sharing would have a strong impact on the diagnostic yield of WES. As of 2014, 23% of positive WES results were found within genes characterized since 2012 (Farwell et al., 2014). In October of 2004 a database of human genes and genetic disorders and traits, known as the Online Mendelian Inheritance in Man (OMIM), listed 1,636 phenotypes with a known molecular cause. Eleven years later in October of 2015, OMIM listed 4,570 Mendelian disorders with gene-disease associations. This was an increase of about 266 entries per year over the past eleven years (Wenger, Guturu, Bernstein, & Bejerano, 2017).

1.8 Whole exome sequencing and reanalysis

Although use of genomic testing has helped expand clinical genetics, this is often not the final chapter in a patient's diagnostic odyssey. For those patients who do not receive a diagnosis from initial WES testing, reanalysis of results may be an option. Reanalysis is accomplished not by obtaining a second blood sample, but by reexamining the initial variants found through a lab's analysis bioinformatics system. Although there are published ACMG guidelines on variant classification in WES, there are currently no published guidelines on WES reanalysis.

Given the fast pace of gene discovery, it is important to realize the need to thoroughly reanalyze WES results (Zhu et al., 2015). One rationale behind a reexamination of the same data after a significant amount of time has passed is that the number of gene-disease associations has improved and thus the likelihood of identifying a causative variant is increased. Other factors that allow for reanalysis include improvement to lab bioinformatics and changes in variant annotation over time. As variant databases grow, laboratories have the ability to update reports and variant

classifications. According to the published ACMG guidelines on the interpretation of sequence variants, previous variant classifications may require modification due to increasing population data (Richards et al., 2015). In some cases, variants previously classified as 'uncertain significance' may now have enough supportive evidence to be re-classified as either 'benign' or 'pathogenic'.

The increasing yearly rate of gene-disease discovery and increasing size of variant databases in combination with recently published WES reanalysis data has validated the usefulness of reanalysis for those who have not received a diagnosis from their initial WES results. Given that WES was first clinically offered in 2011, various laboratories and studies have only recently reported reanalysis diagnostic yield. According to one study, reanalysis of 40 WES data at a two to three-year interval could result in a 10% reanalysis diagnostic yield (Wenger et al., 2017). More recently, Ewans and colleagues (2018) found that reanalysis 12 months following initial WES results could have an 11% diagnostic yield in patients with Mendelian disorders, bringing their study of 54 participants' diagnostic yield from 30 to 41%. Another large-scale study completed in the United Kingdom reanalyzed 1,133 WES data finding a 13% reanalysis diagnostic yield (Wright et al, 2018). This means that up to 13% of families who did not receive a diagnostic result from their initial WES subsequently could receive a diagnostic result from reanalysis at least one year after the initial WES.

1.9 Parental experience with whole exome sequencing

As previously mentioned, WES is indicated in cases of undiagnosed, medically complex individuals whose medical condition has not been identified through previous clinical or molecular investigations. The Undiagnosed Disease Network of the National

Institute of Health stated that "[Undiagnosed] patients have often spent years visiting medical centers and healthcare providers in different specialties across the country, accumulating large amounts of medical notes and test results, often at great emotional and financial cost" (Gahl et al., 2015).

There have been several studies investigating the psychosocial effects of WES. Rosell and colleagues (2016) found that parents view the process of WES as a positive experience resulting in feelings of altruism and hope. In addition, the study found parents may feel a sense of duty to pursue WES to find a diagnosis, and the test can consequently influence medical care and reduce worry. Unfortunately, a positive result from WES or reanalysis may not provide information that may benefit a patient and their family. It is likely that if a causative variant is found from reanalysis of WES results, the condition is either extremely rare or newly discovered, leaving the family with a sense of isolation and frustration (Graungaard & Skov, 2007).

On the other hand, the comprehensive nature of WES may give families false hope and cause feelings of disappointment following nondiagnostic results (Brett et al., 2018). The dichotomy of emotions before versus after testing calls for a balance between hope and realistic expectations. Krabbenborg and colleagues (2016) found that WES results were associated with relief as well as worry, independent of the test result. When families received a conclusive diagnostic WES result, parents reported becoming more accepting, more informed on caring for their child, and better able to cope with perceived guilt. On the other hand, parents identified loss of hope in the recovery of their affected child and loss of social support surrounding the "new label". Some parents felt they no longer belonged to patient organizations they previously participated in. Although some

felt a renewed sense of isolation, many were enabled to search for information regarding the child's conclusive diagnosis, given by WES. While searching, many would come across blogs or Facebook pages and were able to establish new relationships with peers (Krabbenborg et al., 2016).

Although many studies have looked at patient understanding and perception of initial WES, none to the researcher's knowledge, have specifically assessed family response to and understanding of WES reanalysis. Based on the complex and differing perspectives of families living with undiagnosed conditions, it is essential to survey this population to shape current clinical practices and patient experience. In addition, it is important to identify gaps in knowledge and needed areas of growth in current practice when offering WES reanalysis. It is expected that WES reanalysis will continue as genomic testing becomes more accessible and as more information is gained.

This population is unique and most have already completed previous genetic testing. Given that the initial WES process has been found to provide families hope, it was expected that WES reanalysis will yield a similar expectations of reanalysis. Also, it was expected that families will experience negative emotions following nondiagnostic reanalysis results. Those who had received a diagnosis from WES were expected to have had a more positive response to the testing than those who remained undiagnosed. The expected dichotomy of emotions before versus after testing provides an essential need to assess how to best counsel these individuals and families pursuing WES reanalysis.

