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The Utility of Whole Exome Sequencing in Patients With Intellectual Disability and Developmental Delay as a First-Tier Diagnostic Testing Strategy

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THE UTILITY OF WHOLE EXOME SEQUENCING IN PATIENTS WITH INTELLECTUAL DISABILITY AND DEVELOPMENTAL DELAY AS A FIRST-TIER DIAGNOSTIC TESTING STRATEGY

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Abstract

The purpose of this study is to evaluate the diagnostic utility of whole exome sequencing (WES) in patients with intellectual disability (ID) or developmental delay (DD), and to determine which patients may be the best candidates for WES as a first-tier diagnostic test. The diagnostic and clinical utility of WES has emerged to be greater than that of karyotype and chromosomal microarray for patients with ID or DD of unknown etiology, which are currently recommended as first-tier diagnostic tests for these patients. The emergence of next generation sequencing has led to more rapid identification of rare and novel genetic disorders. Diagnosis of such disorders can impact medical management and save money. The value of this study lies in identifying which patients with ID or DD are more likely to receive a diagnosis via WES and therefore should be offered WES as a first-tier diagnostic test. This study is a retrospective review of electronic medical records of patients with ID/DD seen at the Greenwood Genetic Center (GGC) who have had WES. Patients were categorized into diagnosed, undiagnosed, or uncertain categories. Comparisons between patients were made based on delay types, dysmorphic features, birth defects, and comorbid conditions. Neither delay type, number of delays, age of diagnosis, or birth defects had a significant effect on likelihood of diagnosis. Patients with neurological features, tone differences, or eye movement disorders were significantly more likely to obtain a diagnosis by WES. Changes to medical management in diagnosed patients include referrals to new specialists, adjustments in medication

prescriptions, identification of contraindicated medications, and referrals to specialty clinics specific to disease. These data suggest that WES should be considered as a first-tier test in any patient with ID or DD, and WES may have a higher diagnostic utility for those with underlying neurological disorders.

Table of Contents

List of Figures

List of Abbreviations

ACMG.....American College of Medical Genetics

ADHD..... Attention Deficit Hyperactivity Disorder

CMA .....Chromosomal Microarray

DD.....Developmental Delay

FXS .....Fragile X Syndrome

GDD.....Global Developmental Delay

GGC .....Greenwood Genetic Center

ID .....Intellectual Disability

IQ .....Intellectual Quotient

LD ..... Learning Disability

NDD.....Neurodevelopmental Disorder

NGS..... Next Generation Sequencing

NSV.....No Significant Variants

P/LP..... Pathogenic/Likely Pathogenic

VUS..... Variant of Uncertain Significance

WES .....Whole Exome Sequencing

WGS.....Whole Genome Sequencing

Chapter 1

Background

Clinical Diagnosis of Intellectual Disability (ID) and Developmental Delay (DD)

The term developmental delay (DD) is frequently used to describe a child that is failing to meet milestones typical to a developing child his or her age. Four common categories of DD include: 1) cognitive, 2) motor, 3) language and communication, and 4) social/emotional delay (Moeschler, Shevell, & Committee on Genetics, 2014; Petersen, Kube, & Palmer, 1998). Cognitive function refers to the level of cognition a person has, or intellectual function. Learning, thinking, and problem-solving skills are all indicative of cognitive development. Motor skills refer to a person's ability to act physically. Examples of motor delays include not meeting milestones such as grasping, sitting, standing, or walking. Social/emotional delay refers to an individual's ability to interact with others and respond to certain events or actions. This might include a child that does not recognize familiar faces when he or she should. Lastly, delays in language and communication refer to issues communicating with others and conveying information such as pointing to a specific object or the ability to speak at an age-appropriate level ("CDC's Developmental Milestones | CDC," n.d.). Any failure to meet specific age-appropriate milestones in any of these categories can be termed developmental delay. There is clinical importance to the term delay, as it implies that this is a dynamic diagnosis that children can overcome. It is important to note that while not all delays lead

to life-long disabilities, they can be indicative of an underlying neurodevelopmental disorder (NDD) that does lead to additional long-term needs such as autism spectrum disorder, fragile X syndrome (FXS), and various other conditions, many of which have genetic etiologies.

Though there is no age restriction to when a person can be diagnosed with ID, symptoms must arise during the developmental period (American Psychiatric Association. & American Psychiatric Association. DSM-5 Task Force., 2013). Prior to when an accurate assessment of intellectual ability can be ascertained, children who have two or more developmental delays are thought to have global developmental delay (GDD). GDD may or may not manifest into ID later in life but multiple delays are thought to be predictors of ID, and therefore a diagnosis of ID is more indicative of longer term impairment (Michelson et al., 2011; Moeschler et al., 2014).

Intellectual disability (ID) is assessed using tests of adaptive reasoning and standardized testing of intellectual function. Many physicians in the United States use criteria in the Diagnostic Statistical Manual of Mental Disorders, 5th edition (DSM-5, 2013) to make diagnoses of ID. According to the DSM-5, ID is characterized by impairment of abilities that affect adaptive functioning in three different domains:

1) “The conceptual domain includes skills in language, reading, writing, math, reasoning, knowledge, and memory.”

2) “The social domain refers to empathy, social judgment, interpersonal communication skills, the ability to make and retain friendships, and similar capacities.”

3) “The practical domain centers on self-management in areas such as personal care, job responsibilities, money management, recreation, and organizing school and work tasks.” (American Psychiatric Association, 2013)

These adaptive functioning measures are used in conjunction with standardized tests measuring intellectual function (learning, problem solving, and reasoning) like intelligence quotient (IQ) scores to diagnose ID. A person is considered intellectually impaired when an IQ score is two standard deviations below the mean of the population. This is typically an IQ score of 70 or below.

A clinical diagnosis of ID and/or DD is often needed for patient access to resources such as early intervention and special education, as well as insurance coverage of these additional services. Pediatricians, school teachers/psychologists, or parents may be the first to recognize signs or symptoms of ID and DD. Pediatricians can refer children to a pediatric genetics team for evaluation and consideration of genetic testing, as well as a developmental-behavioral pediatrician for further evaluation and clinical diagnosis. Diagnosis of a genetic syndrome can allow patients to follow-up with necessary specialists sooner, leading to faster treatment or potential preventative therapy. It also allows for determination of recurrence risk and reproductive decision making. For these reasons, it is necessary to identify any genetic cause of ID or DD in a timely manner.

Prevalence of ID and DD

In October of 2019 the results of a study using the National Health Interview Survey was released. This study assessed the prevalence of developmental disabilities in individuals aged 3-17 years of age in the United States. Developmental disabilities are a

group of conditions thought to lead to lifelong impairment in physical, learning, language, or behavior areas. The estimated prevalence of any developmental disability, including attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder, blindness, cerebral palsy, ID, learning disability (LD), moderate to profound hearing loss, or other developmental delay, from 2015-2017 in the United States was 17.8 %. ID accounted for 1.10%, LD for 7.74%, and other DD for 4.38% (Zablotsky et al., 2019).

In addition to genetic etiologies, external factors can play a role in ID and DD, particularly maternal exposures, trauma, premature birth and pregnancy complications, or a combination of genetic and environmental factors. For these reasons, when a child presents with developmental delay, it is important for patients to undergo a comprehensive evaluation. This includes a clinical examination including an evaluation of head circumference, height, neurological assessment, ophthalmologic evaluation, otolaryngology evaluation, skin assessment, dysmorphology examination of facial features and extremities, and assessment of any internal malformations. Evaluation should also include an assessment of the patient's full medical history (including the prenatal, perinatal, postnatal, and development period) and a three-generation family history (Moeschler et al., 2014, 2006).

Previous studies have determined that overall, upon evaluation, etiology can be identified in up to 70% of individuals with GDD. Of this 70%, 15% are thought to be syndromic, and up to 40% are thought to have a genetic etiology (Miclea, Peca, Cuzmici, & Pop, 2015; Moeschler et al., 2014). Chromosomal abnormalities account for 25% of these cases, including trisomy 21, trisomy 13, trisomy 18, and structural abnormalities (microdeletions and microduplications). Of this population, 10% are thought to have

monogenic etiologies, the most common being fragile X syndrome (Miclea et al., 2015). These numbers are now thought to be an underestimate of the genetic etiologies, considering WES alone identifies genetic etiologies in 31% of patients with isolated neurodevelopmental disorders (Srivastava et al., 2019).

Genetic Testing Recommendations for ID and DD

Recommendations for genetic testing for children with intellectual disability (ID) and developmental delay (DD) have changed drastically with the onset of new technologies within the last two decades. As of 2005, the recommendation for first-tier testing for any child with ID or DD was cytogenetic G-banding techniques with a resolution of 550-banding as well as analysis for FXS (Shaffer & American College of Medical Genetics Professional Practice and Guidelines Committee, 2005). This was the first guideline set forth identifying testing necessary for any child with ID/DD despite whether another anomaly was present. Those guidelines also recognized that this cytogenetic technique does not provide enough resolution for small microdeletions and microduplications or chromosomal rearrangements that are known to be causative of ID/DD. The 2005 guidelines reserved higher resolution cytogenetic technologies for cases in which patients present with other anomalies such as congenital anomalies or dysmorphic features. In 2010, new guidelines were established recommending chromosomal microarray (CMA) as a first-tier genetic test for any patient with multiple anomalies that do not suggest a specific syndrome; or a patient with ID, DD, or autism of unknown origin (Manning, Hudgins, & Professional Practice and Guidelines Committee, 2010; Miller et al., 2010). As of 2020, patients with ID/DD are still recommended CMA and FXS testing as the initial tests in an attempt to identify causative genetic variants.

The advent of next generation sequencing (NGS) is changing this view. The overall diagnostic rate for individuals with ID/DD solely using targeted NGS panels has increased and in some cases, even provided a corrected clinical diagnosis (Gieldon et al., 2018). In June of 2019 a consensus statement was released by a multidisciplinary group entitled the "Exome Scoping Review Work Group" which states that WES consistently has a higher diagnostic rate than of CMA for NDDs, and proposes a strategy for first-tier testing with WES at the beginning of evaluation of unexplained NDDs (Srivastava et al., 2019).

