GENETIC COUNSELORS' VIEWS AND OPINIONS REGARDING THE POSSIBLE IMPLEMENTATION OF PRENATAL EXOME SEQUENCING

by

Tricia Zion

B.S. in Biological Sciences, University of Pittsburgh, 2012

Submitted to the Graduate Faculty of

the Department of Human Genetics, Genetic Counseling,

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2016

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

This thesis was presented

by

Tricia Zion

It was defended on

March 29, 2016

and approved by

Committee Members

Thesis Director: Robin E. Grubs, PhD, LCGC, Assistant Professor, Director, Genetic Counseling Program, Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh

John R. Shaffer, PhD, Assistant Professor, Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh

Aleksandar Rajkovic, MD, PhD, Professor and Marcus Allen Hogge Chair, Department of Obstetrics, Gynecology and Reproductive Services, University of Pittsburgh, Magee-Womens hospital of UPMC

Elaine C. Weltmer, MS, LCGC, Manager, Neurology Reporting, Ambry Genetics, Aliso Viejo, California

GENETIC COUNSELORS' VIEWS AND OPINIONS REGARDING THE POSSIBLE IMPLEMENTATION OF PRENATAL EXOME SEQUENCING

Tricia Nicole Zion, M.S.

University of Pittsburgh, 2016

ABSTRACT

The utility and effectiveness of whole exome sequencing (WES) have been demonstrated in a pediatric setting, and for this reason, it has recently begun being offered in in the prenatal setting. The scope of exome sequencing and the possibility for incidental findings raises ethical concerns and challenges current resources available to prenatal genetic counselors to implement this testing. This creates a need to understand genetic counselors' opinions toward such a test prior to its implementation. For this reason, a survey focusing on clinical scenarios and factors influencing genetic counselors' opinions on prenatal WES was distributed to clinical prenatal counselors and laboratory counselors through the National Society of Genetic Counselors' student research survey program. One hundred and sixty respondents met criteria for and completed the survey. Results of this survey were analyzed for descriptive statistics as well as comparison of responses using nonparametric analysis. Responses showed that 59.4% of respondents were comfortable with prenatal WES as a diagnostic tool after other diagnostic testing had come back negative or inconclusive. Support for use of prenatal exome sequencing increased significantly ($p=0.0088$) in the context of targeted prenatal exome (TES), with 74.4% of counselors supporting TES after other diagnostic testing was negative or inconclusive. The two largest factors influencing opinions and support of prenatal exome sequencing were the perceived ability of patients to handle the amount of information provided by the testing ($n=125$)

and the clinical utility of exome sequencing in the prenatal setting (n=123). Most genetic counselors (70.7%) were more likely to support prenatal exome sequencing if educational resources were provided to aid in the implementation of the testing. Overall, 46.9% of genetic counselors would support this testing if it were restricted to cases with clear clinical indications, while an additional 31.9% would support prenatal TES only. The public health significance of this study is that, because prenatal WES/TES will likely be clinically available in the near future, understanding genetic counselors' perceptions of testing and barriers to uptake will help to more seamlessly integrate this testing into the clinic to provide the most benefit and least harm to prenatal patients.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I would like to thank all of the individuals who have helped me through the process of completing this thesis and my master's degree. Firstly, I would like to thank my parents, who have always supported me in every way possible in pursuing my interests. I would also like to thank my friends, both near and far, who have lent sympathetic ears during the most difficult and trying times of this process. In addition, I would like to thank my coworkers, who have been so supportive of my goals and helped me to formulate this thesis.

I would like to thank the members of my committee for their input throughout the thesis process, from formulating the survey to analyzing the statistics to the final product. I would also like to thank the genetic counselors who took time out of their busy schedules to help pilot the survey and gave helpful and constructive feedback to strengthen the questions, as well as all of the genetic counselors who responded to the survey.

I would especially like to thank Dr. Robin Grubs and Dr. Andrea Durst for their continued guidance through the program, the thesis process, and everything in between. Lastly, I would like to thank my classmates, without whom I could never have done any of this.

1.0 INTRODUCTION

1.1 BACKGROUND

1.1.1 Exome Sequencing

Exome sequencing is a type of germline genetic testing that can analyze the entire protein-coding region of a genome (exons). This region accounts for approximately 1% of the human genome, while encompassing approximately 85% of disease-causing mutations.[1,2] Exome sequencing may be ordered through many commercial and academic laboratories, either as whole exome sequencing (WES), which analyzes the entire exome, or as targeted exome sequencing (TES), which analyzes only those exons and genes therein with mutations known to be associated with a particular phenotypic expression. The definition of TES may vary from laboratory to laboratory, but generally involves sequencing the entire exome and only reporting out variants related to either the patient's phenotype, or a set of disorders with similar phenotypes (e.g. myopathy, skeletal dysplasia).[3]

Currently, this technology is largely used in the setting of patients for whom all other genetic testing options have been exhausted and is not clinically offered to healthy individuals. Patients who undergo exome sequencing have often endured a drawn-out "diagnostic odyssey" that can be both fiscally and emotionally costly. Most frequently, these patients are children.[4]

Common clinical features in these patients include intellectual disability, developmental delay, epilepsy/seizures, brain MRI abnormalities, and/or multiple congenital anomalies.[5,6] In this setting, exome sequencing can help to identify rare disorders and provide patients and their families with an answer for their symptoms. WES identifies a clinically actionable finding in approximately 30% of cases.[5,6] This diagnostic yield increases slightly in the context of "trio" sequencing, in which two additional blood relatives (typically the proband's parents) are sequenced with the proband. In cases where the entire trio is sequenced, the detection rate can range from 31-37% compared to approximately 23% when only the proband is sequenced.[5,6] Trio sequencing can help to determine segregation patterns of variants of interest, in addition to supporting evidence for or against a particular variant's pathogenicity. Trio sequencing can also aid in variant interpretation and raw data analysis, particularly in areas with otherwise low sequencing coverage.[7]

1.1.2 Incidental Findings

Because WES analyzes the entire exome, this introduces the possibility for incidental findings unrelated to patient phenotype arises. The American College of Medical Genetics (ACMG) defines an incidental finding as "any clinically actionable result that is not associated with the phenotype for which initial testing was conducted."[8] Some ethical controversy has arisen around the return of incidental findings, particularly for adult-onset conditions in pediatric patients. For this reason, the ACMG released recommendations for the return of incidental findings in whole genome and exome sequencing; this includes a list of 57 genes for which it is recommended that pathogenic or likely pathogenic findings always be returned (this list has since been altered to exclude one gene, making it 56 reportable genes, however at the time of the initial

recommendation, 57 genes were listed). Mutations in these genes cause conditions for which there exists some form of treatment, surveillance, or intervention to help prevent or alleviate symptoms.[8] The recommendations laid out by the ACMG indicate that incidental findings should be returned to the clinician by the laboratory performing testing regardless of the age of the patient. The laboratory has a responsibility to report the recommended 57 genes; any genes outside of this minimum list may be reported at the laboratory's discretion. [8] Adequate pre- and post-test counseling should be provided by the clinician to help guide patient understanding of the possibility of incidental findings. Adequate counseling includes informing the patient that they may opt out of incidental finding analysis, as well as the benefits and risks of doing so.[8,9] Targeted exome sequencing eliminates the return of incidental findings by narrowing the sequencing search to genes known to be associated with a particular clinical phenotype.

The National Society of Genetic Counselors (NSGC) has also issued a position statement on incidental findings.[10] This statement advises that a plan for the return of incidental findings results be made during the pre-test counseling appointment, establishing which categories of results will or will not be returned. In addition, it specifies that healthcare providers outside of genetics who are ordering WES for their patients should ensure that the patient has access to a genetics professional, such as a genetic counselor, throughout the testing and results disclosure process.[10]

1.1.3 Informed Consent

The scope of exome sequencing creates a need for a unique counseling process both before and after testing. The ACMG recommends that this counseling be performed by a trained genetic counselor or geneticist and that this counseling process include the following information:

express written documentation of consent either from the patient or the patient's legal guardian; the availability of interventions or treatments for incidental findings; the possible and expected outcomes of testing; which incidental findings should be included as well as the likelihood of identifying incidental findings; the risks, benefits, and limitations of testing as well as implications results will have for family members; a clear delineation between research and clinical testing; and the option to opt in or out of research.[11] These recommendations also state that WES should only be offered to minors in the context of phenotypically-driven diagnostic testing, when results would allow for early monitoring or intervention, and/or in IRB-approved research studies.[11]

There have been studies conducted that further emphasize the importance of informed consent in relation to WES/genomic information. Testing of pre- and post-consent knowledge of genome sequencing showed a significant increase ($p<0.0001$) in understanding of the benefits and limitations of the testing, regardless of sociodemographic characteristics, when informed consent is obtained. General public knowledge about exome sequencing may be limited, highlighting the importance of the informed consent process in educating patients to make the most informed decisions about their own testing choices.[12]

1.1.4 Prenatal Testing

Many testing options are currently available to patients in the prenatal setting. The majority of these tests are screening tests, providing an adjusted risk for two or three of the most common live-born trisomies (13, 18, 21; some tests do not screen for 13), or for neural tube defects. Screening tests available prenatally include first-trimester maternal serum screening, second-trimester maternal serum screening (quad screen), and cell-free fetal DNA (also referred to as

non-invasive prenatal testing/screening, NIPT, or NIPS).[13] Screening comes with a higher potential for false positives, and in the context of genetic counseling, it is usually recommended that the results of these tests be confirmed with diagnostic testing.

It is important to also note that ultrasound imaging is itself a screening procedure. Detailed anatomy ultrasounds performed at 18-20 weeks gestation can provide information about structural fetal anomalies, including cardiac anomalies and brain abnormalities. Ultrasound is able to detect approximately 50% of all cases of Down Syndrome (trisomy 21) and 80-90% of all cases of trisomy 13 and 18.[14] The efficiency of ultrasound screening is dependent upon the technician and/or doctors performing and interpreting the images as well as the age of gestation. Structural anomalies may be more or less difficult to observe earlier or later than 18-20 weeks gestation.[14] Ultrasound is the standard method for obtaining a fetal phenotype, and thus has limitations not seen in pediatric and adult settings, where a much more detailed phenotype can be observed.

Prenatal diagnostic testing typically entails either chorionic villus sampling (CVS) or amniocentesis depending on the gestational age. These tests come with a 1/250 to 1/1000 risk of miscarriage or early labor depending on the facility in which they are being performed and the skill level of the physician performing the procedure.[15] Frequently, diagnosis consists of confirmation of aneuploidy through fluorescence in situ hybridization (FISH) and/or karyotyping.[16] Karyotyping may also detect other structural chromosomal abnormalities such as unbalanced translocations, inversions, and large-scale deletions and duplications (>3MB).[16] Fluorescence in situ hybridization can detect known microdeletions or microduplications no smaller than 190 kb.[16,17] However, sometimes more detailed analysis is warranted. Parents may be known carriers of an autosomal recessive condition or a single parent may have a known

autosomal dominant condition, including monogenic disorders and microdeletion/microduplication syndromes for which the pathogenic mutation is too small for detection via FISH or karyotyping. These conditions may be screened for via single mutation, single gene, or chromosomal microarray analysis (CMA) using CVS or amniocentesis samples.

1.1.5 Chromosomal Microarray Analysis

Chromosomal microarray analysis (CMA) is a type of genetic testing that analyzes chromosomes for deletions and duplications via array comparative genomic hybridization (aCGH) and/or single-nucleotide polymorphism(SNP)-array.[18] Array CGH allows for comparisons of the amount of chromosome material on each chromosome between a patient sample and a control sample. This helps to determine if the patient being tested has any extra or missing pieces of their chromosomes. Using this testing, unique microdeletions and microduplications can be detected that are sometimes associated with a syndromic presentation.[19] The drawback of using aCGH is that it cannot detect copy neutral chromosomal aberrations or triploidy. SNP-based arrays allow for detection of copy-neutral changes such as loss of heterozygosity associated with consanguinity or uniparental disomy (UPD), but have limited ability to detect copy number-variants.[18] For this reason, many clinicians now utilize both forms of microarray at once through a combination platform, allowing for greater detection rates.[18]

Prenatally, CMA is used to detect aneuploidy or UPD, to identify possible CNVs in cases where structural fetal abnormalities have been identified on ultrasound, and to detect known familial microdeletions or microduplications too small for FISH analysis.[19] In cases of structural fetal anomalies, CMA identifies causative deletions or duplications in approximately 6% of cases with normal karyotypes.[19] In cases where the only indication is advanced maternal age, CMA

detects causative deletions or duplications in 1.7% of cases with a normal karyotype.[19] CMA is now frequently being used in lieu of karyotyping for prenatal diagnosis due to this higher detection rate, excluding detection for copy neutral aberrations such as translocations and inversions.[19]

Due in part to concerns for the appropriate use of CMA prenatally, the American Congress of Obstetricians and Gynecologists (ACOG) released a policy statement in 2013 with recommendations regarding this testing in the prenatal setting.[20] These recommendations suggest that in fetuses with one or more structural fetal abnormalities, CMA can replace the need for karyotyping, but that either CMA or karyotyping can be performed in fetuses with no identified structural abnormalities. In addition, it is recommended that this test not be restricted solely to women of advanced maternal age, as most CMA findings are not associated with maternal age. CMA is recommended in cases of fetal demise or stillbirth due to the increased likelihood of obtaining results. Most importantly, ACOG recommends comprehensive pre- and post-test counseling and documented informed consent for all prenatal CMA.[20]

Microarray is perhaps the most analogous testing to exome sequencing that is currently available in the prenatal setting. The broad scope of coverage provided by CMA leads to a higher likelihood for VUS results and incidental findings unrelated to the patient's phenotype.[21] The possibility for more uncertainty or unexpected results leads to a lengthier and more information-heavy pre- and post-test counseling/informed consent process.[20] For this reason, it is important to consider the ethical arguments and clinician responses that followed the introduction of CMA into the prenatal setting, as these may predict how healthcare providers handle the possible introduction of exome into the prenatal setting.

