

**Whole Exome Sequencing in the Cardiac Intensive Care Unit (CICU): Characteristics,
Diagnostic Rates, Time to Diagnosis, and Impact on Treatment**

by

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Submitted to the Graduate Faculty of

Human Genetics

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2020

UNIVERSITY OF PITTSBURGH
GRADUATE SCHOOL OF PUBLIC HEALTH

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University of Pittsburgh, 2020

Abstract

Identifying the correct diagnosis in the shortest time possible is crucial for the treatment of critically ill patients. Previously, patients underwent step-wise testing, testing a single gene/gene panel at a time. Whole Exome Sequencing (WES) bypasses this process by investigating the entire coding region of the DNA. WES could shorten time to diagnosis, improve care, and decrease costs by reducing the number of tests ordered. Ordering appropriate tests is an important public health aspect of medical genetics. Studies have shown that WES is clinically useful in various settings; this has not yet been determined in the Cardiac Intensive Care Unit (CICU).

This study sought to determine the yield of non-negative results, patient characteristics, and outcomes of WES in CICU patients treated at UPMC's Children's Hospital of Pittsburgh (CHP) through a review of electronic medical records of 24 CICU patients who underwent WES. Six patients (25%) had positive results, 2 of which (33%) would not have been detected on a comprehensive cardiovascular panel. Four patients (16.7%) had negative results and 14 (58.3%) had Variant(s) of Unknown Significance (VUS).

The mean time from the initial genetics evaluation to return of results was 119.17 days, with a mean WES processing time of 92.1 days. Thirteen patients (54.2%) had cardiomyopathy,

10 (41.7%) had structural heart defects, and 1 (4.2%) had a channelopathy. Four cardiomyopathy patients (30.8%) were positive, 1 (7.7%) was negative, and 8 (61.5%) had VUS. One patient (10%) with structural heart defect was positive, 3 (30%) were negative, and 6 (60%) had VUS. The patient diagnosed with channelopathy was positive. WES directly impacted management for 13 patients (54.17%) and impacted management for the patient and/or family in 18 cases (75%).

WES may shorten the diagnostic odyssey and improve care. Previous studies have shown WES has a higher non-negative yield in certain settings, bringing to question the utility of WES in the CICU. This study showed WES has high clinical utility in the CICU, leading to changes in management for most patients and/or their relatives. Given the number of VUS requiring interpretation, the use of WES should include medical geneticists and genetic counselors.

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Preface

My time in graduate school has taken some unexpected twists and turns and I could not have done it without the support of so many wonderful people. I am forever grateful to the faculty and staff of the Human Genetics department. My MPH classmates, Jacqueline Cohen, Emma Gyurisin, and Jerome Givi, as well as my genetic counseling classmates were such an important part of my experience at the University of Pittsburgh. To my thesis committee, Lina Ghaloul-Gonzalez, Laura Jenkins, Brenda Diergaarde, and Andrea Durst: thank you for helping me to design and carry out a project that so closely aligned with my interests. To Candy Kammerer, Andrea Durst, and Robin Grubs: thank you for your support, guidance, and compassion through everything. To Lauren Winter: thank you for always being there to talk through assignments, cases, and new card games. To my family: I know these past few years have been difficult, thank you for always believing in me and encouraging me to pursue my dreams. To my dad in particular: thank you for teaching me the value of hard work and a good laugh, and for always encouraging me to figure out the answers. And finally, to my husband, Ian, who promised we could get a dog when I finished my thesis: thanks for following me to Pennsylvania.

1.0 Background

The rise of genomic medicine has been enabled by improvements in genetic testing methodologies. The development of and improvement upon early genetic tests has been a necessary part of the journey towards understanding the role of genetics in health. The use of Whole Exome Sequencing (WES) has the potential to shorten patients' diagnostic odysseys and improve medical care, but is not without its own drawbacks and limitations.¹ The clinical utility of WES has been studied in the Neonatal Intensive Care Unit (NICU) and the Pediatric Intensive Care Unit (PICU), but little is known about the utility of WES in Cardiac Intensive Care Unit (CICU) patients.

1.1 Purpose of the Study

The University of Pittsburgh Medical Center Children's Hospital of Pittsburgh (UPMC CHP) Division of Medical Genetics sees patients, both children and adults, affected by or suspected of having a genetic condition.² In 2017, the clinical genetics team at UPMC CHP had a total of 1,941 patient visits and 1,003 inpatient consultations. Genomic medicine, while a specialty of its own, plays an important role in other specialties and care settings, including in cardiology. Approximately 3% of the general population is affected by an inherited cardiac condition.³ These conditions are genetically heterogeneous and may be hard to distinguish from one another because they share many overlapping features; the use of genetic testing is helpful in establishing a diagnosis and guiding care.³ Many of these conditions exhibit incomplete penetrance,⁴ resulting in

only a portion of individuals with pathogenic variants being affected, or variable expressivity, causing even members of the same family to have different clinical presentations.⁵ WES has been shown to have a high diagnostic rate and impact on the care of infants with heart failure and has led to diagnoses that would not have been made had the patients had testing through cardiomyopathy panels instead of WES.⁶ The use of WES in pediatric cardiac intensive care unit (CICU) patients could streamline testing, leading to faster diagnosis and earlier intervention and reducing morbidity and mortality in these patients. This study seeks to characterize CICU patients who have undergone WES, the types of results obtained, time to diagnosis, and recommendations made based on WES results.

1.1.1 Specific Aim 1: To Determine the Characteristics of CICU Patients Undergoing WES

The first aim of the study is to identify and summarize characteristics of CICU patients on whom WES was completed. This will include presence of family history of similar or related symptoms, age of the patient, and type of cardiac disease. Cardiac disease will be classified as structural heart disease, channelopathy, or cardiomyopathy.

1.1.2 Specific Aim 2: To Assess the Frequency of Relevant WES Results

This study will quantify the types of WES results (negative, positive, uncertain) obtained from CICU patients overall, as well as for each cardiac disease type (structural, channelopathy, or cardiomyopathy) and whether the cardiac disease was isolated or present with other anomalies or dysmorphic features.

1.1.3 Specific Aim 3: To Determine Time to Return of WES Results

The study will calculate overall and within each cardiac disease type the time from the initial genetics evaluation to return of results. Return of results will be defined as the date the test report was released from the laboratory.

1.1.4 Specific Aim 4: To Determine Recommendations Based on WES Results

Finally, the study will identify what, if any, follow-up recommendations were made by genetics providers based on WES results. This will be determined by the genetic providers' medical notes in patients' Electronic Medical Records (EMR).

2.0 Literature Review

The field of Genetics is constantly evolving as additional disease-related genes are identified, more targeted treatments become available, and new testing methodologies are developed. Whole Exome Sequencing (WES) is starting to become more widely used in the clinical setting, replacing serial gene panel testing for patients on a diagnostic odyssey. WES may be able to provide more clinically useful information faster than previous testing strategies and save health-care dollars. However, WES is not without its own challenges and limitations, which must be considered when implementing WES in a clinical setting.

2.1 Whole Exome Sequencing

Whole Exome Sequencing (WES) can provide vast amounts of information about a patient through a single clinical test and is becoming more widely used in clinical settings. WES may reveal information related to a patient's current clinical presentation, as well as information that may not be related to immediate and known health issues, including future health risks, carrier status, and ancestry. When choosing to undergo WES, patients and providers should have a clear understanding of the possible benefits, limitations, and risks of WES.⁷

Whole Exome Sequencing (WES) can be used to look for variants that may explain a patient's phenotype through the use of large-scale sequencing. WES focuses on the exome, which is the part of the genome responsible for coding proteins. The exome accounts for only 1% of the genome⁸, but is the most likely place to find interpretable variants. The aim of WES is to sequence

the entirety of the exome. However, there are limitations to the technology and there are sections of the DNA that may not be sequenced using this method.¹

In a policy statement in 2012 regarding the clinical application of genomic sequencing, the American College of Medical Genetics and Genomics (ACMG) outlined four scenarios in which WES would be an appropriate clinical test. Patients who present with features or a family history that indicate a likely genetic etiology when there is no specific suspected disorder with an available clinical targeted test are appropriate candidates for WES. If a patient has a genetic condition known to have a large amount of genetic heterogeneity, WES can be useful since it can analyze a large number of genes at once. If a patient is believed to have a genetic condition and has undergone genetic tests for genes related to the phenotype but testing has not yet found a diagnosis, WES can be used to look for variants in other genes. Finally, WES can be used in fetuses suspected of having a genetic condition when targeted genetic tests have been uninformative; however the use of WES has significant challenges and limitations when used within the context of a pregnancy, including high false positive and false negative rates, high rates of Variants of Uncertain Significance (VUS), and limitations due to turn-around time of WES.¹

There is an intricate process involved in completing WES before results are returned to patients and acted upon by providers. Testing is typically done using a blood sample to test DNA from peripheral blood leukocytes. Saliva samples can be used if needed, but do not yield the same quality or quantity of DNA and are more likely to have some form of contamination complicating the analysis.⁹ The types of samples accepted varies between laboratories – some laboratories only regularly accept blood samples or buccal swabs, while others may accept additional types of samples, such as skin fibroblasts or purified DNA.^{10,11} Once a sample is received, DNA is extracted and sequenced, the resulting data are aligned with a human genome reference, variant calling is

done, and variants identified in the sequence are annotated. Individuals may have around 25,000 coding variants, and annotated variants must be filtered so that variants potentially related to the phenotype are prioritized. The process of WES is time and labor intensive and filtering allows laboratories to focus on genes that may be relevant to a patient's clinical course, reducing the time spent on variants that are not relevant to the reason the test was ordered. Considerations in filtering may include exclusion of known benign variants, exclusion of variants with minor allele frequencies indicative of a polymorphism (with consideration of inheritance pattern), and analysis of variant segregation within the patient's family when familial samples are included in analysis, known as "trio-WES."⁹

Because of variant filtering, not all genes sequenced in WES will be interrogated further by the laboratory or included in WES test reports. Each laboratory defines their own standards and procedures for what to include in a test report. The ACMG has developed recommendations for variants which should be included in the final laboratory report for WES.¹ Variants typically included in analysis by laboratories are variants in genes known to be associated with at least some aspect of the patient's clinical features, or variants in genes that do not have a known association with the condition but are believed to affect a pathway that may be involved in the patient's condition. Additionally, some laboratories choose to include secondary findings, which are variants in genes that are not related to the patient's current condition or features, but are associated with other health conditions.¹ Secondary findings returned to patients typically include variants in genes included on a list curated by the ACMG, which are associated with medically actionable conditions. There has been some discussion on whether or not patients should be able to decline to learn about secondary findings. The ACMG initially recommended that these findings be reported regardless of patient preference.¹² However, this specific recommendation was amended

after more than 80% of ACMG members who responded to a survey supported patients' rights to decline this information.¹³ A study by Thompson and colleagues (2018) investigated how often parents of children with developmental disabilities chose to receive secondary findings, as well as how often secondary findings were returned. Parents were given the option of receiving all, some, or none of the secondary findings. Of the 455 families in the study, 85% requested that they receive all secondary findings. Only 1.4% of parents in the study were found to have relevant variants in the genes recommended by the ACMG, which the authors noted was consistent with other laboratories, which reported relevant findings in 1-5.6% of patients.¹⁴ In addition to ACMG secondary findings, some laboratories offer to return carrier status and may have their own lists of genes they choose to include as secondary findings.¹⁵ While our study does not specifically investigate patient views on secondary findings or the prevalence of such in our patients, most of the secondary finding genes recommended by the ACMG involve cardiac phenotypes,¹³ and variants in these genes in our patients may have been included in variant interpretation since they may be related to the patients' phenotypes.

After variants have been filtered and a list of genes that may be causing or contributing to the patient's condition has been generated, the variants must be analyzed and classified according to their relationship with disease. Laboratories may begin filtering by identifying candidate genes that may be associated with the patient's phenotype. Filtering may also include consideration of whether a variant has a minor allele frequency (MAF) that suggests a polymorphism, if the variant is synonymous (does not result in an amino acid change), or if variant is present in population databases in unaffected individuals⁹ Once variants have gone through the initial filtering process, they are then classified by the laboratory according to the ACMG guidelines, which are discussed in detail in the next section.

2.1.1 Classifying WES Results

In 2013, the American College of Medical Genetics and Genomics (ACMG), the Association for Molecular Pathology (AMP), and the College of American Pathologists (CAP) developed a workgroup to address the interpretation of genetic sequence variants. The joint position statement developed by the workgroup, published in 2015, included standardized terminology, methods and criteria for variant classification, and result reporting.¹⁶

Using standard terminology across all laboratory, clinical, and research settings allows for a clear understanding of the relationship between a variant and a patients' health. While mutations are not necessarily harmful and polymorphisms are not necessarily benign, these terms are often used incorrectly. It is not uncommon for health care providers to assume that a change referred to as a "mutation" is pathogenic.¹⁶ To remedy this and avoid confusion in the future, the workgroup recommended adopting the term "variant" instead of "mutation" and "polymorphism."¹⁶ Five classifications for variants based on their relationship to a disease were proposed: pathogenic; likely pathogenic; variant of uncertain significance; likely benign; benign. This spectrum of classifications enables more precise communication about the possible effect of a given variant. A mean threshold of at least 90% certainty in regard to how the variant relates to disease is used when classifying a variant as either "likely pathogenic" or "likely benign." The workgroup did acknowledge that the majority of variants will not have enough evidence to reach this threshold.¹⁶

There are several types of evidence included when trying to classify a variant.¹⁶ The categories recommended by the workgroup are: population data; computational and predictive data; functional data; segregation data; *de novo* data; allelic data; other database; and other data. Population data includes how often an allele is found in the general population and may contain information about variant frequency in affected patients versus controls.¹⁶ Computational and

predictive data include the use of various computer modeling programs to predict what effect(s) the variant will have, such as a change in amino acid, which can be compared to known disease mechanisms. Functional data to examine known effects, such as evidence that missense mutations in a specific genetic locus are common and typically pathogenic. Segregation data refer to how the variant segregates with the disease/phenotype in a family, with increased co-segregation increasing the likelihood of pathogenicity. *De novo* data factor in whether the variant is a new mutation that was not inherited (*de novo*) in the patient or if it is also found in the biological parent(s).¹⁶ For *de novo* data, it is important to know if the parents are symptomatic or asymptomatic. The importance of including parental samples is discussed further in 10.2. Allelic data indicate whether alleles are found in *cis* or *trans*, which is particularly important for autosomal recessive conditions.¹⁶ Other databases, such as disease-oriented databases (e.g. the Human Gene Mutation Database (hgmd.org) or sequences databases (i.g. RefSeqGene (ncbi.nlm.nih.gov/refseq/rsg)), can also be useful to find evidence of a variant being benign or pathogenic.¹⁶ Data about other known cases or the specific patient should also play a role in classification. When interpreting variants, the workgroup developed two separate criteria, one set for determining if a variant is pathogenic or likely pathogenic, and another set for determining if a variant is benign or likely benign with clear guidelines to weight these factors in classifying variants.¹⁶ If a variant does not meet any of the criteria or the criteria contradict each other, a variant is classified as having uncertain significance.¹⁶

While these guidelines do exist to standardize variant classification, there may still be discrepancies in interpretation between different laboratories, as well as between laboratories and providers. ClinVar (ncbi.nlm.nih.gov/clinvar) is a publicly available database for information regarding how genetic variation relates to human health.¹⁷ When looking at the discordance

between different laboratories and entries in ClinVar for cardiovascular genetics, Bland and colleagues (2018) found a discordant rate of 82.5%, which was similar to the discordant rate between providers and laboratories in this study.¹⁸ Bland showed that, in the cardiovascular genetics setting, providers' classification of variants differed from the laboratories' classification in 18% of cases (126 out of 688 variants), with providers being less likely to classify variants as pathogenic or likely pathogenic ($P < 0.00001$).¹⁸ The discordant rate ranged from 3-33% depending on the laboratory and 83% of these discrepancies would have had clinical implications.¹⁸ Classification of variants is challenging in the cardiovascular setting due to factors such as reduced penetrance, phenotypic variation, or the presence of multiple variants in cardiovascular genes in a single affected individual.¹⁹ Data sharing between laboratories can improve concordance in variant classification, however it is important that providers review genetic test reports and available data to determine if they agree with the classification of variants and make recommendations or changes in care accordingly.

