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The Exome Clinic and the Role of Medical Genetics Expertise in Interpretation of Exome Sequencing Results

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Abstract

Purpose—Evaluation of the clinician's role in optimal interpretation of clinical exome sequencing (ES) results.

Methods—Retrospective chart review of the first 155 patients who underwent clinical ES in our Exome Clinic and direct interaction with the ordering geneticist to evaluate the process of interpretation of results.

Results—The most common primary indication was neurodevelopmental problems (~66%), followed by multiple congenital anomalies (~10%). The overall diagnostic yield was 36% based on sequencing data. After assessment by the medical geneticist, incorporation of detailed phenotypic and molecular data, and utilization of additional diagnostic modalities, the final diagnostic yield was increased to 43%. Seven patients of our cohort were included in initial case series that described novel genetic syndromes, and 23% of patients were involved in subsequent research studies directly related to their results or involved in efforts to move beyond clinical ES for diagnosis. The clinical management was directly altered due to the ES findings in 12% of definitively diagnosed cases.

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Conclusions—Our results emphasize the usefulness of ES, demonstrate the significant role of the medical geneticist in the diagnostic process of patients undergoing ES, and illustrate the benefits of post-analytical diagnostic work-up in solving the “diagnostic odyssey.”

Keywords

Exome Clinic; Medical Geneticist; Genetic Counseling; Diagnostic Yield; Exome Sequencing

INTRODUCTION

Clinical exome sequencing (ES) has revolutionized the diagnostic work-up in patients with genetic disease and has changed the diagnostic process in medical genetics practice[1]. The increasing utilization of ES has rapidly identified new genetic syndromes and contributed to solving many diagnostic odysseys[2]. Reports of the yield of exome sequencing through diagnostic laboratories have ranged from 25% to 30%[3–5]. Trio sequencing and focusing on specific disease subgroups can raise the diagnostic rate[5,6]. Many (23–30%) of these diagnosed patients were found to have mutations in genes that had been reported in association with the respective phenotype within the prior 2 to 3 years[3,5].

Exome sequencing has provided insights into the genetic and phenotypic heterogeneity (e.g., atypical and milder presentations) of Mendelian disorders, and highlighted the importance of *de novo* mutations and “blended phenotypes” (co-existing diagnoses that combine the clinical features of each) in rare genetic disorders[3–5]. The application of this unbiased whole genome technology has led to shifting of the diagnostic skills of the medical geneticist from focusing on detailed phenotypic characterization to identify the genetic etiology to “next-generation phenotyping”: the interpretation and validation of molecular test results in clinical practice by analyzing observed clinical features[7].

To date, there have only been a few attempts to study the role played by the medical geneticist in interpretation of results as part of the diagnostic process of ES, the concordance rate between the laboratory exome results and the geneticist’s interpretation, and the ability of ES to alter a patient’s or family’s medical management. Duke recently reported that the medical geneticists and the laboratories were 90% concordant in their interpretation of the exome results, and discordance occurred when the medical geneticist reconsidered additional clinical information and/or additional laboratory tests and genotyping of family members[8]. Another study showed that establishing a diagnosis through ES can lead to discontinuation of additional planned studies, screening patients for additional manifestations, altering management, identification of disease in other at risk family members, and reproductive planning[9]. The potential cost-effectiveness of ES has also been evaluated by calculating the cost of previous diagnostic workups, concluding that in some cases it may be most cost-effective to perform ES as a first test[10].

In this study, we present our experience with the “Exome Clinic” with special emphasis on the diagnostic course after ES has been completed by the laboratory. We evaluate the role of the medical geneticist in interpretation of results, auxiliary studies performed to determine pathogenicity of genetic variants, follow up clinical tests, and post-exome enrollment in research studies. We discuss the diagnostic yield of ES in our cohort as a function of

different phenotypic features. The utility of exome reanalysis 1–2 years after the original report is also presented. Finally, we recorded details of the social and financial implications of our exome results, such as determinations of misattributed paternity and the patient's out-of-pocket cost.