1.1.6 Variants of Uncertain Significance

A side-effect of the broad scope of exome sequencing and CMA is the increased likelihood of returning variants of uncertain significance (VUS). These are variants for which not enough data is available to classify the finding as either pathogenic or benign. Variants of uncertain significance can present a challenge in post-test counseling, as it may be difficult to properly explain the meaning of this result. In addition, many patients may leave the session believing that the VUS is a diagnosis, regardless of the skill of the counselor providing post-test counseling.[22] In the context of TES, VUS results will most likely be identified because the gene in which the VUS was found is associated with at least one of the patient's symptoms. Patients or their families may latch onto these similarities, despite not having other major symptoms associated with mutations in that particular gene.[22] This emphasizes the importance of comprehensive pre- and post-test counseling to best educate patients on the ambiguous nature of VUS results.[11]

Variants of uncertain clinical significance are detected in anywhere from 1.5-3.5% of prenatal CMA cases. VUS in the prenatal setting creates unique ethical concerns and counseling complications, as many prenatal patients are anxious about any perceived "abnormal" result from prenatal testing and may alter pregnancy management decisions based on such results. Unlike in the pediatric or adult setting, where phenotype helps to guide VUS interpretation, limited phenotypic information is available in the prenatal setting, making VUS interpretation more difficult. In addition to the concern of VUS results from CMA, parents may wish to test their current pregnancy for a VUS result detected in the context of pediatric or fetal demise testing in a previous child, particularly if the VUS is in a plausible candidate gene matching many of the previous child's symptoms.[23]

1.1.7 Clinician Response to Prenatal Chromosomal Microarray

While CMA is now relatively commonplace in the prenatal setting, its introduction caused some challenges to the genetic counseling process. A major concern expressed by prenatal counselors regarding CMA was the uncertainty of VUS results, and the inability to quantify that risk for patients.[24] According to many counselors, this in turn weakened their confidence in providing patients with guidance on pregnancy management decisions.[24] In addition to the concern with VUS results, counselors stated that counseling was further complicated by a general lack of data in the literature on many of the deletions and duplications found in microarray, which in turn lowered their confidence in explaining the results to patients. Counselors have reported being more comfortable with an expert physician providing the results and/or being willing to confer with them on results.[24] For example, in a study by Bernhardt et al (2014), one genetic counselor stated: "I guess what helps me is that there's somebody else saying the same thing I am and for patients like reinforcing the same information or lack thereof, is I guess can be helpful for them to at least accept the results".[24]

As CMA has become more common in the prenatal setting, additional data has become available and counselors have become more comfortable with unique aspects of the counseling process created by this testing. Genetic counselor response and adaptation to this testing may help to predict and/or guide how genetic counselors will deal with the possible implementation of exome sequencing in the prenatal setting.

1.1.8 Clinician Response to Clinical Exome Sequencing

The implementation of genome and exome sequencing in the pediatric and adult clinical settings brought with it a number of challenges to genetic counselors and other clinicians dealing with this testing. Genetic counselors' views and experiences with the implementation of this testing were assessed in a 2014 study.[25] Some of the challenges specifically expressed by pediatric genetic counselors after the implementation of WES in the clinic included billing issues, the lengthy and information-heavy consent process, and interpretation and disclosure of VUS results and incidental findings. These challenges may increase when considering exome in a prenatal setting. Prenatal counselors, as well as counselors in other specialties such as cancer, expressed discomfort offering the testing to their patients due to lack of clinical utility.[25]

Further response to the integration of exome sequencing in the clinic has centered specifically on the issue of incidental findings, already cited above as a major challenge in implementing this testing. Members of the American Academy of Pediatrics, Section on Bioethics and Section on Genetics and Birth Defects, were surveyed to assess their attitudes toward incidental findings following the implementation of WES in the clinic.[26] Only 34.7% of Section on Bioethics members surveyed agreed with ACMG-proposed mandatory reporting guidelines (highlighted above in section 1.1.2), while 70.8% of Section on Genetics and Birth Defects members agreed with these guidelines. For both groups, roughly 30% of respondents felt that parents should not be allowed access to information on adult-onset conditions in their children. Of all respondents, 80% felt parents should have a right to refuse this information.[26] As highlighted above, these issues will remain in the context of prenatal exome sequencing, and may, in fact, become larger areas of debate. It may, therefore, be beneficial to assess these issues with prenatal providers as well.

1.1.9 The Utility of Prenatal Exome Sequencing

While prenatal exome sequencing has only recently been made available to clinicians, many commercial laboratories do offer exome sequencing on fetal demise samples. In addition, some research studies have been done on the efficacy of exome sequencing in the prenatal setting.

A study conducted in 2014 sought to demonstrate the utility of exome sequencing in the prenatal setting by analyzing 30 parent-fetus trios in which the fetus had at least one structural anomaly on ultrasound.[27] This study showed a diagnostic yield of 10%, finding 3 *de novo* variants likely to be pathogenic. The study found an additional 5 variants (17%) in "plausible candidate genes" that were *de novo*, inherited recessive, or X-linked, which required further validation to determine pathogenicity. A 10% diagnostic yield is relatively comparable to the diagnostic yield associated with CMA and karyotyping in the presence of fetal ultrasound anomalies and lends some credence to the idea that exome sequencing would be clinically useful in a prenatal setting.[27]

A second study conducted by a commercial laboratory analyzed 7 fetal demise samples using whole exome sequencing.[28] Six of these fetuses were from couples who had experienced more than one affected pregnancy. All 7 fetuses had normal karyotypes, and 5 of the 7 fetuses had normal or inconclusive CMA results. After WES analysis, 4 of the 7 fetuses were found to have a relevant alteration (pathogenic or likely pathogenic variants): a diagnostic yield of 57%.[28]

While these studies highlight the fact that WES can be clinically useful in a prenatal setting, especially when considering risk recurrence information for parents, a major drawback of both studies is the small sample size, which likely inflates the actual diagnostic yield. Further studies utilizing larger sample sizes will be important in determining a more accurate estimate of the diagnostic yield of prenatal WES.

1.1.10 Ethical Concerns for Prenatal Exome Sequencing

Any testing in the prenatal setting can raise ethical concerns due to the sensitive nature of pregnancy and pregnancy management decisions. Many of the ethical concerns surrounding prenatal exome sequencing hinge significantly on the context in which it would be used. From a purely technological standpoint, it is possible to use WES as a screening tool in the prenatal population. The argument for using this testing as a screening tool is that it could identify fully penetrant disorders that would likely result in a miscarriage or early death much earlier than ultrasound findings or later pediatric testing could, allowing for early termination of the pregnancy when it may be emotionally easier for the couple making the decision.[29] However, WES as a screening tool could create significant challenges, given the large amount of information returned from WES, the high probability of VUS findings, the limited phenotypic information available for prenatal patients, and the likelihood of returning a mutation result for a gene that may cause a devastating phenotype, but has incomplete penetrance.[29]

Even in the context of a prenatal case with clear ultrasound findings and negative or inconclusive diagnostic testing, ethical issues still arise. Many question whether or not incidental findings should be returned with a prenatal WES test result. There exists some question as to whether or not it would be ethically acceptable to provide information on adult-onset conditions for a fetus. Some argue that if these genes can be screened for using pre-implantation genetic diagnosis (PGD), returning the same result as an incidental finding is not significantly different. Others argue that testing for certain adult-onset conditions, such as most cancer genes, is not availably prenatally from many laboratories who offer this testing (excluding PGD). Because this testing would not normally be an option in prenatal diagnosis, the argument is that these results should therefore, not be returned as an incidental finding. In addition, an incidental finding for an

inherited dominant condition in a fetus would have implications for one of the parents, which can be emotionally difficult on its own without the additional stress of an affected pregnancy.[29] For example, if a *BRCA2* mutation were identified as an incidental finding in a fetus, this would have potentially immediate risk implications for the mother if the mutation had been inherited from her.

The possibility for VUS results also warrants some ethical consideration. While there already exists the possibility for VUS results with prenatal CMA, the argument can be made that it is unethical to offer prenatal exome sequencing due to the high likelihood of a VUS result with a test that has such a broad scope. Variant interpretation relies heavily on phenotypic correlation which is limited or impossible when considering fetal sequencing. As such, the likelihood of a VUS result may be even higher with prenatal WES than it would be in a pediatric or adult setting. Uncertainty may be especially distressing in the context of a pregnancy and pregnancy management decisions.[29] In addition, depending on the education level and level of concern of the couple undergoing sequencing, some couples may consider terminating based on a VUS that may have no real implication for the pregnancy or for the child's health. This can be difficult for couples to deal with on a personal level, and could potentially draw backlash from those who are opposed to termination on broader scale.

Even in the context of "definitive" pathogenic variant findings, there can be ambiguity. Many of the genes and pathogenic variants therein that we consider "fully penetrant" have never been identified prenatally before and, therefore, we do not know how these variants actually present prenatally in terms of phenotype. There are also many disorders for which penetrance and expressivity are highly variable, making it difficult to truly interpret the results when ultrasound can only provide a limited phenotype. This can again lead to termination based on

results which may never manifest in the childhood period, or which may vary drastically in severity from person to person.

1.1.11 Patient Views of Exome Sequencing

While it is important to consider technical and ethical issues surrounding prenatal WES, as well as to consider how genetic counselors have dealt with similar testing in the past, it is just as important to take into account how patients feel about this testing, including its many possible results and how they will utilize these results. To that end, several studies have been conducted to assess patient views of exome sequencing in general as well as prenatal exome specifically.

A 2012 study carried out by the NIH ClinSeq project assessed preferences toward return of results from whole-genome sequencing.[30] The majority of individuals surveyed wished to know their results for preventative means, with roughly 1/3 of respondents citing a desire to inform their family of their risks. Participants in the survey were able to distinguish between different categories of results and showed a preference for clinically actionable results over receipt of variants of uncertain significance. Overall, respondents felt they would value the information provided, and that it would empower them to make decisions about their healthcare. While patient desire to have this information does not automatically mean it should be offered to them, their willingness and ability to participate in the process of this testing is valuable to understand before testing is implemented.[30]

In addition to being willing and interested to receive their genomic information, many patients are willing to share the results they receive with both their healthcare providers as well as family members who may be at similar risks.[31] A 2015 study conducted on 29 participants who received results from the ClinSeq study showed that 72% of these participants shared their

result with at least one healthcare provider, with 31% reporting that the healthcare they received changed in response to this information. In addition, 93% of respondents shared their result with at least one family member. This is directly in line with responses from the aforementioned 2012 study and illustrates that not only are patients interested in receiving genomic information, but they *do* utilize the information the majority of the time.[31]

While these studies are useful in predicting general response to genomic information, it is important to further explore parental opinions specifically. Pediatric patients are the primary population clinically receiving exome sequencing, and therefore parents are the ones utilizing this information (and will be the patients utilizing the information in a prenatal setting).

A 2016 study assessed parental views of WES in the setting of undiagnosed pediatric disorders by interviewing 19 parents of children who had undergone WES.[32] Of the parents surveyed, 14 of 19 had child with a definite, likely, or possible diagnosis while the remaining 5 had no diagnosis. Parents understood and were able to communicate their child's WES findings and recurrence risks to family members and healthcare providers. A major concern of many parents was that a lethal disorder would be identified, but most parents said the hope of a diagnosis outweighed this concern. Those whose children had diagnoses said they felt their child's healthcare was more focused and there was less worry surrounding medical visits. A source of frustration for those whose children had a rare disorder was the limited information available due to the general lack of data on such disorders. This study highlights the realities that parents face in the wake of WES results. Such results could be provided from prenatal exome sequencing as well, and so it is important to understand and prepare for the issues parents undergoing prenatal WES may face.[32]

When considering patient opinions on WES, it is important to consider how they might feel about the possibility of prenatal WES. A study conducted in 2015 sought to assess parental views on prenatal exome sequencing.[33] Responding to a questionnaire while waiting for routine prenatal care, 83% of parents stated that they thought prenatal WES should be offered to patients. Thirty-four percent of parents said they would want WES for their pregnancy even if there was no indication for such testing. When asked about the return of results from WES, the majority of parents said they would want results related to both treatable and non-treatable childhood-onset conditions, both treatable and non-treatable adult onset conditions, and that results would likely influence future family planning decisions.[33] It is important to recognize patient willingness to receive and utilize this information, and again points to the importance genetic counselors will play in adequately explaining the risks, benefits, and limitations of this testing to their patients.

1.2 SPECIFIC AIMS

1.2.1 Specific Aim 1

Conduct an anonymized survey of approximately 200 genetic counselors with prenatal clinical and/or laboratory experience (both academic and commercial laboratories) to ascertain perceptions of different scenarios in which prenatal exome sequencing might be ordered or that might arise as a result of prenatal exome sequencing.

