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Research Article

Clinical Whole Exome Sequencing in an Academic Pediatric Hospital: Analyses of the Diagnostic Odyssey

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Abstract

Background: Whole exome sequencing (WES) is a genetic test that sequences all protein-coding regions, exons, in a patient's genome to identify disease causing mutations. Patients who present with complex phenotypes, may have had previous genetic testing and spent significant time searching for a diagnosis, known as a diagnostic odyssey and WES may help to end the search. WES can also detect secondary findings and/or genetic variants in disease associated genes with uncertain clinical significance. This study is a retrospective chart review designed to describe the diagnostic odyssey of patients who have had WES testing ordered through Cincinnati Children's Hospital Medical Center (CCHMC)

Methods: Electronic medical records were abstracted for 75 pediatric patients (21 years or younger) during July 1, 2013 – September 30, 2014. The Results Group consists of 34 patients who received their WES results by September 30, 2014.

Results: Fifty four patients (72%) in the study population presented with symptoms in infancy and 32 patients (43%) had WES ordered at school age (5-13 years old). Age of onset of symptoms versus age that WES was ordered was significantly different (P<0.0001 using the Wilcoxon signed-rank test). The number of genetic tests ordered prior to ordering WES ranged from 0-12 and the average number was 4.3 tests. In the Results Group, diagnostic yield was 29%, secondary findings were reported for 7 patients (20%) and variants of uncertain significance were reported for 14 patients (41%).

Conclusions: The majority of patients in this study presented with symptoms in infancy and had WES at school age, indicating a long diagnostic odyssey prior to receiving WES testing. This timeframe may, in part, be due to lack of availability of earlier WES testing. The information presented in this study supports the use of WES as a possible first line genetic test when diagnosis is unclear.

Keywords: Whole exome sequencing; diagnostic odyssey; secondary findings; variants of uncertain significance; complex phenotype

Abbreviations

WES: Whole Exome Sequencing;
CCHMC: Cincinnati Children's Hospital Medical Center;
VUS : Variant of Uncertain Significance;
ACMG: American College of Medical Genetics;
ASD: Autism Spectrum Disorder;
MODY: Maturity Onset Diabetes of the Young

Introduction

Whole exome sequencing (WES) is a genetic test that sequences all protein-coding regions, exons, in a patient's genome [1-4]. Approximately 40-80/1000 live births have a genetic disorder [5]. The exact number of undiagnosed patients is unknown; however, according to the National Human Genome Research Institute, it is estimated that 30-40% of children with special needs do not have a genetic diagnosis. WES is used as a diagnostic tool to identify disease causing mutations in patients who present with complex phenotypes, patients that have diseases with genetic heterogeneity or when a genetic condition is suspected and previous genetic testing (i.e. microarray, single gene, karyotype) has not yielded a genetic diagnosis [6-8,10]. In addition, a new or more clearly defined genetic diagnosis due to WES may help guide treatment and result in a better outcome for the patient [4, 9,11]. Patients who are candidates for WES may have undergone previous genetic testing in search of finding a definitive molecular diagnosis. This history of testing is referred to as their diagnostic odyssey.

Recent studies have shown that diagnostic yield of WES ranges from 15-50% [5,12,13]. In addition to finding disease causing mutations, WES can identify secondary findings, variants of uncertain significance (VUS), carrier status and a person's pharmacogenomics profile [8,14]. Secondary findings are defined as pathogenic or likely pathogenic alterations in genes that are not primarily associated with the indication for WES and are also referred to as incidental findings [6,15]. The American College of Medical Genetics (ACMG) released recommendations regarding the reporting of secondary findings. ACMG recommended that patients be consented and understand the strengths, limitations and possible outcomes of the test [16]DNA</keyword></keywords><dates><year>2013</year><pub-dates><date>Aug</date></pub-dates></dates><isbn>1530-0366 (Electronic. In addition, patients have the option to opt in or out of analysis of 57 genes that are not associated with the clinical presentation, which may result in secondary findings [15]. Variants of uncertain significance are changes in the DNA that are not well understood and therefore, have unknown clinical implications until more information is collected [17]. Though in some cases, clinicians may choose to make treatment decisions such as increased screening or further evaluation based on the finding of a VUS. Frequencies of secondary findings and VUS are important information for geneticists and genetic counselors to discuss with their patients during the informed consent process.

The aim of this study is to quantify and analyze the population characteristics and diagnostic odyssey of pediatric patients who have received WES testing. In addition, this study will provide more information about rates of secondary findings and VUS's when performing WES. The information presented provides a better understanding of the clinical utility of WES, which will help to guide its future clinical practices.