MATERIALS AND METHODS

Chart Review and Clinical Evaluation

The Washington University School of Medicine Institutional Review Board approved this study. Clinical data were obtained by retrospective chart review and interview with the ordering medical geneticists and genetic counselors (Supplementary Material 1).

ES Laboratory Results

Exomes for 155 probands were ordered between March 2012 and January 2015. Exomes were performed in 3 different laboratories: 127 were analyzed through GeneDx (Gaithersburg, Maryland), 20 through Ambry Genetics (Aliso Viejo, California) and 8 through Baylor Genetics (Houston, Texas). Laboratories reported genetic variants as *pathogenic*, *likely pathogenic*, or *variants of uncertain significance* (VUS) but did not report *benign* or *likely benign* variants. We refer to this classification as *variant-level assertion*. GeneDx also classified the variants in relation to the patient's phenotype as either *definitively* or *possibly* related and reported potential *candidate genes* for new genetic syndromes, which had not previously been associated with a human phenotype. Ambry Genetics classified variants as either *likely positive*, which we interpreted as *possible*, or *positive*, which we considered as *definitively* associated with the phenotype. Baylor Genetics classified the variants under "disease genes related to clinical phenotype" as either "deleterious" or "VUS." We considered "deleterious" and "VUS" as *definitive* and *possible*, respectively. All three laboratories also reported *incidental* variants. Definitions of these terms were adapted from Retterer *et al.* 2015[6]. We refer to these *definitive, possible, candidate, and incidental* classifications as *case-level assertion*, which is a synthesis of all the molecular data in a single subject specifying whether the test results provide a molecular diagnosis, according to the testing laboratory.

Clinical Assessment of ES Findings

Results of ES were discussed individually with the ordering medical geneticist and exome findings were confirmed or reclassified as needed as *definitively*, *likely*, *possibly*, or *unlikely* causative of the patient's symptoms based on the molecular data (*variant* and *case-level* classifications) and the geneticist's clinical assessment (Supplementary Material 1). We refer to this classification as *clinical-level assertion*. This clinical impression was then categorized as *concordant* or *discordant* with the laboratory's *case-level* assertion to allow us to analyze how the geneticist's interpretation influenced the final diagnosis (Supplementary Material 1). The statistical tools used for data analysis are presented in Supplementary Material 1.

RESULTS

Characteristics of the Cohort

Detailed descriptions of the clinical characteristics and molecular findings of the patients are documented in Supplementary Table 1. Demographic and phenotypic characteristics of our cohort are recorded in Table 1 and Supplementary Material 1. Sequencing cost for Medicaid patients was not covered by their insurance plans, and was either paid for by philanthropic support or absorbed by the hospital that sent the testing. Out-of-pocket costs to families with private insurance and for whom ES was sent as outpatients were available for 82 cases (Figure 1A). 54 of these cases had an out-of-pocket cost of $0, and the average cost was $386.31 with a maximum cost of $4,012.

The average age at which symptoms in patients began was 11 months, with a median of 7 weeks, ranging from birth to 22 years. Of note, 63 patients (41%) had onset of symptoms at birth. Patients were first seen by a medical geneticist at an average age of 3 years, with a median of 14 months and a range from birth to 31 years old.

The primary indications for ES, the most common affected organ systems, and the most common neurodevelopmental findings are presented in Figure 1B, 1C, and 1D, respectively. The average number of organ systems affected in our cohort was 2.6 (median 2 and range 1 to 7 out of 15 possible organ systems). The average number of services (other than genetics) involved in the care of the patients in our cohort was 3.3 (median 3 and range 0 to 10 out of 19 possible services).