Utility of Whole Exome Sequencing

Whole exome sequencing (WES) utilizes NGS technology to analyze/assess the protein-coding portion (exons) of the genome. It has become increasingly instrumental in identifying rare and novel genetic disorders (Bamshad et al., 2011). WES is particularly useful in clinical practice when a patient presents with complex phenotypes that do not point to a particular syndrome of origin. Studies have shown that WES has a significantly higher diagnostic rate overall than that of CMA or NGS panels (Clark et al., 2018; Dillon et al., 2018). Typically, a patient only is offered WES after other technologies are used to rule out known single gene disorders, copy number variants, and chromosome abnormalities. A significant proportion of patients offered WES following other tests present with NDDs and the diagnostic rate by WES for these patients is estimated to be 31% (Srivastava et al., 2019). The increase in diagnostic rate compared to CMA is largely attributed to the ability of WES to pick up variants at a single nucleotide resolution across the protein coding portion of the genome. Currently recommended first-tier technologies such as karyotype and microarray lack this resolution. Targeted panels

using NGS technology to test a number of genes associated with a certain phenotype are also often implemented, but may not cover the entirety of the gene or risk missing a significant variant in a gene not included on the panel. It is also difficult to choose targeted panels when phenotypes appear to be unrelated or are uncommonly seen together. It has been determined that the broader coverage of WES increases its diagnostic utility compared to targeted NGS panels (Dillon et al., 2018)

One of the many arguments against more broadly implementing WES is the financial burden placed on families and the healthcare system. Though more studies need to be done to evaluate the cost-effectiveness of WES in clinical care, it is becoming more commonplace in practice. The estimated cost can range worldwide between $555 and $5,169 for a singleton WES (Schwarze, Buchanan, Taylor, & Wordsworth, 2018). The preferred method of testing is WES trio analysis which includes parental studies to help elucidate inheritance patterns during the initial analysis, increasing those costs to $3,825-$9,304 (Schwarze et al., 2018). Though the cost of WES is high, the collective expense of all other testing leading to WES can easily exceed this. Implementing WES earlier in the diagnostic process may eliminate other costly and unnecessary laboratory tests or other procedures (Córdoba et al., 2018; Monroe et al., 2016; Soden et al., 2014; Stark et al., 2017; Valencia et al., 2015).

ID and DD can be seen with other congenital malformations in syndromic presentation or as an isolated finding. Studies have shown that the more severe phenotype a child presents with and the greater the number of comorbidities presented, the more likely a genetic diagnosis is to be made. This is particularly true of NDDs (de Ligt et al., 2012; Fan et al., 2018). A retrospective study completed in 2018 analyzed the probability

of diagnosis by CMA in patients with DD and ID based on comorbid conditions (Fan et al., 2018). Fan et al. categorized patients based on severity of ID and found that patients with severe ID had a higher diagnostic rate (33%) than those of mild (19%) and moderate (22%) ID patients, though this was not statistically significant. This study also subdivided patients into categories based on common comorbidities present in this population/cohort and found that those with ID/DD were more likely to a obtain diagnosis via CMA when they also presented with congenital heart defects, facial dysmorphism, microcephaly, or hypotonia. Congenital heart defects had the strongest correlation. Neurodevelopmental disorders can range across a wide variety of phenotypes and severities. Studies have focused primarily on grouping children with ID/DD together as a single phenotype and measuring the diagnostic and clinical utility of WES in this way. Current literature neglects to delve into the different types of delay, and separate outcomes of WES based on specific delay phenotypes. These presentations can vary greatly, and severity or type of indication may be an indicative factor of who may be a better candidate for WES over others.

Rationale of study

Prior concerns to implementing whole exome sequencing as a first-tier diagnostic test have included the lack of accessibility due to high cost. Studies have now shown that implementing WES first can decrease the overall cost of the diagnostic odyssey (Monroe et al., 2016). Previous studies that examined the diagnostic and clinical utility of WES for patients with ID/DD have focused on comparing WES to other testing platforms, such as chromosomal microarray (Clark et al., 2018). The cost of WES is decreasing and the ability of WES to pick up certain molecular changes over other testing strategies such as

microarray or targeted panels is greater (Clark et al., 2018; Dillon et al., 2018). However, it is the responsibility of providers to ensure that WES is being used in an appropriate manner. Unrestricted use could lead to inappropriate spending of healthcare dollars or have psychosocial implications for the patient.

Because WES targets so many genes, there is increased chance to find a variant of uncertain significance (VUS) or an incidental finding. Incidental findings such as mutations in a gene unrelated to the indication may illicit psychosocial concerns for the patient (Yang et al., 2014). A VUS may be difficult to interpret or explain to a patient and raise concerns regarding medical management. Additionally, although the cost of WES has decreased it does not ensure that insurance companies will cover such testing. Therefore, it is necessary to identify patients who will benefit from WES over other forms of testing as a first-test strategy. Furthermore, studies have focused on grouping neurodevelopmental disorders or developmental delay together as a single phenotype, but few have delved into the different types of developmental delay associated with likelihood of diagnosis. ID and DD can span a variety of phenotypes and severities and it is necessary to distinguish between varying degrees and types of ID/DD as well as associated anomalies/comorbidities.

Objectives

1. Determine whether type of developmental delay or intellectual disability is associated with increased likelihood of a diagnosis from whole exome sequencing
2. Assess comorbidities present and how these affect the diagnostic yield of WES
3. Assess clinical utility of whole exome sequencing as a first-tier diagnostic test in children with ID/DD by reviewing changes in medical management.

Hypothesis

Patients with certain types and/or multiple types of intellectual disability or developmental delay are more likely to obtain diagnosis by whole exome sequencing. The presence of certain comorbidities impacts the diagnostic yield of WES. Lastly, implementing WES earlier in the diagnostic testing process gives patients faster access to follow-up referrals and necessary resources.

Chapter 2

The Utility of Whole Exome Sequencing in Patients with Intellectual Disability and Developmental Delay as a First-tier Diagnostic Testing Strategy[1]

Introduction

Indications of developmental delay (DD) and intellectual disability (ID) are common referrals to genetics clinics. Children with developmental delays fail to meet milestones typical to a developing child his or her age in various categories including cognitive, language and communication, motor skills, and social domains. Intellectual disability is assessed using tests of adaptive reasoning and standardized testing of intellectual function. Prior to when an accurate assessment of intellectual ability can be ascertained, children who have two or more developmental delays are thought to have global developmental delay (GDD). GDD may or may not manifest into ID later in life but multiple delays are thought to be predictors of ID (Michelson et al., 2011; Moeschler et al., 2014). While not all delays lead to life-long disabilities, they can be indicative of an underlying neurodevelopmental disorder (NDD) that does lead to life-long disability such as autism spectrum disorder, fragile X syndrome (FXS), and various other conditions. Diagnosis of a genetic syndrome can allow patients to follow-up with

[1]Richardson, E., Corning, K., Friez, M., & Walden, K. To be submitted to *Genetics in Medicine*

necessary specialists sooner, leading to faster and/or preventative treatment. It also allows for determination of recurrence risk and reproductive decision making. For these reasons, it is necessary to identify any genetic cause in a timely manner.

The most recent guidelines set forth by the American College of Medical Genetics (ACMG) were released in 2010. These guidelines were established recommending chromosomal microarray (CMA) as a first-tier genetic test for any patient with multiple anomalies that do not suggest a specific syndrome; or a patient with ID, DD (developmental delay), or autism of unknown origin (Manning et al., 2010; Miller et al., 2010). As of 2020, patients with ID/DD are still recommended karyotype, CMA, and FXS testing as the initial tests in an attempt to identify causative genetic variants. The advent of next generation sequencing (NGS) is changing this view due to the increased diagnostic rate of WES for individuals with neurodevelopmental disorders (NDDs) by WES. The diagnostic and clinical utility of WES has emerged to be greater than that of karyotype (~3%, excluding Down Syndrome and recognizable chromosome conditions) and CMA (~15-20%), as well as panel testing (Clark et al., 2018; Dillon et al., 2018). Typically, a patient is only offered WES after other technologies are used to rule out known single gene disorders and chromosomal variants. A significant proportion of patients offered WES following other tests present with NDDs and the diagnostic rate by WES for these patients is estimated to be 31.5% (Nambot et al., 2018). In June of 2019 a consensus statement was released by a multidisciplinary group entitled the “Exome Scoping Review Work Group” which states that WES consistently has a higher diagnostic rate compared to CMA for neurodevelopmental disorders, and proposes a

strategy for first-tier testing with WES at the beginning of evaluation of unexplained NDDs (Srivastava et al., 2019).

The increase in diagnostic rate compared to other testing strategies is largely attributed to the ability of WES to pick up variants at a single nucleotide resolution across the protein coding portion of the genome. Currently recommended first-tier technologies such as karyotype and CMA lack this resolution. Targeted panels using NGS technology to test a number of genes associated with a certain phenotype are also often implemented, but may not cover the entirety of the gene or risk missing a significant variant in a gene not included on the panel. It is also difficult to choose targeted panels when phenotypes appear to be unrelated or are uncommonly seen together. It has been determined that the broader coverage of WES increases its diagnostic utility compared to targeted NGS panels (Dillon et al., 2018)

ID and DD can be seen with other congenital malformations in syndromic presentation or on their own as an isolated finding. Studies have shown that the more severe phenotype a child presents and the greater the number of comorbidities presented, the more likely a genetic diagnosis will be made. This is particularly true of neurodevelopmental disorders (de Ligt et al., 2012; Fan et al., 2018). A retrospective study completed in 2018 analyzed the probability of diagnosis by CMA in patients with DD and ID based on comorbid conditions (Fan et al., 2018). Fan et al. categorized patients based on severity of ID and found that patients with severe ID had a higher diagnostic rate (33%) than those of mild (19%) and moderate (22%) ID patients, though this was not statistically significant. This study also subdivided patients into categories based on common comorbidities present in this population/cohort and found that those

with ID/DD were more likely to obtain diagnosis via CMA when they also presented with congenital heart defects, facial dysmorphism, microcephaly, or hypotonia; congenital heart defects had the strongest correlation.