1.2.2 Specific Aim 2

From survey responses, determine what the prevailing opinions towards prenatal exome sequencing are and what, if any, discrepancies exist between prenatal clinical counselors and laboratory counselors regarding opinions and beliefs towards prenatal exome sequencing.

1.2.3 Specific Aim 3

From survey responses, elicit comfort levels of prenatal clinical counselors with various aspects of prenatal exome sequencing and identify areas of need to be addressed before implementation of this testing.

1.3 SIGNIFICANCE

1.3.1 Specific Aim 1

No study available in the literature has yet been done to determine genetic counselors' views and/or opinions on prenatal exome sequencing. Therefore, the results of this project will fill a gap in the existing research.

1.3.2 Specific Aim 2

Understanding the opinions toward prenatal exome sequencing can help to gauge what the response might be should this testing become widely available to clinicians. In addition,

determining whether or not a discrepancy exists between laboratory counselors and clinical counselors may help facilitate discussion and implementation policy, as the laboratories play a significant role in deciding whether or not to offer this testing to clinicians.

1.3.3 Specific Aim 3

It is important to understand which aspects of prenatal exome sequencing, if any, genetic counselors are uncomfortable with, and to address these issues before implementation of testing. In addition, identifying areas of need is necessary to determine possible resources that could be made available to genetic counselors to enhance their comfort utilizing this technology in a prenatal setting. Because prenatal and laboratory counselors will be at the front line of this testing implementation, it is most important to gauge their needs and concerns.

2.0 MATERIALS AND METHODS

This study was approved as an institutional review board (IRB) exempt study by the University of Pittsburgh IRB. (PRO15080297) (Appendix B)

2.1 SURVEY DESIGN

The study survey was a new design that utilized previous surveys on exome sequencing and prenatal CMA as a reference.[24,25,34] Prior to its implementation, the survey was reviewed multiple times by members of the thesis committee, which includes licensed certified genetic counselors, a research human geneticist with expertise in statistical genetics, and a clinical geneticist. The survey was also piloted by several licensed and certified prenatal and laboratory genetic counselors, who provided feedback on the structure and utility of questions asked. The survey was designed using Qualtrics survey system through the My Pitt student portal. Inclusion criteria for the survey involved currently working as either a laboratory genetic counselor, a clinical prenatal counselor, or both. This inclusion criteria was chosen because these counselors will be the first to deal with implementation of prenatal exome sequencing. The survey included a list of terms that were used in the survey with explanations of these terms: whole exome sequencing, targeted exome sequencing, and incidental findings. The survey included questions to delineate laboratory genetic counselors from prenatal clinical genetic counselors. Two

questions assessed how much time previous or current prenatal clinical counselors spend on average preparing for and seeing patients. Survey questions assessed general comfort levels toward prenatal exome sequencing being offered under different circumstances by using a Likert-scale where 1 was equivalent to “strongly disagree” and 5 was equivalent to “strongly agree”. A subset of questions were shown only to individuals currently practicing as prenatal clinicians assessing their comfort level with counseling on various aspects of exome in a prenatal setting, assessed on the same Likert scale. Additional questions assessed opinions on incidental findings and targeted exome in a prenatal setting, and what resources would influence a counselor to be more supportive of the implementation of this testing. An open-ended question allowed participants an opportunity to explain what influenced their opinions on prenatal exome sequencing. The survey was designed to be anonymous, with no questions revealing identifying information which could be tied back to the participant. A copy of the survey in its entirety is included in **Appendix A.**

2.2 SURVEY DISTRIBUTION

Surveys were distributed to prenatal and laboratory counselors through the National Society of Genetic Counselors (NSGC) student research survey program. Through this service, members of NSGC received an e-mail with a description of the survey, inclusion criteria, and a link to the Qualtrics survey system. An e-mail informing members of the survey was sent on September 3, 2015 with a follow-up reminder e-mail on November 5, 2015. Anyone with an NSGC membership had access to the survey. The survey remained open from September 3, 2015 until November 27, 2015. Please see Appendix A for the cover letter and survey content.

2.3 DATA ANALYSIS

Descriptive statistical analysis was completed for all questions on the survey using the Qualtrics system. Participants who did not qualify (genetic counselors not working in either a laboratory or prenatal clinical setting) and participants who did not complete the entire survey were excluded from analysis. Question 19 allowed for open responses that required coding to group the responses into themes. Responses to this question were read through twice to identify common themes. Some responses touched on several themes. Using these themes, responses were then coded into categories such as: more education is required, time available for sessions, ability of patients to understand material, existing technology is sufficient, limitations of existing technology, targeted exome would be useful in a prenatal setting, targeted exome would not be useful in a prenatal setting, personal/ethical beliefs, clinical utility, VUS/Incidental finding concerns, family planning, and insurance/costs. Some of these categories fit into existing themes provided in the previous question, while some were uniquely identified themes. Coding was done by corresponding colors to the different categories and highlighting sections of the responses that fit these categories and then tallying how many responses fell into each category. If any individual respondent had expanded upon a theme that they also noted in the previous question, the open-ended response was not included in the tally as it was already accounted for in the previous question.

Following descriptive analysis, responses were compared between different subgroups using the Mann-Whitney-Wilcoxon nonparametric test of statistical significance. The responses of laboratory counselors were compared to those of prenatal clinical counselors. In addition, the responses of counselors who have been working 5 years or less were compared to those who

have been working for more than 5 years. All nonparametric analysis was done using MiniTab Express statistical analysis software.

3.0 RESULTS

3.1 DEMOGRAPHICS

A total of 219 genetic counselors responded to the survey. Of these 219 respondents, 48 did not complete the survey and their answers were excluded from statistical analysis. An additional 11 respondents did not qualify for the survey as they were not currently working in either a laboratory or prenatal clinical setting. These respondents were redirected to the end of the survey. One hundred and sixty total responses were evaluated for descriptive statistics and nonparametric analysis. **Table 1** describes the current specialty in which these respondents were working. There were approximately equal numbers of laboratory and clinical prenatal counselors represented.

Table 1. Current Specialty of Respondents

	Total (n=160)	
	n	%
Laboratory Position	73	45.63
Clinical Prenatal Position	80	50
Joint Laboratory and Clinical Prenatal Position	7	4.38

Table 2 describes the demographic information for the 160 respondents who met the study criteria and completed the survey. The majority of respondents were female (96.25%), ages 25-34 (59.75%), and had been working in the field of genetic counseling for 1-5 years (36.48%). Respondents had experience in a variety of fields, with 73.42% having worked in prenatal clinic at some point in time. Of those genetic counselors who had experience in a prenatal clinic, 59.66% said that an average prenatal session lasted between 30-40 minutes.

Table 2. Demographic Breakdown of Survey Respondents

	Total (n=160)	
	n	%
Sex		
Female	154	96.25
Male	6	3.75
Age		
20-24	9	5.66
25-34	95	59.75
35-44	38	23.9
45-54	10	6.29
55-64	6	3.77
65 or older	1	0.63
Length of Time Working as GC		
< 1 year	18	11.32
1-5 years	58	36.48
5-10 years	35	22.01
10 years or longer	48	30.19

3.2 RESPONSES TO PRENATAL WES AND TES

The majority of respondents had never ordered whole exome sequencing for a patient (74.38%). Additionally, most respondents had never analyzed exome sequencing in a laboratory setting (79.38%). Questions focusing specifically on *targeted* exome sequencing revealed 88.75% of respondents had never ordered TES in a clinical setting and 90.63% of respondents had never analyzed TES in a laboratory setting.

Table 3 describes the breakdown of responses to a series of Likert questionnaire items regarding counselor comfort levels for prenatal WES and TES in a variety of different situations. For each response, a value of 1 was equal to “strongly disagree”, 3 was equal to “neutral”, and 5 was equal to “strongly agree”. For prenatal WES, the majority of counselors (59.4%) were comfortable with prenatal exome being offered after other diagnostic testing had been returned either negative or inconclusive. However, when presented with the option of prenatal exome being offered as a first-line diagnostic test, the majority of respondents (81.3%) were not comfortable with this scenario. This was also the only scenario for which no respondent strongly agreed that prenatal exome should be offered. Most counselors (59.4%) were comfortable with using prenatal exome to provide a genetic diagnosis for neonatal management decisions rather than pregnancy management decisions. Most respondents (78.8%) were opposed to allowing prenatal exome sequencing solely based on patient desire to have the testing. T

For prenatal TES, the majority of genetic counselors were comfortable with TES being offered after other diagnostic testing had returned negative or inconclusive (74.4%) and in cases where TES was being used for neonatal, rather than pregnancy, management decisions (64.4%). In contrast, the majority of genetic counselors were not comfortable with TES being provided as

a first-line diagnostic test (68.8%) or solely when a patient expressed interest in having the testing (71.3%).

Table 3. Genetic Counselor Comfort Levels with Various Prenatal WES/TES Scenarios

Please indicate how much you agree or disagree with the following statements regarding prenatal exome sequencing.									
Question - Prenatal exome sequencing should be offered:	Type of Test	1=Strongly Disagree	2=Disagree	3=Neutral	4=Agree	5=Strongly Agree	Total Responses	Mean	Standard Deviation
When there are structural abnormalities on ultrasound and previous diagnostic testing (e.g. microarray) has come back negative or inconclusive	WES	10 (6.3%)	17 (10.6%)	38 (23.7%)	69 (43.1%)	26 (16.3%)	160	3.52	1.08
	TES	5 (3.1%)	8 (5.0%)	28 (17.5%)	87 (54.4%)	32 (20.0%)	160	3.83	0.91
When there are structural abnormalities on ultrasound before other diagnostic testing is offered	WES	77 (48.1%)	53 (33.1%)	16 (10%)	14 (8.8%)	0 (0%)	160	1.79	0.95
	TES	53 (33.1%)	57 (35.6%)	19 (11.9%)	30 (18.8%)	1 (0.6%)	160	2.18	1.11
In the context of providing a genetic diagnosis for neonatal management rather than pregnancy management (e.g. a diagnosis prenatally would help guide medical management decisions in the neonatal period)	WES	12 (7.5%)	19 (11.8%)	34 (21.3%)	69 (43.1%)	26 (16.3%)	160	3.49	1.13
	TES	8 (5.0%)	16 (10.0%)	33 (20.6%)	78 (48.8%)	25 (15.6%)	160	3.6	1.03
Whenever a patient expresses interest in having prenatal exome	WES	77 (48.1%)	49 (30.6%)	21 (13.1%)	10 (6.3%)	3 (1.9%)	160	1.83	1
	TES	70 (43.7%)	44 (27.5%)	27 (16.9%)	16 (10.0%)	3 (1.9%)	160	1.99	1.09

The next series of questions related specifically to counseling situations related to prenatal WES and TES. These questions were only shown to individuals who answered that they currently work in a prenatal setting because they involved counseling scenarios that a laboratory counselor would not typically encounter. **Table 4** describes the responses to these Likert questionnaire items.

In regards to prenatal WES, just over half of prenatal clinical counselors were comfortable with most of the scenarios provided. Specifically, 56.3% of counselors were

comfortable providing pre-test counseling for prenatal WES. Additionally, 52.9% of respondents felt they would be comfortable disclosing and explaining VUS results, 44.2% were comfortable helping a patient to make pregnancy management decisions based on results from a prenatal exome, and 59.7% would be comfortable locating resources specific for their patient's results. It is important to note that while the majority of respondents were comfortable or neutral regarding these situations, a sizeable number of counselors still responded that they would not be comfortable in these scenarios. Anywhere from 25-34% of counselors were uncomfortable with each given counseling scenario.

When posed with the same counseling scenarios provided for prenatal WES, this time in the context of prenatal TES, genetic counselors were similarly mostly comfortable with each of the scenarios. When asked about comfort levels providing pre-test counseling for prenatal TES, 80.5% of counselors were comfortable doing so. Additionally, 60.9% of counselors were comfortable disclosing VUS results from targeted prenatal exome sequencing. In regards to helping patients with pregnancy management decisions based on the results of prenatal TES, 57.5% of respondents felt comfortable with doing so. Finally, 59.8% of respondents felt comfortable locating resources for patients based on results from prenatal TES. It is again important to note that this still leaves a considerable number of counselors who were not comfortable with such scenarios. Anywhere from 18-22% of counselors were uncomfortable with each given counseling scenario.