Methods

This study was approved by the Cincinnati Children's Hospital Medical Center (CCHMC) institutional review board. This study is a retrospective chart review designed to describe the population and time spent searching for a molecular diagnosis of patients who have received clinical WES. Electronic medical records of 75 pediatric patients (21 years of age or younger) who had WES ordered from the CCHMC between July 1, 2013 – September 30, 2014 were abstracted. Thirty four patients had received their WES results by September 30, 2014. This group of patients will be referred to as the Results Group.

Records of patients who have signed the consent forms and underwent WES are maintained by the CCHMC molecular genetics laboratory and were used to identify patients who met the necessary inclusion criteria for this study. Data was abstracted from the requisition form, consent form and electronic medical and laboratory records. Data was collected on demographics, ordering physician information, WES test results, the onset and presentation of symptoms, previous genetic testing ordered and whether the patient opted in or opted out for secondary findings. Demographic information included patient age at the time WES was ordered, gender, and ethnicity. A REDCap database was created to store the information collected and a unique identification number was assigned to each patient [18].

Onset and Presentation of Symptoms

The age of onset of symptoms was collected from the electronic medical records. Age of symptom onset and age that the patient had WES, was divided into the following categories. Infant (0-12 months), toddler (1 year and 1 day to 5 years old), school age (5 years and 1 day to 13 years), teenagers (13 years and 1 day to 18 years) and over 18 (18 years and 1 day to 21 years). Presenting symptoms were documented based off the requisition form submitted by the ordering physician. The requisition form included the following 15 symptom categories, developmental delay including intellectual disability, skeletal, neurological, craniofacial/ophthalmologic/auditory, gastrointestinal, failure to thrive/growth retardation, hematological, immunological/allergy, cardiovascular, dermatological, prenatal/perinatal/maternal, psychiatric/behavior/autism spectrum disorder (ASD), genitourinary, endocrinologic and overgrowth/tall stature. Data were collected on whether a patient had a symptom in any of the symptom categories and whether a patient had any specific symptoms listed in the general history symptom category.

Previous Genetic Testing Ordered

Previous genetic testing for each participant was collected

from the laboratory reports or the electronic medical record. If there were no original laboratory report results or information in the electronic medical record, then the date of previous genetic testing was noted to be as "unknown". Specifically, information on whether the patient had chromosomal analysis, single gene sequencing, multi-gene panel testing, CGH/SNP microarray testing, mitochondrial DNA testing, methylation studies, or FISH studies was collected. The total number of tests completed prior to ordering WES was tallied.

WES Test Results

The data collected on WES test results included whether or not a definitive genetic diagnosis was determined, if the patient chose to opt in or out for secondary findings, if secondary findings were reported when the patient chose to opt in, if variants of uncertain significance were reported, and if the result of the test confirmed or provided a new or better defined genetic diagnosis. Current working diagnosis is defined as the suspected clinical diagnosis that is guiding treatment or the diagnostic findings when a specific diagnosis is unclear. This information was collected either from the requisition form and/or from the primary diagnosis in the electronic medical records.

Statistical Analysis

Summary statistics were calculated using the SAS 9.4 software (SAS, Cary, NC. Categorical variables included counts and frequencies, and continuous variables included means and standard deviations. A Wilcoxon signed-rank test was run on age categories.

Results

Demographics

A total of 75 patients were included in this study; demographic data for these patients are shown in Table 1. Forty one patients (55%) were male and 34 patients (45%) were female. Participants identified as one or more of the following ethnicities; Caucasian (88%), Native American or Alaskan (12%), Pacific Islander (0.0%), African American (8%), Asian American (1%) or Other (15%). Genetics professionals ordered WES for 57 patients (76%). For the remaining patients, ordering health care professionals included immunologists/allergists (n=12), oncologist/hematologist (n=3), PCP (n=2) and nurse (n=1). Overall, 64 patients (85%) had a genetics evaluation either by a geneticist or genetic counselor prior to the ordering of WES.

Onset and presentation of symptoms

The comparisons of the age of onset of symptoms and the age WES was ordered can be seen in Figure 1. Fifty four patients (72%) presented with symptoms in infancy, but only six patients (8%) had WES ordered in this age group. The most frequent age group that WES was ordered was school age (32 patients, 43%). Age of onset of symptoms compared to age that WES was ordered was significantly different (P<0.0001 using the Wilcoxon signed-rank test). The study patient population presented with overlapping spectra of symptoms. The most common symptoms included developmental delay (n=77), neurological (n=42) and skeletal abnormalities (n=42). Figure 2 shows the number of patients with each type of symptom.