Variant Classification and Interpretation

The diagnostic laboratory reported 237 genetic variants, with an average of 1.5 variants reported per patient and a range from 0 to 6. The distribution of genetic variants based on *variant-level* assertion was as follows: 79 *pathogenic*, 37 *likely pathogenic*, and 107 VUS as well as 14 *incidental* findings (Figure S2, Tables S1, S2) that were classified by the laboratory as known *pathogenic* (12) or *expected pathogenic* (2). Among the 155 cases, 56 cases (36%) have *definitive* diagnosis based on *case-level* assertion by the laboratory, 60 cases were reported as *possible*, 10 cases as *candidate*, and 29 cases as negative (Figures 2A, S1, Tables S3). Due to the presence of autosomal recessive (AR) conditions and blended phenotypes among the 56 definitive cases, the number of variants was 71. Definitive diagnoses in 4 genes were identified in more than one unrelated case: *ARID1B* (2), *GABRB2* (3), *NGLY1* (2), and *PTPN11* (2). Eleven cases had mitochondrial genome sequencing completed as part of the ES order but none of these yielded abnormal results. Misattributed or non-paternity was found in two families as a result of ES testing.

Based on the assessment of the ordering medical geneticist, the final diagnosis was changed in 21 subjects (14%) (Figures 2B,S1, S2, Tables S1, S2, S3, 2, 3). The diagnosis in 16 subjects was promoted such that the clinical geneticist determined that the variant was more definitively related to the phenotype, and in 5 subjects it was demoted. Consequently, there was a net gain of 11 additional definitive diagnoses, for a total of 67 cases (43%) definitively diagnosed (Table 2). There were multiple reasons for changing the *case-level* classification (Table 3). First, the clinical geneticist has direct and detailed knowledge of the patient's

phenotype and the opportunity to order follow up studies including biochemical and radiological studies, segregation analysis in relatives, and/or single-gene re-sequencing or deletion/duplication studies to search for a mutation in the second allele. Furthermore, there were variants in candidate genes that were promoted because of subsequent publication of new syndromes, either in other similarly affected patients or contribution of these patients to syndrome discovery themselves[11–16]. Thirty-two (48%) out of the 67 definitive cases had mutations in genes described in 2011 or later. This includes 7 (10%) being described as new genetic syndromes[12–16] (WES038, WES052, WES057, WES062, WES079, WES105, WES121), 3 of which are in the process of being published. Five cases (7.5%) had *definitive* variants in two genes resulting in "blended phenotypes" (WES028[17], WES030, WES060, WES070, WES128). Reanalysis of the exome data was performed in 14 cases by the molecular laboratory, usually 12 to 18 months after the initial report was generated. In 7 cases the reanalysis resulted in no change, in 4 cases it resulted in a new definitive diagnosis (WES013, WES019, WES039, WES131[18]) due to subsequently published new syndromes or functional analysis of variants, and in one case a previously reported variant was demoted (WES002). The remaining two cases (WES099, WES112) involved efforts by the laboratory to identify candidate disease genes for which there have not yet been human phenotypes associated.

We then assessed the relationship between the diagnostic yield, as determined by the medical geneticist, and various demographic and phenotypic characteristics (Table S4). Our results indicated a higher diagnostic yield for females (47%), patients with a craniofacial anomaly (64%), and patients with an abnormal head circumference, specifically microcephaly (50%), but none of these effects were statistically significant. Caucasians had a statistically significant higher rate of diagnosis compared to all other racial groups (46% vs. 24%, p=0.04), which persisted after adjusting for craniofacial anomaly in the multivariable logistic regression model, demonstrating the disproportionately low diagnosis rate for non-Caucasians. The following additional categories were tested for effect on diagnostic rate and were found to be not significant: inpatient versus outpatient status, all other phenotypic categories, death, abnormal height or weight, dysmorphism, and positive family history.

The inheritance patterns in the 72 conditions (67 subjects; 5 with two conditions caused by variants in different genes) that were determined to be definitive are as follows: 42 (58%) autosomal dominant (AD), 24 (33%) AR, and 6 (8%) X-linked. Of the 89 variants that are associated with these 72 conditions, 34 (38%) were de novo, including one variant in each of two cases with AR conditions (Table 2). The average paternal age at delivery of the 42 patients with *de novo* mutations was 32 years with a median of 32 years and a range of 22 to 49 years. For the inherited variants, 25 were passed from the mother, 18 from the father, 4 from both (homozygous for recessive condition), and 8 had unknown inheritance due to at least one parent not being sequenced. We observed reduced penetrance of 5 variants that were associated with AD conditions and inherited from seemingly unaffected parents, although parental cardiac evaluations are pending in 2 of these cases.