Table 4. Prenatal Clinical Counselors' Comfort Levels with Prenatal WES/TES in Various Counseling Scenarios

Please indicate how much you agree or disagree with the following statements regarding prenatal exome sequencing.									
Question - I would be comfortable:	Type of Test	1=Strongly Disagree	2=Disagree	3=Neutral	4=Agree	5=Strongly Agree	Total Responses	Mean	Standard Deviation
Providing pre-test counseling for exome sequencing in a prenatal setting	WES	5 (5.8%)	20 (23.0%)	13 (14.9%)	35 (40.2%)	14 (16.1%)	87	3.38	1.17
	TES	2 (2.3%)	5 (5.7%)	10 (11.5%)	49 (56.3%)	21 (24.1%)	87	3.94	0.89
Disclosing and explaining variants of unknown clinical	WES	6 (6.9%)	16 (18.4%)	19 (21.8%)	35 (40.2%)	11 (12.6%)	87	3.33	1.13
	TES	3 (3.4%)	13 (14.9%)	18 (20.7%)	41 (47.1%)	12 (13.8%)	87	3.53	1.02
pregnancy management decisions based on the results of prenatal exome sequencing	WES	11 (12.8%)	18 (20.9%)	19 (22.1%)	33 (38.4%)	5 (5.8%)	86	3.03	1.16
	TES	7 (8.0%)	10 (11.5%)	20 (23.0%)	42 (48.3%)	8 (9.2%)	87	3.39	1.07
Locating resources (e.g. support groups, specialty clinics) for patients based on the results of prenatal exome sequencing	WES	9 (10.4%)	13 (14.9%)	13 (14.9%)	41 (47.2%)	11 (12.6%)	87	3.37	1.19
	TES	5 (5.7%)	14 (16.1%)	16 (18.4%)	38 (43.7%)	14 (16.1%)	87	3.48	1.12

3.3 COMPARISON OF RESPONSES TO PRENATAL WES AND TES

The responses for prenatal WES were compared to those for prenatal TES using the Mann-Whitney test of statistical significance. For the scenario in which exome was used as a diagnostic test after other testing options had been exhausted, there was a statistically significant difference in responses, with a more favorable response to using TES in this situation ($p=0.0088$). In addition, there was a statistically significant difference in responses toward using prenatal exome as a first-line diagnostic test ($p=0.0013$), with counselors being more comfortable with this situation if TES were to be offered rather than WES, though responses favored disagreement with the use of this testing in such a situation. There was no statistically significant difference in responses for the remaining two scenarios given. For counseling scenarios, there was no statistically significant difference in genetic counselor comfort levels between prenatal WES and

TES, except for the scenario of providing pre-test counseling. For this scenario, there was a statistically significant difference in responses (p=0.0012), with counselors being more comfortable providing pre-test counseling for TES than WES.

3.4 RESPONSES TO INCIDENTAL FINDINGS

The next sets of questions dealt with types of incidental findings that could be reported and counseling scenarios surrounding return of incidental findings. As above, counseling scenario questions were only provided to individuals who responded as currently working in a prenatal clinical setting. **Table 5 and Table 6** describe the responses to these questions.

The majority of respondents (55.6%) believed that incidental findings from the ACMG minimum list should be reported in prenatal exome sequencing cases. In addition, 77.5% of counselors also felt that incidental findings for childhood-onset conditions should be reported. Responses differed in regards to other categories of incidental findings results. Results were split almost evenly for the report of hereditary cancer genes, with 31.9% of respondents against reporting these results, 36.2% neutral, and another 31.9% in favor of reporting. Most respondents were against reporting incidental findings that revealed carrier status (43.8%) and those related to adult-onset conditions (55.6%). Nearly half of all respondents (49.4%) felt comfortable allowing patients to choose freely from any of the listed categories, while 16.9% were neutral, and 33.7% were not comfortable with this scenario.

Most prenatal clinical counselors (72.4%) responded that they would be comfortable discussing incidental findings in pre-test counseling sessions. Comfort levels were less consistently favorable for the other scenarios provided. While many of the respondents were

comfortable (43.6%) or neutral (33.3%) regarding disclosing incidental findings results, only 37.9% were comfortable helping a patient making pregnancy management decisions based on these types of results while 43.6% were not comfortable with this scenario. Regarding locating resources based on the results of incidental findings, 49.4% of counselors felt comfortable doing so, while 30.6% were not comfortable.

Table 5. Genetic Counselor Comfort Levels with Various Categories of Incidental Findings

Please indicate how much you agree or disagree with the following statements regarding incidental findings for prenatal exome sequencing.								
Question - I believe:	1=Strongly Disagree	2=Disagree	3=Neutral	4=Agree	5=Strongly Agree	Total Responses	Mean	Standard Deviation
Incidental findings from the ACMG minimum list should be returned	15 (9.4%)	21 (13.1%)	35 (21.9%)	60 (37.5%)	29 (18.1%)	160	3.42	1.2
Incidental findings for hereditary cancer genes should be returned	16 (10.0%)	35 (21.9%)	58 (36.2%)	41 (25.6%)	10 (6.3%)	160	2.96	1.06
Incidental findings that reveal carrier status should be returned	31 (19.4%)	39 (24.4%)	46 (28.7%)	39 (24.4%)	5 (3.1%)	160	2.67	1.14
Incidental findings for adult-onset conditions should be returned	32 (20.0%)	57 (35.6%)	46 (28.7%)	23 (14.4%)	2 (1.3%)	160	2.41	1.01
Incidental findings for childhood-onset conditions should be returned	5 (3.1%)	8 (5.0%)	23 (14.4%)	82 (51.2%)	42 (26.3%)	160	3.92	0.94
Patients should be able to choose from any of the above categories without restriction	22 (13.7%)	32 (20.0%)	27 (16.9%)	43 (26.9%)	36 (22.5%)	160	3.24	1.37

Table 6. Prenatal Clinical Counselors' Comfort Levels with Various Counseling Scenarios Related to Incidental Findings from Prenatal WES

Please indicate how much you agree or disagree with the following statements regarding incidental findings for prenatal exome sequencing.								
Question - I would be comfortable:	1=Strongly Disagree	2=Disagree	3=Neutral	4=Agree	5=Strongly Agree	Total Responses	Mean	Standard Deviation
Counseling patients about incidental findings in a pre-test counseling session	2 (2.3%)	8 (9.2%)	14 (16.1%)	46 (52.9%)	17 (19.5%)	87	3.78	0.95
Disclosing and explaining incidental findings results in a prenatal setting	6 (6.9%)	14 (16.1%)	29 (33.3%)	28 (32.2%)	10 (11.5%)	87	3.25	1.08
Helping a patient to make pregnancy management decisions based on the results of incidental findings from prenatal exome sequencing	11 (12.6%)	27 (31.0%)	16 (18.4%)	29 (33.3%)	4 (4.6%)	87	2.86	1.15
Locating resources (e.g. support groups, specialty clinics) for patients based on incidental findings from prenatal exome sequencing	10 (11.8%)	16 (18.8%)	17 (20.0%)	30 (35.3%)	12 (14.1%)	85	3.21	1.24

3.5 RESPONSES TO SUMMARY QUESTIONS

Respondents were asked if they would support prenatal exome sequencing being offered by genetic testing laboratories. These responses are highlighted in **Figure 1**. Most respondents stated that they would support labs offering this testing in one of two restricted situations: only if it was restricted to cases with a clear clinical indication (46.9%) or only if it was limited to a phenotype-drive targeted exome (31.9%). An additional 13.1% of respondents would support prenatal exome sequencing in any scenario. Only 8.1% of respondents would not support prenatal exome in any situation.

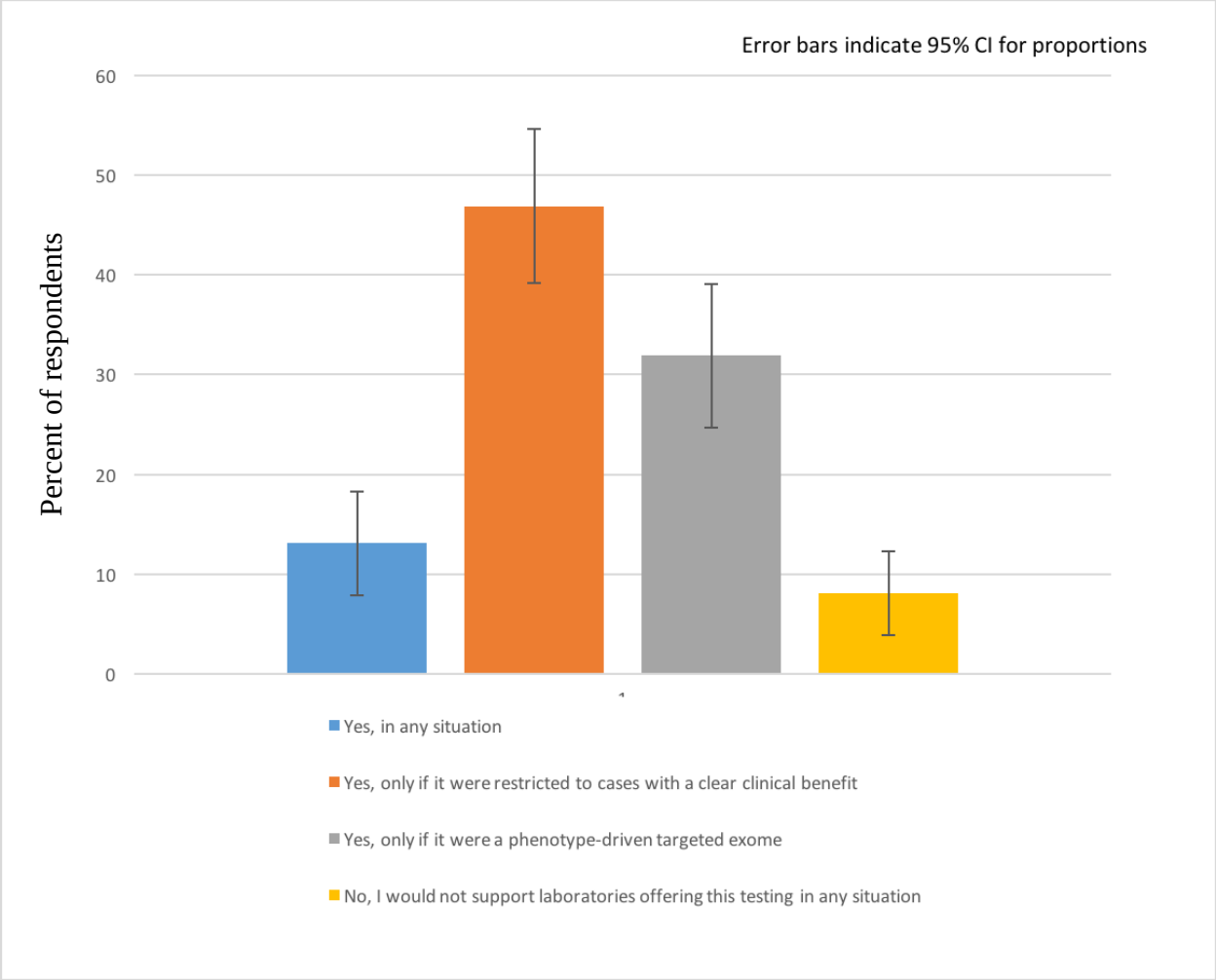

Figure 1. Genetic Counselor Preferences for Laboratories Offering Prenatal Exome Sequencing

As shown in **Table 7**, the majority of respondents reported understanding the clinical utility of exome sequencing in general (95.0%) and felt that it could be clinically useful in a prenatal setting (68.2%). Responses regarding whether or not commercial laboratories should offer this testing to their clients were somewhat divided, with 28.7% disagreeing with this scenario, 34.4% neutral, and 36.9% responding positively. The majority of counselors (69.4%) were more comfortable with the idea of prenatal microarray than they were with prenatal exome. Approximately 50% of respondents stated that they would be more comfortable supporting prenatal exome sequencing if a medical geneticist was there to provide the results with them, while 23.8% were neutral and the remaining 26.2% disagreed that this scenario would make them more comfortable supporting this testing. The majority of respondents (70.7%) were more comfortable supporting prenatal exome sequencing if educational or training opportunities could be made available to them prior to implementation of this testing in a prenatal setting.

Table 7. Genetic Counselors' Understanding of Prenatal Exome and Areas of Need

Please indicate how much you agree or disagree with the following statements regarding targeted prenatal exome sequencing.								
	1=Strongly Disagree	2=Disagree	3=Neutral	4=Agree	5=Strongly Agree	Total Responses	Mean	Standard Deviation
I understand the clinical utility of exome sequencing	0 (0.0%)	2 (1.3%)	6 (3.7%)	67 (41.9%)	85 (53.1%)	160	4.47	0.63
I feel exome sequencing could be clinically useful in a prenatal setting	6 (3.7%)	15 (9.4%)	30 (18.7%)	74 (46.3%)	35 (21.9%)	160	3.73	1.03
I would like commercial laboratories to make this testing available to clients	18 (11.2%)	28 (17.5%)	55 (34.4%)	46 (28.8%)	13 (8.1%)	160	3.05	1.11
I am more comfortable with the idea of prenatal microarray than prenatal exome (WES or TES)	9 (5.6%)	15 (9.4%)	24 (15.1%)	65 (40.9%)	46 (28.9%)	159	3.78	1.13
I would be more likely to support the use of prenatal exome if a medical geneticist was there to help me disclose the results	11 (6.8%)	31 (19.4%)	38 (23.8%)	55 (34.4%)	25 (15.6%)	160	3.33	1.16
I would be more likely to support prenatal exome sequencing if educational/training opportunities were offered prior to its implementation	2 (1.3%)	14 (8.8%)	30 (18.9%)	59 (37.1%)	54 (33.9%)	159	3.94	1

3.6 FACTORS INFLUENCING RESPONSES

For the final component of the survey, participants were given a “check all that apply” option for factors that influenced their decisions, and support of, prenatal exome sequencing. They were then allowed an open-ended question for any additional factors they felt were not covered on the supplied list. Fifty-four genetic counselors provided responses to the open-ended question. Based on themes from responses to this question, the responses were coded into the existing categories listed in the prior question, as well as a few additional categories not covered.