2.1.2 Generalized Diagnostic Yield of WES

As the field of genetics continues to grow with the development and spread of new testing technologies, it is important that genetic tests be evaluated critically to ensure that the intended benefits are reflected in the outcomes of these tests.²⁰ This may include study into the clinical utility of tests, or how likely a test is to lead to improved outcomes.²⁰ Many studies have sought to determine the clinically utility of WES, including the diagnostic yield, in a variety of settings.²¹ WES may be useful for establishing a diagnosis for patients who may have conditions with a large number possible causative genes or for conditions for which the causative gene may be unknown.²²

Yang and colleagues (2013) completed a study of 250 patients with varying phenotypes who had undergone WES. These patients were selected as a convenience sample (the first 250 patients to have WES completed in the group's laboratory) and there was not a specific disease type or patient age targeted. Eighty percent of the patients were children who were being evaluated due to some form of neurological condition. All patients had had some form of genetic testing prior to undergoing WES, such as a chromosomal microarray, and more than half (61%) of the WES tests were ordered by a geneticist. In 75% of the cases, parents also submitted samples to aid in interpretation. The study found an overall diagnostic yield of 25% (95% CI: 20-31). Thirty-three patients were identified as having an autosomal dominant condition, 16 patients were identified as having an autosomal recessive condition, 9 patients were found to have an X-linked condition, and 4 patients were found to have 2 separate genetic conditions. Medically actionable secondary findings were detected in 12% of the patients (30 patients) and 5% (12 patients) were identified as carriers of autosomal recessive conditions included as a screening measure.²³

In a similar study, Lee et al. (2014), investigated the diagnostic yield of WES in patients thought to have genetic conditions, but who had not yet received a diagnosis. Like the Yang study, inclusion in the study was not based on a particular condition. Instead, the study population consisted of the first 814 undiagnosed patients to have WES performed at the University of California, Los Angeles, Clinical Genomics Center. Most (64%) of the patients included in the study were under the age of 18 years and the most common reason for referral across all ages was developmental delay (37% of the patients, 298 patients). In children specifically, developmental delay was the reason for referral for 53% of patients (274 patients). The most common reason for referral for adults was ataxia (26%, 95% CI: 21-32%; 77 patients). The overall diagnostic yield for all patients in the study was 26% (95% CI: 18-27%; 213 patients), with the highest diagnostic yield

being 48% (95% CI: 32-65%; 15 patients) for patients with retinal disorders. Developmental delay had a diagnostic yield of 28% (95% CI: 23-33%; 83 patients) for all cases including developmental delay as a feature. Of the 69 patients with developmental delay and autism, 16% (CI:9-27%; 11 patients) were given a molecular diagnosis. Among patients experiencing developmental delay who also had dysmorphic features, the diagnostic rate of 31% (95% CI: 24-39%; 44 of 144 patients). The difference in diagnostic rates between patients with developmental delay and autism and patients with developmental delay and dysmorphic features was statistically significant ($P=0.03$). When comparing patients whose parents or other family members had also submitted samples for analysis (“trio-CES”) with patients whose samples were submitted alone (“proband-CES”), the trio-CES group had a diagnostic yield of 31% (95% CI; 27-36%; 127 of 410 patients) and the proband-CES group had a diagnostic yield of 22% (95% CI; 18-27%; 74 of 338 patients). The higher diagnostic rate for the trio-CES group compared to the proband-CES group was statistically significant ($P=0.003$). The difference in trio-CES and proband-CES diagnostic yields was even more striking when comparing patients under the age of 5 years old. Trio-CES for patients under 5 years old had a diagnostic yield of 41% (95% CI, 32-51%; 45 of 109 patients), while the diagnostic yield for children in the same age range who underwent proband-CES was 9% (95% CI, 1-28%; 2 of 23 patients). The difference in diagnostic yields was statistically significant ($P=0.002$).²⁴ Lee showed that WES may be particularly useful in younger patients when parental samples are available for analysis.²⁴

A study of 500 probands who underwent WES was completed by Farwell and colleagues in 2015. Probands could either be sequenced alone (141 patients), with 1 first degree relative (21 patients), or with 2 first degree relatives (338 patients). The mean age of the probands was 11.21 years, ranging from prenatal testing to a proband age of 84 years old. The most common clinical

indication for WES was intellectual disability and/or developmental disability (64.4%, 322 patients), 32.6% (105 patients) of whom received a genetic diagnosis via WES. The highest diagnostic rate (44.1%, 26 of 59 patients) was seen in patients with ataxia. Fifteen (3%) patients were referred for WES due to oncologic concerns, which had the lowest diagnostic rate, with 1 patient (6.7%) receiving a genetic diagnosis. Overall, the study observed a positive result in 38.7% of the patients, which included both genes known to be associated with disease, as well as findings in novel genes, or genes not currently associated with a Mendelian genetic condition. One hundred, fifty-two (30.4%) patients had a positive or likely positive result in a gene known to be associated with a Mendelian condition. Uncertain results were returned for 8.6% (43) of patients and 51.7% (215) of patients had a negative result.²⁵ Clinical utility may differ based on specialty or indication, but this study shows that there may be utility for WES in the general patient population.

Performing WES on trios can be useful in interpreting variants found in the proband by determining if a variant is inherited or *de novo*, as well as determining if variants, particularly in recessive conditions, are in *cis* or *trans* with other variants in the same gene. Studies discussed above show the utility of trio WES compared to proband only (singleton) WES. Stark and colleagues (2016) studied the utility of singleton WES as a first-tier test in infants thought to have a monogenic condition. Eighty patients were included in the study, 48 (57.5%) of whom were given a molecular diagnosis related to their phenotype based on WES. While WES was being done on a research basis, standard clinical investigation, including genetic testing, continued in parallel. Standard investigation, without the use of WES, determined a diagnosis for 11 (13.75%) of patients. However, there were 20 genetic tests that were considered but not completed due to issues such as cost of testing or lack of available testing for specific genes. Had these additional tests

been completed, the non-WES diagnostic rate would have been higher (27.5%), but still would have been less than half the rate of WES.²⁶

A larger study of the clinical utility of WES was published by Retterer and colleagues in 2016. This study included 3,040 consecutive patients for whom WES was done in the same clinical laboratory. Seventy-six percent (2,308) of these cases included 2 or more familial samples along with the patient's sample. The most common indication for WES was nervous system abnormalities, which was an indication in 35.6% (1,082) of the patients. Overall, 28.8% of the patients were given a definitive diagnosis based on WES results and 51.8% were found to have a result that was considered a possible or probable diagnosis. When comparing diagnostic yield between cases with only the proband's sample versus cases in which the proband and 2 family members had submitted samples, the diagnostic yield was again significantly higher ($p < 0.01$) when familial samples were included. Proband-only cases had a diagnostic yield of 23.6% while cases with trio analysis had a diagnostic yield of 31%. Twenty-eight patients were found to have 2 or more different genetic conditions. The highest diagnostic yield for definitive diagnosis was 55% in cases where patients had hearing problems, although it should be noted that this group was relatively small ($n=11$). Central nervous system disorders had a diagnostic yield of 31%; neurological diagnoses such as intractable seizures, hypotonia, or ataxia, had a diagnostic yield of 30%.²⁷

WES has clinical utility across a spectrum of medical specialties and indications. The studies discussed above had an overall diagnostic yield (including positive and likely positive results) between 25% and 30.4%. When also including results that could be possible or probable diagnoses, this proportion increases to as much as 51.8% of patients. These studies also show that some indications may be more likely to have a diagnostic WES result than others. For instance,

Lee et al. found a diagnostic yield of only 16% for patients with developmental delay and autism, while Retterer et al. found a 55% diagnostic yield for patients with hearing problems. Additionally, the presence or absence of familial samples, most often parental samples, is important to the diagnostic yield, as evidenced by the difference in diagnostic yield for patients under the age of five years who underwent trio-CES (diagnostic yield=41%) and those whose samples were submitted on their own (diagnostic yield=9%).²⁴ WES can be a valuable tool for patients and providers, however the type of condition, presence or absence of comorbidities or other features, and the availability of familial samples to provide context for variant analysis play a role in the likelihood of finding a diagnosis.

2.1.3 Other Benefits of WES

The use of WES is beneficial over the use of gene panels, even large panels, because of the ability to reanalyze the data as new symptoms develop or as new disease-associated genes are identified.²⁸ This allows for an extensive number of genes to be included in the analysis, reducing the need for ordering providers to identify a subset of specific genes thought to contribute to a patient's clinical features.²² Larger gene panels help reduce how specific a provider needs to be in predicting which gene or genes are involved, but still impose limitations not seen in WES. The use of a panel is limited only to the genes included to the original panel; if a new gene of interest is later added to the panel, testing would need to be redone for the patient. When WES is used, in contrast, the addition of new symptoms or new genomic knowledge does not require new sequencing, but instead requires new analysis.²⁸ Wenger and colleagues (2016) found that reanalyzing WES results lead to a definitive diagnosis in 10% of patients who had previously had non-diagnostic WES results. Patients had undergone the initial WES an average of 20 months prior

to reanalysis. One of the patients included in the Wenger study was diagnosed with Wiedemann-Steiner syndrome due to a missense variant in *KMT2A* after WES reanalysis. The first paper to linking *KMT2A* to Wiedemann-Steiner syndrome was published 2 weeks after the patient's nondiagnostic WES report, and information about *KMT2A* mutations was added to the Human Gene Mutation Database (HGMD) the day the paper was published.²⁹ When the patient had their WES results reanalyzed, the understanding of the role *KMT2A* plays in disease development had changed, and the patient was finally diagnosed without needing to do additional genetic testing. The ability to reanalyze WES without having to retest a patient is a key benefit that is not seen with single-gene or gene panel testing, and may be necessary with the ever-growing genetic knowledgebase.

In addition to reanalysis, WES has the ability to make multiple diagnoses in the same patient. A small subset of patients may have features suspicious for “multiple potentially relevant findings,” or MPRF.³⁰ These patients may have features that do not fit a single, unifying diagnosis, which may be due to an expanding clinical phenotype of one condition or the presence of multiple conditions.³⁰ Smith and colleagues (2019) completed a study on 7,698 patients who underwent WES through the commercial laboratory Ambry, 23% of which (1,792 patients) received at least one diagnosis.³⁰ Of those receiving a diagnosis, 8.5% of patients (153 patients) received multiple diagnoses, 11 of whom had variants in 3 genes.³⁰ WES has the ability to detect multiple diagnoses, which may or may not have overlapping clinical features, although it may not be possible to predict which patients will have multiple diagnoses.³⁰

Many patients have had multiple genetic tests, incurring significant health care costs, prior to undergoing WES.³¹ Initiating WES earlier in care may be cost effective because it has the potential to reduce the number of uninformative genetic tests that would ultimately be

encompassed within WES, as well as reducing the use of other clinical diagnostic tools such as biopsies or various imaging methodologies.³¹ Stark and colleagues (2017) examined the cost of per diagnosis in 40 patients at a children’s hospital in Australia; patients were included because they had previously been enrolled in a study regarding the clinical utility of WES.³² The study found that the cost of each positive diagnosis through standard care was approximately US\$21,099; while the cost of each diagnosis made through singleton WES was US\$3,937.³² It is challenging to quantify the cost effectiveness of WES because improvements in technologies, such as algorithms used to sort variants, continues to lead to a reduction in cost.³¹

2.1.4 Limitations of WES

WES yields a large amount of data that must be carefully considered before any clinical action can be taken. This requires a combination of bioinformatic tools, variant databases, and human interpretation. While ACMG provides guidelines on how to classify variants, this is still a labor-intensive process that must be done for each patient. One challenge in the interpretation of WES data is that data may be spread out among different sources, such as multiple journal articles, scattered throughout different publications and different years.³³ There are efforts to create and promote the use of shared variant databases so that clinical data about specific variants can be more readily shared amongst the scientific community. This includes databases and repositories such as the NIH-designated data repositories dbGaP, Decipher, and ClinVar, which include information about variant function and clinical effects of those variants.³³ Other countries have developed larger networks for laboratories to share variant information. A system known as “SymBioSys” is used in Belgium to create a network between laboratories to share sequencing data; data can then be aggregated by study parameters or specific variant. Sharing within a country

is helpful, however, a system that allows for efficient, international clinical data sharing would be a larger improvement still. Some of the international efforts include the use of GeneMatcher (genematcher.org) and VariantMatcher (variantmatcher.org), which are freely accessible web sites designed to enable connections between clinicians and researchers from around the world who share an interest in the same gene or genes as well as same variant or variants with the ability to include phenotypic information in the search, widening the results to functionally-similar variants rather than just the specific variant of interest.³³ An additional challenge lies in laboratories' use of proprietary databases. These databases may also contain data pertinent to clinical interpretation of genetic variants, but may only be accessed by the laboratory that owns the database. Some commercial laboratories do contribute to public databases, such as ClinVar, but do not typically include specific information about the data used to come to their conclusions.³³ The time and labor that goes into researching variants for classification is an important limitation of WES, and accuracy of interpretation could be affected if not all the information related to a variant is accessible. The principal goal for public databases is to ensure information is available is to help classify variants and subsequently increase diagnostic yield in WES and other genetic testing.

Even with the recent efforts to share data to improve interpretation across the board, there are still differences between the laboratories performing WES. This may include technical aspects of the actual sequencing, such as coverage of specific genes. It may also include differences in interpretation. As laboratories continue to test samples, they are able to build their own, proprietary databases by compiling data from genetic testing done on patient samples. Differences in databases can lead to differences in interpretation- if a laboratory has tested multiple samples for patients with a given phenotype and have found the same or functionally similar variant in each of those patient samples, this may be seen as evidence pointing towards pathogenicity of the variant. On

the other hand, if a variant has not previously been detected in patients with a given phenotype by a laboratory and the variant is, therefore, not in the laboratory's database, the lack of previous cases will make it harder to classify and may result in a VUS.³⁴ In reviewing more than 6,000 variants in the ClinVar database that had been classified by at least 2 of 4 laboratories participating in the study, Harrison and colleagues (2017) found that 88.3% of variant classifications were concordant between laboratories.³⁵ After the discordant variants were identified, laboratories reviewed these to see if their classification had been updated within the respective laboratory based on more recent data; laboratories identified subsets of discordant variants, shared internal data about these variants between laboratories, and discussed the rationale behind the classifications. Each laboratory then independently reviewed the classifications and updated them accordingly, which led to a new concordance rate of 91.7%.³⁵ Concordance rates of variant interpretation between laboratories can be improved by data sharing.