In 9 cases, ES was sent prior to the implementation of the 2013 ACMG guidelines for reporting *incidental* findings[19]. Of the remaining 146 cases, 5 (3%) families opted out and

141 (97%) families elected to receive the findings. 14 patients (10%) had one incidental finding each. Incidental findings were found in the following genes from the ACMG-recommended list of 56 genes: *BRCA2* (2), *FBN1*, *LDLR* (2), *MYBPC3* (4), *MYH7*, *RET*, *SCN5A*, *TTN* (2) (Table S5). Although the laboratories' reports indicate that these *incidental* variants are *known pathogenic* in 12 cases, only 5 of these 12 are uniformly classified as *pathogenic* in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) and the remainder have conflicting interpretations of pathogenicity, with some submitters even identifying two of these variants as *likely benign* (Table S5). Follow up assessment or evaluation was done based on established guidelines and protocols for these cases and their carrier relatives (Table S5).

The Effect of Exome Results on Auxiliary Tests, Management and Research Studies

We examined whether the exome results affected subsequent diagnostic work-up or changed patient management. Additional diagnostic studies were performed in 84 subjects (54%), including molecular studies (proband or family members) in 37 (24%), imaging studies in 29 (19%), and biochemical and/or chemistry tests in 22 (14%). The distribution of the 84 cases based on *clinical-level* assertion was the following: 48 were *definitive*, 4 were *likely*, 8 were *possible*, 20 were *unlikely*, 1 was *incidental* only, and 3 had completely negative results, but had follow up genetic testing performed due to concerns regarding poor coverage of the exome data at particular genes of interest (Supplementary Material 1, Table S6). In 12 out of the 84 cases, these follow up studies were due to the discovery of an ACMG-designated *incidental* finding. An echocardiogram was performed in 19 (12%) probands or family members, 7 of which were due to *incidental* findings. In addition, cancer surveillance protocols were initiated in 7 probands or related family members due to variants found by ES, 2 of which were *incidental*. Three families used the ES information for prenatal or pre-implantation genetic diagnosis.

In 8 out of the 67 definitive cases (12%), clinical care was directly altered due to primary ES findings, as follows: 1) discontinuation of levothyroxine (WES113, *SLC16A2*); 2) cardiac ablation in an asymptomatic patient (WES118, *TBX3*) found to have Wolff-Parkinson-White syndrome on the EKG that was ordered based on ES results; 3) prophylactic thyroidectomy and Hirschprung's diagnosis (WES018, *RET*); 4) neuropsychology evaluation because of known deficits associated with this condition, although not obviously present in this case, which showed ADHD and anxiety disorder and resulted in an atomoxetine prescription (WES057, *WAC*); 5) orthopedics referral of a patient (WES025, *PHF6*) with a condition known to cause musculoskeletal phenotypes which led to diagnosis and surgical repair of her scoliosis; 6) amantadine trial initiated for ataxia telangiectasia (WES126, *ATM*); 7) a trial of methylene blue and vitamin C in a patient (WES050, *CYB5R3*) with methemoglobinemia; and, lastly, 8) serine prescription for serine-responsive seizures (WES059, *PHGDH*). Thirty-six patients were enrolled in research studies related to their ES results. These include efforts to characterize the potential functional effect of a particular variant, as well as reanalysis of otherwise negative clinical exome data for research purposes.