Gender	**n**	**%**
Male	41	55
Female	34	45
Race/Ethnicity	**n**	**%**
Caucasian	66	88
Native American/Alaskan	9	12
Pacific Islander	0	0
African American	6	8
Asian American	1	1
Latino/Hispanic	3	4
Ashkenazi Jewish	2	3
Other	11	15
Had Genetics Evaluation Prior to ordering WES	**n**	**%**
Yes	64	85
No	11	15

*Patients may have identified as one or more race/ethnicity

Table 1. Demographics of patient population.

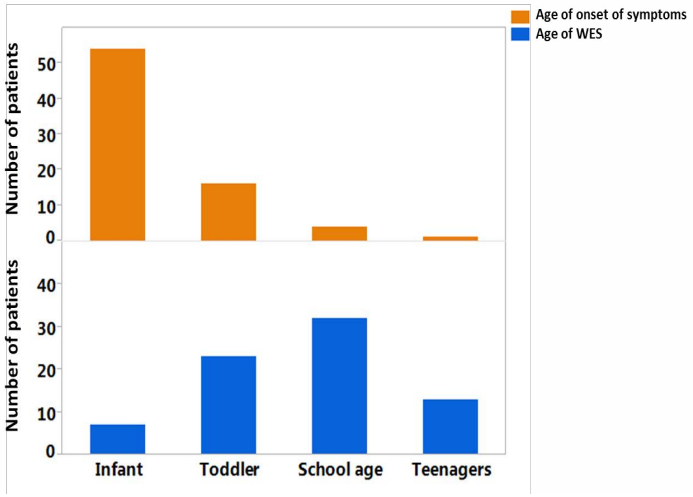
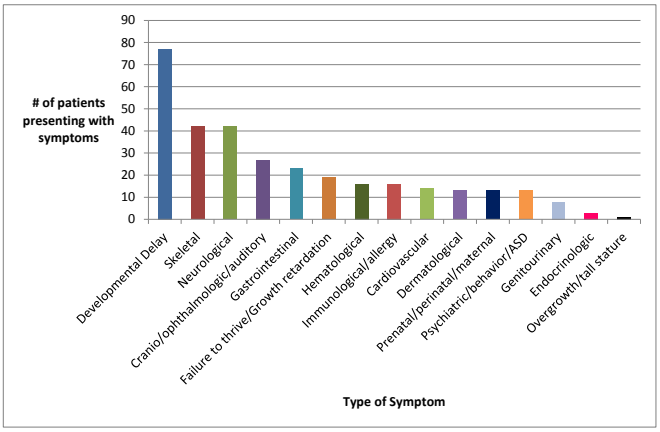


Figure 1. Age symptom Onset vs. Age WES was ordered. Age of onset of symptoms versus age that WES was ordered was significantly different. *P<0.0001 using the Wilcoxon signed-rank test. Fifty four (of 75) patients (72%) presented with symptoms in infancy and 32 (of 75) patients (43%) had WES ordered or had a diagnosis from WES at school age.



Presenting symptoms. The most common presenting symptoms includes developmental delay (n=77), neurological (n=42) and skeletal abnormalities (n=42). Developmental delay includes intellectual disability, developmental regression, speech delay, gross motor skill delay and fine motor skill delay.

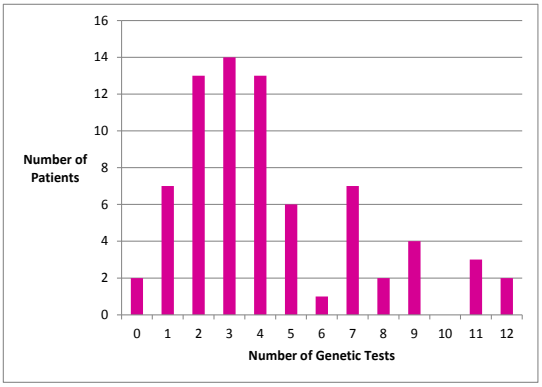
Figure 2. Presenting symptoms. The most common presenting symptoms includes developmental delay (n=77), neurological (n=42) and skeletal abnormalities (n=42). Developmental delay includes intellectual disability, developmental regression, speech delay, gross motor skill delay and fine motor skill delay.