Patients and providers who are not familiar with genetic testing may, understandably, assume that WES encompasses all information about a patient's exome and will be the last genetic test needed. Even before WES is reported to the ordering clinician, additional genetic testing is typically done in the form of Sanger sequencing to confirm variants. There are also types of genetic variation that are not detected with WES. WES does not detect copy number variation (CNVs), larger insertions/deletions (InDels),³³ structural rearrangements that conserve base sequence, changes in methylation, intronic variation, or tri-nucleotide expansion.³⁶ WES may not be able to detect low-level mosaicism.²⁸ Following WES, a patient may still need an array CGH, deletion/duplication studies, methylations studies, tri-nucleotide expansion testing or other testing. Familial testing to try to determine pathogenicity of variants may be pursued if a VUS is found, but is often not covered by health insurance. Laboratories do, in some cases, offer familial testing

for a VUS at no cost, but this is not routinely available. Additionally, if a provider does want to pursue familial testing for a VUS, family members may be unavailable or unwilling to provide a sample.³⁷ The return of a VUS can complicate clinical management and create feelings of anxiety and distress for patients.³⁷

Finally, while the aim of WES is to sequence protein coding regions in all known genes, analysis is directed to a subset of genes related to the patient's current clinical features and past medical history and to the variants found in these genes. If a patient develops a new symptom after WES has been completed, there may be genes left out of the analysis and final report, which could mean missing a diagnosis. Providers are able to request a reanalysis of a patient's WES if new symptoms have developed or after a period of time has lapsed to allow for new discoveries. Laboratories often offer one free analysis per patient, so providers must be careful in when they choose to request reanalysis.²⁹

2.2 WES in Specialty Medicine

Genetic conditions play a role in the morbidity and mortality of the pediatric population.³⁸ Gonzaludo and colleagues (2019) sought to measure the burden of genetic conditions on the pediatric population through the analysis of inpatient discharges among pediatric patients in the United States in 2012.³⁹ The study analyzed the 2012 Kids' Inpatient Database (KID) to determine the proportion of discharges which involved genetic conditions, as determined by identifying ICD-9 codes associated with potential genetic conditions. National and regional estimates of discharges were determined by weighting KID data, result in an estimated 5.85 million inpatient discharges of patients under the age of 18 years old.³⁹ The authors estimated that genetic conditions were

associated with 2.6-14% of inpatient discharges; discharges associated with genetic conditions were associated with higher numbers of diagnoses and medical procedures than those not associated with genetic conditions ($P < 0.0001$).³⁹ Discharges with genetic conditions were also associated with total costs that were \$16,587 higher than discharges without genetic condition, illustrating that genetic conditions have a large impact in both the number of inpatient visits, and subsequent discharges, and health care costs in the pediatric population.³⁹

Through a review of medical records, Stevenson and Carey (2003) analyzed deaths of patients at the Primary Children's Medical Center in Utah, between 1994 and 1998. They found that 34.4% of all deaths (180 out of 523 deaths) at the Primary Children's Medical Center in that time period were related to malformations and/or a presumed genetic condition.³⁸ An additional 2.3% of patients who died in the NICU had malformations and/or genetic conditions, but died of causes other than the malformation or genetic condition. The study by Stevenson and Carey illustrates the importance of genetic factors in the development of disease and, ultimately, the mortality in the pediatric setting, even in cases when a specific genetic etiology has not yet been identified.³⁸ Genetic testing to identify underlying genetic causes can identify new diagnoses and confirm suspected diagnoses, shedding light on causes of mortality. With the increase in genomic knowledge and advances in technology, many of the individuals classified as having died from non-genetic causes may have ultimately been diagnosed with genetic conditions had they been born today. WES can be used to test for a multitude of conditions concurrently, saving the time and cost of multiple genetic tests, such as sequential genetic panels. As seen in the Yang and Retterer studies previously discussed, there may be indications for which WES has a higher diagnostic yield, making WES more clinically useful. In the Yang study, retinal diseases had a higher diagnostic yield than other indications,²³ while Retterer found the highest diagnostic yield

to be in cases of hearing loss.²⁷ The use of WES has been studied in the context of a variety of medical concerns, including neurodevelopmental disorders and other neurological disorders.

2.2.1 WES in Patients with Neurological Indications

Neurodevelopmental conditions, or developmental disabilities, are conditions which affect different aspects of daily living, including learning, behavior, or the ability to perform self-care tasks.⁴⁰ According to the Centers for Disease Control (CDC), approximately 7% of all children in the United States have, at some point in their lives, been diagnosed with some form of neurodevelopmental disorder.⁴⁰ The term “neurodevelopmental condition” includes a wide variety of different conditions, many of which include spectrums of severity. The phenotypic and genetic heterogeneity can make it difficult to pinpoint genetic causes in individuals with neurodevelopmental conditions. Chromosomal microarray (CMA) is the current first-tier test for these patients,³⁶ even though CMA does not detect single gene mutations, balanced chromosomal rearrangements, trinucleotide expansion disorders, or low-level mosaicism.²⁸

Neurologists may see patients with a wide variety of conditions and symptoms, including patients with seizures, hypotonia, or developmental delays. A study (Nolan and Carlson, 2015) of 135 patients evaluated for WES at the University of Michigan Hospital System’s Pediatric Neurology clinic looked at the uptake and utility of WES.³⁶ Of those evaluated, 53 patients underwent WES. Slightly more than half (51%) of the patients who did not undergo WES did not have the testing due to insurance denial. The primary indication in patients who received WES was developmental delay, impaired cognition, or Autism Spectrum Disorder, with 88% of patients falling into this group. Analysis of WES results was restricted to the 50 patients (out of 53) for whom results were available. The diagnostic rate of WES was 48%, which included 15 individuals

with pathogenic variants and 9 individuals with likely pathogenic variants. VUS or other variants that did not fit the phenotype were found in 44% (22) of patients. Nolan and Carlson compared this to a 25% diagnostic rate for pediatric neurodevelopmental disorders in individuals who were diagnosed through other tools, such as chromosomal microarray, karyotype, or based on clinical features, and who did not have WES completed.³⁶

In a retrospective study, Soden and colleagues (2014) analyzed the use of WES in children with neurodevelopmental disorders who had not yet had an underlying genetic etiology identified.⁴¹ Included in the study were one hundred families, totaling 113 patients, 103 of whom underwent WES. WGS was completed for 6 children who had negative WES results; 16 children were acutely ill and underwent rapid WGS. The most common presentation was global developmental delay or intellectual disability (seen in 52 children), followed by seizures (seen in 39 children). The combined diagnostic yield for exome and genome sequencing for the study was 45%, with 53 children receiving a molecular diagnosis. Nine additional families were identified as having mutations in potential candidate genes. *De novo* mutations accounted for 51% of diagnoses and were present in 62% of families without a prior history of neurodevelopmental disorders. Patients who had intrauterine growth restriction or failure to thrive were more likely to receive a diagnosis than those who did not ($P < 0.05$). Older children whose conditions were non-acute had had significant genetic testing prior to undergoing WES, with this group having had an average of 13.3 prior genetic tests, including panels. The number of prior genetic tests in the non-acute group ranged from 4 to 36 prior tests. The acute group had an average of 7 prior genetic tests, ranging from 1 to 15.⁴² Soden and colleagues showed that children with neurodevelopmental disorders often have *de novo* mutations and can benefit from the use of WES, which has a high diagnostic yield and can reduce the number of uninformative genetic tests performed.

Srivastava and colleagues (2019) completed a meta-analysis of 30 different studies to compare diagnostic rates of CMA and WES in individuals with neurodevelopmental disorders as part of their work to develop a multidisciplinary consensus statement.²⁸ The meta-analysis did not include individuals with multiple congenital anomalies, unless there was also a diagnosis of Global Developmental Delay, Intellectual Disability, or Autism Spectrum Disorder. Select articles are discussed in more detail in this section. Overall, Srivastava et al. found a diagnostic yield of 36% (CI: 30-43%) for WES of individuals with isolated neurodevelopmental disorders; they found a diagnostic yield of 53% (CI: 41-46%) for WES of individuals with neurodevelopmental disorders and an additional condition. The overall range of diagnostic yield of WES was 30-43%. Comparing this diagnostic yield for WES to a 15-20% diagnostic yield for CMA, Srivastava's group recommended that WES, including samples from both of the proband's parents, be used as first-tier testing in individuals with unexplained neurodevelopmental disorders. If WES is non-diagnostic, they recommend then moving on to CMA and periodic WES re-analysis, as well as other testing (such as Fragile X or metabolic testing) if necessary.²⁸ This recommendation of having WES as a first tier test for neurodevelopmental disorders is a paradigm shift in how the medical field is moving in regards to genetic testing and diagnosis.

2.2.2 WES and Fetal Anomalies

There is concern for a genetic etiology in patients presenting with multiple congenital anomalies. Patients may have a constellation of features not consistent with or explained by a single genetic condition, or they may undergo genetic testing for suspected conditions only to receive negative results. Tools such as prenatal ultrasounds, fetal echocardiograms, and amniocentesis are used to try and identify congenital anomalies prenatally in an effort to make a

diagnosis prior to birth. CMA is often used in cases of multiple fetal anomalies, although only 10% of families receive a diagnosis using this method.⁴³ WES may be able to provide a genetic diagnosis before the patient is ever born. However, collecting information about fetal phenotype is challenging and may limit the ability to interpret WES results.⁴⁴

Meier and colleagues (2018) completed a study of 19 families, which included 26 fetuses and 1 child who were found to have severe anomalies on ultrasound, to learn more about the clinical utility of WES in the prenatal setting.⁴³ CMA was negative for each fetus. WES identified variants that could potentially explain the anomalies in 12 families. Three of these families had twin pregnancies in which both twins were affected and shared the same variant, but had different phenotypic presentations. Variants were primarily autosomal recessive, although 3 were *de novo* in autosomal dominant genes and one was an X-linked recessive condition in a male. The authors note that the preponderance of autosomal recessive conditions in the study may be due to how participants were selected since families with a recurrent phenotype were specifically included in the study, or due to the possibility that autosomal recessive conditions may be tied to earlier lethality.⁴³ The Meier study does show that there is likely utility in WES in the prenatal setting when there is a recurrence of a phenotype within a family or when there are multiple anomalies that may cause early death.

A study by Petrovski and colleagues (2019) further investigated the use of WES in fetuses with structural anomalies.⁴⁵ The study enrolled couples whose fetus had been identified as having any structural anomaly on an ultrasound between 11 and 35 weeks of pregnancy, and for whom there were no known teratogenic exposures or family histories of genetic conditions. If a CMA or karyotype established a diagnosis, or if the ultrasound was suggestive of Down syndrome, the fetus was excluded from the study. Samples from both parents were also required for participation in

the study. A diagnosis was reached in 10% (24 out of 234) of the fetuses using trio WES, 63% of which were due to *de novo* mutations. Fetuses with multiple anomalies had a significantly higher diagnostic yield than those with only 1 anomaly (19% and 6%, respectively; $P=0.005$). Fetuses whose lymphatic or effusion systems were affected had a diagnostic yield of 24%, as did fetuses whose skeletal system was affected. Fetuses with cardiac anomalies had a diagnostic yield of only 5%. While WES led to a diagnosis for some families, the diagnostic yield reported by Petrovski and colleagues is lower than seen in other settings. This may be due in part to the study design, since a wide range of anomalies were included in the study. Studies with very specific inclusion criteria for the type of anomalies can be designed to better target phenotypes known to be related to genetic conditions, thereby increasing the likelihood of having a high diagnostic yield. The low diagnostic yield in this study may also be due to the limitation of fetal phenotyping previously discussed.⁴⁵

2.3 WES in the NICU

Since 1965, babies with serious, life-threatening conditions have received specialized care in Neonatal Intensive Care Units (NICU).⁴⁶ Today, NICUs continue to see an increase in the number of admissions. In 2012, the admission rate of newborns weighing at least 500 grams to a NICU was 77.9 admissions per 1,000 live births. This was a 22% relative increase from 2007, when the admission rate was 64 admissions per 1,000 live births ($P<0.001$).⁴⁷ In 2013, the United States had an infant mortality rate of 5.96 deaths per 1,000 live births. Collectively, congenital malformations, deformities, and chromosomal abnormalities are the leading cause of infant deaths, with a rate of 121.5 deaths per 100,000 live births. The neonatal period, defined as the first 28 days

of life, had a morality rate of 4.04 deaths per 1,000 live births.⁴⁸ It is difficult to estimate the actual incidence of genetic conditions responsible for NICU admissions since genetic evaluations or testing are not always done during admission.⁴⁹ However, given the large role genetics plays in neonatal deaths, as well as the rise in NICU admissions, genomic technologies may be particularly important in the rapid diagnosis and treatment of NICU patients.

2.3.1 Genetic Evaluation and Morbidity in NICU Patients

The potential for an increased disease burden of genetic conditions in the NICU compared to other health care settings may make the NICU a more appropriate place for routine use of WES. Wojcik and colleagues (2018) studied infants who had been in the NICU and who did not live past 5 years old in order to better understand the genetic diagnostic process.⁵⁰ Of the 2,670 infants admitted into the NICU between January 1, 2011, and December 31, 2015, 170 passed away before the age of 5 years old and were subsequently included in the study. Ninety-three (55%) patients died while in NICU. Eighty-seven (51%) patients had had a genetics or metabolism consult while in the NICU. The 2 most common reasons for consultation request being multiple congenital anomalies, which accounted for 53% (46 patients) of the consults, or suspected metabolic condition, which accounted for 21% (18 patients) of the consults. Ninety-six (57%) patients included in the study had 1 or more genetic or metabolic tests performed.⁵⁰

Wojcik and colleagues found that 28% (47 patients) of the patients had a confirmed genetic diagnosis; 30% (14) of those diagnoses were made after death. Single gene testing was more frequently performed than any other test, with 47 patients having a total of 94 gene panel tests; the second most common test was chromosomal microarray, with 51 microarrays completed. Single gene testing had only a 13.3% diagnostic yield. However, because single gene testing was more

frequently used, it was also the most common method that lead to a molecular diagnosis with 13 of the 40 diagnoses (33.33%) established by a single gene test. Twenty patients had a total of 25 gene panels, with a diagnostic rate of 16%. Positive results were returned for 20% (6 out of 30) of karyotypes, 22% (2 out of 9) of FISH studies, 16% of chromosomal microarrays, 19% (5 out of 27) of deletion and duplication studies, and 14% (1 out of 7) of mitochondrial gene tests. All methylation and triplet repeat studies returned negative results. WES or WGS returned a positive result in 43% of cases, a negative result in 29% of cases, and VUS in 29% of cases.⁵⁰

Often a key focus of and reason behind pursuing genetic testing is to determine a diagnosis so that the patient can be managed appropriately. A high proportion (94%) of patients in in Wojcik's study who had a laboratory-confirmed genetic diagnosis prior to their death had their care redirected towards end-of-life comfort care after receiving the confirmed genetic diagnosis. However, this was not significantly different from those who did not receive a confirmed genetic diagnosis prior to death (81%, $P=0.32$). The study did not clearly describe if the patients without confirmed genetic diagnoses had been clinically diagnosed.⁵⁰

2.3.2 Medical Management

For many NICU patients, serial gene testing is not efficient enough to be useful in NICU management. This testing strategy also requires providers with intricate knowledge of which genes may be associated with a patient's phenotype. Some conditions have variable expressivity, incomplete penetrance, more subtle associated features, or may not be expected to fully manifest until later in life, which can complicate the diagnostic process when using serial testing. WES can consolidate multiple genetic tests for monogenic disorders into a single test, which can affect decisions about care, both in the long and short term.⁴⁹

A recent study (Meng et al., 2019) was done on the clinical utility of WES in 278 patients who had been admitted to Texas Children's Hospital of Houston within the first 100 days of life. Patients underwent proband-only exome, trio exome analysis, or critical (rapid) trio exome as part of their clinical care.⁵¹ The majority of patients (68.3%) were in the NICU at the time WES was initiated. The study did not delineate results based on service setting or type of condition. There was not a specific condition for inclusion in the study, but rather inclusion was based on the patient being less than 100 days old, had been referred by Texas Children's Hospital for WES during the study period, and underwent WES through Baylor Genetics in Houston, Texas. The study found an overall diagnosis rate of 36.7%, with 102 infants being diagnosed with a total of 106 conditions.⁵¹ Four additional infants received partial diagnoses, but were not considered as receiving a positive diagnosis in the analysis since not all of the symptoms could be explained by WES results. Critical trio exome, which is the same testing but given higher priority and a shorter turn-around time, yielded the highest proportion of patients with a positive diagnosis, with 50.8% (32 of 63) of patients receiving a positive diagnosis. Several of the patients (38%; 39 of 102 patients) who received a diagnosis were reported to have had an atypical or otherwise unrecognized presentation of their disorder. Ten percent of those with an atypical presentation were only diagnosed after WES reanalysis. In patients receiving a diagnosis, medical management was directly affected by WES results 52% of the time. A diagnosis led to a change in the medical management in 71.9% (23 of 32) of the patients who underwent critical trio exome. Comparatively, proband-only and non-critical trio exome analysis combined led to a change in the medical management of 42.9% (30 of 70) of patients in either of these groups. This difference was statistically significant ($P < 0.01$). Of those who did not receive a positive result, 88% underwent chromosomal microarray and 50% underwent mitochondrial genome sequencing, however no

additional diagnoses were made. Meng's study shows that there is clinical utility in the use of WES, particularly in the care of critically ill infants admitted to the hospital at a young age.⁵¹

2.4 WES in the PICU

Children may be admitted to the PICU for a variety of medical concerns, both acute and chronic. One study found that the majority (67.1%) of patients admitted to the PICU had 1 chronic health condition and nearly a third had 2-4 chronic health conditions.⁵² Patients affected by chronic health conditions typically have longer PICU admissions. Patients with chronic health conditions have significantly longer stays than those without chronic conditions; the difference in the length in stays increases as the number of chronic conditions a patients has increases.⁵² In 2010, each day in an ICU setting had a cost of approximately \$4,300, although this amount can vary greatly by location, hospital system, and needs of the patient.⁵³ The chronic nature of genetic conditions, coupled with the high cost of ICU care increases the importance of making an accurate diagnosis as soon as possible.