Prior concerns to implementing whole exome sequencing as a first-tier diagnostic test have included the lack of accessibility due to high cost. Studies have now shown that implementing WES first can decrease the overall cost of the diagnostic odyssey (Monroe et al., 2016). The cost of WES is decreasing and the ability of WES to pick up certain molecular changes over other testing strategies such as microarray or targeted panels is greater (Clark et al., 2018; Dillon et al., 2018). However, it is the responsibility of providers to ensure that WES is being used in an appropriate manner. Unrestricted use could lead to inappropriate spending of healthcare dollars. Additionally, identification of a variant of uncertain significance (VUS) or a secondary or incidental finding in a gene unrelated to the indication may raise psychosocial implications for the patient. Therefore, it is necessary to identify patients who will benefit from WES over other forms of testing as a first-test strategy. Furthermore, studies have focused on grouping neurodevelopmental disorders or developmental delay together as a single phenotype, but few have delved into the different types of developmental delay associated with likelihood of diagnosis. ID and DD can span a variety of phenotypes and severities and it is necessary to distinguish between varying degrees and types of ID/DD as well as associated anomalies/comorbidities. Presentations can vary greatly, and severity or type of indication may be an indicative factor of who may be a better candidate for WES over others.

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Patients with certain types and/or multiple types of intellectual disability or developmental delay are more likely to obtain diagnosis by whole exome sequencing. The presence of certain comorbidities impacts the diagnostic yield of WES. Lastly, implementing WES earlier in the diagnostic testing process gives patients faster access to follow-up referrals and necessary resources.

Methods

Participants

Participants included in this study are patients that had a clinical evaluation at the Greenwood Genetic Center (GGC) and had whole exome sequencing through the Greenwood Diagnostic Lab. Patients must have a documented diagnosis of intellectual disability and/or developmental delay.

Research Methods

This study is a retrospective review of electronic medical records. Cases that had whole exome sequencing in the years 2017 or 2018, with an indication of developmental delay or intellectual disability were considered for inclusion. A total of 111 cases from

2017 and 142 cases from 2018 were reviewed. Cases were included from two consecutive years to capture a representative sample across a longer period of time. Of the 253 cases reviewed, eight were excluded for lack of additional information.

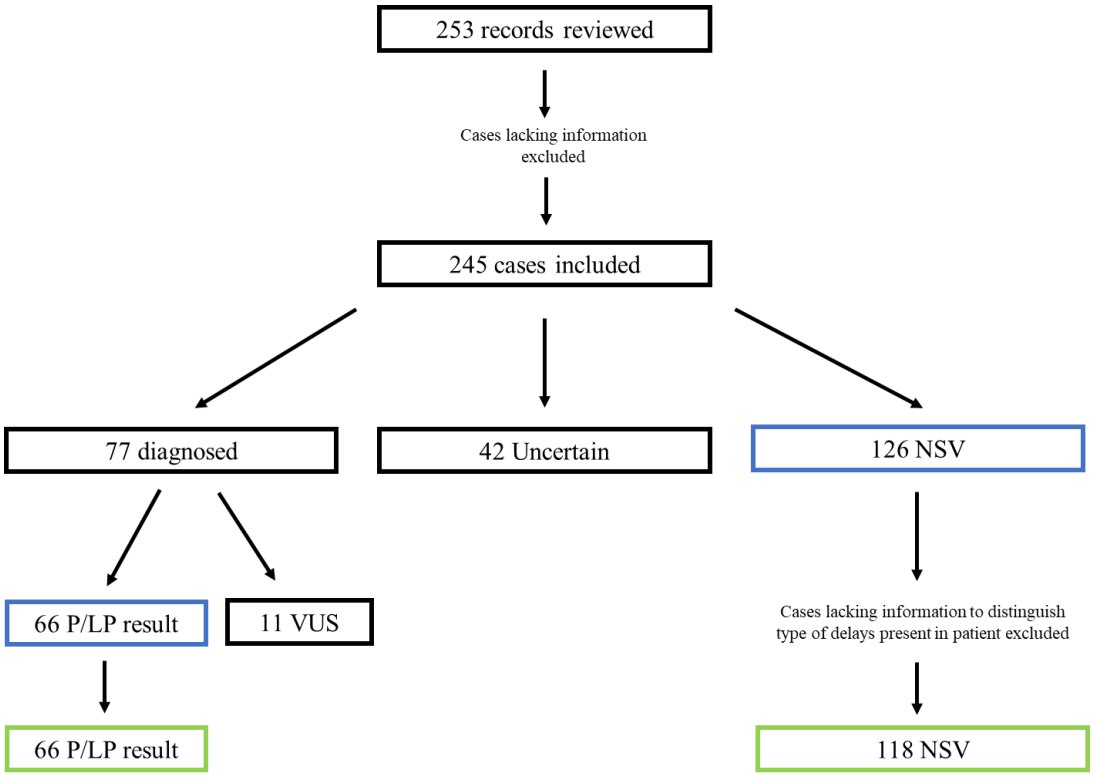

Figure 2.1. Methods by which cases were filtered to assess for features. Cases included in the blue boxes were assessed for dysmorphic features, birth defects, and comorbid conditions. Cases included in the green boxes were additionally assessed for different types of delay.

Patient records were reviewed to collect all relevant clinical, medical, and laboratory data including, primary indication for genetics consultation, detailed phenotypic data, and laboratory test results. Records were reviewed to ascertain type of developmental delay present, for example: cognitive, motor, social/emotional, language delay, or multiple delays. This information was determined by reviewing behavioral tests from professionals such as developmental pediatricians or psychologists, any school records available, and the assessment of the medical geneticist as documented in the

clinic notes. Intellectual Quotient (IQ) scores were recorded for patients when they were available.

Patients were categorized into three result groups: diagnosed, undiagnosed, and uncertain. The diagnosed group consists of patients that received a result from WES that explained their clinical features. These results were delivered as a diagnosis by the physician and patients are being managed accordingly; this includes cases with pathogenic and likely pathogenic alterations as well as 11 cases who received a variant of uncertain significance (VUS). The undiagnosed patients received results that did not explain their condition. These results included those with a pathogenic mutation or variant of uncertain significance in a gene that did not fit the phenotypic description. It also included identification of a single variant in a gene that must be present *in trans* with a second pathogenic variant to be disease-causing. More commonly, in the undiagnosed cohort, no significant variants were identified. Patients were considered part of the uncertain category if a VUS was identified in a gene that is suggestive of the clinical picture, but more evidence is needed to definitely confirm the diagnosis.

Other phenotypic data was collected and organized into three categories 1) dysmorphic features, 2) birth defects, and 3) comorbid conditions. Dysmorphic features include physical differences documented in notes from clinic visits such as differences in head shape, tonicity, facial features, stature, hands, and feet. Birth defects include congenital anomalies such as structural defects in the brain, heart, and genitalia. Comorbid conditions include additional diagnoses and conditions such as autism, attention deficit hyperactivity disorder (ADHD), seizures, coordination/balance issues, premature birth, vision loss, and hearing loss. For a full list of conditions included in

these categories see Appendix A. Categories were analyzed to determine if having any of these features affected the likelihood of receiving a likely pathogenic or pathogenic variant by WES. For the sake of analysis of phenotypic data including different categories of developmental delay, dysmorphic features, birth defects, and comorbid conditions, the 11 patients with a VUS in the diagnosed category, as well as patients with an uncertain result (42) were removed from this portion of the study. This left 192 cases that were assessed for these features. Additionally, no phenotypic data was analyzed if that feature was present in less than 5 patients total, or if a feature was present in the undiagnosed category of patients, but was not present in the diagnosed category. There were no features present in patients of the diagnosed category that were not present in patients of the undiagnosed category. For this part of the analysis, patients were placed into two groups. The patients categorized as diagnosed with a pathogenic or likely pathogenic result are referred to as the P/LP population and patients for whom no significant variants were identified and are considered undiagnosed are the no significant variants population (No Sig. Variants, or NSV.)

The impact of a molecular diagnosis on medical management changes was also assessed. No identifying patient information, including name, medical record number, or date of birth, were recorded when data was collected. Each patient was assigned a study-specific identifier.

Statistical Analysis and Statistical Methods

The data collected required both quantitative and qualitative analysis to reach the objectives of this study. The majority of the data collected were categorical, and therefore descriptive statistics (percentages, frequencies, and odds ratios) were calculated. To

compare categorical variables, Pearson's chi-square test for independence was used. Statistical significance was determined from a two-tailed exact value. For quantitative analysis to compare means, a two-tailed student's t-test was used. For descriptive statistical analysis, Microsoft Excel was used. Microsoft Excel was also used as a database for the collected information, and de-identified data was exported to IBM Statistical Package for Social Sciences (SPSS) version 25 for quantitative analysis as well as for calculation of odds ratios with 95% confidence intervals. Figures and tables were constructed using Microsoft Excel, Microsoft PowerPoint, and GraphPad Prism version 8.3.1.

Results

The overall diagnostic rate was determined for this patient population, the results are displayed in Figure 2.2. Of the 245 patients included in this study, 77 received a diagnosis (31.4%). Eleven of those patients have a variant(s) of uncertain significance (VUS) that were delivered to the family as a diagnosis and are being medically managed for the genetic syndrome identified. The remaining 66 patients received a definitive diagnosed by a likely pathogenic or pathogenic variant(s) (P/LP Population). Forty-two patients (17.1%) had uncertain results, which consisted of a VUS that fits the clinical picture and is suspected to be causative but more information is needed to confirm a diagnosis. For the remaining 126 patients (51.4%), no variants identified were thought to be significant (No Sig. Variants or NSV Population).