Chapter 2: Parental experience with whole exome sequencing reanalysis and its impact on the diagnostic odyssey[1]

2.1 Abstract

Advances in genomic technology and an increase in the number of gene-disease associations have helped reduce the number of individuals living without a diagnosis. Whole exome sequencing (WES) analyzes the entire human exome in an attempt to determine if there is a molecular etiology for individuals who remain undiagnosed after other clinical or molecular investigations. Still, WES leaves most individuals undiagnosed, resulting in feelings of disappointment and uncertainty. Individuals who remain undiagnosed after WES can subsequently undergo WES reanalysis later due to improvements in bioinformatics, software updates, and an increase in known gene-disease associations. This is the first study, to the investigator's knowledge, which investigates parental perspective of those undergoing the most current genetic testing available. This study recruited parents of undiagnosed individuals who have completed WES and subsequent reanalysis through the Greenwood Genetic Center to investigate their response to and experience with WES reanalysis while on their diagnostic odyssey. Six semi-structured interviews were conducted, recorded, and transcribed verbatim. Transcripts were analyzed using grounded theory and assigned codes to meaningful segments of text. Results showed most participants had lower expectations of reanalysis

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compared to the initial WES and felt it would not lead to a diagnosis. Most participants responded to nondiagnostic reanalysis results with feelings of disappointment and worry about the future. However, some exhibited a difference in the degree to which they negatively responded. Most participants recognized that reanalysis has been unhelpful for their child but expressed willingness to contribute to science if it will assist future individuals on a diagnostic odyssey. Despite feelings that reanalysis was unhelpful, most participants would consider reanalysis again for their child. Considering the apparent comprehensive nature of genomic testing, these results show there is a need to balance hope and realistic expectations during counseling and consent of WES reanalysis. In addition, parents desired ongoing medical support which can be offered through reanalysis.

2.2 Introduction

Advances in genomic technology and an increase in the number of gene-disease associations have helped reduce the number of individuals living without a diagnosis. Receiving a medical diagnosis can be beneficial for many reasons. A diagnosis can help direct treatment, aid in anticipatory guidance, determine prognosis, and influence family planning (Carmichael et al., 2015). The lack of a diagnosis can have various adverse effects for individuals and their families. This may include the inability to receive certain medical or educational services provided and covered by insurance or the state. Additionally, families may emotionally feel isolated, unable to connect with others living with a similar diagnosis, or have difficulty in coping with an uncertain future (Graungaard & Skov, 2007).

The journey and search for a diagnosis is referred to in the clinic and literature as a “diagnostic odyssey” (Carmichael et al., 2015). Many individuals searching for a

diagnosis have been on a diagnostic odyssey for years. A diagnostic odyssey can be emotionally exhausting and financially costly for individuals and their families. A recent study found that undiagnosed individuals can be spending up to $21,000 searching for a diagnosis (Stark et al., 2017).

Genetic testing is a quickly evolving field that many undiagnosed individuals have pursued. Whole exome sequencing (WES) first was offered clinically in 2011 and is just one example of genetic testing that undiagnosed individuals may pursue (Atwal et al., 2014). WES reads the entire human exome, which is the portion of all the human genome that codes for proteins made within the body. Therefore, WES analyzes a critical portion of the human genome.

Indications of WES include cases of undiagnosed, medically complex patients whose medical conditions are unidentified through previous clinical or molecular investigations. Due to cost and amount of data sequenced, WES is recommended as one of the last steps in the search for a diagnosis (Stark et al., 2016). As WES and data analysis become more efficient and cost effective, its use in clinical genetic testing will become increasingly accessible. Currently, WES gives a molecular diagnosis in about 25-40% of cases (Baldridge et al., 2017; Farwell et al., 2014; Lee et al., 2014; Yang et al., 2014). Therefore, up to 75% of individuals pursuing WES remain undiagnosed.

Patients who remain undiagnosed after WES can subsequently undergo WES reanalysis later. After a significant amount of time has passed, theoretically various factors such as the number of gene-disease associations have improved thus increasing the likelihood of finding a diagnosis. A database of human genes and genetic disorders and traits, known as Online Mendelian Inheritance in Man (OMIM), increased their

database with about 266 new gene-disease associations per year between 2004 and 2015 (Wenger et al., 2017). As variant databases grow, laboratories will have the ability to update previous reports and variant classifications thus underscoring the importance of reanalysis of WES results.

Although there are currently no published guidelines on WES reanalysis, laboratories may use their own guidelines for WES reanalysis, such as waiting at least one year between the initial test and the reanalysis (Williams et al., 2016). A recent study found that reanalysis at a one to two-year interval could result in a 13% reanalysis diagnostic yield (Wright et al., 2018). In other words, 13% of individuals who did not receive a diagnostic result from their initial WES were diagnosed after reanalysis. The increasing yearly rate of gene-disease discovery and increasing size of variant databases in combination with recently published WES reanalysis data has validated that reanalysis is useful in those who have not received a diagnosis from their initial WES results.

It is further necessary to explore patient, or family, perspective of those undergoing WES and reanalysis. WES is not first-tier testing; therefore, this population was unique and had already completed previous genetic testing. Parents feel a sense of duty to pursue WES to find a diagnosis. Even when an individual receives a diagnosis from WES, the condition may be rare, leaving the family with feelings of frustration and continuing lack of anticipatory guidance (Graungaard & Skov, 2007).

Because WES analyzes much more data than most other clinical and genetic testing options, the test can give these families hope after previous genetic testing has been inconclusive (Rosell et al., 2016). On the other hand, the comprehensive nature of WES may give families false hope and cause feelings of disappointment following

nondiagnostic results (Brett et al., 2018). The dichotomy of emotions before versus after testing calls for a balance between hope and realistic expectations.

Although there have been several studies investigating parental perspective for those going through WES for the first time, none to the researcher's knowledge, have assessed the impact that WES reanalysis has on undiagnosed individuals and their families. This study aimed to gain further insight into individuals and families who had received a negative, or nondiagnostic, test result from their initial WES and subsequently completed reanalysis. Compared to previous research on the topic, this qualitative study aimed to gain more in-depth knowledge regarding family emotions experienced after reanalysis, to understand the impact of the process and the results on the undiagnosed individual's care, and to obtain the family's response to the testing experience.

Understanding factors associated with WES reanalysis may help medical professionals specifically address the needs of individuals pursuing reanalysis and help the families gain fulfillment and satisfaction from genetic services. Genetic counselors and other healthcare providers help counsel, interpret, or explain reanalysis results. By asking individuals and families about motivations, reactions, and the emotional impact of reanalysis, this study highlighted patient and family perceptions of the value this type of genetic testing has to offer. Identifying themes and experiences for those undergoing reanalysis may help genetic counselors to understand the needs to be addressed for this unique patient population. Therefore, this study aimed to provide guidance for genetics healthcare providers working with individuals and families pursuing the recent technology of reanalysis.