DISCUSSION

Although there have been several studies reporting clinical ES results, most of these reports are from diagnostic laboratories and do not focus on the medical geneticists' interpretation of the findings. The main purpose of this study was to evaluate the medical geneticist's role in the optimal interpretation of the exome results, and how this might alter the final diagnostic yield. The overall definitive diagnosis rate of clinical ES in our cohort was 36% based on laboratory sequencing data but this increased to 43% after the integration of the molecular and phenotypic data by the medical geneticist as well as the incorporation of additional diagnostic modalities. Fifty-four percent of patients in our cohort underwent "post-analytical" auxiliary diagnostic studies, including biochemical analyses, imaging studies, complementary molecular tests (e.g., deletion and duplication analysis of a specific gene or Sanger sequencing of a gene with low exome coverage), and/or genotyping affected and unaffected family members for segregation analysis. Furthermore, each genetic variant was evaluated by a thorough literature review and searching databases such as ExAC and ClinVar. This extensive post-exome assessment by the clinician is time consuming and illustrates that ES results as reported by the molecular laboratory require clinical context. The laboratory identifies sequence changes and provides information about suspected pathogenicity, but the medical geneticist must compare the expected phenotype associated with the molecular finding to the patient's phenotype to determine if they align, and whether the molecular finding may account for the patient's clinical presentation. In 5 cases, we determined that the molecular finding was not consistent with the patient's phenotype, and the genetic variant was considered to be either benign or not completely explanatory. In 16 other cases, the classification was promoted to a more definitive category and ultimately the final diagnosis was modified (Table 3). However, in other patients the final diagnosis is still uncertain and pathogenicity of the variants is difficult to establish due to lack of functional data, inability to perform segregation analysis, incomplete explanation of the phenotype by the variant, or candidate gene status. These limitations pose challenges to the clinician and demonstrate that receiving the exome results can be the beginning of a continuing exploration process rather than the end of the "diagnostic odyssey."

As evidenced by large-scale research studies that use ES as a tool for discovery such as the Deciphering Developmental Disorders[20], the rate of discovery of new genetic syndromes is rapidly increasing. Therefore, reanalysis of previously reported clinical ES data has the potential to increase the sensitivity of the test. In fact, 48% of definitive cases in our study had mutations in genes with associated syndromes described in 2011 or later. Subsequent reanalysis of the exome data, either at the request of the medical geneticist or at the prompting of internal reanalysis by the diagnostic laboratory, directly resulted in 7 additional definitive diagnoses than would have otherwise been obtained, illustrating the need to perform ongoing data mining for previously submitted cases with negative exome results.

The increased diagnostic yield in our cohort relative to previously reported clinical series[3–6,8–10] can be in part attributed to the selection process we apply for subspecialty referrals for the Exome Clinic, including an ES-specific referral form (Supplementary Material 2) and review of the suitability of the case by a medical geneticist. It is also possible that there was a selection bias toward the most severely affected patients referred to

a tertiary medical center, reflected by a relatively high number of organ systems, services involved, as well as highly skewed growth parameters and high rate of dysmorphism in the probands when the test was initially implemented in our institution. We cannot exclude the contribution of other factors such as a high trio rate (83%), different categories of indications, or differences in sample size.

This study has a number of important limitations. For example, ES was ordered through 3 different laboratories that used different terminology to classify the variants in relation to patient's phenotype, which limits cross-case comparison. In addition, the laboratories' data analysis processes changed over time as algorithms have improved and ACMG guidelines have been implemented. However, there were no statistically significant differences among the three different diagnostic laboratories regarding the number of cases with *incidental* findings, the proportion of cases that received a *definitive* diagnosis at the *case-level*, and whether the *case-level* classification was revised by the clinician (Supplementary Material 1). Another factor limiting the generalizability of our findings is that these patients were all part of a highly selected population that was evaluated at a tertiary medical center.