2.4.1 The impact of WES on PICU Patients and Families

Having as much information about a child's condition is important for their care and management, as well as for the psychological well-being of their family. The admission of a child into the PICU is a stressful and uncertain time for many families; providing parents with information about their child's condition can reduce stress and alleviate feelings of helplessness.⁵⁴ In many cases, WES can be used to provide additional information into the cause of a child's

condition, possible additional concerns, and potential treatment options by establishing a diagnosis. Lakhani and Pierce (2019) outlined 4 ways in which identifying a genetic diagnosis, often found via WES at Yale's Pediatric Genomics Discovery Program (PGDP), can be useful in the pediatric intensive care setting.⁵⁵ The first benefit is in being able to tailor care based on the patient's diagnosis. Changes in care can vary widely, from a change in medication or diet, to the involvement of additional specialists, and even determining the appropriateness of major surgical interventions. The second benefit is the comfort a family may find by having the closure of receiving a diagnosis, ending what may have been a long, arduous journey. In addition to helping the family of the immediate patient, finding a genetic diagnosis may identify new genetic conditions, which may benefit other families. Finally, this testing may lead to the discovery of new genes, which can improve scientific understanding of genetic disease.⁵⁵

2.4.2 Rapid WES in the PICU

Accurate and rapid diagnosis of genetic conditions can alter medical management in the PICU. This is important not only in the treatment of individual patients but also in decreasing costs. Wu and colleagues (2019) employed WES, including the use of an algorithm they designed to efficiently prioritize variants, to study rapid WES in pediatric patients suspected of having genetic conditions.⁵⁶ Forty patients from 10 different Taiwanese hospitals were enrolled on the study, with a goal of limiting turnaround time for results to less than 1 week. Twenty-seven (67.5%) were PICU patients, the rest were referred due to abnormal newborn screening (6 patients, 12.5%), or by doctors from transplantation or other units. The most common indication was neurological conditions (14 patients, 25%), followed by cardiac conditions (9 patients, 35%). Mean turnaround time for WES results was 6.2 working days, with a range of 4.3-9 days. The

overall diagnostic yield was 52.5% (21 of the 40 patients); 10 patients (45.5%) were identified as having a condition for which a specific medication could be prescribed. PICU patients specifically had a 37% diagnostic yield. The authors noted that many of these diagnoses represented the first time these conditions had been recognized in Taiwan, which they believe makes it less likely that these diagnoses would have been made if not for the use of WES.⁵⁶ Wu and colleagues illustrated the usefulness of WES in PICU patients, particularly for patients with conditions with which providers may be unfamiliar and otherwise less likely to recognize or diagnose.

2.5 Cardiac Genetics and WES

Cardiac abnormalities in infants can be divided into 3 categories: structural heart defects, cardiomyopathies, and channelopathies. Pediatric cardiac conditions can be devastating and develop unexpectedly in families with no prior known history of cardiac disease. A study by Ritter and colleagues (2019) set out to investigate the clinical utility of WES in infants experiencing heart failure.⁶ The study included 15 patients who were diagnosed with heart failure before the age of 1 year old. The most common cardiac diagnosis was dilated cardiomyopathy, which was seen in 60% of patients; however patients with severe structural heart diseases were not included. Ten of the 15 patients (66.7%) received a molecular diagnosis via WES, which included all 6 of the patients who had extra-cardiac symptoms prior to WES. Seven of the 10 patients who received a diagnosis would not have been diagnosed on cardiomyopathy or arrhythmia gene panels commonly used at the time of the study. Two of the 10 patients who received a molecular diagnosis had mutations in genes that were primarily related to cardiac conditions. Only 1 patient had a close family member (first or second degree relative) who had been diagnosed with a

cardiomyopathy prior to the patient's diagnosis. Overall, 53% of the patients in the study had changes in their medical management as a result of WES.⁶

The study by Lee and colleagues, discussed previously in Section 102.1.2 , included the analysis of WES of 814 patients.²⁴ Of these patients, 39 were referred due to a cardiomyopathy and arrhythmia. The overall diagnostic yield for patients with cardiomyopathy and arrhythmia was 26% (10 of 39 patients, 95% CI: 14-41%).²⁴ When only the proband's sample was analyzed, the diagnostic yield for patients with cardiomyopathy and arrhythmia was 30% (7 of 23 patients, 95% CI: 15-51%); when both parental samples were available, the diagnostic yield was 21% (3 of 14 patients, 95% CI: 7-48%). This is in contrast with the overall results of the study, which showed a significantly higher diagnostic yield for trio-CES (P=0.003).²⁴ While studies have shown a higher diagnostic yield when including familial samples, that was not the case for cardiomyopathy and arrhythmia patients in Lee's study, showing that WES can be useful for patients experiencing cardiomyopathy and arrhythmia, even in the absence of familial samples.

Cardiovascular conditions, including arrhythmias and cardiomyopathies, can follow Mendelian or complex inheritance patterns and are a common cause of morbidity and mortality.⁵⁷ The Yale Program for Cardiovascular Genetics (YPCG) completed a study on the use of WES in 200 adult cardiovascular patients, presenting with various cardiovascular conditions.⁵⁷ Pathogenic or likely pathogenic variants were found in 26.5% of patients (53 patients), providing a molecular diagnosis. The authors compared these results to commercially available panels, considering any gene present on 2 out of 3 commercial panels to be standard genes. Only 18% of patients (36 patients) would have been identified as standard genes on a commercial panel.⁵⁷ The YPCG showed that WES is an appropriate tool in adult cardiovascular patients.

2.5.1 Structural Heart Disease

Approximately 3% of all babies born in the United States are born with a genetic condition or major structural birth defect.⁵⁸ Heart defects that are present at birth, often termed congenital heart disease (CHD), are the most common form of birth defect.⁵⁹ This group of conditions is incredibly heterogeneous and includes numerous subtypes, ranging from mild to severe. Between 2010 and 2017, there was an approximately 10% increase in the prevalence of CHD.⁵⁹ The most common CHDs were mild defects (ventricular septal defects, atrial septal defects, and patent ductus arteriosus), which accounted for 65.3% of CHDs between 2010 and 2017. The prevalence of mild defects had increased ($P < 0.001$) compared to a prior study done for 1970-1974, at which point mild defects accounted for 49.2% of CHDs. This increase may be, at least partially, due to improvements in the ability to detect more mild structural heart defects. Additionally, the prevalence of right ventricular outflow tract obstructions (RVOTO) has increased from 0.355 per 1,000 births in 1970-1974 to 0.767 per 1,000 births in 2010-2017, and the prevalence of left ventricular outflow tract obstructions (LVOTO) have decreased from 0.689 per 1,000 births to 0.392 per 1,000 births for the same time periods. The decrease in prevalence of LVOTO may be due to improvements in prenatal diagnoses, allowing for the choice of termination of affected fetuses.⁵⁹

Left-sided lesions (LSL) are a heterogeneous group of heart defects that result in obstructed or altered flow of blood through the fetal heart.⁶⁰ LSL includes hypoplastic left heart syndrome, aortic valve stenosis, coarctations of the aorta, interrupted aortic arch (Type A), mitral valve atresia, mitral valve stenosis, and Shone's complex.⁶⁰ In a study of 342 unrelated patients with an LSL who did not have any apparent extra-cardiac malformations, Li and colleagues (2017) used WES to look for likely damaging variants in genes associated with cardiovascular malformations.

The most common LSL was hypoplastic left heart syndrome (136 cases), followed by coarctation of the aorta (130 cases). The study team compiled an a priori list of 1,712 genes thought to contribute to cardiovascular malformations before analyzing patient data. After variants were filtered through consideration of population allele frequency, potential for Loss of Function (LOF), and functional gene constraints, variants were further filtered by the a priori list. Through this process, 7.9% (27 participants) of the study participants were found to have novel candidate pathogenic variants, each in a different gene. When these 27 genes were compared to the Baylor Genetics clinical diagnostic exome sequencing database, 79 individuals had LOF variants in 1 of the genes identified in the study population. In total, patients in Baylor's database had variants in 17 of the 27 newly identified novel genes.⁶⁰ This study highlights the genetic heterogeneity seen in structural heart diseases, which may not be fully captured on gene panels. The study also illustrates the benefit of being able to reanalyze a patient's WES to reflect updated knowledge without needing to do additional genetic testing.

Tetralogy of Fallot (TOF) accounts for approximately 7-10% of congenital heart defects and is the most common cyanotic congenital heart defect.⁶¹ TOF is a complex defect resulting in the obstruction of blood flow to the lungs and is often seen in the presence of genetic syndromes, particularly in patients with 22q11.2 microdeletions.⁶¹ Eighty percent of TOF cases, however, are non-syndromic and may follow multifactorial inheritance.⁶² A study of 829 patients with TOF (Page et al., 2019) sought to investigate unique and deleterious variants that may contribute to the development of TOF through the use of WES.⁶² The study found that variants were clustered into specific genes, with two genes, *NOTCH1* and *FLT4*, reaching genome-wide significance of $P < 5 \times 10^{-8}$. Variants in these two genes were identified in 6.9% of the patients in the study, with no patient having variants in both genes.⁶² There were clusters of deleterious variants in other genes

(*RYR1, TBX1, ZFPM1, ZNF717, DLX6, PCMI, CAMTA2*), which were found in a combined total of 1.2% of the patients.⁶² Approximately 20% of patients with TOF have an underlying genetic syndrome, while genetic causes of non-syndrome TOF has been left largely unexplained. The study by Page and colleagues suggests that there is wide genetic heterogeneity in patients with non-syndromic TOF, with *NOTCH1* and *FLT4* contributing to a higher proportion of TOF cases than other candidate genes.⁶²

2.5.2 Channelopathies

Channelopathies are cardiac conditions that cause arrhythmias, or abnormal heart rhythm, leading to the risk of cardiac events in structurally normal hearts. Channelopathies can be due to mutations affecting several types of ion channels, including sodium channels, sodium related channels, potassium channels, potassium related channels, calcium channels, or calcium related channels.⁶³ In many cases, the first presentation of a channelopathy may be sudden cardiac death, which may be described as Sudden Unexplained Death (SUD) if the condition was not previously known in the deceased. Channelopathies are thought to account for as many as 1/3 of cases of infantile or juvenile cases of SUD, and between 10 and 25% of cases of adult SUD.⁶⁴ Identifying the type of channelopathy and the specific gene involved plays a role in the management of patients since risk factors can vary based on causative gene. For example, cardiac events in Long QT Syndrome Type 1 (LQTS1), caused by mutations in *KCNQ1*, and Long QT Syndrome Type 2 (LQTS2), caused by mutations in *KCNH2*, are similar conditions, but have different risk factors and variable treatment. Both LQTS1 and LQTS2 may be treated by beta blockers, but LQTS2 is more commonly treated with an implantable cardioverter defibrillator (ICD) than is LQTS1.⁶⁵ An ICD is a device that is implanted under the skin to monitor heart rhythms and deliver an electric

shock to the heart if an abnormal rhythm is detected in order to return the heart to a normal rhythm.⁶⁶

2.5.3 Cardiomyopathies

The term “cardiomyopathy” refers to a group of conditions which affect the myocardium, causing mechanical and/or electrical problems with the heart.⁶⁷ There are multiple types of cardiomyopathies, they can be isolated or secondary to other conditions, and may be genetic or acquired.⁶⁷ The ACMG, in collaboration with the Heart Failure Society of America (HFSA) (2018), has developed recommendations for genetic testing in the context of cardiomyopathies.⁶⁸ Cardiomyopathies, when detected, have well-established interventions that reduce morbidity and mortality and lead to a higher quality of life. Genetic testing has implications for the management of patients with cardiomyopathy, especially in the setting of some specific variants. Ideally, genetic testing should be initiated when the patient is first diagnosed with a cardiomyopathy. Within a family, genetic testing is recommended for the family member who carries the most definitive cardiomyopathy diagnosis or is the most severely affected family member. If a patient had previously had negative genetic testing, retesting may be appropriate since the number of genes included in cardiac genetic panels is increasing rapidly. Genetic testing may also be appropriate for newborns or infants with cardiomyopathy; these patients may require specialized evaluation since cardiomyopathy in this age range is often associated with inborn errors of metabolism or other syndromes.⁶⁸ If a pathogenic or likely pathogenic variant is identified, cascade testing for at-risk family members should be offered. In many cases, the first symptom of a cardiomyopathy in a family is sudden cardiac death, making it crucial to identify asymptomatic individuals harboring the variant. Preventive measures, including life style changes or the use of an implantable

cardioverter defibrillator (ICD) can be put in place to manage risks in pre-symptomatic individuals if identified early.⁶⁸ A full genetic evaluation for patients with cardiomyopathy includes collecting a three-generation pedigree, screening for cardiomyopathy in at-risk family members, referrals to expert centers (when necessary), genetic counseling, and treatment/therapy based on phenotype and/or identified genetic variant.⁶⁸ While these recommendations were established specifically for cardiomyopathies, they are generally applied to structural heart diseases and arrhythmias as well.

The recommendations for genetic evaluation set forth by the ACMG and HFSA focus on the use of gene panels.⁶⁸ Large gene panels are beneficial in that there are a large number of genes associated with cardiomyopathies, which are a feature of multiple genetic conditions, so more extensive gene panels are more likely to identify a variant when one is present. Differences between laboratories in which genes are included on a panel further complicate test selection since there is a risk of missing a pathogenic variant if a gene is not included on a chosen panel. Large panels also increase the chance of identifying a variant in a second gene, which can affect targeted familial testing.⁶⁸ However, increasing the number of genes tested also increases the likelihood of finding a VUS and some cardiomyopathy genes have been more well-studied than others, so there may be disparities in the amount of information available for variants in different genes.⁶⁸ The recommendations acknowledge that exome sequencing may identify a genetic cause for cardiomyopathy in families with multiple affected family members, but do not specifically recommend for or against the use of WES.⁶⁹

Recently, Ramchand and colleagues (2020) completed a study into the diagnostic yield of WES in patients with dilated cardiomyopathy (DCM).⁷⁰ Genetic testing has been less successful in patients with DCM when compared to hypertrophic cardiomyopathy, for which a genetic cause may be identified in up to 60% of patients.⁷⁰ This study included 83 patients with either idiopathic

or familial DCM, most of whom were adults. When variant interpretation strictly adhered to ACMG guidelines, 12% of patients received positive results; 6% of patients had variants which were classified as VUS by ACMG guidelines, but which were classified as pathogenic or likely pathogenic by a clinical laboratory.⁷⁰ Two (20%) of the pathogenic or likely pathogenic variants were found in genes that would not have been included on a commercially available DCM gene panel. Truncating variants in the gene *TTN* may represent a challenge in classification because approximately 3% of healthy individuals may carry a truncating variant in this gene, making it difficult to establish if such a variant is pathogenic.⁷⁰ Ten percent of patients in this study had truncating *TTN* variants that were classified as VUS. The use of WES in patients with DCM has a lower diagnostic yield than is seen in other patient populations, however this may be, in part, due to the proportion of patients with truncating *TTN* variants.⁷⁰

2.5.4 Gaps in Knowledge

Previous studies have not focused on the use of WES in CICU patients, although some studies have included CICU patients in their cohorts. A study by Kingsmore and colleagues, done at Rady Children's Hospital, published in 2019, included 213 patients undergoing either ultra-rapid Whole Genome Sequencing (urWGS), rapid Whole Genome Sequencing (rWGS), or rapid Whole Exome Sequencing (rWES), 26.7% (57 patients) of which were CICU patients. The study also included NICU patients (143 patients, 67.1%), PICU patients (11 patients, 5.2%), and other patients (2 patients, 1%). To be enrolled in the study, patients had to be less than 4 months old, be suggestive of a genetic condition, and have an expectation that a genetic diagnosis may potentially influence management. Conditions were considered suggestive of a genetic diagnosis if the time from admission, the time from having an abnormal response to a standard therapy, or the time

from development of the feature (including laboratory test result suggestive of a genetic condition) was less than 96 hours prior to enrollment. Patients deemed “gravely ill” underwent urWGS (n=24), while the rest of the patients were randomized to receive either rWGS (n=94) or rWES (n=95). Eleven of the 24 patients (46%) who underwent urWGS received a diagnosis. This was significantly higher than rWGS and rWES, which had a combined diagnostic yield of 20% (P<0.01). Of the 94 patients who underwent rWGS, 18 (19%) were given a genetic diagnosis. This did not significantly differ from those who underwent rWES, of which 19 (20%) were given a genetic diagnosis (P=0.88). One of the diagnoses made by rWGS would not have been made had that patient undergone rWES. The diagnostic yield of rWGS and rWES in this study were lower than seen in other studies, which may be due to the broader eligibility criteria set by this study than has been used in previous studies.⁷¹ While this does provide insight into the diagnostic yield of rWGS compared to rWES, as well as the diagnostic yield of these technologies overall, it does not delineate results by care setting, and therefore does not give information about the percent of patients in the CICU with positive results.