Given that WES has been found to provide families hope before testing, it was thought that WES reanalysis would have similar expectations preceding results. WES reanalysis is a facet of an already complicated, non-specific test. Therefore, it was difficult to know what their perception of and understanding behind reanalysis would be. Most families do not receive diagnostic results from WES, and even fewer receive diagnostic results from reanalysis. This study was expected to find frustration as a significant emotional response to nondiagnostic reanalysis results. It was expected that those who received a diagnosis from reanalysis would have a more positive response to the testing than those who remained undiagnosed. This was primarily an exploratory study, and its goal was to provide insight into a population that might benefit from meeting with medical professionals and to highlight unique areas of concern or interest that could be addressed by genetic counselors and other healthcare providers.

2.3 Materials and Methods

The University of South Carolina Institutional Review Board reviewed the protocol and designated it as exempt from review in June of 2017. Greenwood Genetic Center's (GGC) Clinical Genomic Sequencing Program Director, Dr. Julie Jones identified eligible participants. When WES reanalyses were completed, Dr. Jones sent a secure email to the ordering clinicians and genetic counselors, to recruit patients to the study. Eligible participants were recruited by phone using an original script (see Appendix A). The recruitment process took place from August 14, 2017 until February 14, 2018. For sample size calculation, the total number of eligible participants was provided to the primary investigator (PI) in aggregate by GGC.

The inclusion criteria were as follows:

- Individual, or caretaker of an individual, who has completed reanalysis of whole exome sequencing through Greenwood Genetic Center
- Individual, or caretaker of an individual, who has received a diagnostic OR nondiagnostic reanalysis of whole exome sequencing
- For individuals under the age of 18 who have completed reanalysis, their caretaker could participate
- Individual who speaks fluent English

The exclusion criteria were as follows:

- Individuals under the age of 18

The phone number and name of interested participants were obtained by their respective clinician and given to the PI through an encrypted email. Afterward, interested participants were contacted by the PI to determine a time for the interview.

Semi-structured interviews were completed over the phone. Participation consent was obtained verbally at the beginning of each interview phone call by the PI reading aloud a standard script (Appendix B). Telephone interviews lasted up to 40 minutes. Demographic variables were collected and included gender, age, ethnicity, highest level of education attained, relationship status, location of residence, and number of children. In addition, participants were asked if their child's reanalysis was diagnostic or nondiagnostic as well as when the initial WES and reanalysis was completed. GGC confirmed, or clarified, when each WES and reanalysis was truly completed. Key topics explored for qualitative analysis included participant understanding and expectation of reanalysis, response to reanalysis results, and any advice for medical professionals offering reanalysis.

Interviews were recorded on the PI's password protected computer using Microsoft Voice Recorder. Next, interviews were transcribed verbatim by the PI into a Microsoft Word document. For the responses collected from interviews, grounded theory methods were used to analyze the qualitative data. There were no preset themes for the study's focus. The PI and an assistant independently identified and coded apparent themes from the participants' responses and reported on their frequency. Kappa coefficient was calculated to be 0.605. To address the goals of this study, themes were identified among participant responses to compare them to previous literature published on parental experience with WES. Quantitative data was described by counting response types and through descriptive statistics (percentages and means). All identified themes and representative quotes can be found in Appendix C.

2.4 Results

There were 25 total whole exome sequencing (WES) reanalyses completed by GGC of which 23 were eligible for this study. One of the 23 eligible families had significant psychosocial issues that their clinician and counselor felt would not lend themselves well to participate and, therefore, were not contacted. Thus, there were 22 eligible participants for which contact was attempted by GGC clinicians and counselors between August 2017 and February 2018. The PI received eight verbal consents and successfully contacted six of the eight (75%). Therefore, this study successfully recruited six of the total 22 eligible (27%). All participants completed telephone interviews. Length of the interviews was between 14 and 38 minutes.

The sample had an age range of 22 to 45 years (average age of 34) and all were Caucasian females. The majority were married, had between 3 and 4 children, resided in South Carolina, and were college educated. There was a gender balance between males

and female children. None verbally reported that their affected child had a molecular diagnosis. One participant's child had received a clinical diagnosis of Autism Spectrum Disorder (ASD) after reanalysis completion. She felt that clinical diagnosis explained her child's full phenotype. Table 2.1 provides participant demographics.

Table 2.1 Participant demographics (*N*=6)

Characteristics	**Response**	***n***	**(%)**
Gender	Male	0	(0)
	Female	6	(100)
Age	20-29 years	2	(33)
	30-39 years	3	(50)
	40-49 years	1	(17)
	50+ years	0	(0)
Ethnicity	Caucasian	6	(100)
Highest level of education	Some High School	1	(17)
	High School	1	(17)
	Technical Degree	1	(17)
	Bachelor's Degree	2	(33)
	Master's Degree	1	(17)
Relationship status	Married	4	(66)
	Single/Committed	1	(17)
	Divorced	1	(17)
Current residence	South Carolina	4	(66)
	Georgia	1	(17)
	Kentucky	1	(17)
Number of children	0-2	2	(33)
	3-4	4	(67)
	5+	0	(0)
Sex of affected child	M	3	(50)
	F	3	(50)
Affected child has molecular diagnosis	Yes	0	(0)
	No	6	(100)

On average, parents recalled expressing concern for their child's symptoms at two months of age with a range from birth to six months. Data provided by GGC on the sample interviewed showed the youngest age at which initial WES results were reported was at 6 weeks and oldest 15.5 years of age (average age of 6). The youngest that

reanalysis results were reported in the sample interviewed was at 2.3 years and oldest 16.9 years of age (average age of 7.8). The average time between a child's initial WES and reanalysis was 1.75 years. All samples' initial WES reports were issued between 2015 and 2016. All reanalyses were reported between 2016 and 2018. Individual timelines can be visualized in Figure 2.1.