Several themes emerged that were not previously identified in the “check all that apply” question. One of the biggest concerns stemmed around dealing with VUS and incidental findings results (n=11). One respondent said,

“My main concern about offering whole exome prenatally would be the possibility of identifying variants of uncertain significance, and what that would mean for pregnancy management and counseling. Additionally, ACMG secondary findings that are related to cancer/adult onset conditions would have to be carefully considered and discussed with the patient.” (Laboratory Counselor)

Along the same lines, another respondent discussed the difficulty incidental findings might create in the counseling process:

“A thought that crossed my mind while answering this survey was that if we offer PGD for something (essentially offering the patient a "way out," if you will), then is it really fair that we wouldn't disclose results of a same caliber from exome sequencing? I mean, I'm definitely uncomfortable with thinking about disclosing BRCA results from a prenatal exome, but when thinking about what should be included on a report, I'm a little torn.” (Clinical Prenatal Counselor)

Other responses focused on variants in the context of technological limitation, with 8 of the 54 respondents who answered the open-ended question citing that they felt there would be too much uncertainty around variants, and limited ability to gather fetal phenotype to aid in variant interpretation with current technological capabilities.

“All my experience with WES has been in the pediatric setting. Results are not always definitive. We receive lots of likely pathogenic result. We frequently use the results to explain the phenotype we observe. In the prenatal settings we fear that we could receive lots of results with unclear clinical significance and this will cause additional stress and anxiety for the pregnant woman...” (Clinical Prenatal Counselor)

Another factor affecting opinions on prenatal exome identified through the open-ended question was the need for further education before testing is implemented (n=6), not just for genetic counselors, but for any prenatal provider who might be involved in the process. As one genetic counselor said:

“There needs to be a lot more education offered for genetic counselors and medical professionals about the clinical utility of exome sequencing. I hear other medical professionals (outside of genetics) discussing how great exome sequencing would be and I feel that they don't

really understand the type of information their patients may receive from these results." (Clinical Prenatal Counselor)

Another respondent echoed and elaborated upon these sentiments:

"In addition to having concern about patients' ability to handle the amount of information, I have concern about the ability of many physicians to understand, interpret, and communicate results with patients and have had experience in prenatal clinics where the OBs did not understand information about Fragile X carrier test results, NIPT testing, or testing for recessive conditions and these seem like they should be "basic" concepts for physicians from different backgrounds (especially OBs), not just geneticists." (Laboratory Counselor)

Issues surrounding TES and its utility emerged as well. More respondents felt that the technology would be useful, and perhaps even preferable, to WES in a prenatal setting (n=3).

"Targeted exome in prenatal is the only appropriate option. All adult onset and hereditary cancer syndromes should be excluded." (Clinical Prenatal Counselor)

"…targeted exome seems more appropriate, although I think diseases we could test for would be fairly limited based on U/S findings" (Laboratory Counselor)

"The idea of a targeted WES is appealing and would reduce the risk of incidental findings (such as WES for skeletal dysplasias as multiple gene panels are very expensive)" (Clinical Prenatal Counselor)

One respondent, however, felt the TES had even less utility in the prenatal setting than WES:

“I also don't feel that "targeted" or "phenotype-driven" exome sequencing is useful. Analyzing only known disease genes would potentially miss causative novel disease genes that could be identified in trio based whole exome sequencing. Furthermore, the effort/cost/time that goes into whole exome sequencing vs. targeted sequencing is negligible. I don't think targeted exome is useful in any circumstance actually.” (Laboratory Counselor)

A final theme that emerged from the open-ended question was the importance that prenatal exome could play in discussions of family planning and recurrence risk (n=6), particularly in the setting of couples who had multiple similarly affected pregnancies. Several respondents had statements regarding the utility of prenatal exome for this purpose:

“I think it's important to have this technology available for families who cannot get a prenatal diagnosis by other methods (microarray, sequencing panels, etc). It's important that families understand the causes for their baby's anomalies (and prognosis) and if there is expected to be an associated recurrence risk.” (Clinical Prenatal Counselor)

“I think in the event that a patient has a fetus with multiple anomalies, with normal genetic testing (microarray and suspected single gene) an exome could be considered. I think it could be useful in cases where a child may pass away shortly after birth and performing an exome prenatally would be the most efficient and cost effective way (if covered by insurance) to provide the family with a potential answer and RR.” (Clinical Prenatal Counselor)

The responses were coded and categorized into themes. These themes were ultimately labeled as: more education required, targeted exome would not be clinically useful in a prenatal setting, targeted exome *would* be clinically useful in a prenatal setting, family planning for recurrence risk or termination, concerns about VUS and/or incidental findings results, and technological limitations (such as turnaround time or variant interpretation).

Other responses simply elaborated on themes from the "check all that apply" question and were therefore either added to the tally for those questions (if the participant had not checked the corresponding box), or not added to the tally (if the participant had already checked the corresponding box). The answers provided, while already related to factors in the "check all that apply" question, were nonetheless beneficial in highlighting *why* these factors influenced genetic counselors' opinions.

Tallied results to these questions are highlighted in **Figure 2.**

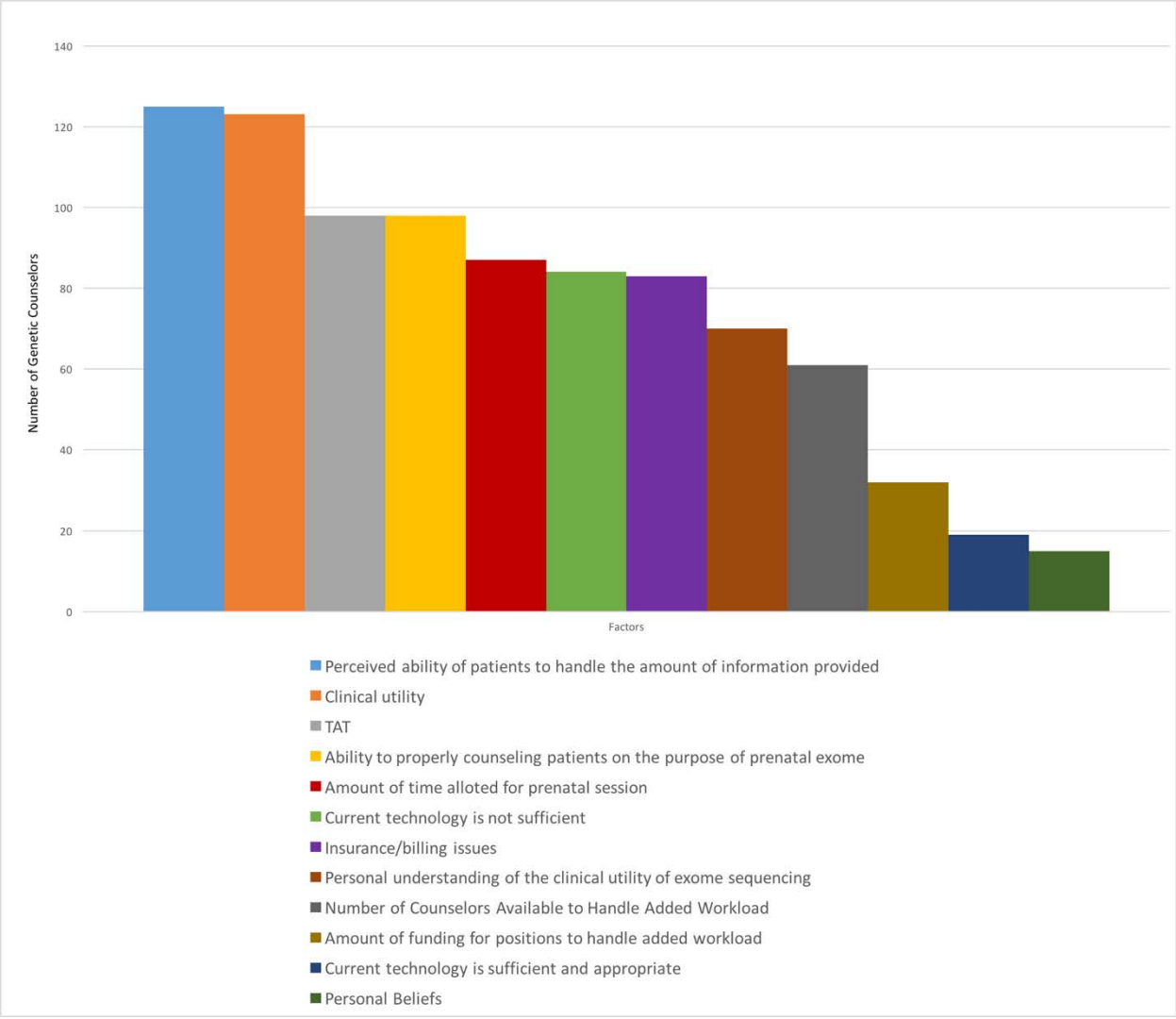

Figure 2. Factors Influencing Genetic Counselors' Opinions on Prenatal Exome Sequencing

The two factors most largely influencing respondents' views on exome sequencing were the perceived ability of patients to handle the amount of information provided by the testing (n=125) and the clinical utility of exome sequencing in the prenatal setting (n=123). Many of the responses to the open-ended question elaborated on responses given in the prior question and how these items factored into individual opinions toward exome sequencing.

Several answers to the open-ended question provided some clarification of how respondents felt about the perceived ability of patients to handle the amount of information provided by the testing. Some respondents felt that the amount of information would be too difficult to handle during an already stressful time:

"I'm concerned about the amount of information that would be provided to parents at a time when they are particularly stressed. If incidental results were found, I would be concerned about parents getting that information while also trying to process the issues more immediately facing them during pregnancy that is abnormal in some way." (Prenatal Clinical Counselor)

Other respondents were concerned about a patient's ability to fully comprehend results, especially uncertain ones:

"I think a lot of patients would have a difficult time dealing with VUSes. I previously worked in the prenatal setting with very high-maintenance patients, who nit-picked every possibility. I could see those type of people grappling with terminating over VUSes or adult on-set conditions." (Laboratory Counselor)

Additionally, respondents had comments regarding the issue of clinical utility of exome sequencing. Some responses elaborated that there was limited utility to prenatal exome sequencing, while others argued that the utility was clear. One respondent arguing *for* its utility said:

"I would be hesitant to offer this testing to all individuals in a prenatal clinic, but I do think exome sequencing is a clinically useful test that will be incorporated into our standard of care offerings. I think prenatal exome can be a useful tool but should be used with discretion. Patients need to be fully informed of the benefits and limitations and providers should be aware of the benefits and limitations." (Clinical Prenatal Counselor)

Another respondent argued that the utility of prenatal exome was not quite as clear:

"I think the greatest utility of exome sequencing may actually be on POCs [parents of the child], to provide couples planning to have more children with recurrence risk information. With current technology, the actual utility prenatally to make a decision about the pregnancy seems limited. ***just because it is possible or available does not mean it is a good idea to implement it***." (Clinical Prenatal Counselor)

3.7 COMPARISON OF PRENATAL CLINICAL COUNSELOR AND LABORATORY COUNSELOR RESPONSES

When all descriptive analysis was complete, the responses of lab counselors were compared to those of clinical counselors using the Mann-Whitney test of statistical significance. For the purposes of analysis, individuals who identified as currently working in both lab and clinical positions were grouped with prenatal clinical counselors and included in the clinical responses.

Responses were significantly different for several questions. In regards to prenatal WES scenarios, there was a statistically significant difference for use of prenatal WES as a first-line diagnostic test ($p=0.0016$), in addition to use of prenatal WES as a means for guiding neonatal management ($p=0.0236$). For these scenarios, a larger number of laboratory counselors agreed that prenatal WES should be offered when compared to the number of clinical counselors favoring its use in these situations. There was no statistically significant difference in responses between groups for questions regarding the types of incidental findings that should be returned for prenatal WES. For scenarios involving prenatal TES, laboratory counselors were again more likely than clinical counselors to agree that this testing should be offered as a first-line diagnostic test ($p=0.0214$). There was no statistically significant difference in any other responses regarding prenatal TES.

More laboratory counselors agreed that they understood the clinical utility of exome than clinical counselors ($p < 0.0001$), with 98.6% of lab counselors agreeing or strongly agreeing with this statement compared to 91.9% of clinical counselors. Regardless, there was not a statistically significant difference in whether or not these counselors felt exome sequencing could be clinically useful in a prenatal setting. Significantly more clinical counselors than laboratory counselors were more comfortable with the idea of prenatal microarray than prenatal exome sequencing ($p=0.0003$). There was no statistically significant difference found between these groups for any other responses in the survey.

An additional analysis was conducted to determine if responses from counselors who had been working for less than 5 years were statistically different from those who had been working for 5 years or more. More counselors who had been working for less than 5 years agreed with the statement “I am more comfortable with the idea of prenatal microarray than prenatal exome”

(82.9%), while responses from counselors working for 5 years or more were more spread out, with the majority agreeing with the statement (58.3%) and the remaining 41.7% disagreeing or neutral. The difference in responses was statistically significant with a p value of 0.0014. There was no statistically significant difference found for any other responses in the survey.

4.0 DISCUSSION

4.1 ANALYSIS OF SURVEY RESPONSES

Exome sequencing (WES and TES) is utilized in pediatric and adult settings and uptake into these settings occurred relatively soon after the testing became clinically available.[25] Uptake of newer, more unfamiliar testing has traditionally taken longer to reach the prenatal setting, but using testing such as microarray as an example, it is expected that exome sequencing will be more widely offered in the prenatal setting in the near future, bringing with it several situations that complicate the genetic counseling process and patient understanding of results.[25,29] This study helped to elucidate situations in which genetic counselors might be more or less inclined to offer prenatal WES or TES, comfort levels with various counseling issues that could arise as the result of prenatal WES or TES, areas that need to be addressed prior to testing implementation, and factors influencing these opinions.