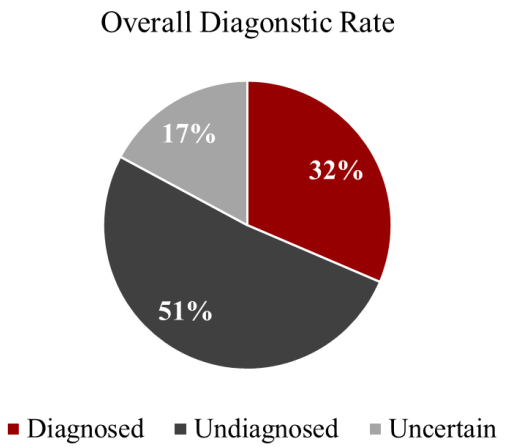

Figure 2.2. Overall Diagnostic Rate. Overall diagnostic rate of WES in this study's patient population.

Age of the patient when the WES report was disclosed from the laboratory to the physician was recorded. A comparison of the mean age of patients at the date of WES report in diagnosed, undiagnosed, and uncertain categories revealed no significant difference between result groups (Figure 2.3).

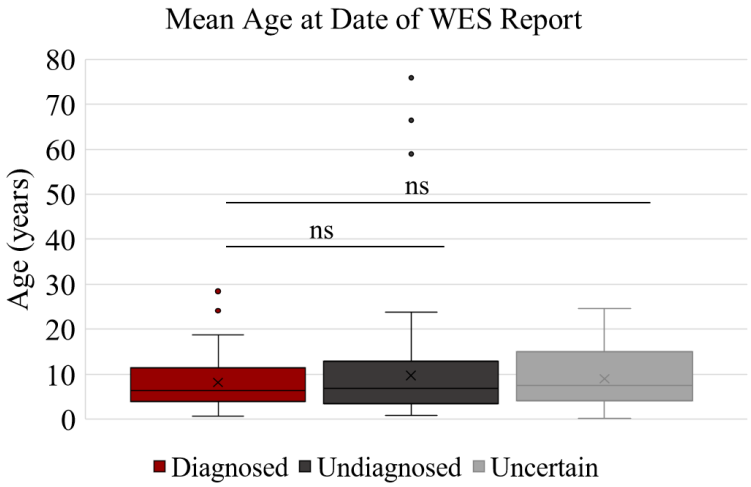

Figure 2.3. Mean age at date of WES report. Comparison of the mean age of patients at the date of WES report in Diagnosed (8.13y) vs. Undiagnosed (9.71y) and Uncertain (8.95y) WES results. A two-tailed student's t-test revealed no significant difference between result groups.

IQ scores were recorded from records when available. This study revealed no significant difference between patient IQ scores in each result category.

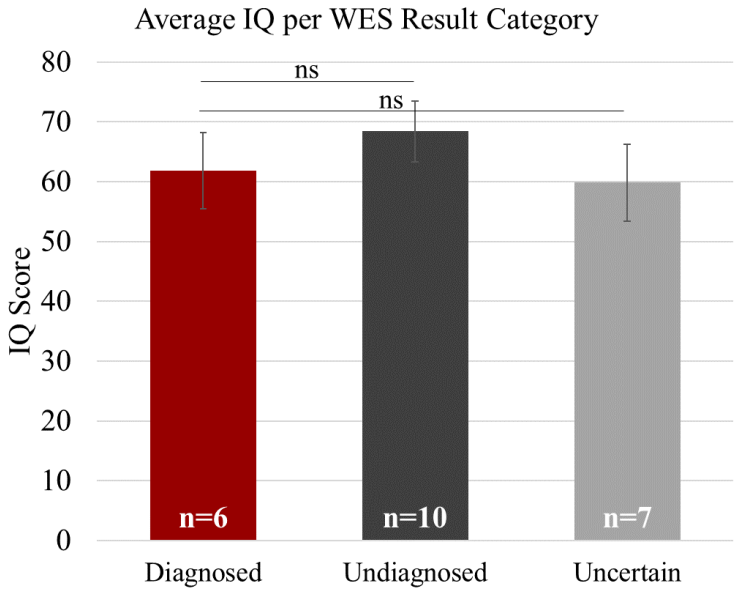

Figure 2.4. Average IQ per WES result category. Comparison of known IQ scores in patients with Diagnosed, Undiagnosed, and Uncertain WES results. A student's t-test (two tailed) revealed no significant difference in IQ score between result groups.

The following data was assessed excluding patients in the diagnosed category that had a VUS and all patients in the uncertain result category. Patients who received a diagnosis with a pathogenic or likely pathogenic variant were placed in the P/LP Population category. Patients for whom WES was not diagnostic because no significant variants were identified were placed in the No Significant Variants (No Sig. Variants or NSV) category. Odds ratios were determined for how likely a patient is to be in the NSV category if particular features were not present.

Number of delays, prevalence of each type of delay, and odds ratios were assessed for this patient population (Figures 2.5-2.7). Neither type of delay nor number of delays between the P/LP and NSV populations were significantly different.

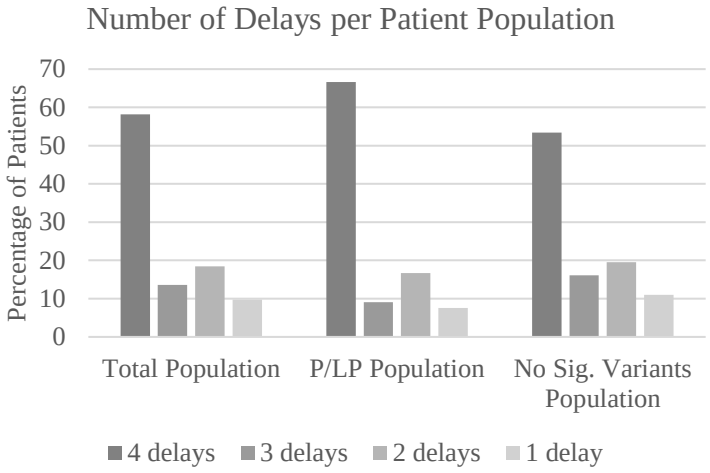

Figure 2.5. Number of delays per patient population. The majority of patients studied for delay type had delays in all four areas assessed.

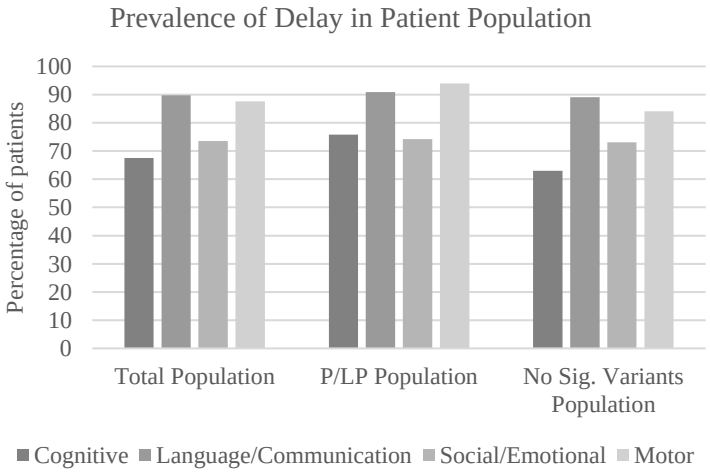

Figure 2.6. Prevalence of delay in patient population. The percentage of patients with a specific type of delay in each result population (i.e. 68% of the Total Population (P/LP + NSV Populations) had cognitive delay).

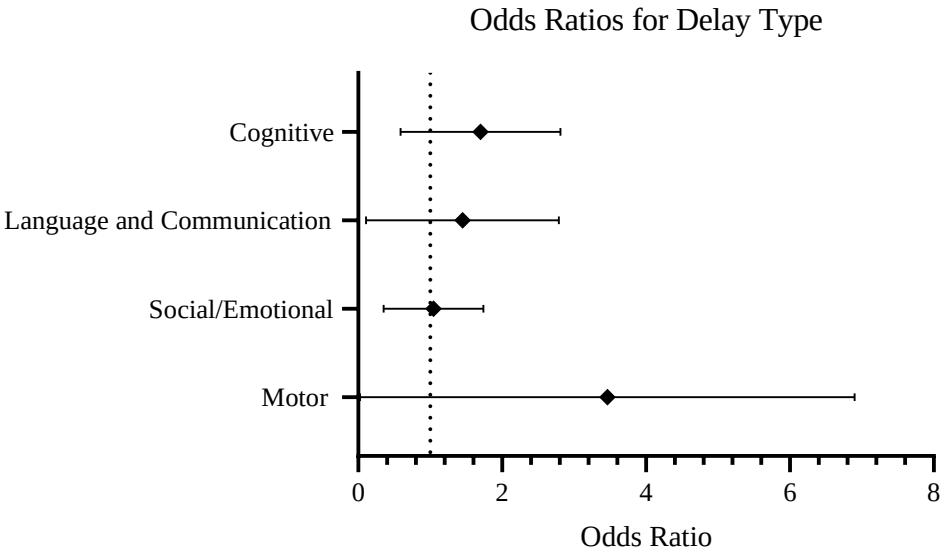

Figure 2.7. Odds ratios for delay type. Odds ratios with 95% confidence intervals for being in the NSV Population if a patient does not have a particular type of delay. Pearson's chi-squared test revealed there was no significant difference between delay type in determining odds of being in the NSV category.

Different dysmorphic features (Figures 2.8-2.9), birth defects (Figures 2.10-2.11), and comorbid conditions (Figures 2.12-2.13) were assessed among the P/LP and NSV patient populations. Prevalence of the feature in the P/LP and total populations (P/LP + NSV populations) are displayed, as well as odds ratios for being in the NSV Population category if a particular feature is not present in a patient. Chi-squared analysis revealed the only significant features were differences in tonicity (primarily hypotonia), hypotonia alone, and eye movement disorders. Neurological features were then assessed separately (Figures 2.14-2.15). Patients who did not exhibit any neurological feature were 2.7x more likely to be in the NSV category (p=0.011). Patients who did not have a tone difference were 2.1x more likely to be in the NSV category (p=0.019). Patients who did not exhibit hypotonia were 1.89x more likely to be in the NSV category (p=0.055). Lastly, patients who did not exhibit eye movement disorders were 2.11x more likely to be in the NSV

category (p=0.054).