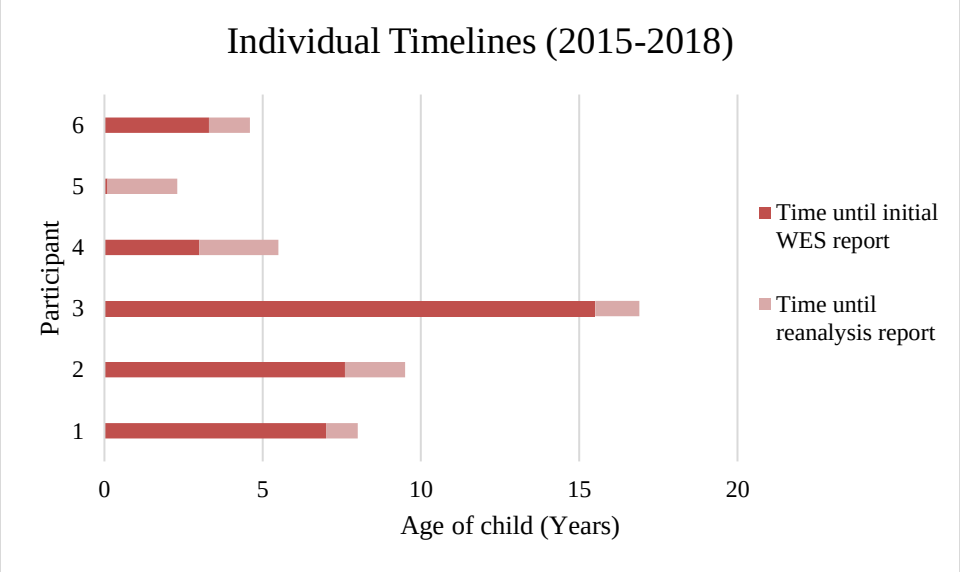

Figure 2.1 Individual timelines for each participant's child. Figure adapted from Rosell et al., 2016.

The PI received unidentifiable WES and reanalysis results for the interviewed sample of six. Three of the six had normal initial WES results. The other three initial results detected variants but none explained the child's phenotype. One child's initial WES results found two variants in one gene; one pathogenic and the other a variant of uncertain clinical significance (VUS). These two variants were part of an ongoing research study taking place outside of GGC. The study's goal was to investigate the effect of the two variants. The outside study had recently concluded that the two variants detected on the child's initial WES were responsible for the patient's full phenotype. Although this child now has a molecular diagnosis, the study participant and PI were

unaware of these findings at the time of the interview therefore, this participant's child was undiagnosed during data collection. Individual WES and reanalysis results can be found in Table 2.2.

Three of the reanalyses did not detect any new variants. One of the initial WES that was negative detected a new VUS and a variant that partially explained the child's phenotype. This was not reported verbally to the PI by the participant during the interview. The other two reanalyses detected new information including variants of uncertain significance (VUSs) and a heterozygous pathogenic variant associated with autosomal recessive disease. Neither of those two reanalyses detected variants thought to be responsible for the child's phenotype. Overall, half of the reanalyses gave the family new information but none leading to a complete explanation of phenotype.

Presented below are five main themes found describing parental understanding of reanalysis, response to, and impact of reanalysis results. Key themes found include expectation of reanalysis, negative emotional response to results, acceptance, altruism, and support. Subthemes are described in each section.

2.4.1 Expectation of reanalysis. Five of six participants understood why reanalysis of initial WES data might yield a diagnosis after previous WES results had not. One was unsure why the reanalysis was useful. Four participants explained that there have been new advances in technology and new gene discoveries over the last few years.

> Why don't we just do this again to make sure there is nothing else, you know. And there were some new advances in the past couple of years. That maybe it could ...detect things that it couldn't detect a couple years ago. *Participant 4, 5.5-year-old daughter*

Table 2.2 WES results, provided by GGC (*N*=6)

Case	Age initial WES report issued	Initial WES report	Age reanalysis report issued	Reanalysis report
1	7yrs (2016)	1 hemizygous X-linked VUS	8yrs (2017)	Diff. hemizygous X-linked VUS; 1 heterozygous pathogenic variant assoc. with AR disease
2	7.6yrs (2015)	Normal	9.5yrs (2017)	Normal
3	15.5yrs (2015)	Normal	16.9yrs (2016)	1 *de novo* likely pathogenic variant explaining partial phenotype; 1 VUS assoc. with AR disease
4	3yrs (2015)	2 VUS assoc. with AR disease; 1 VUS assoc. with XLR disease; 2 heterozygous variants *in trans* in one gene (1 pathogenic, 1 VUS)	5.5yrs (2017)	1 VUS reclassified benign
5	6wks* (2015)	Normal	2.3yrs (2017)	Normal
6	3.3yrs (2016)	1 hemizygous VUS	4.6yrs (2018)	Normal

*WES was completed on amniocytes
Abbreviations: Diff=different; assoc=associated; AR=autosomal recessive; XLR=X-linked recessive

Similar responses acknowledged advancements in science as the main reason for missing anything the first time. Those responses explained reanalysis as a way to "double check" results as opposed to advancements in technology driving reanalysis. Overall, all but one response described that reanalysis had the ability to detect or reclassify a previously undetected or unknown variant. One of the participants opted in to secondary findings during the reanalysis. Participant reasons for reanalysis can be found in Figure 2.2.

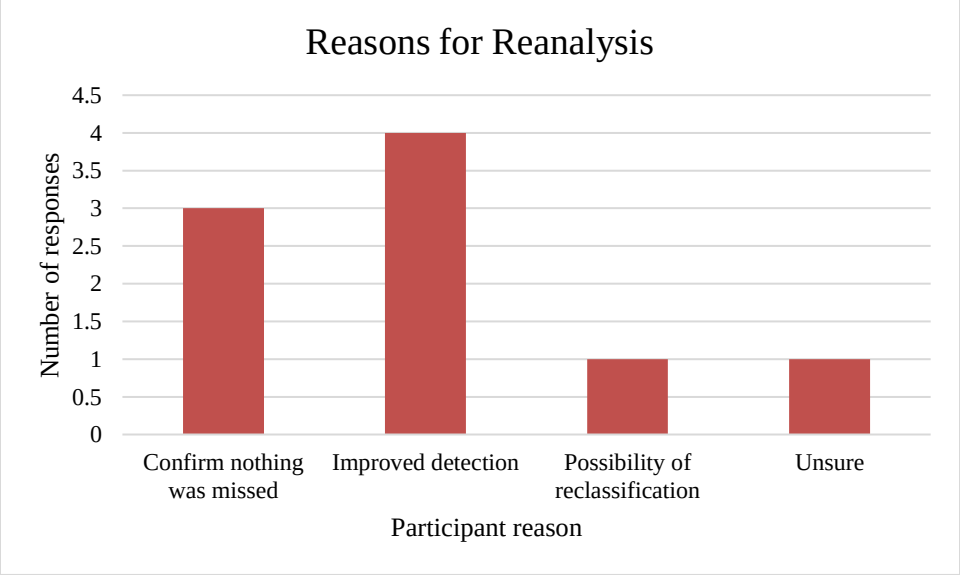

Figure 2.2 Participant reason for reanalysis. Improved detection included gene discovery and improvements in technology.