Despite the controversial nature of offering exome in a prenatal setting, the majority of genetic counselors (59.4%) were, at the very least, comfortable with prenatal exome as a last option diagnostic test for patients when other diagnostic testing was negative or inconclusive. Many counselors were also comfortable with the use of prenatal exome to guide neonatal management decisions (59.4%). Importantly, comfort levels were much lower for both prenatal WES and TES as a first-line diagnostic test or as an effective screening test (any time a patient

expresses interest in having the testing), indicating that genetic counselors have a clear preference for how and when the test is utilized in clinical settings. Discrepancies existed between laboratory and clinical counselors, with more laboratory counselors responding favorably to both WES and TES as a last option diagnostic test. This may be related to the fact that a significant difference ($p < 0.0001$) was observed in the number of respondents who agreed that they understood the clinical utility of exome sequencing, with more laboratory counselors also responding affirmatively to this question. However, this explanation is unlikely given that there was no significant difference observed between clinical and laboratory counselors' opinions on whether or not this testing *could* be useful in a prenatal setting. It is possible that the difference could instead be explained by the fact that laboratory counselors would not have to deal with the complications this testing would create in the clinical setting, such as counseling on incidental findings and VUS results, and would primarily be dealing with analysis and variant interpretation, more positively coloring their views regarding the testing. It is also possible that laboratory counselors have implicit bias in regards to supporting newer forms of testing because laboratory revenue comes from implementing and marketing new testing. However, it is important to distinguish that this bias may differ even amongst laboratory counselors, as some work directly with sales while others have little or no stake in marketing and sales of tests.

While definitions were given for whole exome sequencing, targeted exome sequencing, and incidental findings prior to respondents completing the survey, many prenatal clinical counselors responded that they had no experience ordering or utilizing exome sequencing. This may in turn account for discrepancies between laboratory and clinical counselors, as having more experience with, and understanding of, the technological aspects of exome sequencing may influence opinions and comfort levels with the testing. Regardless of the reasons for

discrepancies, it is important to note these differences, and that laboratories should consider these results when deciding when and how to offer testing to prenatal clinical genetic counselors and other prenatal clinicians.

The majority of respondents (59.66%) who had worked in a prenatal setting before stated that their average prenatal session lasted 30-40 minutes. Existing literature has shown that the extensive informed consent process for WES and TES lengthens and complicates genetic counseling sessions.[25] Consideration should be given to this discrepancy in time available to prenatal counselors. Counseling for prenatal exome may require multiple sessions, increasing the risk of losing patients to follow-up, in addition to the more obvious concern as to whether or not enough resources exist to take on this extra caseload.

Most respondents were comfortable with offering childhood-onset and ACMG minimum list incidental findings genes and less comfortable with offering incidental findings for genes associated with carrier status, adult-onset conditions, and cancer genes. Congruent with the principle of autonomy, the majority of respondents still agreed that patients should have the option to choose freely from any of the categories, providing any incidental findings are returned on a prenatal exome. Many of the responses to the open-ended question elaborated that answers to the questions regarding incidental findings were not straightforward. Previous literature has shown that ethical concerns exist around more controversial incidental findings such as adult-onset and cancer genes being returned in a prenatal setting.[29] Answers highlighted in the results of this research echo previous discussions in the literature. In existing prenatal testing, it is generally considered acceptable to test for adult-onset disorders/cancer genes using pre-implantation genetic diagnosis. In addition, in the current pediatric setting, individuals undergoing exome can still receive these incidental findings results. Therefore, some individuals

argue that incidental findings for adult-onset conditions should be returned in prenatal WES as well.[29] Participant responses agreed with this consensus, but many respondents were still uncomfortable with the idea of returning, for instance, *BRCA* results from a fetal exome analysis. The option to return incidental findings with prenatal WES seemed to be an issue that generated some of the greatest concern for respondents. Clinical counselors were less comfortable with counseling scenarios regarding the return of incidental findings in this context than they were with the generalized counseling scenarios of returning exome results, including VUS results.

Several respondents expressed concern about how parents might make pregnancy management decisions based on incidental findings unrelated to the fetus' phenotype. A suggestion to avoid such a difficult scenario was provided by at least one respondent, "If the disclosure of incidental findings were agreed to be held off until after delivery (like 3-6 months), then I would be more agreeable to prenatal exome sequencing". While this idea could alleviate some of the immediate difficulties surrounding prenatal exome sequencing and the counseling process, the onus of providing these results would still fall onto someone. It would then need to be decided if these results should be provided by the same counselor, a pediatric counselor, or another health care provider and would require patient follow-up for disclosure. This may be even more challenging should the parents be dealing with a child with multiple medical needs. These results highlight the importance of careful consideration as to whether or not incidental findings should be offered at all and the process of disclosure.

Differences in responses between prenatal WES and TES show a statistically significant preference for TES in this setting ($p=0.0088$), likely because it eliminates the likelihood of incidental findings and could potentially lower the likelihood of VUS results if the genes reviewed are limited to those related to the fetus' phenotype. While some respondents argued

that utilizing TES as a replacement for, as an example, skeletal dysplasia panels could ultimately be faster and cheaper than ordering an actual skeletal dysplasia panel, others argued that the limited phenotypic information provided by ultrasound would render TES useless. A consideration could be made that TES would be the best way to first introduce exome sequencing to the prenatal setting to alleviate the burden of dealing with incidental findings and examine the efficacy of such a test. Most studies of exome's efficacy have shown that the detection rate increases with the use of a trio.[5,6] While utilizing TES in a prenatal setting would not exclude the use of a trio to determine gene segregation, there would still be the potential to miss novel gene findings and limited ability to utilize phenotypic information given by ultrasound. Use of a trio would also potentially add to turnaround time, which is already high for exome sequencing of any type, and is a greater concern in a prenatal setting where time is limited. In addition, all studies on the efficacy of exome in the prenatal setting have utilized WES. These studies are promising, but still only show a detection rate comparable to existing technology such as microarray.[27,28] This detection rate would be more likely to decrease in the context of prenatal TES. It is therefore important for genetic counselors, both laboratory and clinical, to consider the utility of both prenatal WES and TES when considering what they may offer to clients in the future, especially given that results of this study show a preference for TES on the part of respondents.

A variety of responses affected genetic counselors' opinions on prenatal exome sequencing suggesting these factors are areas that would be important to address before or during implementation of WES/TES in the prenatal setting. Major factors contributing to opinions included concerns over technological limitations (turn around time, ability to analyze and interpret variants, etc.), ability of patients to handle the amount of information provided by

testing, difficulty in properly counseling patients about prenatal exome sequencing, difficulty in dealing with insurance or billing issues, and clinical utility. Interestingly, while many of these factors are what caused counselors to be hesitant about utilizing prenatal exome, some counselors cited favorable responses toward clinical utility. Some of those who recognized that prenatal WES/TES had clinical utility still had reservations due to the limited phenotype provided in the setting. Many respondents indicated that the factors affecting their responses were significant and should be addressed before this test could be efficiently and usefully offered to patients. As one respondent said,

"I would love to be able to potentially provide them with a diagnosis so that we can offer more effective prenatal diagnostic testing rather than just fall back on saying there's a 25% recurrence risk for an unspecified disorder. I think there are *several significant hurdles that need to be overcome before prenatal or targeted exome sequencing should reach the prime-time clinical setting.*"

The factor, perceived ability of patients to handle the amount of information provided by exome sequencing, was cited by the most respondents as affecting their opinions and perceptions of prenatal WES/TES. Respondents expressed concern that some findings, including incidental findings, could have phenotypic uncertainty/variable penetrance. This could potentially be difficult for patients to understand or deal with because it becomes extremely challenging to predict how severely affected their child might be. As one respondent said:

"The one concern is our limited knowledge of genetics. I was at a meeting in which a leading edge researcher made a comment that on whole exome testing she was found to have a mutation for an autosomal dominant genetic condition that she clearly does not express. So had her parents been given that result, they would have been told that she would have this very well

understood condition - but she doesn't have any symptoms of this condition at all…if patients terminate due to abnormal findings (a very common decision in my region) we won't know how many of those kids never express symptoms (like the researcher I mentioned).” (Clinical Prenatal Counselor)

This echoes existing research by Bernhardt et al assessing counselor response to prenatal chromosomal microarray (CMA),[24] in which counselors interviewed for the study discussed that the phenotypic uncertainty of some copy number variants caused significant distress for patients. One respondent in the Bernhardt study said this about the uncertainty:

“It’s frustrating especially as this technology has taken off and there’s just not a lot of data about these esoteric deletions or duplications. All you can do is go to a family and say, “Well, we found a change, but there are only 3 cases in the literature and one’s walking around and fine and the other 2 have severe mental retardation.”[24]

The results from this study builds upon previous research on CMA testing in the prenatal setting and supports the idea that a major concern among genetic counselors is the potential to encounter significant uncertainty with the test results. Uncertainty is complicated in the prenatal setting, as highlighted by the responses above, by the inability to accurately predict phenotype and provide parents with an accurate risk assessment for their unborn child.

In another study by Machini et al (2014) assessing genetic counselors’ views and experiences with the integration of WES and whole genome sequencing into the clinical setting, respondents who did not offer WES after its implementation were asked to cite their reasons for not offering the testing. While the respondents to this survey were not limited to genetic counselors, clinical utility was one of the most cited reasons for not offering this testing.[25] This is consistent with the data highlighted in the results section of this document, with 123/160

respondents citing clinical utility as a factor that influenced their opinions on prenatal WES/TES, the second most-cited answer after patient ability to understand results. Most of the responses in the open-ended question that elaborated on this issue focused on the uncertainty of a phenotype provided by ultrasound and how important it would be to have the information immediately rather than after the child was born. Nearly 50% of respondents to the Machini survey had not offered WES because of their area of practice, with prenatal clinicians making up only 20% of all respondents.[25] It is perhaps unsurprising that these clinicians would find less clinical utility for WES than in the pediatric setting and interestingly, two years later, the data from this research suggests clinical utility is still a major factor influencing how prenatal genetic counselors feel about WES in this setting. Since the Machini paper was published, however, two separate studies have provided promising, if limited results on the clinical efficacy of WES in the prenatal setting.[27,28] With these studies in mind, and as exome sequencing becomes a more commonly used test, it is interesting to note that 68.2% of respondents to this survey felt exome sequencing could be clinically useful in the prenatal setting. This suggests that perceptions about the clinical utility of exome in a prenatal setting may be gradually shifting. It is important to consider that while 123 respondents cited clinical utility as a factor influencing their opinions on prenatal WES/TES, these respondents include both those who felt negatively about the clinically utility of prenatal WES/TES as well as those who felt positively.

Some of the additional factors complicating the integration of WES into the clinical setting in the Machini study were the same factors that influenced opinions of prenatal WES in this study. These included insurance/billing issues, results interpretation (here grouped with technological limitations), and turn-around time.[25] Both studies highlighted a need for further training to assist in a more seamless integration of exome sequencing in the clinic. It is important

to note that problems surrounding exome sequencing highlighted 2-3 years ago are still concerns genetic counselors have about this testing and that these issues could be amplified in the prenatal setting. It may be more difficult for insurance to cover such novel testing with a limited phenotype to warrant the testing in a prenatal setting, and along those lines limited phenotype hinders the ability of laboratories and genetic counselors to interpret results. While turn-around time for exome sequencing has been reduced since its implementation, it may still take months to receive results once insurance is approved, which, as stated with discussion of trio testing above, may even be more critical when pregnancy management decisions are being made.

It is important to also consider prenatal clinical counselors' comfort levels with scenarios that would arise as a result of prenatal exome sequencing. No studies were identified that assessed counselor response to prenatal exome, but responses of this research can be compared to existing research on counselor response to prenatal chromosomal microarray (CMA) as well as the integration of exome/genome sequencing into the pediatric and adult clinics. In a 2014 study by Bernhardt et al assessing genetic counselors' perceptions and experiences with CMA in the prenatal setting,[24] 60% of respondents were comfortable with counseling on uncertain results that could result from a microarray.[24] The data from the research highlighted in the results section of this document suggests that, regardless of the test being discussed, comparable amounts of counselors, when compared to the results of the Bernhardt study, feel comfortable counseling in such situations, with 52.9% of respondents to this survey citing that they would be comfortable counseling on uncertain results for a prenatal exome. Interestingly, similar numbers of respondents expressed that they would be comfortable helping a patient make pregnancy management decisions. In the 2014 Bernhardt study, only 43% of respondents were comfortable with such a situation in the context of uncertain CMA results[24], comparable to the 44.2% of

respondents who were comfortable with helping patients to make pregnancy management decisions based on *any* results of prenatal exome sequencing. While the question in the Bernhardt study specifically addressed *uncertain* results, the comparable numbers could indicate that there is, in general, more uncertainty around exome in the prenatal setting, which would cause fewer genetic counselors to be comfortable assisting the pregnancy management decisions of patients. This is especially likely when consideration is given to the fact that 80% of respondents in the Bernhardt study were comfortable helping patients to make pregnancy management decisions in the context of a clearly abnormal CMA results,[24] much higher than the 44.2% who were comfortable with the same scenario in the context of prenatal exome. This discrepancy may also be attributed to the fact that this study was conducted *prior* to implementation of WES/TES in the prenatal setting, therefore few prenatal counselors have had access to such testing and its results in order to become comfortable with these scenarios, while the Bernhardt study was conducted *following* implementation of CMA in the prenatal setting. The factors stated as affecting opinions in this research, and the number of counselors citing these factors, are items that could be considered prior to introducing this testing in the clinic.