Previous genetic testing ordered

In the patient population, the average number of genetic tests ordered prior to ordering of WES was 4.3. There was no statistically significant difference between genders for the number of genetic tests completed prior to WES. For this study, genetic testing is includes chromosomal analysis, single gene sequencing, multi-gene panel testing, CGH/SNP microarray testing, mitochondrial DNA testing, methylation studies, or FISH. For many patients, previous genetic testing had reported abnormal results; however, no diagnosis had been determined. The median number of genetic tests ordered was 4. The number of genetic tests ordered prior to WES ranged from 0-12, with WES being the first test ordered in two cases (Figure 3). The total number of genes tested from multi-gene panels ranged from 3 to 679 genes. Figure 4 shows the types of genetic tests ordered. Forty eight (64%) of the patient population had a microarray prior to having WES, making it the most common genetic test ordered. The second most common genetic test ordered prior to WES was for a single gene, with forty six (61%) patients receiving single gene tests. The number of single gene tests ordered for each patient ranged from 1 to 10. The average number of single genes tests ordered was 3.7.

WES Test Results

Figure 5 shows the WES test results for the Results Group. Diagnostic yield was 29% (10 of 34 patients). All 10 patients that received diagnoses were found to have a pathogenic or likely pathogenic variant that better defined or fully explained the pathogenesis of their condition. When given the choice whether to opt in or opt out of secondary findings, 12% of patients (n=4) opted out. Twenty percent of patients (n=7) were reported to have secondary findings. Variants of uncertain clinical significance were reported for 41% (n=14) of the Results Group. For five patients in the Results Group, WES provided a new or better defined diagnosis, and for 5 patients WES confirmed the suspected diagnosis. Table 2 contains information about all 10 patients with either a confirmed or better defined diagnosis.



Number of genetic tests ordered prior to WES. The median number of genetic tests ordered was 4. The number of genetic tests ordered prior to WES ranged from 0-12, with WES being the first test ordered in two cases.

Figure 3.Number of genetic tests ordered prior to WES. The median number of genetic tests ordered was 4. The number of genetic tests ordered prior to WES ranged from 0-12, with WES being the first test ordered in two cases.

Gender	Working Diagnosis/Clinical Symptoms	Disease Causing Gene	WES Diagnosis
Female	White Matter Abnormality on MRI of Brain	NPC1	Niemann Pick Type C
Male	Chiari I Malformation	COL5A1	Ehlers-Danlos Syndrome Type I
Female	Moebius Syndrome	CHRNE	Congenital Myasthenic Syndrome
Male	Skeletal Dysplasia	NPR2	Acromesomelic Dysplasia Maroteaux Type (AMDM)
Male	Pierre Robin Sequence	ASXL3	Bainbridge-Ropers syndrome
Male	Common Variable Immune Deficiency	NFKB2	Immunodeficiency, common variable
Male	Syndromic Intellectual Disability	DYRK1A	Mental retardation, autosomal dominant
Female	Unspecified Immunity Deficiency	CTPS1	Immunodeficiency
Female	Intellectual Disability	STXBP1	Epileptic encephalopathy, early infantile
Male	Diabetes Mellitus	INS	Maturity-onset diabetes of the young, type 10

Table 2. Diagnoses of WES test results for Results Group. For 5 patients, WES provided a new or better defined diagnosis, and for 5 patients WES confirmed the suspected diagnosis.

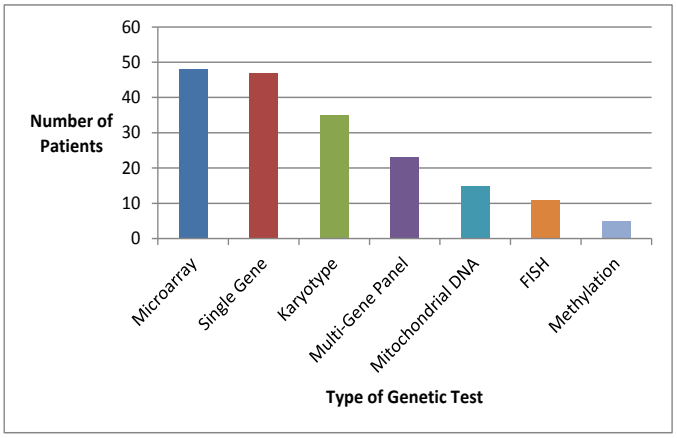


Figure 4.Type of Genetic Test Ordered. Forty eight (64%) of the patient population had a microarray prior to having WES, which was the most common type of genetic test ordered. The second most common genetic test ordered prior to WES was for a single gene, with forty six (61%) patients receiving single gene tests. The number of single gene tests ordered for each patient ranged from 1 to 10. The average number of single genes tests ordered was 3.7.