Although all but one participant noted they remained hopeful of finding a diagnosis, some had differing levels of expectations which ranged from low to high expectations that reanalysis would provide a diagnosis. A low level of expectation was assigned when the participant did not expect that reanalysis would lead to a diagnosis. A moderate level of expectation was assigned when the participant did not feel reanalysis

would yield a diagnosis but still felt there was a chance. A high level of expectation was assigned when the participant reported that they felt reanalysis would lead to a diagnosis. Finally, a neutral level of expectation was assigned when the participant could not comment either way on level of expectation. Level of expectations of each participant can be seen in Table 2.3. One participant held high expectations due to her understanding of reanalysis.

> ...because they were like, uhm, you know, we've made leaps and bounds in two years and you know, hopefully something is going to come up or not come up so we can know it's not there, you know. I think I put even more into it this time because it's been two years and they've made a ton of progress. *Participant 2, 2-year-old daughter*

Two of the participants expressed moderate levels of expectation for reanalysis. One felt that reanalysis was an afterthought, leading to a low expectation.

> Reanalysis was kind of like okay were going to do that again, no big deal. So, it wasn't as big of a deal. *Participant 4, 5.5-year-old daughter*

Two had neutral expectations, one of which was unsure about why reanalysis was completed and the other had a similar confusion about reanalysis.

Table 2.3 Participant expectation of reanalysis

Sex of child	Age of child at interview	Time between initial WES and reanalysis	Expectation of reanalysis
M	8 years	1 year	Moderate
F	9 years	2 years	High
F	18 years	1 year	Neutral
F	5.5 years	2.5 years	Low
M	2 years	2 years	Neutral
M	4 years	2 years	Moderate

Participant hope for a diagnosis changed from WES to reanalysis. One participant recalled how expectations were different during the initial WES compared to reanalysis.

> I guess I should say expectations were different. I was hoping on the first one that we would get answers and on the second one felt more like I didn't expect that we would find anything. So, by that point we've tried everything we can...so I see that I was really hopeful we would get answers. *Participant 6, 4-year-old son*

In addition to retaining hope in the presence of lower expectations, was the obligation to pursue testing. All but one participant would reconsider reanalysis again for their child if it had a chance of finding a diagnosis. When asked if they would consider reanalysis again, participants responded that they feel they should try everything, especially if a medical professional feels it might be beneficial. They felt a duty to complete any testing that might lead to a diagnosis, including reanalysis. These responses expressed some degree of hope that reanalysis may lead to diagnosis at some time. One participant who was unsure if they would pursue reanalysis further explained her child was diagnosed recently with ASD and felt there was no need to pursue further genetic testing.

Similarly, when participants were asked to provide advice to other families pursuing reanalysis many emphasized the role of positivity and hope, even if expectation of WES had decreased. Two of the participants expressed a duty and obligation to pursue all recommended testing and to keep trying.

> Keep trying, I mean that's all you can do. *Participant 2, 2-year-old daughter*

2.4.2 Negative emotional response to results. All participants verbally stated WES and reanalysis had not provided a diagnosis for their child. When participants were asked to describe their reaction to the reanalysis test results, most recalled an immediate negative emotional response to the results. The overall negative emotion associated with these results was disappointment. Disappointment was accompanied by frustration, guilt, and worry.

> I know it's not my fault but I feel like it's my fault... I remember being a little upset when I got the results back because they could say, again, you know, that it was from me. *Participant 1, 8-year-old son*

Common concerns following results included worry about the life expectancy, treatment, and recurrence risk. In this way, reanalysis elicited more questions than answers just as the initial WES had.

> Also felt like his future was really uncertain. So like some genetic disorders you find out and like they have a low life expectancy. They'll never, you know...and like knowing that that was possible, that we had no way of knowing, felt really disconcerting. And it probably took us a few months to say out loud, "will he ever leave our house? Or will he be an adult needing to be cared for?" ...sort of what we have found is that there's more questions than answers. *Participant 6, four-year-old son*

A subtheme noted was a difference in the degree to which a negative response was elicited compared to the initial WES results. Most participants did not have as much of a negative emotional response following reanalysis results compared to the initial WES.

> I felt like we got the big blow of that there is something happening the first time. So that like scariness of okay this is real, happened the first time. And so the second time I didn't have that same surprise of like okay there is something going on. Before I didn't think there was. *Participant 6, 4-year-old son*

Participants felt that they had been through this testing before; therefore, receiving negative, nondiagnostic results was not as disappointing as the first WES results.

On the other hand, one participant felt she had been given false hope and, therefore, had an enhanced negative emotional response of frustration.

> Frustrated! Because we haven't really asked for any of this stuff...it's not my fault that the geneticists haven't caught up yet, to find out what she has to give it a name. *Participant 2, 2-year-old daughter*

Two participants noted that lack of financial burden affected how they perceived this testing. It is important to note that GGC did not bill insurance or the patient for reanalysis. Therefore, these participants did not have a financial burden from reanalysis. Although cost associated with the initial WES was not a focus of this study nor discussed during interviews, participants mentioned that finances would affect decision-making during the course of WES and reanalysis. One admitted that if they had paid for reanalysis themselves, they would not make the test a priority because they do not have much faith in the test to find a diagnosis after negative initial and reanalysis results.

I don't think we would have run the reanalysis if…I know we wouldn't have run the reanalysis if there had been a financial piece on us. *Participant 6, 4-year-old son*

The others knew that they would have been even more disappointed with the nondiagnostic results but knew they would pursue reanalysis again if the doctors ever felt reanalysis would be helpful.

If I had paid the big bucks, I probably would have been very disappointed ...you still feel like you threw your money away. But it is worth it, 'cause you want to know. *Participant 4, 5.5-year-old daughter*

Furthermore, GGC does not bill for reanalysis, therefore none of the participants had paid for reanalysis.