While most respondents were comfortable with all counseling scenarios given for both WES and TES, significant numbers of respondents still expressed discomfort with these scenarios. While genetic counselors may always have some level of discomfort with difficult scenarios such as counseling on VUS results in a prenatal setting, it is worthwhile to address this discomfort and provide tools and resources which may increase the number of counselors who are comfortable with these situations. Over 70% of respondents stated that they would be more likely to support prenatal exome if educational resources were provided before its implementation. This is congruent with existing literature assessing prenatal counselors'

responses to the introduction of CMA to this setting.[24] The study by Bernhardt et al (2014) addressed specific areas of education or training related to prenatal CMA and gauged how many respondents would be interested in such training. More than 80% of respondents in that study were interested in receiving further education on locating resources for up-to-date information on specific test results, dealing with uncertain results, and communicating abnormal or uncertain results to patients.[24]

These are scenarios identified as major areas of concern for prenatal exome as well. Perhaps, priority could be given to the factors that the majority of respondents cited. Because genetic counselors will likely be the first line of prenatal providers offering this testing, efforts could be made to utilize this information to identify and address areas of concern to best ease the process of introducing WES/TES to the prenatal setting. This effort could fall under the purview of either the organizations responsible for the education and training of genetic counselors and/or the laboratories seeking to offer this testing to their clients. Many genetic testing laboratories already offer such resources, either in the form of client and patient pamphlets for panel tests, or in the form of field specialists, i.e. genetic counselors who help to educate their clients about new testing. If laboratories wish to begin offering this testing, it may be in their best interest to provide this education (as more educated clients may be more likely to utilize the testing), but it is also in the patient's best interests, as a more educated clinical provider may be more comfortable and confident in the information they provide to their patient as well as in identifying when this testing is appropriate to offer.

4.2 LIMITATIONS AND FUTURE RESEARCH

One limitation to this study was the fact that demographic information was provided at the end of the survey, limiting the ability to determine if there were any significant factors affecting participation and completion of the survey. This decision was made to address the concern that demographic questions may 'prime' participants to respond according to a certain identity. Ultimately, those who did not complete the survey were excluded entirely from analysis. Because these participants did not reach the end of the survey, they did not complete the demographic questions and no analysis could be done on these individuals.

While 160 responses provided useful data and is a significant response rate for a field the size of genetic counseling, it by no means encompasses the entire population of prenatal and laboratory genetic counselors in North America. There may be many genetic counselors with significantly different opinions and responses not represented by this survey, which is a limitation created by any questionnaire-based research as data is limited to individuals who decide to take the time to participate and respond. Prenatal clinical counselors make up approximately 35% of all clinical counselors, while laboratory counselors make up roughly 26% of all non-clinical counselors, indicating that there are hundreds of genetic counselors whose opinions are not accounted for in this survey.[35]

While Likert-scale questions are validated as research tools, this specific survey is new and therefore has not been validated as a research tool. Some questions of the survey may have been ambiguous, which in turn may have altered the responses to these questions. One such example is the question "I would like commercial laboratories to make this testing available to clients". In this question, "clients" was meant to refer to genetic counselors and physicians who order testing from commercial laboratories. However, it is possible that clinical genetic

counselors interpreted “clients” to mean patients and therefore thought that the question was asking whether or not they would effectively support labs offering this testing in a direct-to-consumer fashion. The interpretation of this question may have altered how respondents answered the question. In addition, clinical utility, in the context of the survey, was not well defined and was therefore open to interpretation. The exact meaning of clinical utility that was intended was that prenatal exome sequencing has diagnostic value (comparable to existing prenatal diagnostic tools, as discussed in the background section of this document) and could be beneficial to patients in the clinical setting to provide answers for fetal anomalies/recurrent miscarriages. Any respondent may have had their own definition of clinical utility in mind, which would alter how they answered the question and how they discussed clinical utility as a factor influencing their opinions.

This survey was limited to current prenatal and laboratory genetic counselors because they will be the first genetic counselors dealing with this testing in the prenatal setting. Due to the flexible nature of the genetic counseling field, many counselors who are not currently in either of these roles may likely transition to one or both in the future. Future research may consider these questions in a wider scope, addressing genetic counselors in all specialties. Genetic counselors who did not meet criteria for this survey may still be involved with policy decisions about the implementation of this testing and it is therefore important to gauge their opinions as well. Future research should also consider the knowledge base and opinions of other prenatal providers such as medical geneticists and OBGYNs, as they will undoubtedly play a role in the future of exome sequencing in the prenatal setting.

4.3 PUBLIC HEALTH SIGNIFICANCE

Genetic counselors often serve as gatekeepers to important genetic testing. The American College of Medical Genetics and Genomics (ACMG) and the National Society of Genetic Counselors (NSGC) explicitly mention the importance of involving genetic counselors in the testing process in many of their policy statements, with some statements even recommending that testing not be provided without genetic counselor involvement.[9,11,10] While prenatal exome sequencing is currently offered on a limited basis only by a few genetic testing laboratories outside of a research setting, it is highly likely that this testing will be widely available in the near future.[28,29] It is therefore important to gauge the opinions of genetic counselors toward this testing as they will be the ones at the front-lines when it is implemented. WES and TES have already been shown to have utility in the prenatal setting,[27,28] and could therefore be useful in providing patients with information about recurrent abnormal pregnancies as well as risks for miscarriage, while giving more accurate predictions of recurrence risk. This research has identified several barriers that would be important to address prior to implementation of WES or TES in the prenatal setting, as well as identifying overall opinions regarding appropriate clinical situations for testing, which may help to guide policy decisions and recommendations about such testing. Addressing the issues identified by this research can help to more seamlessly introduce WES and/or TES to the prenatal setting, providing the most benefit to patients.

4.4 CONCLUSIONS

Genetic counselors' views and opinions toward prenatal exome sequencing were gauged by providing specific scenarios in which this testing might be offered, types of incidental findings that might be reported, counseling scenarios that could arise as a result of this testing, and factors associated with this testing that affected these opinions. Demographic information was collected in order to determine if any significant differences in opinions existed based on this information. More than half of respondents would at least support prenatal exome sequencing (WES or TES) after other diagnostic testing had returned as negative or inconclusive. The majority of respondents would support prenatal exome sequencing as long as it was either limited to clinically indicated cases or phenotypically-driven TES. Perceived patient ability to understand the scope of the testing and the perceived clinical utility of exome in the prenatal setting where the two largest factors influencing opinions on prenatal exome sequencing. More than 70% of respondents would be more likely to support prenatal WES or TES if educational resources were provided.

The results of this research help to identify areas of need to be addressed prior to implementation of this testing. This may help to facilitate a discussion between laboratories wishing to offer this testing and clinical genetic counselors who will be providing this testing to patients, as well as help guide management guidelines about when the testing should be offered. There are a significant number of factors that need to be considered as this testing becomes more widely available commercially, including important ethical concerns that should also be addressed.

APPENDIX A – COVER LETTER AND SURVEY

A.1 COVER LETTER

Survey - Student research project - *Genetic counselors' views and opinions on the possible implementation of prenatal exome sequencing*

I am a second year graduate student in the genetic counseling program at the University of Pittsburgh and I am conducting research to assess the views and opinions of prenatal and laboratory genetic counselors about the possible implementation of exome sequencing in a prenatal setting. This research project has been approved by the University of Pittsburgh IRB.

You are invited to participate in this study by taking part in an online survey. Counselors who are currently practicing in the prenatal clinical setting (part-time or full-time) as well as counselors currently working in either academic or commercial testing laboratories (part-time or full-time) are eligible to participate in this survey.

Because of the speed at which new genetic testing technology has previously been implemented for use in prenatal diagnosis/screening, exome sequencing could soon be available to clinicians who provide prenatal care. Your answers to this survey will help to better understand the needs and opinions of prenatal and laboratory counselors before the possible availability of prenatal exome sequencing through testing laboratories.

Please forward this survey to any genetic counselors who are not NSGC members, who fit the above eligibility criteria, and who would be interested in participating in this study.

This survey takes approximately 15-20 minutes to complete. Please follow the link below to access the survey: You may contact me with any questions regarding this survey: tnz1@pitt.edu

Thank you in advance for your participation in this study, Tricia Zion, Genetic Counseling Student, University of Pittsburgh Graduate School of Public Health.

A.2 SURVEY

Introduction and Consent

Thank you for participating in this survey. The survey will take approximately 20-30 minutes to complete. Your participation is completely voluntary and can be discontinued at any time. You may skip any questions you are uncomfortable with answering. By completing this survey, you are consenting to participate in this research study.

Terms that will be used in this survey:

Exome Sequencing

Exome sequencing analyzes only the protein-coding region of the genome. It is currently typically used for cases in which all other genetic testing options have been exhausted and no clinically significant findings have been detected. It may also be used for patients with multiple findings not consistent with any one specific syndrome. Currently, exome sequencing finds a

clinically significant genetic change in approximately 30% of cases. Similar to chromosomal microarray analysis, the large scope of this testing means there is a greater likelihood of detecting variants of unknown clinical significance. In addition, incidental genetic findings unrelated to patient phenotype may also be discovered.

Incidental Findings

Because exome sequencing looks at all of the protein coding genes, it is possible that genetic findings unrelated to the patient's clinical phenotype will be detected. The American College of Medical Genetics has issued a recommended list of 25 genes in which clinically actionable variants should be reported for all patients undergoing exome sequencing. (https://www.acmg.net/docs/IF_Statement_Final_7.24.13.pdf)

Patients are able to opt in or out of receiving incidental findings and may be able to choose specific categories of genes in which they do or do not want to receive results.

Targeted Exome Sequencing

Targeted exome sequencing allows the laboratory to focus on genes known to be associated with a patient's clinical phenotype. This essentially results in a custom-made panel that can reduce interpretation time, reduce the likelihood for variants of unknown clinical significance, and eliminates the option for incidental genetic findings. Targeted exome sequencing is currently being offered through several genetic testing laboratories and helps to reduce the length and difficulty of the consent/disclosure process for the clinical counselor.

Q1. Do you currently work in a laboratory (commercial or academic) position or a clinical prenatal position?

[ ] Laboratory position [ ]Clinical Prenatal Position [ ] I work in both laboratory and prenatal positions [ ] I do not work in either a laboratory or a clinical prenatal position

Q2. Have you ever ordered whole exome sequencing for a patient?

[ ] Yes [ ] No

Q3. Have you ever analyzed whole exome sequencing in a laboratory setting?

[ ] Yes [ ] No

Q4. If you work/have worked in a prenatal clinic – On average how much time do you spend *preparing* for each case you see?

[ ] 10-20 minutes [ ] 20-40 minutes [ ] 40-60 minutes [ ] Longer than 1 hour [ ] I have never worked in a prenatal clinic

Q5. If you work/have worked in a prenatal clinic – On average, how long do your sessions typically last?

[ ] 20 minutes [ ] 30 minutes [ ] 40 minutes [ ] 50 minutes [ ] 60 minutes or more

Q6. Please indicate how much you agree or disagree with the following statements regarding *prenatal exome sequencing.* (1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree)

Prenatal exome sequencing should be offered:

-When there are structural abnormalities on ultrasound and previous diagnostic testing (e.g. microarray) has come back negative or inconclusive

-When there are structural abnormalities on ultrasound before other diagnostic testing is offered

-In the context of providing a genetic diagnosis for neonatal management rather than pregnancy management (e.g. a diagnosis prenatally would help guide medical management decisions in the neonatal period)

-Whenever a patient expresses interest in having prenatal exome

Q7. Please indicate how much you agree or disagree with the following statements regarding *prenatal exome sequencing.* (1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree)

I would be comfortable:

-Providing pre-test counseling for exome sequencing in a prenatal setting

-Disclosing and explaining variants of unknown clinical significance

-Helping a patient make pregnancy management decisions based on the results of prenatal exome sequencing

-Locating resources (e.g. support groups, specialty clinics) for patients based on the results of prenatal exome sequencing

Q8. Please indicate how much you agree or disagree with the following statements regarding *incidental findings for prenatal exome sequencing.* (1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree)

I believe:

-Incidental findings from the ACMG recommended list should be reported

-Incidental findings for hereditary cancer genes should be reported

-Incidental findings that reveal carrier status should be reported

-Incidental findings for adult-onset conditions should be reported

-Incidental findings for childhood-onset conditions should be reported

-Patients should be able to choose from any of the above categories without restriction

Q9. Please indicate how much you agree or disagree with the following statements regarding *incidental findings for prenatal exome sequencing.* (1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree)

I would be comfortable:

-Counseling patients about incidental findings in a pre-test counseling session

-Disclosing and explaining incidental findings results in a prenatal setting

-Helping a patient to make pregnancy management decisions based on the results of incidental findings from prenatal exome sequencing

-Locating resources (e.g. support groups, specialty clinics) for patients based on incidental findings from prenatal exome sequencing

Q10. Have you ever ordered *targeted* exome sequencing for a patient?

[ ] Yes [ ] No

Q11. Have you ever analyzed a *targeted* exome in a laboratory setting?

[ ] Yes [ ] No

Q12. Which of the following would you support in a prenatal setting?