The study by Wu and colleagues (2019), previously discussed in Section 2.4.2, examined the use of rapid WES in patients suspected of having a genetic condition, but who had not yet been positively diagnosed. Nine (22.5%) of patients enrolled in the study were being treated for cardiac conditions, though were not specifically being treated in a CICU setting. The diagnostic yield for WES of these patients was 33%. This included the diagnosis of a patient with familial restrictive cardiomyopathy, whose ultimate approval for cardiac transplant was influenced by the WES results.⁵⁶

Some of these studies have focused on rapid WES, which is more costly than standard WES. The difference in cost of rapid and standard WES may be prohibitive in many situations.

Hospitals and medical providers must weigh the potential costs and limitations of different testing options with the potential benefits in order to take the most appropriate actions. Other studies discussed above focused on specific types of cardiac conditions and were not specific to patients in the CICU. More information is needed about the clinical utility of WES, both standard and rapid, in the CICU setting so that providers can choose if and when WES is appropriate in this patient population.

3.0 WES in the CICU

3.1 Background

Whole Exome Sequencing (WES) is a genetic test that allows for the sequencing of nearly all genes and the interpretation of variants in genes currently known to be associated with a patient's phenotype, as well as genes included in the ACMG secondary findings list, depending on laboratory policy and the patient's choice to receive these findings. The goal of WES is to reduce laboratory costs and provide a diagnosis to improve outcomes for patients. Because WES sequences the entire exome, it may reduce the number of genetic tests a patient undergoes,⁴¹ which can save health care dollars that would traditionally be spent on standard methods, such as step-wise single gene or gene panel testing.³² According to the American College of Medical Genetics (ACMG), WES is warranted particularly in cases when: (a) a patient's features are concerning for a genetic cause but there is not a specific, testable condition suspected; (b) a patient's features could be explained by a large number of genes; (c) a patient with a possible genetic condition has undergone other genetic testing without finding a diagnosis; or (d) a fetus is suspected of having a genetic condition and targeted testing has been uninformative.¹ When WES is performed, the laboratory sequences the exome of the patient, then filters variants to genes known to be potentially associated with the patient's phenotype, as well as secondary findings recommended by the ACMG, if the laboratory offers this analysis and the patient chooses to receive these results. Variants in genes related to at least some of the patient's phenotypic features are then analyzed by the laboratory and classified based on the expected relationship between the variant and the disease. The ACMG has published criteria for

classifying variants as “pathogenic,” “likely pathogenic,” “variant of uncertain significance,” “likely benign,” and “benign.”¹⁶

Ideally, samples from patients are submitted with samples from 2 other relatives, typically the patient’s biological parents. With this trio approach, the 2 additional samples can be used to clarify classification of variants found in the patient. For instance, if a patient has a variant in a gene known to have autosomal dominant inheritance and the variant is also found in an unaffected parent, the variant may be less likely to be contributing to the phenotype. Conversely, if a variant is found in a patient but not in either unaffected parent, the variant may be more likely to be contributing to the phenotype. Studies have shown higher diagnostic yields for trio analysis by 7.4-16.7 % points when compared to proband/patient only analysis.^{24,25,27}

Table 1 Diagnostic Yield of WES

Study	Study population	n	Diagnostic Yield (95% CI)
Yang et al. (2013) ²³	First 250 patients undergoing WES in laboratory	250	25.5% (20-31%)
Lee et al. (2014) ²⁴	First 814 patients undergoing WES in laboratory	814	Overall: 26% (18-27%)
			PO: 22% (18-27)
			Trio: 31% (27-36%)
Soden et al. (2014) ⁴¹	Pediatric patient with neurodevelopmental disorders	103	45%
Farwell et al. (2015) ²⁵	First 500 patients undergoing WES in laboratory	500	Overall: 30.4%
			PO: 20.6%
			Trio: 37.3%
Nolan & Carlson (2016) ³⁶	Pediatric neurology patients	50	48%

Table 1 Continued

Retterer et al. (2016) ²⁷	3,040 patient samples sent to the laboratory	3,040	Overall: 28.8%
			PO: 23.6%
			Trio: 31.1%
Stark et al. (2016) ²⁶	Infants under the age of 2y with multiple congenital abnormalities/dysmorphic features, or other features of monogenic disorder	60	57.5%
Meier et al. (2018) ⁴³	Prenatal WES, anomalies identified on ultrasound	19 families, 26 fetuses	63.2%
Meng (2018) ⁵¹	Patients undergoing WES in the first 100 days of life	278	Overall: 36.7%
			Trio: 50.8%
Kingsmore et al. (2019) ⁷²	Infants under the age of 4 months who were acutely ill and admitted to Rady Children’s Hospital (San Diego, CA) or developed a feature suggestive of a genetic condition <96 hours before study enrollment	213	urWGS: 46%
			rWGS: 19%
			rWES: 20%
Petrovski et al. (2019) ⁴⁵	Fetuses with anomalies identified on ultrasound between 11 and 35 weeks gestational age	234 parent-fetus trios	10%

Diagnostic rate of WES sequencing from various studies published between 2013 and 2019. PO = proband

only; urWGS = ultrarapid Whole Genome Sequencing; rWGS = rapid Whole Genome Sequencing; rWES = rapid Whole Exome Sequencing. Soden et al. included both WES and WGS in the overall diagnostic rate.

The diagnostic yield of WES varies greatly between different care settings, indications, and patient populations (see Table 1 Diagnostic Yield of WES. WES has shown a diagnostic yield as much as 4 times greater than that of traditional diagnostic methods.²⁶ The diagnostic yield of

WES may vary based on factors such as clinical indication, the presence or absence of dysmorphic features, and the age of the patient.^{24,25,27} In addition to the diagnostic yield, there are several benefits to WES. The broad nature of WES reduces the need for providers to identify a subset of genes for testing, as is needed with gene panels.²² This also increases providers' ability to diagnose conditions with which they may not be familiar.⁵⁶ WES can identify novel genes, or genes not currently associated with Mendelian conditions, that would not be included in gene panel testing.⁶⁰ In addition, as our knowledge of genetics continues to grow, patients who have undergone WES with negative or uncertain results do not need to be re-sequenced but may be diagnosed through re-analysis instead.²⁹ Finally, patients often undergo a number of genetic tests prior undergoing WES; WES may reduce the number of tests performed, reducing the overall; cost per genetic diagnosis.^{31,32}

While there is promise in the clinical utility of WES, it is important to acknowledge the limitations of this approach. There are limitations in the types of mutations that can be detected with WES. WES cannot detect copy number variations (CNVs), insertions/deletions (indels), structural rearrangements, methylation changes, intronic variants, or trinucleotide repeat expansion.³⁶ WES also generates a lot of data, which must be filtered and interpreted before results are ever reported to clinicians. Public databases, such as those supported through the NIH (i.e., dbSNP, dbGaP, Decipher, and ClinVar), are available to assist in the interpretation of the data generated.³³ However, commercial laboratories often have their own, proprietary databases that are not available to other laboratories or researchers. Differences in these databases can lead to discrepancies in how variants are classified; for example one laboratory may classify something as “pathogenic” or “likely pathogenic,” while another lab classifies the same variant as a “Variant of Uncertain Significance (VUS).”³⁴ A study by Harrison and colleagues showed an increase in

variant classification concordance from 88.3% when laboratories do not share data, to 91.7% when there is data sharing and open communication between laboratories.³⁵

Cardiac conditions can be classified as cardiomyopathies (a disease of the heart muscle), channelopathies (heart disease causing abnormal rhythm), or structural heart defects.^{63,67} Cardiac conditions have a high level of genetic heterogeneity and may be due to variants in novel genes.^{24,60} These conditions often have well-defined management guidelines to reduce the associated morbidity and mortality.⁶⁸ Previous studies have been done on WES in the Neonatal Intensive Care Unit (NICU) and the Pediatric Intensive Care Unit (PICU); the clinical utility of WES in diagnosis and management of these patients has been demonstrated.^{50,51,55,56} To date, the use of WES has not been thoroughly studied in the Cardiac Intensive Care Unit (CICU). In this study, we aim to provide insight into the diagnostic yield and clinical utility of WES specifically in the CICU.

3.1.1 Purpose of the Study

The introduction of new genetic testing methodologies and strategies comes with the possibility of decreasing the time it takes to determine a diagnosis, allowing for improving care. It is important to evaluate these tests to determine their clinical utility. The clinical utility of WES in the NICU, PICU, and specific medical subspecialties has previously been studied, but the utility of WES in the CICU has not yet been closely investigated. Clinical utility, in our study, was defined as whether or not a test has an impact on the management or care. This study sought to provide characteristics of patients undergoing WES in the CICU, as well as to determine the amount of time between when the patient was initially evaluated by Medical Genetics and when a diagnosis was made. This study assesses the outcomes of WES by determining how often a relevant result was found; it also classifies the recommendations made following the WES results.

3.2 Study Design

3.2.1 Ethical Considerations

This study was approved by the University of Pittsburgh Institution Review Board (IRB), under Principal Investigator Lina Ghaloul-Gonzalez, MD. Documentation is included in Section 1.01(a)(i)Appendix B. All data were collected through chart review and the study was determined to have no greater than minimal risk.

3.2.2 Study Population

Data were abstracted from the electronic medical records of patients who had undergone Whole Exome Sequencing (WES) at University of Pittsburgh Medical Center (UPMC) Children's Hospital of Pittsburgh (CHP) between 2012 and 2018. A list of individuals who had undergone WES (including TES) was generated by UPMC Children's Utilization Management team from billing data. The list included 965 patients. Patients' Electronic Medical Records (EMR) were subsequently individually reviewed to determine the patients' locations when WES was initiated (in the CICU or not in the CICU). Data collection, as described below, continued for all patients, but only those who met the inclusion criteria were included in this study. The only inclusion criteria used for this study is that the patient's sample had to have been drawn and sent to a laboratory for clinical WES during in-patient stay in the CICU while under the care of the CICU team. Due to the extended turn-around time currently associated with WES and TES, patients were typically discharged before results were returned. Patients were not required to be in the CICU when results were returned. In total, twenty-four patients met the criteria and were included in this study.

3.2.3 Data Collection

Data abstracted includes information about the patients' clinical presentation, ethnicity, sex, age at first genetic evaluation and when the patients' sample was sent to a laboratory for WES, and other genetic testing completed. Family history was considered positive if there was at least one close relative with similar medical conditions or clinical phenotypes, or a family history suggestive of an inherited cardiac condition. For example, one patient had a second degree relative who died from Sudden Infant Death Syndrome (SIDS), which can suggest an inherited arrhythmia. WES reports and medical notes by the medical geneticist and/or genetic counselor were reviewed to collect data on types of results (categorized as positive, negative, or variant of uncertain significance) and genes involved. After initial data collection, all patients were reviewed by a medical geneticist to ensure appropriate disease and result classifications. Only genes related to cardiac disease were considered in determining if results were positive, negative, or uncertain. Patients were considered to have a positive result if they had a likely pathogenic or pathogenic variant as determined by the laboratory and clinicians in genes related to the cardiac phenotype. If a patient's WES listed a variant as a VUS but the variant was interpreted by the Medical Genetics department as pathogenic or likely pathogenic without further testing or evaluation, testing was considered positive. If the Medical Genetics department thought the variant could be contributing but were not certain without taking additional steps, the variant was counted as a VUS. Patients who had no results listed on their WES, or those who had variants of any classification on their report but none of which were related to their cardiac phenotype, were considered to have a negative result.

The types of changes in management were based on recommendations made, which were determined based on notes written by the medical geneticist and/or genetic counselor in the EMR.

Recommendations were also taken from the patient letter written by the genetic counselor, which was also found in the EMR. Management changes were then put into 6 categories: 1.) change in diagnosis; 2.) change in medication; 3.) additional testing for patient; 4.) referral or re-referral for the patient; 5.) family members tested; and 6.) family members referred for care (see Table 2 Types of Management Changes).

Table 2 Types of Management Changes

Management Change	Definition
Change in diagnosis	Diagnosis determined via WES, or an additional diagnosis was added.
Change in medication	Recommended a medication to be started or discontinued.
Additional genetic or other testing for the patient	Additional testing recommended for the patient. For example, deletion/duplication studies for a VUS in a gene associated with an autosomal recessive cardiac disease-related condition. This also includes tests recommended by Medical Genetics but not completed (i.e. testing denied by insurance) and requesting an expanded WES report from the laboratory.
Referral or re-referral for the patient	Recommended the patient be evaluated by another specialty. Includes patients who had previously seen a specialist but were not following with the specialty at the time.
Additional testing for family members	Recommended additional testing for family members. This includes testing parental samples if not included in the initial analysis.
Family members referred for care	Recommended family members be evaluated by a specialist or that they have specific evaluations through their primary care provider. Typically, recommendations are made for family members to seek prenatal genetic counseling if concerned of their own reproductive risks. Because the generalized recommendation for prenatal counseling in this context is

Table 2 Continued

	routine, this recommendation was not considered as a family member referral.
Any change for patient	At least one of the following occurred: a change in diagnosis; change in medication; additional genetic or other testing for patient; referral or rereferral for patient.
Any change for patient and/or family members	At least one of the above changes was made following WES.

3.2.4 Comparison to Gene Panel Testing

The genes related to cardiac features in patients were found to have variants were compared to a comprehensive cardiovascular panel available through Fulgent Genetics, which includes 252 genes.⁷³ This panel was chosen by searching the NCBI Genetic Testing Registry (www.ncbi.nlm.nih.gov/gtr) to search available tests using the search term “comprehensive cardiovascular.” Results were then filtered to only include CLIA certified laboratories in the United States, then selecting the largest available panel.

3.2.5 Data Analysis

Descriptive statistics such as proportions, means, etc. were calculated in Excel. Initially, this was done for all patients collectively. Patients were also stratified by cardiac disease type (cardiomyopathy, structural heart defect, or channelopathy) for comparisons between the disease groups and the analyses were repeated (see Figure 1 WES Results and Disease Classifications). If a patient had both a structural heart defect (excluding patent foramen ovale or small patent ductus

arteriosus, which are relatively common) and a cardiomyopathy, they were classified as having a structural heart defect since the structural heart defect likely led to the cardiomyopathy. Only one patient in the study was treated for a channelopathy, so while their results are reported (see Cardiac Disease Type), statistical comparison was limited to cardiomyopathies and structural heart defects. Two sample proportion tests were used to compare the proportion of patients receiving each type of WES results (positive, negative, or variant of uncertain significance) and changes in management between patients with cardiomyopathies and patients with structural heart defects. A two-sample t-test was used to compare the ages of patients, the time between the first genetics consult and when WES was sent, and the time between when WES was sent and when results were returned.