2.4.3 Acceptance. Present in all the interviews was a theme of acceptance. At some point on their diagnostic odyssey, these families have accepted they will likely not receive a reason or name for their child's diagnosis. Because these families have been undiagnosed for years, they have been learning to cope with uncertainty. Results from reanalysis are coming at a time when they have already dealt with the initial shock that their child will be living with a medical condition for the rest of their lives. In this way they have become resilient to disappointing test results.

It's just been a long 18-year journey with her. And I just pretty much went on ahead and accepted her for the way she was. And how she was going to be. And I just took it day by day with her…I don't get my hopes up for

> nothing, because I've learned that when she was a baby. So I just take it as it comes. *Participant 3, 18-year-old daughter*

By the time of reanalysis they have accepted this is how their child will be and shifted the majority of their focus on treating their child, rather than fixating on a diagnosis.

> 'Cause, you know, it's like the first time you're really talking about like there's definitely something wrong with my kid, like it's a fact and you're still accepting it. I think by the time exon sequencing results come in we'd been dealing with this for a couple of years. And we kind of hardened, and kind of like much more like not surprised by stuff. *Participant 4, 5.5-year-old daughter*

Although participants exhibited acceptance of not finding a diagnosis, participants noted that staying positive is how they cope with nondiagnostic results.

> Well I'm trying to look at it in a positive way, and not findings some answers have been a good thing... So I'm trying to look at it that way, that no answer is a good answer... I'm still staying positive on it...we're just going to keep doing what we're doing. Hopefully one day we'll figure it all out. *Participant 1, 8-year-old son*

2.4.4 Altruism. All participants expressed that WES and reanalysis has been unhelpful for their own child. Although the testing was felt to be unhelpful, none regretted completing the testing. Half expressed if the testing was not hurting their child and data might help future families then it was worth it.

I think eventually if it doesn't help [our daughter] it'll help someone else ...if it keeps one other person, eventually down the road, from having this then, or from having to deal with it without a name, then it's worth it.

Participant 2, 9-year-old daughter

Some understood that this testing may aid in new discoveries which was enough reason to pursue testing.

We both sort of got to a place of like I mean that's fine we can keep digging in for science sake but it doesn't seem to be really helping [our son]. *Participant 6, 4-year-old son*

2.4.5 Support. When asked what was helpful during WES reanalysis, participants expressed the important of immediate and ongoing communication from the genetics community. Immediate support was desired in the form of clear communication and time spent explaining the test during appointments. Three of the participants felt their genetic counselor and geneticist clearly communicated why reanalysis might be beneficial. When families felt the medical professionals would take the time to explain WES and reanalysis, participants noted an appreciation for honesty and realistic expectations.

I think the biggest thing is being clear up front, which they were. Is that you may not get anything out of this. You still may not have an answer.

Participant 4, 5.5-year-old-daughter

Not only was immediate support during appointments important but ongoing support was desired through email and telephone. One participant felt strongly about her genetic counselor's ongoing availability to answer questions.

> And I could call 50 times and she would still answer the questions. And I think that's really big because sometimes when we come in and we talk to you guys and you tell us all this stuff and we're just trying to process that our kid has something and it might not be that day that we realize we have a question. We need to know that we can call back and ask those questions and you're not going to be upset and that you're going to answer them.
>
> *Participant 2, 9-year-old daughter*

2.5 Discussion

The population interviewed is unique because these families have pursued many clinical and genetic tests which have not led to a diagnosis for their child. When one of the most comprehensive genetic tests available such as WES reanalysis does not lead to a diagnosis for their child, parents may find themselves with more questions than answers. This poses new challenges not only for families undergoing reanalysis but also for medical professionals offering reanalysis. Similar to previous studies investigating parental experiences with WES, responses supported that reanalysis can give families hope of findings a diagnosis (Rosell et al., 2016). In addition, responses supported that a negative emotional response follows nondiagnostic test results (Brett et al, 2018; Krabbenborg et al., 2016).

This is the first study to identify parental understanding, response to, and impact of WES reanalysis that the principal investigator is aware of. Although this study was exploratory and completed on a small sample, it provides insight on essential aspects including psychosocial implications and parental experience that should be taken into consideration when offering reanalysis to individuals and families.

2.5.1 Practice implications. Most participants had some level of understanding as to why reanalysis might lead to a diagnosis after previous WES had not. In accordance with a previous study investigating parental understanding of initial WES, this study indicated that this sample received effective pre-test counseling that explained reanalysis (Tolusso et al., 2016). Responses also showed parental understanding of reanalysis likely influenced their expectation that reanalysis would lead to a diagnosis. It appeared that those who appropriately understood that reanalysis might not lead to a diagnosed reported a low to moderate expectation. For the majority of participants, their expectations of testing were not higher than their initial WES.

On the other hand, one participant reported a high expectation due to her understanding that there have been numerous advances made within the last few years. She had a similar, if not higher, level of expectation to the initial WES. This perception of reanalysis led to a feeling of false hope. Brett and colleagues (2018) recently identified balancing hope and expectations during the course of genomic testing being a significant counseling challenge. These results further emphasize the importance of balancing hope with realistic expectations while counseling these families. Being honest with parents during pre-test counseling is equally important as instilling hope. One suggestion might be to present the diagnostic yield of reanalysis being approximately 10% based on recent research (Wenger et al., 2017; Wright et al., 2018). In this way one can inform them that reanalysis may not lead to a diagnosis and can let them develop an informed perception of reanalysis.

Similar to initial WES results, when reanalysis did not lead to a diagnosis there was an immediate negative emotional response (Brett et al., 2018; Graungaard & Skov,

2007). Although the type of emotional response did not change from the initial WES to the reanalysis, the degree to which the results elicited a negative response did change. Most participants expressed there was less of a negative emotional response following reanalysis than the initial WES. There were no new emotions identified. Participants likely had a different degree of response because they had previously experienced nondiagnostic results from this testing and were prepared to receive similar results. Although these participants seem to already possess the ability to cope with these types of results, it is still necessary to prepare them for nondiagnostic reanalysis results. This should be done to avoid exacerbated negative feelings that follow false hope.

Furthermore, the degree to which parents negatively responded differed from their initial testing depending on their perception of reanalysis. This was observed in the participant who expressed high expectations of reanalysis. When holding high expectations of reanalysis, she experienced a heighted negative emotional response to reanalysis. This further bolsters the need to give realistic expectations of reanalysis. Although one cannot predict how a parent will respond to negative results, medical professionals should consider how they can present reanalysis to help families properly respond to nondiagnostic results.