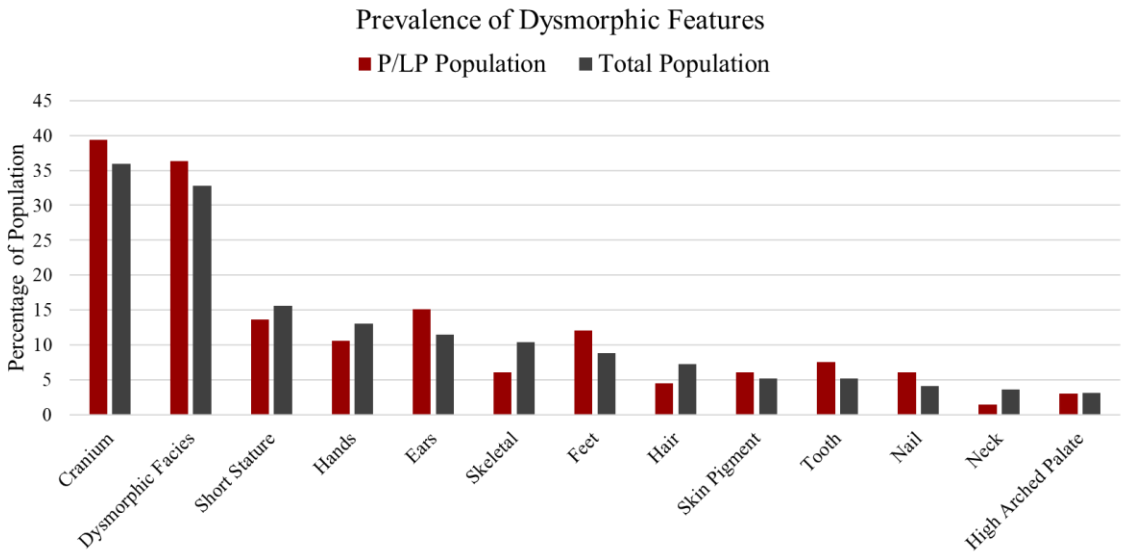

Figure 2.8. Prevalence of dysmorphic features. Prevalence of dysmorphic features in the P/LP Population and Total Population.

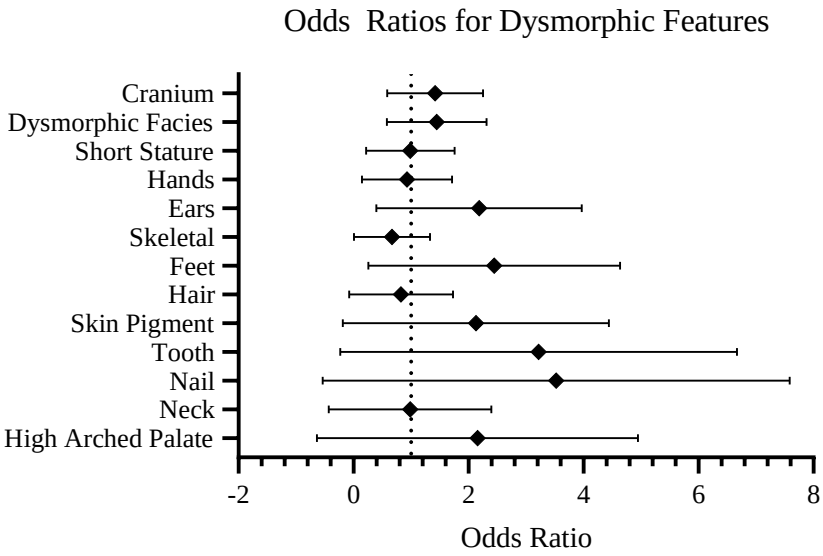

Figure 2.9. Odds ratios for dysmorphic features. Odds ratios with 95% confidence intervals for being in the NSV Population if a patient does not have a particular dysmorphic feature. None of the features listed were statistically significant.

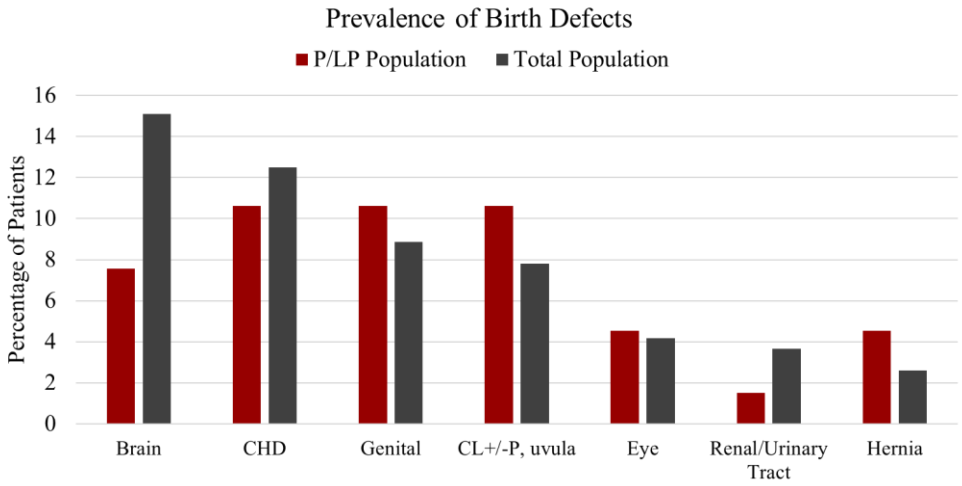

Figure 2.10. Prevalence of birth defects. Prevalence of birth defects in the P/LP Population and Total Population.

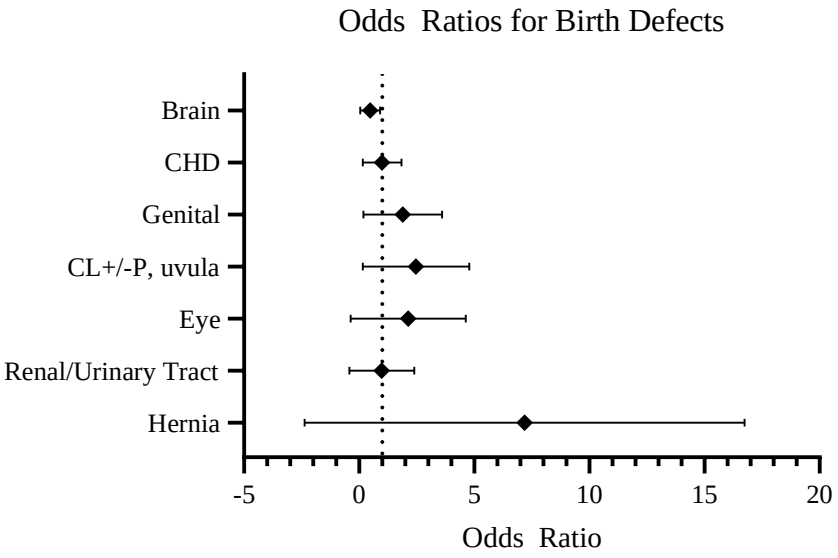

Figure 2.11. Odds ratios for birth defects. Odds ratios with 95% confidence intervals for being in the NSV Population if a patient does not have a particular birth defect. None of the features listed were statistically significant.

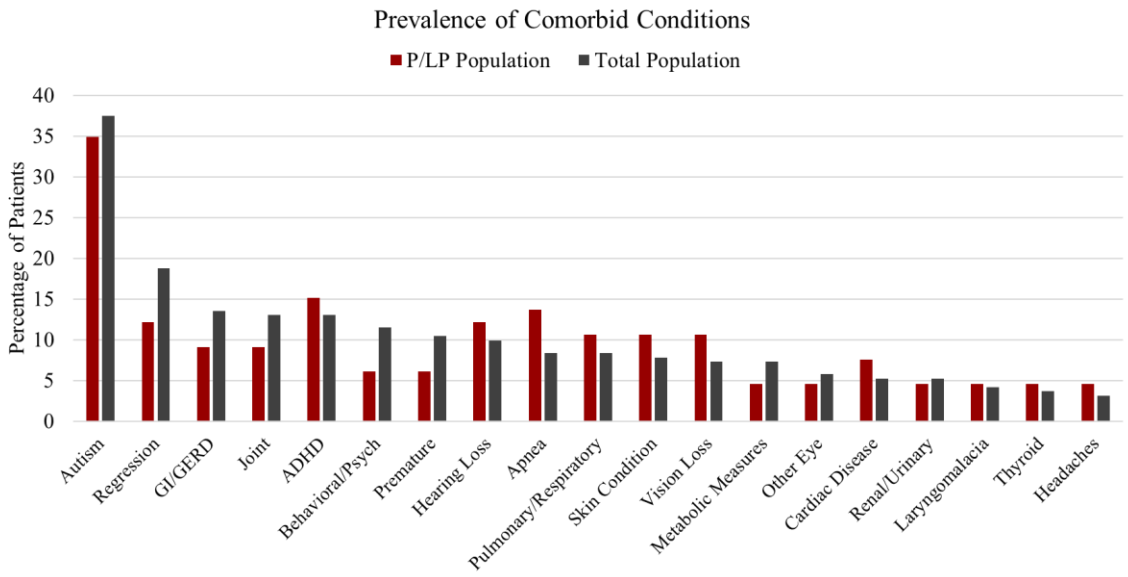

Figure 2.12. Prevalence of comorbid conditions. Prevalence of comorbid conditions in the P/LP Population and Total Population.