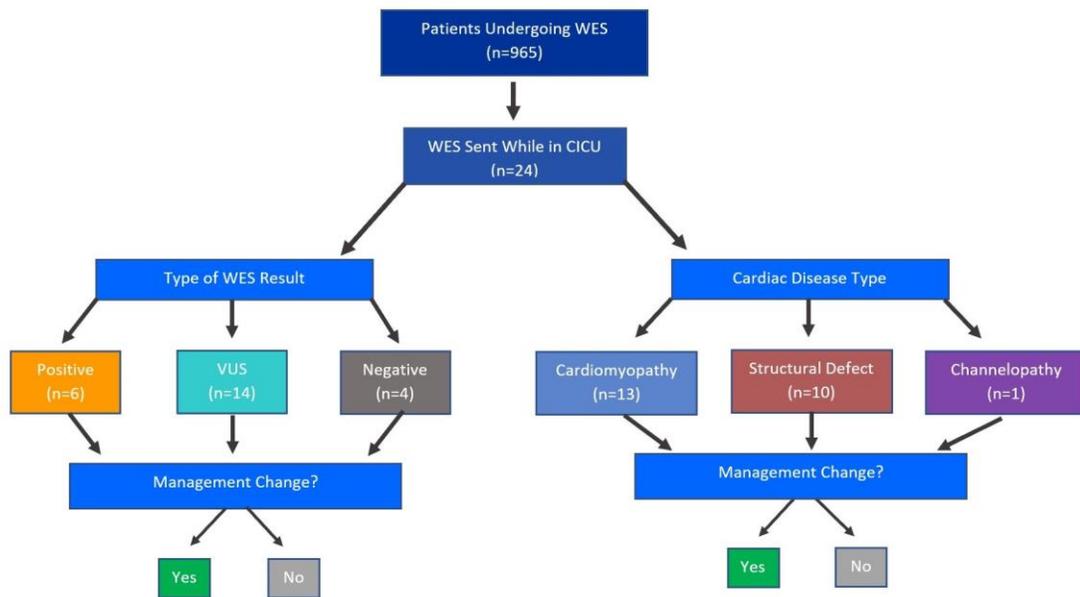


Figure 1 WES Results and Disease Classifications

Patients were also stratified by type of test result. We chose to stratify patients by type of WES result separately from type of cardiac disease to identify trends that may be specific to a particular type of testing result or cardiac disease type.

The age of patients at the first genetics evaluation, time from the first evaluation to when WES was sent, and WES processing time were compared between the 3 types of results using one-way ANOVA tests. Pearson’s Chi-squared tests were used to compare changes in management between the 3 groups. For all analyses, P values <0.05 were considered statistically significant.

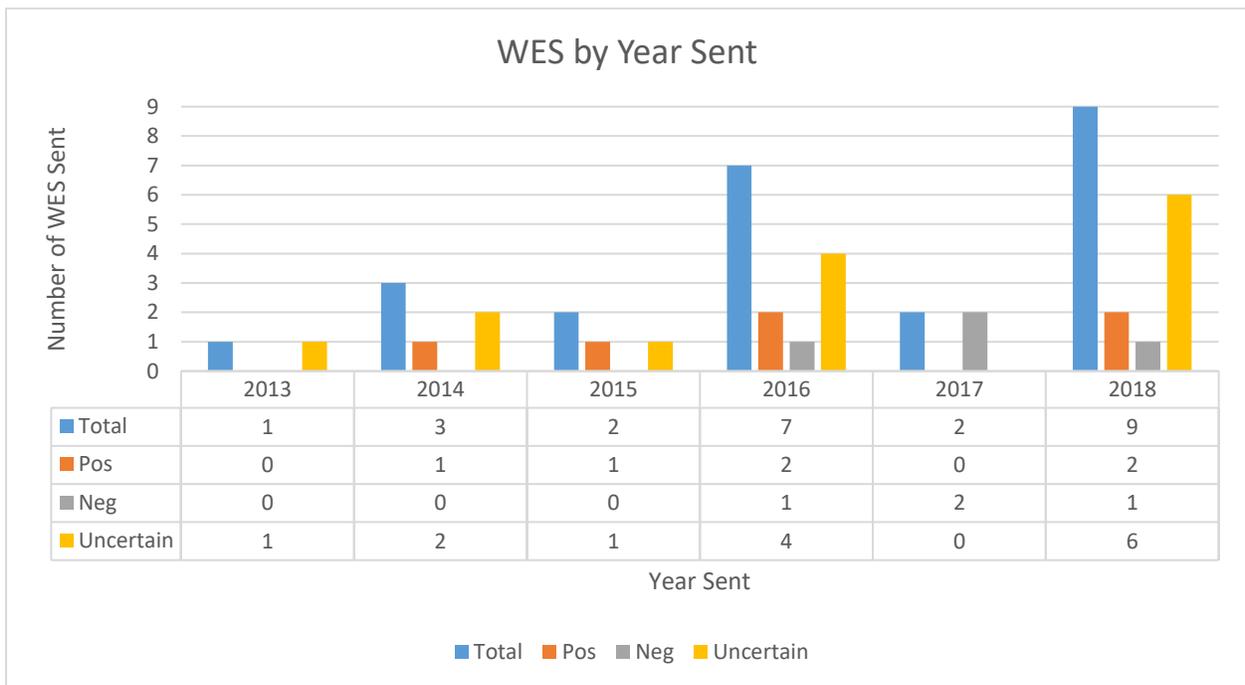


Figure 2 WES Results by Year Sent

Patients were also classified by when WES was sent to the laboratory for analysis. Details for each year can be seen in Figure 2 WES Results by Year Sent. Patients were stratified as having WES sent for analysis between prior to 2016 (WES sent in 2013, 2014, or 2015) or 2016 or later. This grouping was chosen for 2 reasons. The first being that variant interpretation guidelines were

published in May of 2015, potentially signaling a change in how laboratories were interpreting results and providing more standardized framework for WES. The second reason was the shift towards using WES clinically more often. Two patients in the study underwent WES in 2015, while 7 underwent WES in 2016. This may have indicated that providers were becoming more comfortable ordering WES in the CICU setting. The time between the first genetic consult and when WES was sent for analysis, the time between when WES was sent and when results were returned, and the total time between when the first genetics consult and the return of results were compared based on when WES was sent using a two-sample t-test.

Finally, patients were classified by the presence or absence of dysmorphic features, as well as the presence or absence of multiple congenital anomalies, as determined by the medical geneticist reviewing the patients. Two-sample tests of proportion were used to compare cardiac disease type and WES result type between patients who had dysmorphic features and those who did not. The same analyses were done to compare patients with and without multiple congenital anomalies.

3.3 Results

The current study included a total of 24 patients. Most patients (N=16 patients, 66.7%) were of European decent and 5 patients (20.3%) reported mixed ethnic backgrounds, such as European and African American heritage (see Figure 3 Patient Ethnicities). Three patients had their samples analyzed without any parental samples (Singleton analysis); 3 patients had their samples and a sample from 1 parent analyzed (Duo WES analysis); and 18 patients had their sample and samples from both parents analyzed (Trio WES analysis).

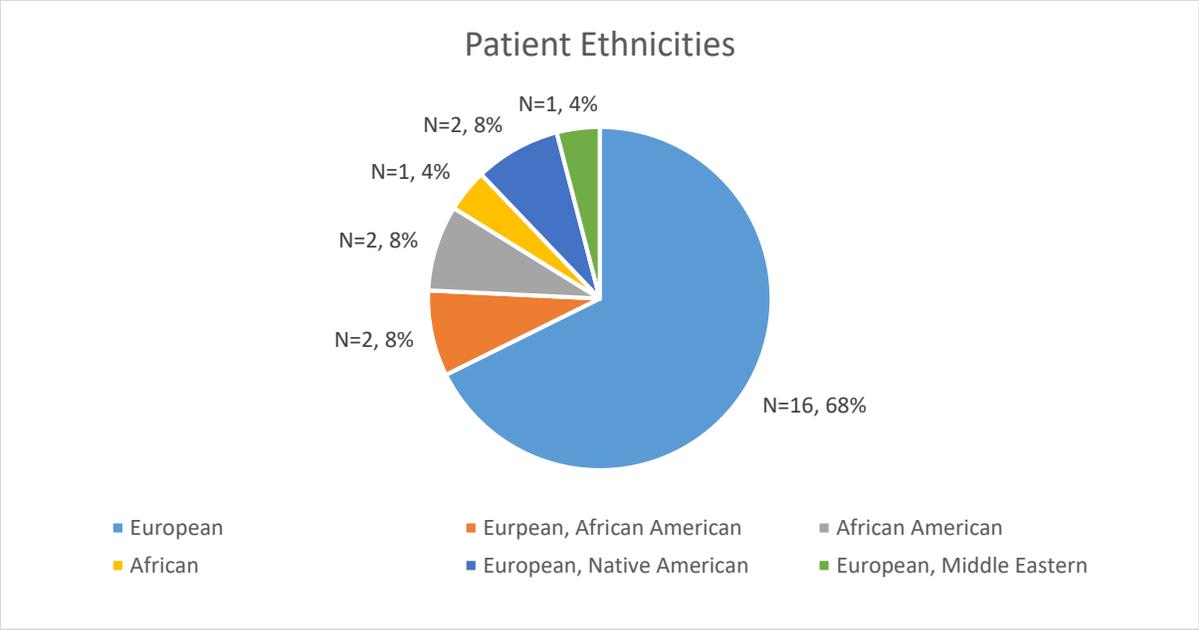


Figure 3 Patient Ethnicities

Table 3 Frequency of Disease and Result Classification

	All patients (n=24)
Female (%)	8 (33.3)
Positive (%)	6 (25)
Negative (%)	4 (16.7)
VUS (%)	14 (58.3)
Cardiomyopathy (%)	13 (54.2)
Structural Heart Defect (%)	10 (41.7)
Channelopathy (%)	1 (4.2)
Mean age at first consult (range)	3.1y (DOL1-25.8)
Mean age when WES sent (range)	3.2y (DOL2-25.9y)
Positive Family History (%)	14 (58.3)

Prior to WES, 13 patients (54.2%) had cardiomyopathy, 10 patients (41.7%) had a structural heart defects, and 1 patient (4.2%) had a channelopathy (see Table 3 Frequency of Disease and Result Classification). Six patients (25%) received positive results, 4 patients (16.7%) received negative results, and 14 (58.3%) received VUS. Six patients (25%) passed away before the results of their WES were returned, 2 patients passed away shortly after WES results were available (see Section 1.01(a)(i)A.2). Changes in management were determined based on genetics notes for all patients, as defined in Table 2 Types of Management Changes One deceased patient did not have any genetics notes following WES, and was counted as not having changes to their management. As illustrated in Figure 4 Management Change by Disease Type, 18 of the 24 patients (75%) had a change in their management and/or a change in the management of family members following WES, which included 13 patients (54.2%) who had a direct change in their management. Ten patients (41.7%) had additional testing recommended. This included 4 (40%) patients who had a recommendation of deletion/duplication studies; for 3 patients these were negative and this testing was not done for the fourth patient due to insurance denial. Two patients had additional enzymatic testing after WES, 1 of which had an enzyme activity level below detectable amounts and the other was done through research and results were not available. One patient had post-mortem testing on skeletal muscle, which showed likely Combined Oxidative Phosphorylation Deficiency 24 (COXPD-24), which was consistent with the patient's WES results.

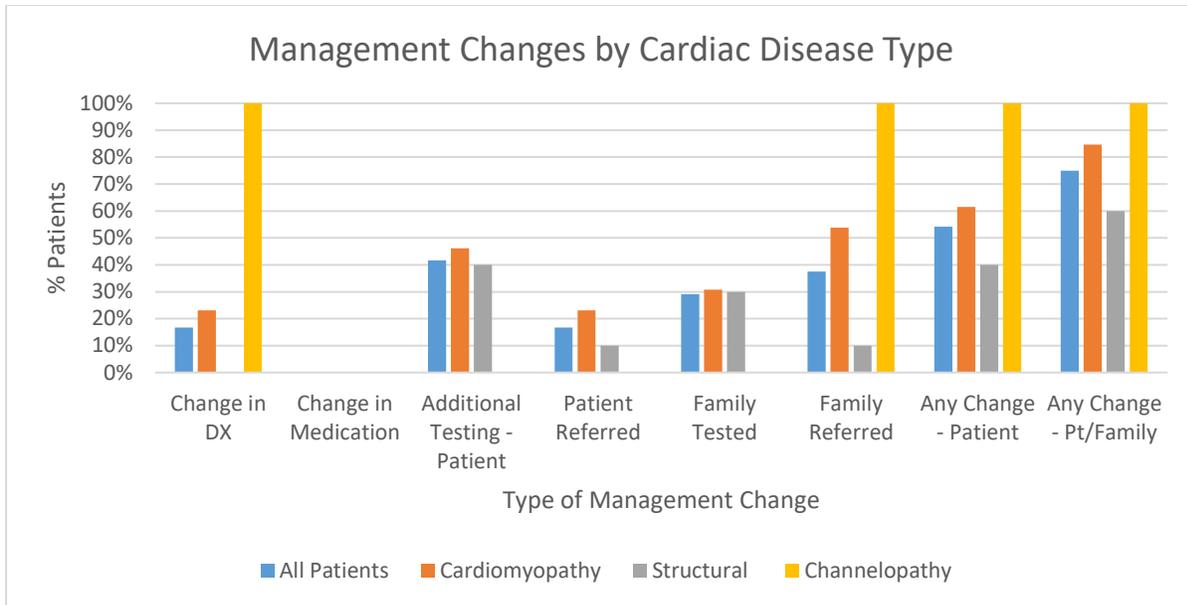


Figure 4 Management Change by Disease Type

3.3.1 Cardiac Disease Type

Due to having only one patient in the study treated for a channelopathy, comparisons of outcomes between cardiac disease type were limited to cardiomyopathies and structural heart defects. Selected details regarding results by cardiac disease type can be found in 76. Patients with cardiomyopathies were significantly older at the time of the first genetic evaluation than were patients with structural heart defects ($5.6y \pm 9.5y$ vs. $0.2y \pm 0.2y$, $P=0.04$). Of patients with cardiomyopathies, 4 patients (30.8%) had positive results, 1 patient (7.7%) had a negative result, and 8 patients (61.5%) had uncertain results (VUS). One patient (10%) with structural heart diseases had a positive result, 3 patients (30%) had negative results, and 6 patients (60%) had VUS. There was no significant difference in the proportion of subjects who received a positive result between patients with cardiomyopathies and patients with structural heart defects ($z=1.38$,

P=0.17), nor was there a difference in the proportions of patients receiving a negative ($z=-1.40$, $P=0.16$), or VUS ($z=0.08$, $P=0.94$).

There was no significant difference in the proportion of patients who had a change in their management following the return of WES results ($z=0.07$, $P=0.94$) between cardiac disease types. Eight (61.5%) cardiomyopathy patients and 4 patients (40%) with structural heart defects had changes to their direct care. Eleven patients (84.6%) with cardiomyopathies had a change in their management and/or the management of family members, while 6 patients (60%) with structural heart defects had a change in their management and/or the management of their family members, this difference was not significant ($z=1.39$, $P=0.16$). There were no significant differences in whether or not a patient underwent additional testing ($z=-0.10$, $P=0.92$) and no significant difference in proportion of patients who had recommendations for family members to undergo additional genetic testing ($z=0.42$, $P=0.67$). Patients with cardiomyopathies were significantly more likely to have a change in diagnosis on WES, although this difference was at the threshold of being insignificant ($z=1.63$, $P=0.05$). There was no significant difference in proportion of patients referred to other specialists ($z=0.82$, $P=0.41$). However, family members of patients with cardiomyopathies were more likely to be referred to a specialist or for an evaluation than were family members of patients with structural heart defects ($z=1.65$, $P=0.05$).