[ ] Whole exome sequencing [ ] Targeted exome sequencing [ ] Whole exome sequencing AND targeted exome sequencing [ ] Neither

Q13. Please indicate how much you agree or disagree with the following statements regarding *targeted* prenatal exome sequencing. (1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree)

Targeted prenatal exome sequencing should be offered;

-When there are structural abnormalities on ultrasound and previous diagnostic testing (e.g. microarray) has come back negative or inconclusive

-When there are structural abnormalities on ultrasound before other diagnostic testing is offered

-In the context of providing a genetic diagnosis for neonatal management rather than pregnancy management (e.g. a diagnosis prenatally would help guide medical management decisions in the neonatal period)

-Whenever a patient expresses interest in having targeted prenatal exome

Q14. Please indicate how much you agree or disagree with the following statements regarding *targeted* prenatal exome sequencing. (1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree)

I would be comfortable:

-Providing pre-test counseling for exome sequencing in a prenatal setting

-Disclosing and explaining variants of unknown clinical significance

-Helping a patient make pregnancy management decisions based on the results of prenatal exome sequencing

-Locating resources (e.g. support groups, specialty clinics) for patients based on the results of prenatal exome sequencing

Q15. Would you support genetic testing laboratories offering prenatal exome sequencing?

[ ] Yes, only if access was restricted to cases with a clear clinical benefit

[ ] Yes, only if it were a phenotype-driven targeted exome

[ ] Yes, in any situation

[ ] No, I would *not* support laboratories offering this testing in any situation

Q16. Please indicate how much you agree or disagree with the following statements about *exome sequencing.* (1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree)

-I understand the clinical utility of exome sequencing

-I feel exome sequencing could be clinically useful in a prenatal setting

Q17. Please indicate how much you agree or disagree with the following statements about *prenatal exome sequencing.* (1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree)

-I would like commercial laboratories to make this testing available to clients

-I am more comfortable with the idea of prenatal microarray than prenatal exome sequencing (whole exome sequencing or targeted exome sequencing)

-I would be more likely to support the use of prenatal exome if a medical geneticist was there to help me disclose results

-I would be more likely to support prenatal exome sequencing if educational/training opportunities were offered to help prepare for its implementation

Final Questions – the following two questions are the last exome related questions in the survey. This will include an option for you to express specific or detailed answers, after which you will be linked to demographic questions and the survey will end.

Q18. Which of the following aspects of prenatal exome sequencing contributed to your answers to this survey (Check all that apply)

-Perceived difficulty (or lack of difficulty) in properly counseling patients on the purpose of prenatal exome sequencing

-The number of genetic counselors available to handle the added workload of exome sequencing in a prenatal setting

-The amount of funding for positions to handle the added workload of exome sequencing in a prenatal setting

-The amount of time allotted for a prenatal session

-Perceived ability of patients to handle the amount of information provided from this testing

-Turnaround time of exome sequencing tests

-Perceived difficulty (or lack of difficulty) in dealing with billing/insurance issues

-Current technology (noninvasive prenatal testing, chromosomal microarray analysis) is sufficient and appropriate for identifying genetic causes of abnormal ultrasound results

-Current technology (noninvasive prenatal testing, chromosomal microarray analysis) IS NOT sufficient for identifying genetic causes of abnormal ultrasound results

-Clinical utility for exome sequencing in a prenatal setting

-Personal understanding of the clinical utility of exome sequencing

-Personal beliefs (please explain)

-Other (see below)

Q19. Are there any additional reasons you think exome sequencing should or should not be offered in a prenatal setting? (This question is optional, but responses are highly encouraged to fully encompass the scope of factors affecting opinions on prenatal exome)

D1. Gender

[ ] Male [ ] Female

D2. Age

[ ] 20-24 [ ] 25-34 [ ] 35-44 [ ] 45-54 [ ] 55-64 [ ] 65 or older [ ] Prefer not to say

D3. How long have you been working as a genetic counselor?

[ ] Less than 1 year [ ] 1-5 years [ ] 5-10 years [ ] 10 years or longer

D4. What specialties of genetic counseling have you worked in? (Check all that apply)

[ ] Prenatal (specify how many years) [ ] Pediatric (specify how many years) [ ] Cancer (specify how many years) [ ] Adult (specify how many years) [ ] Cardiology (specify how many years) [ ] Metabolic (specify how many years) [ ] Academic Laboratory (specify how many years) [ ] Commercial Laboratory (specify how many years) [ ] Other – please specify (specify how many years)

D5. What specialty of genetic counseling do you currently work in? (Check all that apply)

[ ] Prenatal [ ] Pediatric [ ] Cancer [ ] Adult [ ] Cardiology [ ] Metabolic [ ] Academic Laboratory [ ]Commercial Laboratory [ ]Other – please specify

D6. How long have you been working in your current position?

D7. In what state/province do you currently practice? (skip this question if you would prefer not to answer/your region is not on the list)

APPENDIX B – IRB APPROVAL LETTER

University of Pittsburgh

Institutional Review Board

Memorandum

To: Tricia Zion

From: IRB Office

Date: 9/2/2015

IRB#: PRO15080297

Subject: Genetic counselors' views and opinions on the possible implementation of prenatal exome sequencing

---

The above-referenced project has been reviewed by the Institutional Review Board. Based on the information provided, this project meets all the necessary criteria for an exemption, and is hereby designated as "exempt" under section

45 CFR 46.101(b)(2)

Please note the following information:

- Investigators should consult with the IRB whenever questions arise about whether planned changes to an exempt study might alter the exempt status. Use the "**Send Comments to IRB Staff**" link displayed on study workspace to request a review to ensure it continues to meet the exempt category.

- It is important to close your study when finished by using the "**Study Completed**" link displayed on the study workspace.

- Exempt studies will be archived after 3 years unless you choose to extend the study. If your study is archived, you can continue conducting research activities as the IRB has made the determination that your project met one of the required exempt categories. The only caveat is that no changes can be made to the application. If a change is needed, you will need to submit a NEW Exempt application.

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

BIBLIOGRAPHY

1. Ng SB, Turner EH, Robertson PD, et al. Targeted capture and massively parallel sequencing of 12 human exomes. *Nature*. 2009;461(7261):272-276. doi:10.1038/nature08250.

2. Rabbani B, Tekin M, Mahdieh N. The promise of whole-exome sequencing in medical genetics. *J Hum Genet*. 2014;59(1):5-15. doi:10.1038/jhg.2013.114.

3. DaRe JT, Vasta V, Penn J, Tran N-TB, Hahn SH. Targeted exome sequencing for mitochondrial disorders reveals high genetic heterogeneity. *BMC Med Genet*. 2013;14:118. doi:10.1186/1471-2350-14-118.

4. Iglesias A, Anyane-Yeboa K, Wynn J, et al. The usefulness of whole-exome sequencing in routine clinical practice. *Genet Med*. 2014;16(12):922-931. doi:10.1038/gim.2014.58.

5. Farwell KD, Shahmirzadi L, El-Khechen D, et al. Enhanced utility of family-centered diagnostic exome sequencing with inheritance model–based analysis: results from 500 unselected families with undiagnosed genetic conditions. *Genet Med*. 2015;17(7):578-586. doi:10.1038/gim.2014.154.

6. Retterer K, Juusola J, Cho MT, et al. Clinical application of whole-exome sequencing across clinical indications. *Genet Med*. December 2015. doi:10.1038/gim.2015.148.

7. Retterer K. Why trios are better for exome sequencing. *GeneDx*. 2014. http://www.genedx.com/genedx-blog/why-trios-are-better-for-exome-sequencing-april-22-2014/.

8. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med*. 2013;15(7):565-574. doi:10.1038/gim.2013.73.

9. Medicine G. ACMG policy statement: updated recommendations regarding analysis and reporting of secondary findings in clinical genome-scale sequencing. *Genet Med*. 2015;17(1):68-69. doi:10.1038/gim.2014.151.

10. National Society of Genetic Counselors : Position Statements : Incidental Findings in Genetic Testing. NSGC.org. http://nsgc.org/p/cm/ld/fid=14#incidental. Published 2013. Accessed March 7, 2016.

11. Board of Directors A. Points to consider for informed consent for genome/exome sequencing. *Genet Med*. 2013;15(9):748-749. doi:10.1038/gim.2013.94.

12. Kaphingst KA, Facio FM, Cheng M-R, et al. Effects of informed consent for individual genome sequencing on relevant knowledge. *Clin Genet*. 2012;82(5):408-415. doi:10.1111/j.1399-0004.2012.01909.x.

13. Latendresse G, Deneris A. An update on current prenatal testing options: first trimester and noninvasive prenatal testing. *J Midwifery Womens Health*. 60(1):24-36; quiz 111. doi:10.1111/jmwh.12228.

14. Nyberg D a, Souter VL. Sonographic markers of fetal trisomies: second trimester. *J Ultrasound Med*. 2001;20(6):655-674.

15. Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2015;45(1):16-26. doi:10.1002/uog.14636.

16. Bishop R. Applications of fluorescence in situ hybridization (FISH) in detecting genetic aberrations of medical significance. *Biosci Horizons*. 2010;3(1):85-95. doi:10.1093/biohorizons/hzq009.

17. NIEUWINT AWM. Rapid detection of microdeletions using fluorescence in situ hybridisation (FISH) on buccal smears. *J Med Genet*. 2000;37(6):4e - 4. doi:10.1136/jmg.37.6.e4.

18. Wiszniewska J, Bi W, Shaw C, et al. Combined array CGH plus SNP genome analyses in a single assay for optimized clinical testing. *Eur J Hum Genet*. 2014;22(1):79-87. doi:10.1038/ejhg.2013.77.

19. Wapner RJ, Martin CL, Levy B, et al. Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis. *N Engl J Med*. 2012;367(23):2175-2184. doi:10.1056/NEJMoa1203382.

20. Hillman S, McMullan DJ, Maher ER, Kilby MD. The use of chromosomal microarray in prenatal diagnosis. *Obstet Gynaecol*. 2013;15(2):80-84. doi:10.1111/tog.12007.

21. Stark Z, Gillam L, Walker SP, McGillivray G. Ethical controversies in prenatal microarray. *Curr Opin Obstet Gynecol*. 2013;25(2):133-137. doi:10.1097/GCO.0b013e32835ebb67.

22. Kiedrowski L a., Owens KM, Yashar BM, Schuette JL. Parents' Perspectives on Variants of Uncertain Significance from Chromosome Microarray Analysis. *J Genet Couns*. 2015. doi:10.1007/s10897-015-9847-3.

23. Westerfield L, Darilek S, van den Veyver I. Counseling Challenges with Variants of Uncertain Significance and Incidental Findings in Prenatal Genetic Screening and Diagnosis. *J Clin Med*. 2014;3(3):1018-1032. doi:10.3390/jcm3031018.

24. Bernhardt BA, Kellom K, Barbarese A, Faucett WA, Wapner RJ. An exploration of genetic counselors' needs and experiences with prenatal chromosomal microarray testing. *J Genet Couns*. 2014;23(6):938-947. doi:10.1007/s10897-014-9702-y.

25. Machini K, Douglas J, Braxton A, Tsipis J, Kramer K. Genetic counselors' views and experiences with the clinical integration of genome sequencing. *J Genet Couns*. 2014;23(4):496-505. doi:10.1007/s10897-014-9709-4.

26. Barajas M, Ross LF. Pediatric Professionals' Attitudes about Secondary Findings in Genomic Sequencing of Children. *J Pediatr*. 2015;166(5):1276-1282.e7. doi:10.1016/j.jpeds.2015.01.032.

27. Carss KJ, Hillman SC, Parthiban V, et al. Exome sequencing improves genetic diagnosis of structural fetal abnormalities revealed by ultrasound. *Hum Mol Genet*. 2014;23(12):3269-3277. doi:10.1093/hmg/ddu038.

28. Alamillo CL, Powis Z, Farwell K, et al. Exome sequencing positively identified relevant alterations in more than half of cases with an indication of prenatal ultrasound anomalies. *Prenat Diagn*. 2015:n/a - n/a. doi:10.1002/pd.4648.

29. Mackie F, Carss K, Hillman S, Hurles M, Kilby M. Exome Sequencing in Fetuses with Structural Malformations. *J Clin Med*. 2014;3(3):747-762. doi:10.3390/jcm3030747.

30. Facio FM, Eidem H, Fisher T, et al. Intentions to receive individual results from whole-genome sequencing among participants in the ClinSeq study. *Eur J Hum Genet*. 2013;21(3):261-265. doi:10.1038/ejhg.2012.179.

31. Lewis KL, Hooker GW, Connors PD, et al. Participant use and communication of findings from exome sequencing: a mixed-methods study. *Genet Med*. November 2015. doi:10.1038/gim.2015.133.

32. Rosell AM, Pena LDM, Schoch K, et al. Not the End of the Odyssey: Parental Perceptions of Whole Exome Sequencing (WES) in Pediatric Undiagnosed Disorders. *J Genet Couns*. February 2016. doi:10.1007/s10897-016-9933-1.

33. Kalynchuk EJ, Althouse A, Parker LS, Saller DN, Rajkovic A. Prenatal whole exome sequencing: parental attitudes. *Prenat Diagn*. 2015:n/a - n/a. doi:10.1002/pd.4635.

34. Bower MA, McCarthy Veach P, Bartels DM, LeRoy BS. A survey of genetic counselors' strategies for addressing ethical and professional challenges in practice. *J Genet Couns*. 2002;11(3):163-186. doi:10.1023/A:1015275022199.

35. Dunn K, Cohen L, Fay AM, Myers M. 2014 Professional Status Survey: Salary & Benefits. *Natl Soc Genet Couns*. 2014:1-59.