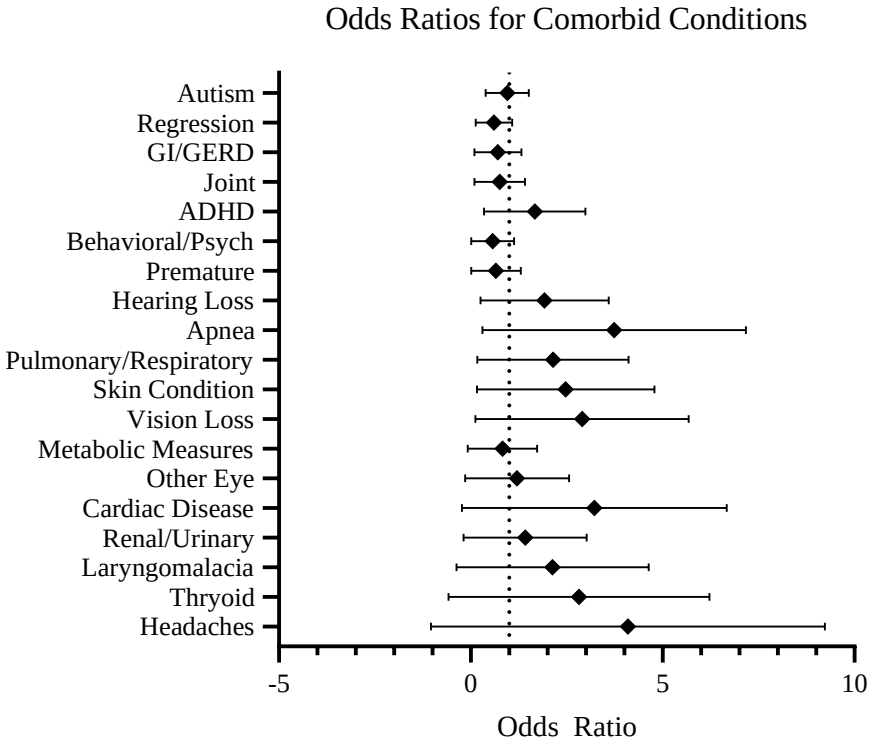

Figure 2.13. Odds ratios for comorbid conditions. Odds ratios with 95% confidence intervals for being in the NSV Population if a patient does not have a particular comorbidity. None of the features listed were statistically significant.

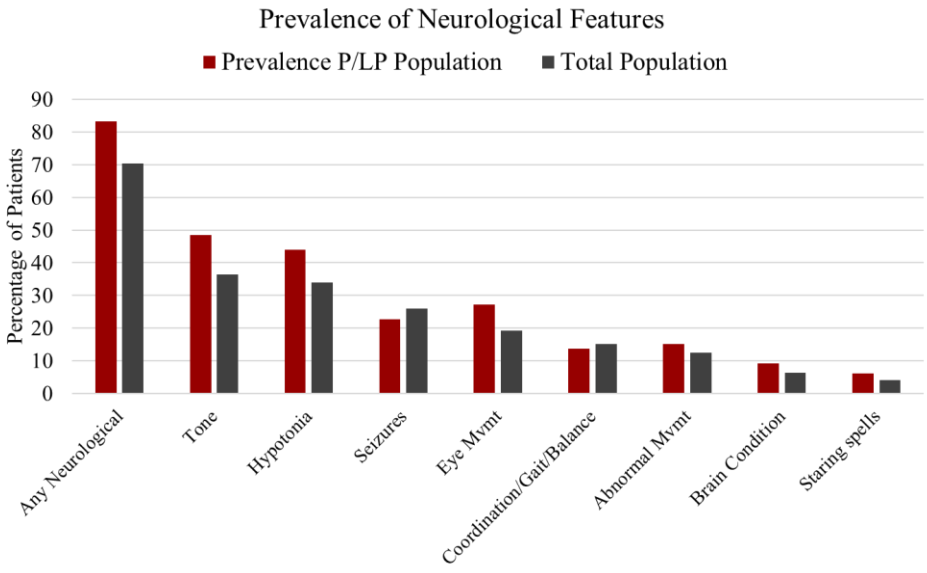

Figure 2.14. Prevalence of neurological features. Prevalence of features than can be indicative of underlying neurological disorders in the P/LP and Total patient populations.

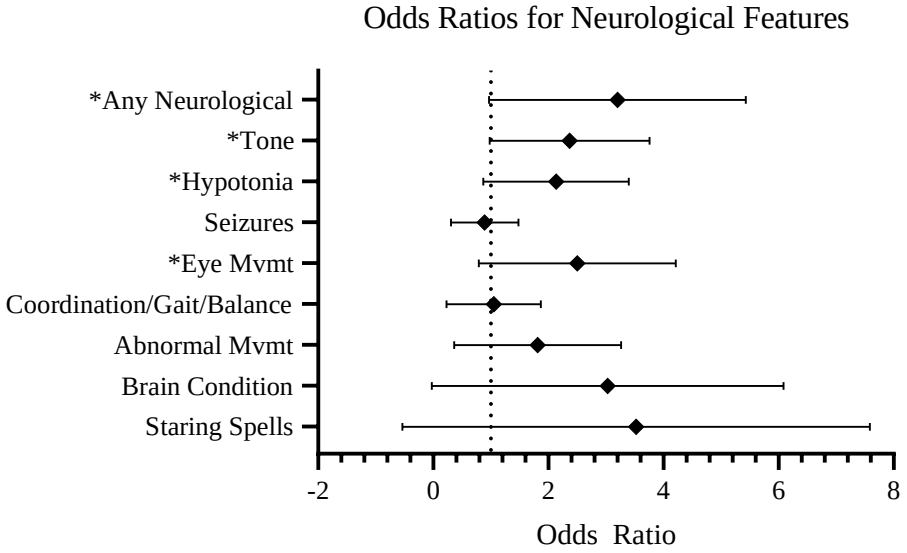

Figure 2.15. Odds ratios for neurological features. Odds ratios with 95% confidence intervals for being in the NSV Population if a patient does not have a neurological feature. *Features which were statistically significant based on two-tailed exact p-values of ≤0.05 using Pearson's chi-squared analysis.

Medical management changes were assessed for all 77 of the patients who

received a diagnosis from WES sequencing results. Of those patients, 25 patients

received a new referral immediately after diagnosis. Four patients were referred to specialty clinics for their specific syndrome for expert care. Two patients were prescribed additional medications, and three were instructed to avoid contraindicated medications. Recurrence risk was determined for 69 of these families.

Secondary and incidental findings were discovered in 12 patients in the genes listed in Figure 2.16.

Gene	**Classification**	**Associated condition**
APOB	Likely pathogenic	Familial hypercholesterolemia
ATM	Likely pathogenic	Breast cancer and possibly other cancers
ATM	Likely pathogenic	Breast cancer and possibly other cancers
BRIP1	Pathogenic	Ovarian cancer
DSP	Likely pathogenic	Arrhythmogenic right ventricular dysplasia
KCNH2	Likely pathogenic	Type II long QT syndrome, Romano Ward Syndrome
PALB2	Pathogenic	Breast cancer and other cancers
PALB2	Likely pathogenic	Breast cancer and other cancers
RAD51C	Pathogenic	Ovarian cancer
RAD51D	Likely pathogenic	Ovarian cancer and possibly breast cancer
RAD51D	Likely pathogenic	Ovarian cancer and possibly breast cancer
SPAST	Likely pathogenic	Hereditary spastic paraplegia 4 (SPG4)*

Figure 2.16. Secondary and incidental findings revealed by WES. These findings do not fit the patient's clinical picture at the time WES was run, but imply health risks that could develop later and patients should be monitored accordingly.

Lastly, age of each patient on the date of the WES report was recorded for each of the patients in this study. Of these patients, 35.4% were under the age of five years old at the date of the WES report disclosure to the physician, while 64.2% were under the age of 10 years old. For 163 patients, the age of the patient at the date the CMA results were recorded. The average age of a patient at CMA return of results was 6.61 years old, and the average age at return of WES results was 9.17 years old; 20% of patients had less than six months between return of the CMA result and return of the WES result.

Discussion

Overall Diagnostic Rate

This study revealed an overall diagnostic rate of 32% (Figure 2.2); a rate similar to those previously reported for patients with neurodevelopmental disorders (Srivastava et al., 2019). This does not include the 17% of patients who received results that may be diagnostic and require some functional studies to verify pathogenicity (uncertain results). These uncertain results are variants of uncertain significance (VUS) in genes implicated in diseases that may fit the clinical picture of the patient, but insufficient evidence about the variant itself is available to deem it pathogenic. Some of the variants discovered by these studies are being evaluated by functional studies through GGC's research division, or contributed to publications of novel genetic disorders. Further studies, such as functional analysis, will work to verify pathogenicity of particular variants in these patients, resulting in confirmed diagnoses for these individuals and will contribute to helping diagnose others presenting with similar phenotypes among undiagnosed patients all over the world.

Age of diagnosis

The average age of patients at return of WES results did not vary significantly between result groups (Figure 2.3). The majority of patients in this study were under the age of 10 years old at the date WES results were returned (64%), with 35% of patients under the age of 5 years old. It was initially hypothesized that older patients, who have likely already exhausted testing options may have a higher diagnostic rate by WES. This study is limited by the fact that most of the individuals included, regardless of age, have

already had at minimum a karyotype and a microarray, ruling out diagnoses from structural chromosome abnormalities, microdeletions, and microduplications. These results support that once these abnormalities have been ruled out, it is a logical next step to move to WES, regardless of age of the patient.

Role of IQ in diagnostic rate

Some have theorized that more severe forms of intellectual disability are more likely to be genetic (Ropers, 2010). This study did not reveal a statistically significant difference on the diagnostic rate based on IQ score (Figure 2.4). However, the relatively small sample size that had a documented IQ may not be enough to make this determination. A large proportion of patients included in this study were under the age of 5 years old, and thus were not of appropriate age to assess IQ. It is also important to note that IQ scores can vary by testing strategy and change as individuals age. The IQ scores collected during this study were tested at various ages and by various testing strategies, for which we were unable to control.