The patient with a channelopathy presented with wide complex tachycardia and did not have dysmorphic features or multiple congenital anomalies. Wide complex tachycardia is defined as a QRS width greater than 120ms and a heart rate greater than 100 beats per minute.⁷⁴ WES returned a positive result, with 2 variants in *SCN5A*, conveying a diagnosis of Sick Sinus Syndrome.⁷⁵ Individuals with Sick Sinus Syndrome may be managed through the placement of a pacemaker, catheter ablation of atrial fibrillation, or antiarrhythmic medications, although the

complexity of the condition complicates management.⁷⁶ While Sick Sinus Syndrome is autosomal recessive, individuals with one pathogenic or likely pathogenic variant in *SCN5A* are also at risk for arrhythmias, including Brugada syndrome, and may benefit from medical intervention.⁷⁷ Unfortunately, this patient passed away before WES results were returned.

3.3.2 Types of Test Results

We also assessed differences in age and timelines between patients based on the type of WES result (positive negative, or VUS) using one-way ANOVA. There were no significant differences in patient age at first genetic evaluation (positive=0.5y±0.7y; negative=0.1y±0.1y, VUS=5.1y±9.3y, F=1.21, P=0.32), nor was there a difference in the time between the first genetic consultation and when WES was sent (positive=83.3d±35d; negative=9.3d±7d, VUS=28.8d±45d, F=0.51, P=0.61). Management changes were compared between types of WES test results using Pearson's Chi Squared tests. Details about outcomes by WES result type can be found in Table 4 Changes in Management by Result Type4. Four patients (66.7%) with positive results had a change in diagnosis, while no patients with negative or VUS results did ($X^2=14.4$, P=0.001). The proportion of patients referred to other specialists significantly differed by WES result type ($X^2=6.5$, P=0.04), as did the proportion of patients with changes to their own management ($X^2=7.2$, P=0.03) and patients with changes to their management and/or management of their family members ($X^2=7.4$, P=0.02).

Table 4 Changes in Management by Result Type

	Pos (n=6)	Neg (n=4)	VUS (n=14)	X ² (P-value)
Change in Diagnosis (%)	3 (50)	0 (0)	0 (0)	10.3 (0.006)
Change in Medication (%)	0 (0)	0 (0)	0 (0)	
Additional Testing for Patient (%)	4 (66.7)	1 (25)	5 (35.7%)	2.2 (0.33)
Referral for Patient (%)	3 (50)	0 (0)	1 (7.1)	6.51 (0.04)
Testing for Family Members (%)	2 (33.3)	0 (0)	5 (35.7)	2.0 (0.37)
Referral for Family Members (%)	2 (33.3)	0 (0)	7 (50)	3.4 (0.19)
Any Change for Patient (%)	5 (83.3)	1 (25)	6 (42.9)	7.2 (0.03)
Any Change for patient and/or Family (%)	6 (100)	1 (25)	11 (78.6)	7.4 (0.02)
Results Returned After Death (%)	1 (16.7%)	1 (25)	4 (28.6%)	0.3 (0.85)

The genes reported on the patients' WES reports were compared to Fulgent Genetics' Comprehensive Cardiovascular NGS Panel, which includes 252 genes,⁷³ to determine if a panel approach would have been as effective in detecting variants. Excluding genes without a cardiac phenotype, 54 pathogenic variants, likely pathogenic variants, or VUS were found in the 24 patients in the study. A total of 32 genes were involved. Some patients had multiple variants in the same gene, for example Patient Q had 4 VUS in *TTN* and Patient U had 5 VUS in *TTN*. Fifteen variants (27.8%) in 11 genes detected on WES would not have been detected on the Fulgent

Genetics panel, including 3 variants in 2 patients that led to positive diagnoses (see Table 5 Positive Results). Neither of these 2 genes are currently available on gene panels designed specifically for cardiovascular conditions available from CLIA certified laboratories in the United States, although they are available on gene panels for other indications, such as gene panels for mitochondrial conditions or lysosomal storage disorders.^{78,79}

Table 5 Positive Results

Patient	Positive Results	Included on Panel
Patient A	<i>NAGA</i> (c.973G>A, p.E325K, homozygous)	No
Patient B	<i>RRAS</i> (c.116_118dupGCG, p.G39dup)	Yes
Patient C	<i>NARS2</i> (c.749G>A, p.R250Q) <i>NARS2</i> (c.325G>C, p.E109Q)	No
Patient G	<i>SCN5A</i> (c.673C>T, p.R225W) <i>SCN5A</i> (c.3840+1G>A, n/a)	Yes
Patient L	<i>TPM1</i> (p.R160H; c.479G>A)	Yes
Patient S	<i>ELAC2</i> (c.297-2_297-1delinsT, Intron 3) <i>ELAC2</i> (c.2245C>T, p.H749Y)	Yes

3.3.3 Time to Diagnosis

There was a mean of 27 days (± 40 days) between the first genetics consult and when WES was sent. However, there was a wide range in this time period (see Figure 5 Days from First Evaluation to WES Result), with 2 patients having WES the day of the evaluation and 2 patients with more than 100 days between the first evaluation and WES (range: 0-152 days). Cardiomyopathy patients had a significantly longer time between the first genetic consultation and when WES was sent for analysis when compared to patients with structural heart defects (see Table

6 Timelines by Cardiac Disease Type). The time between the first genetics evaluation and when WES was sent did not differ by the type of WES result received (see Table 7 Timelines by WES Result Type), or by when WES was sent (either 2012-2015, or 2016-2018) ($t=-1.26$, $P=0.22$).

Table 6 Timelines by Cardiac Disease Type

	All Patients (n=24)	Cardiomyopathy (n=13)	Structural Heart Defect (n=10)	T-statistic (P-value)
Time from first genetics consult to WES sent (days) (range)	27d (0-152)	43d (1-152)	8d (0-16)	2.26 (0.02)
WES processing time (days)	80d (7-234)	78d (7-234)	84d (34-119)	-0.24 (0.81)
Time from first genetics consult to WES results (days)	107d (14-254)	121d (14-254)	91d (34-135)	1.16 (0.26)

The mean time between when WES was sent for analysis and when results were returned (WES processing time) for WES was 80 days (± 51 days; range:7-234). Mean processing time did not differ by type of WES result or the type of cardiac disease, nor did it differ by when WES was sent ($t=-0.69$, $P=0.50$). The time from the first genetic consultation to the return of WES results was did not differ by cardiac disease type, the type of WES result, or by when WES was sent ($t=-1.42$, $P=0.17$).

Table 7 Timelines by WES Result Type

	Positive	Negative	VUS	F-statistic (P-value)
Time from first genetics consult to WES sent (days) (range)	35d (1-90)	9d (0-16)	29d (0-152)	0.51 (0.61)
WES processing time (days) (range)	78d (7-176)	112d (34-234)	72d (9-104)	0.98

Table 7 continued

				(0.39)
Time from first genetics consult to WES results (days) (range)	113d (16-254)	121d (34-135)	70d (14-193)	0.20
				(0.82)

Three patients had rapid WES with a processing time under 30 days (1 patient at 7 days, 2 patients at 9 days), all of which were sent in 2018. All three patients were being treated for cardiomyopathy; 2 of the patients received positive results and 1 received VUS. The mean time between the initial genetics consult was 34 days, which ranged from 5 to 90 days. All 3 patients underwent additional testing following WES results. One patient passed away 2 days after WES results were returned.

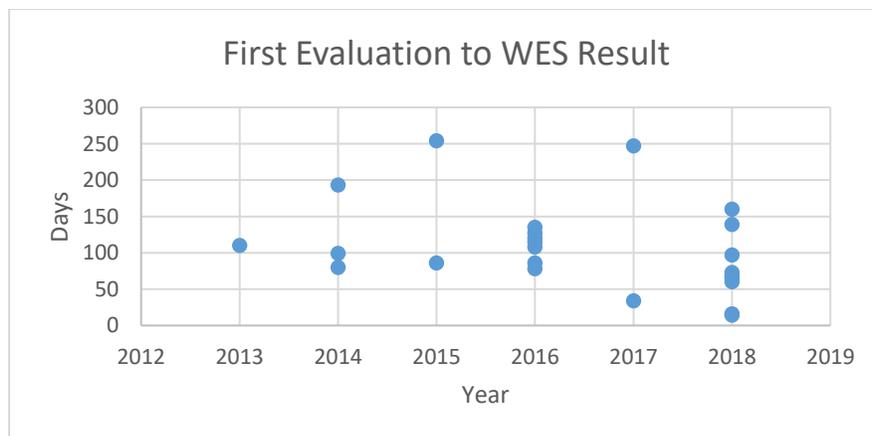


Figure 5 Days from First Evaluation to WES Result

3.3.4 Presence of Other Anomalies

Fourteen patients (58.3%) had dysmorphic features while 10 (41.7%) did not. Table 8 Dysmorphic Features and Congenital Anomalies shows the type of cardiac disease, as well as the

type of WES test result based on the presence of dysmorphic features and/or congenital anomalies. There was no significant difference in the proportion of patients receiving a positive result based on the presence or absence of dysmorphic features ($z=-1.5$, $P=0.15$). Patients with dysmorphic features were more likely to have a negative WES result than those without dysmorphic features ($z=1.9$, $P=0.03$). Patients with dysmorphic features were more likely to have a structural heart defect than patients without dysmorphic features ($z=2.6$, $P<0.01$). Conversely, patients without dysmorphic features were more likely to have a cardiomyopathy than were patients with dysmorphic features ($z=-2.1$, $P=0.02$). Eight patients (33.3%) had multiple congenital anomalies. Patients without multiple congenital anomalies were more likely to receive a positive diagnosis than those with multiple congenital anomalies ($z=-2.0$, $P=0.02$). Patients with multiple congenital anomalies were more likely to have structural heart defects than those without multiple congenital anomalies ($z=2.3$, $P=0.01$), while patients without multiple congenital anomalies were more likely to have cardiomyopathies ($z=-2.04$, $P=0.02$).

Table 8 Dysmorphic Features and Congenital Anomalies

	Dysmorphic Features Present (n=14)	Dysmorphic Features Absent (n=10)	Congenital Anomalies Present (n=8)	Congenital Anomalies Absent (n=16)	Dysmorphic Features and Congenital Anomalies Present (n=7)
Positive (%)	2 (14)	4 (40)	0 (0)	6 (38)	0 (0)
Negative (%)	4 (29)	0 (0)	2 (25)	2 (13)	2 (29)
VUS (%)	8 (57)	6 (60)	6 (75)	8 (50)	5 (71)
Cardiomyopathy (%)	5 (36)	8 (80)	2 (25)	11 (69)	2 (29)
Structural Heart Defect (%)	9 (64)	1 (10)	6 (75)	4 (25)	5 (71)
Channelopathy (%)	0 (0)	1 (10)	0 (0)	1 (6)	0 (0)

3.4 Discussion

Six of the 24 patients (25%) received a positive WES result. The clinical utility of WES in the CICU, as measured by positive results, is similar to that seen in other settings.²³⁻²⁵ The majority of patients (75%) undergoing WES while in the CICU had a change in their management and/or in the management of family members. All types of test results led to additional management recommendations from the medical genetics team. Our study shows that WES can be useful in the management of CICU patients and their families regardless of the type of test result returned.

One of the benefits of WES is the ability to test more genes than are included on a gene panel. Patient results were compared to the comprehensive cardiovascular gene panel available from Fulgent Genetics, which includes 252 genes.⁷³ Two positive results in our study (33.3%) would not have been revealed using this panel. Gene panel testing for these patients could potentially have led to poorer outcomes for those 2 patients by failing to identify a diagnosis. Additionally, family members of those patients would not have been able to tailor their own care based on a known genetic cause, which could lead to inadequate screening for those at risk, or unnecessary medical care for family members who do not carry the causative mutation. Table 5 Positive Results lists the positive results identified on WES and whether those results would have been detected on the Fulgent panel. A total of 27.8% of variants in genes with a cardiac phenotype would not have been reported on this panel. Gene panels are continuously being updated and expanded as more information becomes available. Given that the patients in the study underwent WES as far back as 2013, it's likely that more diagnoses would have been missed had the gene panels available at the time been used instead of WES, particularly for patients who underwent WES earlier on, when gene panels included fewer genes.

The gap between diagnoses made through the use of WES and those made using gene panel tests was evident in multiple studies. When comparing the 9 candidate genes for Tetralogy of Fallot found in the study by Page and colleagues⁶² to the same comprehensive cardiovascular panel used for comparison in our study, only 2 of the 9 genes identified in the study were included on the panel. The study by Ramchand and colleagues (2020) noted that 20% of positive results in DCM patients who underwent WES would not have been found using a commercially available DCM panel.⁷⁰ Similarly, the study by Ritter into the study of WES in infantile heart failure patients found that 7 of 10 heart-failure patients had a diagnosis made on WES that would have been missed on a gene panel used at the time.⁶ This gap is also seen in the adult cardiovascular patients, as illustrated by the YPGC, which found diagnostic yield of 26.5% through WES and a potential diagnostic yield 18% for standard gene panel testing in the same patients.⁵⁷ The heterogeneity of cardiac conditions, including less well-known candidate genes, should be an important consideration in deciding the appropriate genetic testing methodology for a patient. Our study is consistent with the current literature in illustrating that WES is useful in diagnosing cardiac patients who would not have received a diagnosis through gene panel testing. However, it is also important to note that 1 patient, discussed further in Section 3.4.2 , would have had a molecular diagnosis using this panel that was not made by WES.

Once a WES report is published, it is important that clinicians evaluate the report in the context of the patient. While this is done by the laboratory prior to releasing the report, the clinicians treating the patient have the most up-to-date and complete clinical picture and ultimately decide upon appropriate clinical management. Multiple patients had additional variants reported in genes not related to their cardiac symptoms, and it is important that clinicians be able to interpret WES results in the context of each, individual patient to determine if the results truly explain the

phenotype versus what is incidental. This genetics expertise is also needed in patients who have only a VUS in a gene related to an autosomal recessive condition to decide if additional testing is warranted. If a patient was felt to have features consistent with conditions for which they were identified as carriers (or possible carriers if the variant was a VUS) on WES, additional studies, such as deletion/duplication analysis, were recommended. Some genes reported on WES and found to be suspicious for contributing to the patient's phenotype by the medical genetics team, such as *CAP2*, are not associated with any disease in the Online Mendelian Inheritance in Man (OMIM) database, in which case interpretation may be less straight-forward.⁸⁰ It is not uncommon for clinical providers and laboratories to disagree on the classification of a variant.¹⁸ WES is a valuable tool for patients in the CICU when the results can be interpreted by clinicians with genetics expertise.

The 3 timescales used (time from first evaluation to WES sent; processing time; and total time from first evaluation to WES result) did not vary significantly between the years analyzed or by the type of result received. Patients were grouped by when WES was sent (2013-2015 or 2016-2018) due to small sample size. A larger study sample would allow analysis by individual year and may show different results.

While studies done on other patient populations have reported that the presence of dysmorphic features or multiple congenital anomalies may increase the diagnostic yield of WES^{24,45}, this was not the case for patients in this study. There was no difference in the proportion of patients receiving a positive or uncertain result based on the presence of dysmorphic features. The lack of difference in diagnostic yield based on the presence of dysmorphic features or multiple congenital anomalies may be due, at least in part, to all of the patients being evaluated by a medical geneticist. This study only included patients who had had WES and did not include information

about how many CICU patients were evaluated by a medical geneticist and were not offered WES. Specific dysmorphic features or congenital anomalies, or the certain combinations of such, may be suggestive of a particular genetic condition. In that case, the medical geneticist may have chosen to send more specific genetic testing if they had suspicion for a condition or group of conditions instead of casting a wider search through the use of WES.

Patients in the CICU are faced with serious, often life-threatening conditions. It is imperative that clinicians be able to diagnose these patients as quickly as possible in order to provide the correct treatment in a short time. The yield of positive WES results observed in our study is similar to that in other settings, but WES also led to additional next steps for patients receiving negative or uncertain results. The families of patients who passed away either before or shortly after the return of WES results had changes in their management by identifying family members who may be at risk of similar conditions. This study showed that WES has an impact on the management of patients and their families, may lead to diagnoses that would be missed using even large gene panel testing, and is useful in patients with cardiomyopathies, structural heart defects, and channelopathies. The involvement of medical genetics and the use of WES should be considered early on in the care of CICU patients with unexplained cardiac conditions.