A second aspect found to influence the response to reanalysis results was where the parents were on the timeline of their diagnostic odyssey. At some point these families have accepted they may not find a diagnosis for their child's condition. Acceptance may come at different times for different families but by the time they are pursuing reanalysis they have dealt with disappointment from previous experiences with testing. Through these disappointing experiences, they have learned to accept an uncertain future.

Although these families have accepted an uncertain future, pursuing reanalysis can remind them that the genetic community has not given up on finding a diagnosis for their child.

While many participants felt reanalysis was unhelpful for their child this study found that most would consider reanalysis again for various reasons. Like Rosell and colleagues (2016), this study found that parents feel an obligation to try everything possible that could help diagnose their child's condition. The hope for a diagnosis outweighed their negative response to the results. A second reason for pursuing reanalysis was a desire to help families in the future. This indicated altruism and a desire to help others even when testing has been unhelpful for themselves. Despite a negative emotional response, participants were motivated by the possibility to help future families in similar situations to them. It may be beneficial for all ordering providers to explain how their child's reanalysis may aid in future discoveries and help future families receive a diagnosis. This may be through reanalysis or offering data be used in research.

Interestingly, a reason participants noted that they might not pursue reanalysis again is if there was a financial burden. None of the participants paid out-of-pocket for reanalysis and, therefore, there was no cost burden to outweigh the possible benefits of testing. Some mentioned that if they had to pay they would have struggled more with the decision to pursue reanalysis. With a diagnostic yield of 13%, these families may not feel reanalysis would be worth it if they had to pay. Although GGC does not currently charge for reanalysis, this may be pertinent for families completing reanalysis through other labs as some will charge for reanalysis or have differing billing policies surrounding reanalysis. This might change the extent to which these families feel reanalysis is an

option. In addition, it is possible families would have greater expectations or negative emotional responses if they are financially, not only emotionally, invested in the testing.

Importantly, participants noted an appreciation for immediate and ongoing support during their experience with reanalysis. Immediate support was desired during appointments by medical professionals taking the time to explain reanalysis and identify risks versus benefits. This is similar to any pre-test counseling offered to these families. Ongoing support was appreciated through knowing a medical professional was available for these families to reach out to when needed. In this study, a genetic counselor was the medical professional managing their testing and fielding questions from these participants. It was important that these families knew they could call or email to ask any medical question, as these questions frequently arose after appointment times. After experiencing care with other specialties, these participants felt genetics understood the need for immediate and ongoing support. It was comforting to the families that a medical professional recognized the need for support outside of their appointment. This ongoing support seemed to reduce some emotional burden found to be associated with being undiagnosed.

Different from any other testing available, reanalysis itself is a form of ongoing support for these families after comprehensive WES results have not led to a diagnosis. The test lets families know there may be something more to offer them in the future. It acts as a reminder that their care team has not forgotten about them. Results indicated that although they remained hopeful for a diagnosis, they did not feel a diagnosis was essential at this point in their child's life. At this point in their odysseys most had accepted that they will not receive a name for their child's condition. Although the results

from reanalysis are important when yielding actionable results, these families may be best served by the support they feel from the medical community through reanalysis.

2.5.2 Study limitations. Several factors may have influenced these results. First, the study had a small sample size that were eligible through one institution, GGC. GGC is one lab of many who offers WES and reanalysis. All labs have unique WES and reanalysis procedures, billing policies, and diagnostic yields. As discussed previously, financial burden may largely influence the expectation and response to reanalysis results. Surveying a sample that has completed reanalysis through a different lab may yield different responses. Similarly, all reanalyses were offered by geneticists and genetic counselors employed by GGC. Therefore, pre-test and case management is likely similar for this entire sample. In reality, many different geneticists and genetic counselors are offering reanalysis; therefore, those pursing reanalysis may have different experiences than this sample. All these factors, including the small sample successfully recruited, makes these results difficult to generalize to all who have completed reanalysis. Finally, this study did not gather responses from reanalysis that resulted in a diagnosis; therefore, data were unable to establish differences between diagnostic and nondiagnostic reanalysis results.

2.5.3 Future research. A study including more participants, with reanalysis ordered from different institutions, and through different laboratories would be worthwhile as it would allow for greater insight and generalizability of results. Similarly, collecting responses from families who have received a diagnosis through reanalysis to compare nondiagnostic and diagnostic reanalysis experiences would be helpful. Based on responses gathered in this study, it would also be useful to survey the role of financial

burden of reanalysis. Finally, it would be interesting to evaluate counselor presentation versus family perception of reanalysis to assess what patients are being told about reanalysis compared to what they are truly retaining.

2.6 Conclusions

Whole exome sequencing and subsequent reanalysis is one of the most comprehensive tests that can be offered to individuals who are living undiagnosed with complex conditions. Families of those living undiagnosed can be accompanied by adverse emotions including uncertainty, worry, and feelings of isolation. Due to an uncertain future and feelings of isolation, support for these families is essential. Genetics is a unique facet of healthcare that interact with these families. Genetic counselors and other genetics professionals are in a pivotal role to offer immediate and ongoing support to the undiagnosed population. Although these families may have accepted that they will not find a diagnosis, they should not be forgotten. From this research it is important to recognize that these families see reanalysis as a form of ongoing support.

Despite negative emotional responses to initial WES results, the hope for a diagnosis was still present enough to pursue testing such as reanalysis. Most of the participants noted that they did not have as high of expectations for reanalysis to lead to a diagnosis as they did the initial WES. These families also recognized that reanalysis may not be helpful for their child but were willing to complete reanalysis with the idea the data may help future families. These results emphasized a need to balance parental hope and realistic expectations of reanalysis. Participants appreciated honesty regarding reanalysis. Specifically informing parents that reanalysis has a relatively low diagnostic yield may help mitigate negative responses to nondiagnostic results.

Although this study cannot be generalized to all completing reanalysis, it provides preliminary insight into parental experiences with reanalysis. As more WES tests are completed, reanalysis will become more frequent. Knowing how to navigate complex factors such as parental emotions and questions regarding reanalysis is key to providing these families with the support they need.