Role of delay type in diagnosis

A goal of this study was to assess the likelihood of diagnosis based on type of delay or number of delays present in an individual. Often, children present early on with a single delay, such as language and communication, and others may become apparent later in development. Assessment of both the number of delays present in each patient in each result category (Figure 2.5) and the percentage of patients with of each type of delay in each result category (Figure 2.6) revealed no significant effect on likelihood of diagnosis based on type of delay or number of delays present in an individual. Likewise,

the odds ratios for the likelihood of being in the category of patients without a significant variant identified (No Sig. Variants) if a patient does not have a particular delay, is not significant (Figure 2.7). Each delay type revealed an odds ratio close to one, indicating no difference between delay type and whether patients were in the P/LP or NSV categories. These results indicate that individuals with any type or number of delays present should be considered for WES. The results of this study may be limited by the relatively small sample size for individuals with fewer than four delays. The majority of patients had a delay in all four categories assessed. It is important to note that all patients were evaluated by medical geneticists, and have essentially been preselected as good candidates for WES where a diagnosis may be likely. Many patients were seen within the first two years of development, when certain delays may be difficult to assess without standardized testing strategies. These testing strategies are not implemented in the short time allotted for a genetics evaluation. Similarly, there are long wait-lists for developmental pediatric evaluations where these delays are formally evaluated. Though developmental pediatric assessments were reviewed, records were not always available or patients may not have been seen by the time of review. Therefore, those with a single delay or fewer delays may have had delays in all areas, but these delays were not apparent at the time. Given the long wait times for developmental pediatric assessments this data suggests that all patients with developmental delays are good candidates for WES, and it is not necessary to wait for these assessments and delay genetic testing.

Association of dysmorphic features, birth defects and comorbid conditions with diagnostic rate

A large number of dysmorphic features, birth defects, and comorbid conditions were present in the patients included in this study (Figures 2.8-2.15). Patients recommended for WES typically have complex phenotypes that do not point to a particular syndrome. Given there were no patients included in this study that did not have any additional features in at least one of the categories studied, this population has an inherent bias for more complex phenotypes. Overall, the most common features were differences in tonicity (primarily hypotonia), dysmorphic head size/shape (primarily microcephaly), dysmorphic facial features, and autism; each of which were present in greater than 30% of the total population of patients. The statistically significant features were differences in tone (primarily hypotonia) and eye movement disorders (nystagmus, strabismus, exotropia, esotropia). Hypotonia is a common referral for genetic evaluation. Both hypotonia and eye movement disorders are common features of underlying neurological disorders, many of which can be genetic. For these reasons, neurological features were further assessed for this cohort. Figure 2.14 displays other neurological features present in this cohort. Tone differences were further assessed by addressing patients with hypotonia specifically. A chi-squared analysis revealed that any tone difference, hypotonia, eye movement disorder, or any of these neurological features assessed were statistically significant (Figure 2.15). Thus, these data support previous knowledge that WES has higher diagnostic potential for neurodevelopmental disorders, specifically those with neurological features in addition to developmental delays.

Changes to Medical Management

For each of the 77 diagnosed patients, clinic notes were reviewed for changes to medical management following diagnosis. Many patients were already followed by several specialists, and the geneticist did not feel it necessary to make further referrals. For these patients where disease associated phenotypes were not a current concern, pediatricians should be aware of the newly diagnosed condition to monitor for future concerns. For 25 patients, a referral to another specialist was made immediately after diagnosis. These specialty referrals included: nephrology, ophthalmology, cardiology, audiology, neuropsychology, dentistry, endocrinology, metabolic, developmental pediatrics, orthopedics, otolaryngology, and neurology. An additional four patients were referred to specialty clinics for their specific syndrome for expert care. Two patients were prescribed additional medications, and three were instructed to avoid contraindicated medications.

Aside from the important changes to medical management that were identified, there were also important implications to family members. Many patients were children of parents who sought to have more children and would benefit from a recurrence risk estimate. For these families, finding the cause of their child's symptoms holds important information for future pregnancies and reproductive decision making. This allows for prenatal testing, preimplantation genetic testing, and can aid families in decisions regarding use of egg or sperm donors, or adoption. Recurrence risk was determined for 69 of the 77 families. Lack of one or both parental samples made it impossible to determine the recurrence risk for the remaining eight patients. Obtaining a diagnosis can also have positive psychosocial implications for families. Studies have shown that parents

who receive a diagnosis for their child have better coping mechanisms, are more aware of their child's particular needs, and have the opportunity to reach out to families affected by the same or similar conditions (Krabbenborg et al., 2016).

Secondary and Incidental Findings

There are many ethical considerations in implementing WES, especially in young children. Proper consent should be given, and families should be informed of the possibility for uncertain, secondary, and incidental findings. Secondary and incidental findings were identified in 12 patients in this study (Figure 2.16.) Though these findings do not offer an explanation for the clinical picture for which the WES was indicated, they have important management implications for the patient and family members. Many mutations discovered were in genes related to hereditary cancer syndromes that do not have medical management implications until adulthood. Identifying these mutations in young children reduces patient autonomy, and parents should be properly informed of this potential consequence. On the other hand, identification of these mutations also allows for testing of other family members, increased screening earlier in life and is potentially lifesaving.

WES as a First-tier Test

WES is not currently a recommended first-tier test for any of the patients included in this study, therefore each of the patients diagnosed in this study received numerous laboratory tests before WES. For many of these patients, changes to medical management would have been implemented sooner had WES been ordered sooner. It is clear that some physicians are already turning to WES quickly after karyotype and CMA when these

first-tier tests do not reveal a cause for symptoms. At least 20% of patients received a result from WES less than six months after return of CMA results. For some of these patients, CMA and WES were ordered at the same time. Though the diagnostic yield varies between CMA and WES, it is clear that understanding both copy number variants (CNVs) and single nucleotide variants is important and helpful in diagnosing individuals with neurodevelopmental disorders. For several patients who received a result revealing a pathogenic variant in a gene implicated in an autosomal recessive disorder, without a second variant *in trans*, CMA was utilized to determine if there was a CNV on the opposite chromosome.

Costs are not only decreasing for WES, but laboratories are working quickly to implement whole genome sequencing (WGS) in the clinical setting. Additionally, many patients had a finding on CMA, such as a deletion or duplication considered a VUS, or an inherited variant. It is unclear whether these findings are significant, or if the combination of these variants with other types of variants may play a role for a polygenic effect on the phenotype. We may learn more about how these variants interact with each other in the future as we learn more about genetics in general. As the costs decrease, and analysis tools improve, WGS could be used as a single test in place of CMA and WES. WGS has the capability of picking up copy number variants and single-nucleotide variants, as well as variants missed by WES like deep intronic variants and variants in regulatory elements.

Healthcare providers consider many factors in trying to select the best test for their patients. These data indicate that it is reasonable and beneficial to consider WES as a first-tier test for patients with developmental delays. Additionally, this study supports

the idea that patients with a variety of additional features like dysmorphic features, congenital anomalies, and comorbid conditions benefit from implementing WES sooner in the diagnostic process. This would allow for earlier implementation of treatment and potentially increased screening of comorbid disorders. It would allow for increased access to services at earlier ages, and save families money from uninformative tests. Giving families answers could lead to reproductive decision making such as preimplantation genetic testing, egg or sperm donors, or adoption. Knowing the genetic risks could lead to prenatal testing, prenatal and perinatal management of any future pregnancies.

Limitations

There are limitations to consider when interpreting the results of this study. The phenotypic information collected in medical records did not always reflect what was included on the test requisition and used for WES analysis. A key element to analysis of variants discovered by WES is knowing all relevant clinical information. This information is taken into account to determine the pipeline through which variants are filtered. If information recorded in this study was not present on the test requisition form, it is likely that this information was not used during analysis, and causative variants may have been overlooked. This study also neglects to address that these features typically present as a constellation of features in a patient. Most of the patients used to study the different effects of type of delay and various co-occurring features had multiple features that did not fit into a single category. Additionally, patients had other features that were not addressed in this study primarily due to the low population of patients in this study with that particular feature.

Types of delay present in a child were often recorded based on experience and expertise of the medical geneticist using only what was discussed in clinic visit notes. Many children had yet to be evaluated by a developmental pediatrician, or records from those visits and standardized tests were not available. When testing records were available, the most recent evaluations may not have been included. There were cases where clinic notes were compared to the Denver Developmental Milestones to determine if a delay was present if it was not explicitly mentioned in the clinic note. Though these milestones are used by most pediatricians to determine the developmental progress of a child, it is out of the scope of practice of a genetic counselor to make these determinations and this was only done for the sake of this study.

CHAPTER 3

CONCLUSIONS

Developmental delays are fairly common in children. Though they do not mean that a child will have life-long needs, it is necessary to determine if there is an underlying medical condition that needs to be addressed. Earlier diagnosis has numerous benefits in that it could lead to faster and/or preventative treatment, increased screening, and financial savings. Determination of inheritance pattern of genetic diseases could lead to reproductive decision-making such as family planning, use of egg or sperm donors, preimplantation genetic testing, or adoption. For families who choose to have children knowing their genetic risk, it could allow for prenatal testing and change in medical management prenatally and perinatally. It may also give families time to prepare emotionally and gather what they might need to care for a child with special needs.

Genetic testing options have evolved rapidly in the past decade. Karyotype, CMA, and FXS testing are currently the recommended genetic tests for any patient presenting with developmental delays or intellectual disability (Manning et al., 2010; Miller et al., 2010). Studies have shown that WES has an increased diagnostic rate compared to each of these tests for individuals with NDDs who have had other anomalies ruled out from the recommended first-tier testing options (Srivastava et al., 2019). This study supports those previously determined diagnostic rates, with an overall diagnostic rate of 32%. The majority of patients included in this study had developmental delays in each of the four

categories assessed. These patients also had at least one additional feature in dysmorphic features, congenital anomalies, or comorbid conditions. Neither type of delay or age of the patient had a significant effect on the likelihood of diagnosis. For the majority of the additional features assessed, there was no significant difference in likelihood of diagnosis for patients who had that feature versus those who did not. The exceptions being patients with any neurological feature, tone differences, or eye movement disorders. This study suggests that WES is a good test for any individual with a history of developmental delays or with any additional feature. It may have higher diagnostic potential for patients with underlying neurological disorders, though more studies are necessary to definitively make this conclusion.