3.4.1 Limitations of the Study

This study sought to classify recommendations and discuss outcomes of WES. However, the study only describes what follow-up recommendations were made by the medical genetics team. Whether or not this follow-up occurred is not included in this study. This could over-estimate the actions taken based on WES results since some patients and/or other providers could choose not to follow these recommendations.

The generalization of these results to all CICU settings may be limited. The patients included in the study were evaluated by a medical geneticist, who determined that WES was appropriate for the care of each patient. There may be patients in the CICU for whom WES would not be deemed appropriate by a medical geneticist. For example, 15% of cases of Tetralogy of Fallot (TOF) are due to 22q11.2 deletion syndrome,⁶² which would not be detected on WES, making a chromosomal microarray a more appropriate test for patients with TOF. Medical genetics evaluations typically include describing even minor dysmorphological features, which are important for how the laboratories filter variants in WES. Not all providers are trained in dysmorphism. Since WES for each of these patients was ordered through a medical geneticist, the sample population is more targeted than the general pediatric CICU patient population, and may have more detailed phenotypic information available than if WES were ordered without the involvement of a medical geneticist. The use of WES in CICU patients not evaluated by a medical geneticist may have a lower diagnostic yield. If this were the case, it could be that general CICU patients may be less likely to have a genetic etiology that would be detected on WES, or potentially due to differences in phenotypic details reported by medical geneticists compared to cardiologists or other physicians. Further study is needed to determine how well WES performs in the broader CICU patient population.

Additionally, the sample size of the CICU patients who underwent WES at UPMC's CHP is small in comparison to other studies done in NICU settings. Our study included 24 patients over a period of 6 years, and we recognize that a higher number of patients would have led to a stronger result conclusion. Had the sample size been larger, we could have stratified the patients by cardiac disease type, then within those groups stratified by WES result type. However, given the sample size, this stratification would not have been very informative.

3.4.2 Limitations of WES

The limitations of WES were previously discussed, but are also highlighted by 1 patient in the study. Patient W had an initial Genetics evaluation due to concerns of left ventricular non-compact cardiomyopathy. This patient had medical concerns that included hypotonia, seizures, and developmental delay. While WES was in process, providers also sent enzyme testing and *GAA* full gene sequencing. Patient W had a single base deletion, as well as a second, larger deletion detected on the full gene sequencing. This led to the diagnosis of infantile Pompe disease, initiation of enzyme replacement therapy, and removal from the heart transplant waitlist. When WES results were available, only the single base deletion was identified; based on the WES result, Patient W would have been considered a carrier for Pompe disease when they were actually affected. No management changes were made based on WES results, as the molecular single gene testing of Pompe disease revealed the diagnosis in this patient prior to receiving the WES results. Had providers not chosen to send the additional testing, WES would have provided uncertain results. At that time, providers may have chosen to send additional testing based on carrier status, but the delay in diagnosis and treatment would have allowed further uncontrolled progression of the condition. Providers unaware of the limitations of WES may have viewed Patient W's *GAA* mutation as an incidental finding that was non-contributory to the clinical picture. While WES is a valuable tool in many cases, it is important that providers understand the limitations of WES. This information should also be conveyed to patients, who may perceive WES as the final step in genetic testing.

3.4.3 Future Studies

Further study is needed to determine if WES has a comparable clinical utility when done on the general pediatric CICU population when patients are not selected by a Medical Geneticist, as well as in the adult CICU setting. Previous WES studies have suggested that younger patient age is associated with an increased diagnostic rate for WES. This study was conducted in the pediatric setting, which may have a higher diagnostic rate for WES than in the adult CICU. A study by the YPCG showed a similar diagnostic yield for WES in adult patients with cardiovascular conditions, however patients in the study were reviewed by cardiovascular geneticists, which may have led to a higher diagnostic yield in this study than would be seen in the absence of a cardiovascular geneticist.⁵⁷ Future study into the use of WES in the general CICU population, both pediatric and adult, may be helpful in determining the overall usefulness of WES in these populations.

3.4.4 Study Conclusions

Patients in the CICU may benefit from the use of WES, when deemed medically necessary by a medical geneticist. The presence of dysmorphic features or multiple congenital anomalies did not increase the likelihood of receiving a positive WES result, nor did the type of cardiac disease. This suggests that there may not be specific patient characteristics that affect the likelihood of determining a diagnosis through WES. Twenty-five percent of patients in the study received a positive result, showing that the diagnostic yield in this population is similar to that seen in other settings (see Table 1 Diagnostic Yield of WES). A total of 33% of positive results would not have been detected on a comprehensive cardiovascular panel. The detection of variants in cardiac genes

through the use of WES that would not have been detected on gene panel testing has been seen in other studies as well.^{6,70} Given the potential for missed diagnoses, as well as lack of characteristics that may predict which patients will receive a positive result, the use of WES should be considered early on in the care of pediatric CICU patients, as an alternative to gene panel testing.

4.0 Genetic Counseling and Public Health Significance

4.1.1 Relevance to Genetic Counseling

Having a child in intensive care is stressful and anxiety-inducing for families.⁵⁴ Families of patients being treated in intensive care units need not only timely information about their child's care and privacy to process the situation, but also psychosocial support and resources.⁸¹ A study by Dinc and Terzioglu (2005) used semi-structured interviews to investigate the psychological impact genetic testing may have on parents. The Spielberger's State and Trait Anxiety Inventory (STAI), a 40 item scale, was used to measure the parents' level of anxiety. Parents reported feeling distressed before their child's genetic testing in 93% of cases, particularly over what the result may mean for the health and futures of their children. Parental state anxiety (the level of anxiety felt by the parents in reaction to their child needing genetic testing) was higher than parental trait anxiety (the level of anxiety felt by the parents in a stable setting) ($P=0.01$). Parents cited a need for genetic counseling after their child's genetic test results were returned in 78.5% of cases.⁸² Genetic counselors are specifically trained to support families in regards to all aspects of genetic disease, including supporting patients in understanding medical information and in intensive care units and adapting to possible psychosocial implications.⁸³ Given the severity of illness of patients in the CICU and the complexity of WES results, we may expect anxiety levels of parents awaiting genetic test results to be even higher, making genetic counseling for these patients and their families more crucial to their care.

When counseling patients, genetic counselors must balance families' hopes that genetic testing will lead to a diagnosis with the possibility that genetic test results may be uninformative

or uncertain. It is necessary to establish realistic expectations about what genetic testing may reveal with families prior to testing. Diagnostic yields have not been well established for all tests or patient populations, which can be challenging for the genetic counselors supporting these families.⁸⁴ While CICU patients have been incorporated into studies regarding the diagnostic yield of WES in general, WES specifically in CICU patients has not been previously studied. This study provides more targeted information about the use of WES for patients being treated in the CICU, which may be incorporated into clinical genetic counseling in order to establish clear expectations with families about WES.

4.1.2 Relevance to Public Health

The evaluation of the effectiveness of health services is an essential service of public health and ensures that impactful interventions are put in place, while ineffective interventions are discontinued.⁸⁵ New genetic tests are continuously being introduced, and it is important that tests be evaluated to determine whether or not they are clinically useful. Our study shows that WES in the CICU had an impact on the management of patients being treated for cardiomyopathies and structural heart defects. Testing of patients in the CICU may benefit not just the patient, but their relatives as well. WES results also affected the management of patients' families, such as additional genetic testing or specialty referrals for at-risk relatives. When possible, patient samples for WES are submitted along with parental samples, allowing laboratories to determine if variants may be *de novo*. This is done to help interpret the variants, but also identifies which side of the family may be at risk of developing the same condition. WES is useful in diagnosing disease and, by extension, identifying at-risk family members.

Ordering appropriate genetic testing can reduce the cost to the health care system and decrease time to diagnosis for patients. Genetic counselors are often involved in evaluating the appropriateness of genetic tests prior to ordering.⁸⁶ Genetic counselors at Seattle Children's Hospital reviewed 3,441 genetic testing orders over a 57 month period. Modifications were made to the testing in 32% of cases, which resulted in total cost savings of \$972,000. Similarly, genetic counselors at the Regions Hospital/HealthPartners health care system, located in Minnesota and western Wisconsin, reviewed 904 genetic test orders. This group modified or cancelled 13.5% of genetic test orders, which saved \$263,000.⁸⁷ Reducing the number of genetic tests could reduce costs. As seen in the study by Soden and colleagues (section 2.2.1), WES can reduce the number of genetic tests a patient undergoes before finally reaching a diagnosis.⁴¹ Health care systems have developed and continue to develop policies regarding genetic testing in an effort to reduce inappropriate or superfluous genetic tests. Our study shows that WES is reasonable for CICU patients. Policies regarding the use of WES for patients with cardiomyopathies or structural heart defects should reflect the knowledge that WES is clinically useful in this population.

In addition to reducing the number of genetic tests done and saving health care costs, WES could provide molecular diagnoses for patients who would go undiagnosed if other genetic tests were used. Landry and colleagues (2018) conducted a study of 5,729 patients with cardiomyopathy who had had genetic testing between October 2003 and December 2017. Twenty-nine percent (1,314 patients) of Caucasian patients had positive genetic test results while only 18.4% (155 patients) of underrepresented minorities had positive results. The difference between these groups was significant ($P < 0.001$), however the proportion of Caucasian patients who received positive results and Asian patients (25%, 87 patients) did not significantly differ ($P = 0.12$).⁸⁸ The cases of cardiomyopathy in patients who did not receive a molecular diagnosis who are underrepresented

minorities could, potentially, be due to genes not included on the panels previously used if mutations in the causative genes have not been reported in the Caucasian population. The use of WES helps to alleviate this disparity because WES is not limited to the genes included on the chosen panel. WES can identify variants in a wider set of genes, including novel genes, which are not currently associated with a Mendelian condition. This could lead to a higher diagnostic yield for underrepresented minorities, as well as identification of new candidate genes.

In the context of the public health, the use of WES is appropriate and effective for patients in the CICU when recommended by a medical geneticist. It has the potential to diagnose disease and alert families to the need for other family members to be evaluated. It may save health care systems money by reducing the number of genetic tests ordered. Finally, by expanding the number of genes investigated beyond what is possible using gene panel testing, WES may help improve diagnostic rates in underrepresented minority populations.

Appendix A Selected Results

A.1 Selected Results By Cardiac Disease Type

	All patients (n=24)	Cardiomyopathy (n=13)	Structural Heart Defect (n=10)	Channelopathy (n=1)
Female (%)	8 (33.3)	6 (46.2)	2 (20)	0 (0)
Positive test result (%)	6 (25)	4 (30.8)	1 (10)	1 (100)
Negative test result (%)	4 (16.7)	1 (7.7)	3 (30)	0 (0)
VUS test result (%)	14 (58.3)	8 (61.5)	6 (60)	0 (0)
Mean age at first consult (range)	3.1y (DOL1-25.8)	5.6y (DOL1-25.8y)	0.2y (DOL2-0.7y)	DOL4
Mean age when WES sent (range)	3.2y (DOL2-25.9y)	5.7y (DOL8-25.9y)	0.2y (DOL2-0.7y)	DOL22
Positive Family History (%)	14 (58.3)	9 (69.2)	5 (50)	0 (0)
Change in diagnosis (%)	4 (16.7%)	3 (23.1)	0 (0)	1 (100)
Change in medication (%)	0 (0)	0 (0)	0 (0)	0 (0)
Additional Testing for Patient (%)	10 (41.7)	6 (46.2)	4 (40)	0 (0)
Referral for Patient (%)	4 (16.7)	3 (23.1)	1 (10)	0 (0)
Testing for Family Members (%)	7 (29.2)	4 (30.7)	3 (30)	0 (0)
Referral for Family Members (%)	9 (37.5)	7 (53.8)	1 (10)	1 (100)
Any Change for Patient (%)	13 (54.2)	8 (61.5%)	4 (40)	1 (100)
Any Change for patient and/or Family (%)	18 (75)	11 (84.6)	6 (60)	1 (100)

A.2 Deceased Patients

	Deceased Patients (n=8)
Result returned after death (%)	6 (75)
Female (%)	2 (25)
Positive (%)	3 (37.5)
Negative (%)	1 (12.5)
VUS (%)	4 (50)
Mean age at first consult (range)	0.3y (DOL2-0.7y)
Mean age when WES sent (range)	0.3y (DOL2-0.7y)
Positive family history (%)	3 (37.5)
Change in diagnosis (%)	3 (37.5)
Change in medication (%)	0 (0)
Additional testing for patient (%)	2 (25)
Referral for patient (%)	1 (12.5)
Testing for family members (%)	3 (37.5)
Referral for family members (%)	3 (37.5)
Any change for patient (%)	4 (50)
Any change for patient and/or family (%)	6 (75)

Appendix B IRB Approval

B.1 Initial Approval

University of Pittsburgh
Institutional Review Board

Human Research Protection Office
3500 Fifth Avenue, Suite 106
Pittsburgh, PA 15213
Tel (412) 383-1480
www.hrpo@pitt.edu

APPROVAL OF SUBMISSION

IRB:	STUDY18100140
PI:	Lina Ghaloul Gonzalez
Title:	Chart Review of Patients Who Have Undergone Whole-Exome Sequencing
Funding:	None
Date:	January 9, 2019

On 1/9/2019, the Institutional Review Board reviewed and approved the above referenced study. The study may begin as outlined in the University of Pittsburgh approved application and documents.

Approval Documentation

Review type:	Initial Study
Risk Level:	No greater than minimal risk
Approval Date:	1/9/2019

Determinations:	<ul style="list-style-type: none">• Waiver of HIPAA authorization• Waiver/alteration of the consent process• Children
Approved Documents:	<ul style="list-style-type: none">• Peds Sci Review Signed Vockley

As the Principal Investigator, you are responsible for the conduct of the research and to ensure accurate documentation, protocol compliance, reporting of possibly study-related adverse events and unanticipated problems involving risk to participants or others.

Continuing review (CR) can be submitted by clicking "Create Modification/CR" from the active study at least 5 weeks prior to the expiration date.

If this trial meets the definition of a clinical trial, accrual cannot begin until it has been registered at clinicaltrials.gov and a National Clinical Trial number (NCT) provided. Contact ctgov@pitt.edu with questions.

Clinical research being conducted in an UPMC facility cannot begin until fiscal approval is received from the UPMC Office of Sponsored Programs and Research Support (OSPARS).

If you have any questions, please contact the University of Pittsburgh IRB Coordinator, [Carolyn Ivanusic](mailto:Carolyn.Ivanusic@pitt.edu).

Please take a moment to complete our [Satisfaction Survey](#) as we appreciate your feedback.

B.2 Modification Approval

University of Pittsburgh Institutional Review Board

Human Research Protection Office
3500 Fifth Avenue, Suite 106
Pittsburgh, PA 15213
Tel (412) 383-1480
www.hrpo@pitt.edu

MODIFICATION APPROVAL

IRB:	MOD18100140-001
PI:	Lina Ghaloul Gonzalez
Title:	Chart Review of Patients Who Have Undergone Whole-Exome Sequencing
Date:	January 25, 2019

On 1/25/2019, the Institutional Review Board reviewed and approved the above referenced Modification through the expedited review procedure authorized by 45 CFR 46.110 and 21 CFR 56.110.

The proposed modifications are well justified and appropriate and do not alter the risk to benefit considerations of the study. The requirements under CFR 46.111 continue to be met.

Approval Documentation

Approval Date:	1/25/2019
Determinations:	<ul style="list-style-type: none">• Waiver of HIPAA authorization• Waiver/alteration of the consent process• Children
Approved Documents:	<ul style="list-style-type: none">• Peds Sci Review Signed Vockley

As the Principal Investigator, you are responsible for the conduct of the research and to ensure accurate documentation, protocol compliance, reporting of possibly study-related adverse events and unanticipated problems involving risk to participants or others. The HRPO Reportable Events policy, Chapter 17, is available at <http://www.hrpo.pitt.edu/>.

Research being conducted in an UPMC facility cannot begin until fiscal approval is received from the UPMC Office of Sponsored Programs and Research Support (OSPARS). Contact OSPARS@upmc.edu with questions.

If you have any questions, please contact the University of Pittsburgh IRB Coordinator, Carolyn Ivanusic at ivanusic@pitt.edu.

Please take a moment to complete our [Satisfaction Survey](#) as we appreciate your feedback.

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