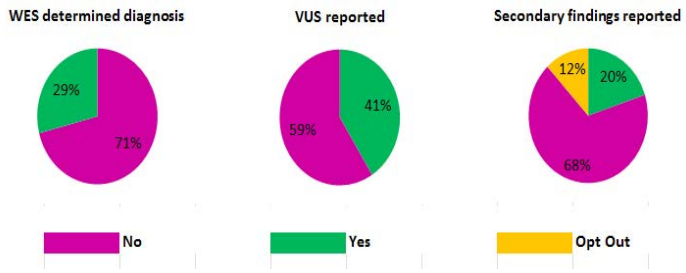


Figure 5. WES test results for Results Group. Diagnostic yield was 29% (n=10). Variants of uncertain clinical significance were reported for 41% (n=14).When given the choice whether to opt in or opt out of secondary findings, 12% of patients (n=4) opted out. Twenty percent of patients (n=7) were reported to have secondary findings.

Discussion

In the study pediatric population, the majority of patients presented with symptoms in infancy, while school age was the most frequent age range when WES was ordered or a diagnosis determined, indicating a long diagnostic odyssey. For those patients who had long diagnostic odysseys, a likely explanation is not having the option for WES when symptoms initially presented. Future studies could compare diagnostic odysseys of pediatric patients with recent onset of symptoms to pediatric patients that presented in the past. One example of a patient who has had a long diagnostic odyssey was a 17 year old female who presented to the genetics clinic with global developmental delay since infancy and several dysmorphic features. This patient had seven genetic tests prior to WES, none of which determined diagnosis. Previous genetic testing included a karyotype, microarray, methylation study for Prader-Willi and Angelman Syndrome, and four single gene tests (MECP2, CDKL5, STK9, UBE3). The patient's diagnostic odyssey began in 1997, within the first year of her life. WES detected a de novo mutation in the STXBP1 gene, which is known to be associated with severe intellectual disability and early onset epileptic encephalopathy. Although this finding did not change treatment, the family was counseled on recurrence risk and was given an end to the diagnostic odyssey.

The diagnostic yield of WES in this pediatric population was 29%, which is similar to previously reported yields for other laboratories. One of the first laboratories to offer clinical WES reports a diagnostic yield of 30% for WES for a cohort of 500 pediatric and adult patients [13]. A recent study reported the WES diagnostic yield for a population of 504 that included both pediatric and adult patients was 25.2% [14]. An example of a patient who had no previous genetic testing and WES determined a molecular diagnosis for his condition is a 6 year and 3 month old male. The patient presented to the genetics clinic with cataracts, unusual presentation of insulinopenic diabetes and hepatomegaly as a toddler. The symptoms did not fit into any classic diabetes pattern. After the completion of WES, a de novo mutation was detected in the *INS* gene, which is associated with maturity onset diabetes of the young (MODY). This finding was believed to explain the patient's symptoms and avoid running unnecessary genetic testing and spending extra time looking for a diagnosis.

Secondary findings for our study population were reported in 20% of patients, which is higher than the previously reported range of 1-7% [14,15]. If clinicians are able to tell patients the estimated risk of secondary findings, then they can make a better informed decision as to whether they would like to opt in or opt out during the consent process. One possible explanation for this number being large and a limitation in this study is the small cohort. In addition, the CCHMC laboratory's policy is to report findings that are pathogenic or likely pathogenic regardless if they have been previously reported. Determining if a variant is disease causing is based on the laboratory's algorithm, which includes the type of mutation, presence in literature and databases, as well as the use of prediction models. Other laboratories may have different criteria, which may explain the difference in reportable secondary findings.

Our study cataloged the patients' diagnostic odyssey based on genetic testing. Other diagnostic tests such as MRI and metabolic testing is important in determining diagnosis and that was not addressed here. Other testing could be included in future studies as well as cost analysis of WES for patients who have spent a significant time searching for diagnosis to determine the cost-effectiveness of WES. WES is an example of the continuing advances in genetic testing technology, specifically

an increase in speed, accuracy and the amount of data that a test is able to analyze. The goal for improved genetic testing is to find a diagnosis as quickly as possible and decrease the time gap from presentation of symptoms to diagnoses as well as the cost burden.

Conclusions

WES is a useful tool in clarifying a diagnosis or elucidating a diagnosis not previously considered and should continue to be used in clinic. The information presented in this study supports the use of WES as a possible first line genetic test when diagnosis is unclear. In addition, this study provides information that may be helpful in determining clinical utility and possible outcomes of WES.

Competing interests

The author(s) declare that they have no competing interests

Authors' contributions

RF participated in the design of the study, collected data and drafted the manuscript. JC participated in the design of the study and edited the manuscript. KC participated in the design of the study and edited the manuscript. VP performed the statistical analysis. KZ participated in the design of the study and edited the manuscript. All authors discussed the results and implications and commented on the manuscript at all stages. All authors read and approved the final manuscript.

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