Chapter 3: Conclusions

Whole exome sequencing and reanalysis is one of the most comprehensive tests that can be offered to individuals who are living undiagnosed with complex conditions. Families of those living undiagnosed can be accompanied by adverse emotions including uncertainty, worry, and feelings of isolation. Due to an uncertain future and feelings of isolation, support for these families is essential. Genetics is a unique facet of healthcare that interact with these families. Genetic counselors and other genetics professionals are in a pivotal role to offer immediate and ongoing support to the undiagnosed population. Although these families may have accepted that they will not find a diagnosis, they should not be forgotten. From this research it is important to recognize that these families see reanalysis as a form of ongoing support.

Despite negative emotional responses to initial WES results, the hope for a diagnosis was still present enough to pursue testing such as reanalysis. These results emphasized a need to balance parental hope and realistic expectations of reanalysis. Participants appreciated honesty regarding reanalysis. Although families may experience a negative emotional response to nondiagnostic reanalysis results similar to that of initial WES, they likely will not feel the emotional response to the same extent felt after the initial results. Specifically informing parents that reanalysis has a relatively low diagnostic yield up to 13% may help mitigate negative responses to nondiagnostic results.

Interestingly, these families also recognized that reanalysis may not be helpful for their child but awere willing to complete reanalysis with the idea the data may help future

families. Providers can inform parents how their child's reanalysis data may be used in research and subsequently help future families.

Although this study cannot be generalized to all completing reanalysis, it provides preliminary insight into parental experiences with reanalysis. As more WES tests are completed, reanalysis will become more frequent. Knowing how to navigate complex factors such as parental emotions and questions regarding reanalysis is key to providing these families with the support they need.

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Appendix A – Consent completed by GGC healthcare provider

You are agreeing to be contacted by Nicole Larsen, a genetic counseling graduate student, with the interest in participating in a school research project. Your phone number and name will be given to Nicole Larsen upon consent. Nicole will contact you to set up a time for a phone interview. Your participation in this project is voluntary. Consent to participate will be completed upon the beginning of the interview phone call.

Name of clinician or genetic counselor obtaining consent: _____

Name of interested participant: _____

Phone number of interested participant: _____

Date and time of consent: _____

Upon consent, please send this form in an encrypted to Nicole Larsen at **Nicole.larsen@uscmed.sc.ed**

Appendix B – Participant consent completed during interview

Consent statement

You are agreeing to participate in a telephone interview as a part of a genetic counseling graduate school research project. This interview will last approximately 45 minutes to 1 hour. Your participation in this project is voluntary. You may withdraw from the study at any time. If at any time there is a question you are not comfortable answering, please let me know and we can proceed on to the next question. While no direct benefit may be observed, this study may provide future benefit to others pursuing WES reanalysis and medical professionals working with them. The risk for participating in this study are minimal.

With your consent, this conversation will be recorded and transcribed. All responses gathered from the interviews will be kept anonymous and confidential. If a quotation is used from this interview, all identifying information will be removed and you will be assigned an alternative name.

If you have any questions regarding this research, you may contact either myself or my faculty advisor, Emily Jordon, MS, CGC. If you have any questions about your rights as a participant, you may contact the Office of Research Compliance at the University of South Carolina at (803)777-7095.

Do you consent to this research study? Date: _____ Time: _____

Appendix C – Themes

Table C.1 Overall themes

Theme	**Subtheme**
Expectation of reanalysis	Understanding of reanalysis, hope for a diagnosis, obligation
Negative emotional response to results	Disappointment, worry, guilt, degree of response elicited
Acceptance	Uncertainty, time spent on diagnostic odyssey
Altruism	Future families, aiding in gene discovery
Support	Immediate and ongoing communication, availability

Table C.2 Themes with representative quotes

Theme	Subtheme	Example
Expectation of reanalysis	Understanding of reanalysis	"Yeah there is always something new coming up so...everybody is always making a breakthrough on something." Participant 3
		"I guess they just wanted to make sure they wasn't missing anything. I guess, I don't know." Participant 5
	Hope	"I was still just hopeful but more patient this time." Participant 1
		"I was hoping on the first one that we would get answers and on the second one felt more like I didn't expect that we would find anything." Participant 6
	Obligation	"[we would consider reanalysis] if they ever felt like it would be worth it for them to reanalyze, or it would be help." Participant 2
Negative emotional response to results	Disappointment	"...hoping that there was one magic bullet that was going to explain everything...and there isn't. So just disappointment with that. Participant 6
		"What are you supposed to do if you can't tell somebody what's wrong...it's been very heartbreaking to see that and not put a name on it." Participant 2
	Worry	"My reaction was like "that's fine but what do we do from here?" Participant 6
		"Not that I ever thought we'd get a diagnosis and be like "oh let's fix her." But more to be able to say, "Okay, this is what she has, let's looks at other people with the same condition. What is their life expectancy, what other organ systems get involved?" You know, what are some things that come up in the future?" Participant 4
	Guilt	"Seeing that something came from me that I could see potentially in him was a little bit scary to see." Participant 6

	Degree of response	"A little disappointment, but I kind of knew a little bit going into it that. Kind of went into it knowing that it may not give us an answer. So I think we'd already come prepared for that. So a little disappointment but also expecting it at the same time." Participant 4
Acceptance	Uncertainty	"I think some days I would want to know this is the path, even it's the worse news possible.... but when he's progressing I don't want that at all. I'm like "He can do anything! We're totally good." But when it feels stale like when he's not progressing or when we just have more concerns than we have answers then yes. I just would rather just have answers even if it's worse case scenario." Participant 6
	Time spent on odyssey	"It really didn't bother me, because it's just been a long 18-year journey with her. And I just pretty much went on ahead and accepted her for the way she was." Participant 3
		"I think we are much more resistant. By the time we had got the results, we had kind of weren't as impacted by things as we used to be." Participant 4
Altruism	Future Families	"…but if it keeps one other person, eventually down the road, from having this then, or from having to deal with it without a name, then it's worth it." Participant 2
	Gene Discovery	"They're constantly finding out new things. You know, that trying again is never really a bad thing. Even if you've done it once and found nothing. You can do it again and find more information." Participant 1
Support	Immediate	"I appreciate that in every appointment I feel like they've taken a lot of time with us. And that was really helpful because it is really heavy information and...they've always done a really good job at slowing it down. And explaining to us, without talking down to us." Participant 6
	Ongoing	"Trust that everyone has your child's best interest in mind, even if it's information that's hard to hear." Participant 6