In our patient population, providers have already started to order WES quickly after, or in tandem to CMA. The financial burden placed on patients can be large as insurance companies are not necessarily covering such tests. Studies like this, establishing WES as a first-tier test may provide insurance companies evidence to show that covering such tests is beneficial to the patient and the insurance provider, by limiting the number of uninformative tests performed. As quickly as WES has become more commonplace in practice, WGS is also quickly emerging in clinical care. Despite the difference in diagnostic rate, it is clear that CMA and WES have their place in clinical care of patients with DD and/or ID. It may be that as WGS becomes faster and more affordable, this test will come to replace both CMA and WES due to its increased coverage of the genome and ability to detect copy number variants and single nucleotide variants.

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APPENDIX A

FEATURES INCLUDED IN EACH CATEGORY

Dysmorphic Features	
Short Stature	Short stature
Dysmorphic Facies	Any mention of a feature of the face that is dysmorphic including forehead, eyes, nose, lips, chin, facial structure
Cranium	Microcephaly, macrocephaly, relative microcephaly, turricephaly, dolichocephaly, trigonocephaly, acquired microcephaly, plagiocephaly, narrow cranium, relative macrocephaly, borderline microcephaly
High Arched Palate	High arched palate
Neck	Short neck, wide neck, broad neck, torticollis
Feet	Short toes, 2-3 toe syndactyly, small feet, flat feet, toe contractures, abnormality of foot, sandal gap toes, webbing of toes, wideness of forefoot, pes planovalgus, broad great toes, brachydactyly, hypoplasia of the toes, dorsal puffiness of feet, splaying of toes
Skeletal	Bowing of lower legs, positional scoliosis, hip dysplasia, pectus carinatum, pectus excavatum, kyphoscoliosis, chondrodysplasia punctata, underdeveloped tibias, radial clubbing of hands, scoliosis, vertebral anomalies, low bone density
Skin pigmentary changes	Café au lait macules, mongolian spots, hypopigmented macules, skin changes, at birth pigment on legs, swirling pigmentation, vitiligo, ash leaf spot, variable pigmentation of the skin
Tooth	Tooth anomalies, dental anomalies, dental abnormalities, missing adult tooth, brittle teeth, diastema in central upper incisors, wide spaced teeth, small unusually shaped teeth
Nail	Nail anomalies, brittle nails, concave nails, deep-set nails, fingernail hypoplasia, hyperconvex fingernails, small nails, thin nails

Hands	Small hands, short hands, stub thumbs, clinodactyly, single transverse palmar crease, bridge palmar crease, single palmer crease, horizontal crease on left hand, fetal fingertip pads, square-shaped thumbs, short metacarpal bones, camptodactyly, short metacarpals, short 5th finger(s), prominent fingertip pads, long thin tapered fingers, brachydactyly, hypoplastic thumbs, single flexion crease on 5th fingers, adducted thumbs
Hair	Increased hair on back, thick eye brows, synophrys, abnormal hair pattern and growth, thin hair, low anterior hairline, sparse hair in parietal areas, sparse blonde hair, increased hair on arms, low anterior hairline, abnormal eyebrows
Ears	Low set ears, abnormal cartilage of external ears, preauricular tag, over-folded helices, large ears, mildly cupped ears, low set ears, dysmorphic ears, thick ear helices, dysplastic semicircular canals, misshapen right ear, posteriorly rotated ears, simple helices, left ear abnormality with prominent tragus/extra tissue, abnormally shaped ears, cupped ears, small ears, thick helices and antihelices

Birth Defects	
Genital anomalies	Penile torsion, undescended testes, shawl scrotum, penile chordee, hypoplastic labia, genital anomalies, cryptorchidism, hypospadias, small vaginal area, undescended testicle, small uterus with no connection of cervix to vagina, hypoplastic vagina
Congenital heart defects (CHD)	Patent foramen ovale, ventral septal defect, tricuspid valve defect, mild supravalvular aortic narrowing, atrial septal defect, left sided superior vena cava, congenital heart defect, patent ductus arteriosus, atrioventricular canal defect, coarctation of the aorta, short aortic arch, tetralogy of Fallot, aortic root dilation, left ventricular enlargement, small internal carotid artery, hypoplastic aortic arch
Hernia	Bilateral hernia, hernia, umbilical hernia, diaphragmatic hernia, inguinal hernia and hydrocoele
Eye	Chorioretinal colobomas, ocular anomalies, optic nerve hypoplasia, eye anomalies, hypoplasia of fovea centralis, optic nerve abnormalities, optic nerve atrophy, congenital macular scar, microphthalmia
CL+/-P, uvula	Submucosal cleft palate, bifid uvula, Pierre Robin sequence, cleft lip and palate, pseudo-cleft of the upper lip

Brain	Congenital brain anomalies, agenesis of corpus callosum, cerebellar dysplasia, enlarged cerebellum, enlarged vermis, small cysts, small peduncles, fused cerebellum, underdevelopment of left frontal lobe, dysgenesis of corpus callosum, hypoplastic septum pellucidum, small corpus callosum, holoprosencephaly, cortical dysplasia, Dandy-Walker variant, shortened corpus callosum, cerebral ventriculomegaly, polymicrogyria, cerebellar white matter abnormalities, periventricular leukomalacia (MRI in NICU), hemimegaloencephaly, interhemispheric brain cysts, cortical dysplasia, thin corpus callosum, brain malformations, small cerebellum, Chiari malformation, hydrocephalus, cerebral ventriculomegaly, cerebellar ectopia, periventricular leukomalacia found shortly after birth, agenesis of corpus callosum, polymicrogyria, midline arachnoid cyst, schizencephaly, heterotopias, brain stem underdevelopment, changes in cortical sulcation and opercularization patterns, spinocerebellar atrophy, craniosynostosis, cerebral atrophy, brain tumors
Renal/Urinary Tract	Surgery for ureter repair, hydronephrosis, small right kidney, underdeveloped kidney, ureteropelvic junction obstruction

Comorbid Conditions	
Premature	Born prior to 37w
Thyroid	Hypothyroidism, thyroid disease
Other Eye	Familial exudative vitreoretinopathy, heterochromia, chorioretinal scarring, photophobia, pupil dilation abnormalities, problems with tracking, lazy eye, retinal pigmentary changes, corneal abrasions
Vision Loss	Vision loss, retinopathy of prematurity, cortical vision impairment, severe myopia, visual impairment
Skin Condition	Eczema, dry skin, stretchy skin, dermal histiocytosis, irregular capillary vascular malformation of the skin, hemangioma, keratosis pilaris, soft skin
Hearing Loss	Hearing loss, auditory neuropathy, sensorineural hearing loss, conductive hearing loss

Metabolic Measures	Electrolyte problems, concern for mitochondrial disorder, low blood glucose, selective IgA deficiency, ketotic hypoglycemia, mildly elevated CK, elevated plasma homocitrulline, elevated lactate, hyperlipidemia, hyperglycemia, mitochondrial abnormalities, vitamin d deficiency, mitochondrial abnormalities, metabolic abnormalities, elevated lactic acid, elevated alkaline phosphatase, mitochondrial dysfunction
Cardiac Disease	Dilated cardiomyopathy, heart murmur, mild cardiac hypertrophy, heart-left bundle block, heart murmur, bradycardia, mitral valve prolapse, postural orthostatic tachycardia syndrome
Renal/Urinary	Renal tubular acidosis, microscopic hematuria, vesicoureteral reflux, history of hydronephrosis, vesicoureteral reflux, chronic kidney disease, history of kidney issues and surgeries, kidney disease, neurogenic bladder
Apnea	History of apneic spells, sleep apnea, obstructive sleep apnea
Pulmonary/Respiratory	Asthma, pulmonary problems, chronic lung disease, choanal atresia, respiratory distress, velopharyngeal insufficiency, congenital hypoventilation syndrome, respiratory distress, asthma, recurrent respiratory infections, respiratory issues
GI/GERD	Gastroesophageal reflux disease, GI complications, constipation, recurrent intestinal obstruction, GI issues, delayed gastric emptying, eosinophilic esophagitis, chronic diarrhea, gastroparesis, gastrocutaneous fistula, gastrointestinal dysmotility, eosinophilic esophagitis
Laryngomalacia	Laryngomalacia
Joint	Hyperreflexia, joint pain, joint laxity, stiff joints, joint hypermobility, hyperreflexia, elbow stiffness, hyporeflexia, increased deep tendon reflexes, elbow laxity, hyperextensibility, progressive stiff joints, deep tendon reflexes, hyperreflexia, hyperextensibility, mild elbow stiffness
Behavioral/Psychiatric	Sensory processing issues, behavioral issues, anxiety, social anxiety, separation anxiety, self-injurious behaviors, obsessive compulsive disorder, head banging, depression, psychiatric concerns, bipolar disorder, oppositional defiant disorder, under-socialized conduct disorder, aggression, mental health issues
Headaches	Migraines, hemiplegic migraines, headaches
Regression	Any history of regression of skills
Autism	Diagnosis of autism spectrum disorder
ADHD	Diagnosis of attention deficit hyperactivity disorder

Neurological Features	
Any Neurological	Any of the features listed in any of the other categories in this table
Tone	Hypotonia, hypertonia, low tone, mixed tone abnormalities
Hypotonia	Hypotonia
Seizures	Any history of seizures
Eye Movement Disorders	Abnormal eye movements, nystagmus, exotropia, esotropia, strabismus
Coordination/Gait/Balance	Unsteady gait, episodic ataxia, ataxia, abnormal ambulation, history of in-toeing, toe-walking, waddling gait, wide based gait, abnormal gait, mild gait imbalance, uncoordinated gait inability to walk, coordination impairment, poor balance, balance issues
Abnormal Movements	Jerky upper body movements, spasticity, abnormal twitching and jerking, abnormal movements, dystonia, clonus, spastic quadriplegia, infantile spasms, tremors in hands and feet, benign shuttering attacks
Brain Condition	Brain hemorrhage, white matter atrophy, abnormal brain MRI, leukodystrophy, brain cyst, cerebral atrophy, benign external hydrocephalus, hemimegaloencephaly, pseudotumor cerebri, delayed myelination, leukomalacia
Staring Spells	History of staring spells