Our study shows that clinical ES is a powerful diagnostic tool especially for atypical and mild presentations of well-established genetic syndromes. For example, none of the patients who received diagnoses of CHARGE, Noonan, ataxia telangiectasia and LADD syndromes met clinical diagnostic criteria, but rather exhibited partial phenotypes. Furthermore, the discovery of five patients in our cohort having "blended phenotypes", as similarly described in other cohorts[3,21,22], should change our traditional diagnostic approach. ES is a valuable gene discovery tool as illustrated in 7 patients who were included in initial case series that described novel genetic syndromes. Other unexpected exome results were related to potential germline mosaicism in one case (WES057) and uniparental disomy in another case (WES050). This information about non-Mendelian modes of inheritance was very important for providing accurate recurrence risks in future pregnancies. ES also uncovered non-paternity in two cases, which required a consultation with our institutional ethics committee and ultimately led to altered strategies for pretest counseling regarding this complicated issue.

Incidental findings present in 9% of our cohort patients often resulted in additional interventions in both the probands and their carrier relatives. This number is higher than we would have expected by comparison to previous cohorts.[3–5,23] However, based on the conflicting assertions in ClinVar (Table S5), it is clear that the performing laboratories over called incidental findings and the actual rate is 3.8% (6/14). These data illustrate the challenges in variant classification and the need for simple and consistent criteria for classification based on variant-specific databases and knowledge bases[23]. We speculate that this lack of uniformity may be due to changes in how variants are classified over time, especially after the release of the 2015 ACMG guidelines[24]. The role of the medical geneticist in following up these incidental results is as important as it is for following up primary results because subsequent monitoring, such as cancer screening and cardiac monitoring, can have life-saving consequences for the patients and their relatives. However, the conflicting interpretations of the data as presented here and the workup performed for patients with uncertain incidental findings (Table S5) illustrate the challenges the medical

geneticist faces and reveal one of the significant drawbacks of ES related to false positive incidental findings, which could lead to substantial harmful consequences including performing unnecessary and potentially harmful tests and procedures, increased healthcare costs due to performing unnecessary follow-up evaluations, and causing anxiety among a percentage of patients undergoing ES[23,25,26]. These are important points that should be carefully considered prior to ordering ES and during pretesting counseling.

For many patients, ending a diagnostic odyssey limits additional expensive, time-consuming, and potentially invasive diagnostic procedures. It also allows precise determination of recurrence risk and prognosis. ES results were used by 3 families from our cohort for prenatal diagnosis testing. Although the discovery of a treatable condition can dramatically change the clinical outcome, the exome resulted in specific treatments in only a limited number of our patients. Nevertheless, clinical management was directly altered due to primary ES findings in 8 patients, which is 5.2% of all patients who underwent ES. It is also possible that careful clinical assessment for part of these cases would detect clinical findings that might ultimately change the management even without the molecular data.

The correlation of diagnostic yield in our cohort with various demographic and phenotypic characteristics showed a higher yield for Caucasians, females, patients with craniofacial anomalies, and patients with abnormal head circumference, but none of these reached statistical significance except for ethnic background (Supplementary Material 1, Table S4). It is important to note that patients from minority populations are under-represented in our cohort, suggesting a need for increased access to ES for individuals from these backgrounds. While the average out-of-pocket cost for ES was $386 per family and although we do not have detailed socioeconomic data for our cohort, we speculate that economic factors may play a role in this discrepancy. Publicly funded insurance plans do not routinely provide coverage for ES and families with high out-of-pocket costs sometimes self-selected not to pursue this testing. Compounding this situation, non-Caucasians achieved a significantly lower diagnostic rate of only 24%. This finding may be due in part to an underrepresentation of minority populations in variant databases, causing challenges in interpreting the clinical significance of variants found in these populations.

Taking into account the work involved in interpreting and following up both primary and incidental exome findings, the complex phenotype of patients referred for ES, as well as the constantly evolving nature of these results due to re-analysis and publication of new genetic syndromes, medical geneticists serve an essential role in this complex diagnostic process. This study shows that the partnership of the clinician with the molecular laboratory can increase the diagnostic yield by 7%. An accurate molecular diagnosis ends a diagnostic odyssey, allows for precise genetic counseling, and has the potential to change clinical management. It is also the launching point for the development of targeted pharmacologic therapies, which can hopefully translate these discoveries into efficacious novel treatments to achieve the promise of personalized genomic medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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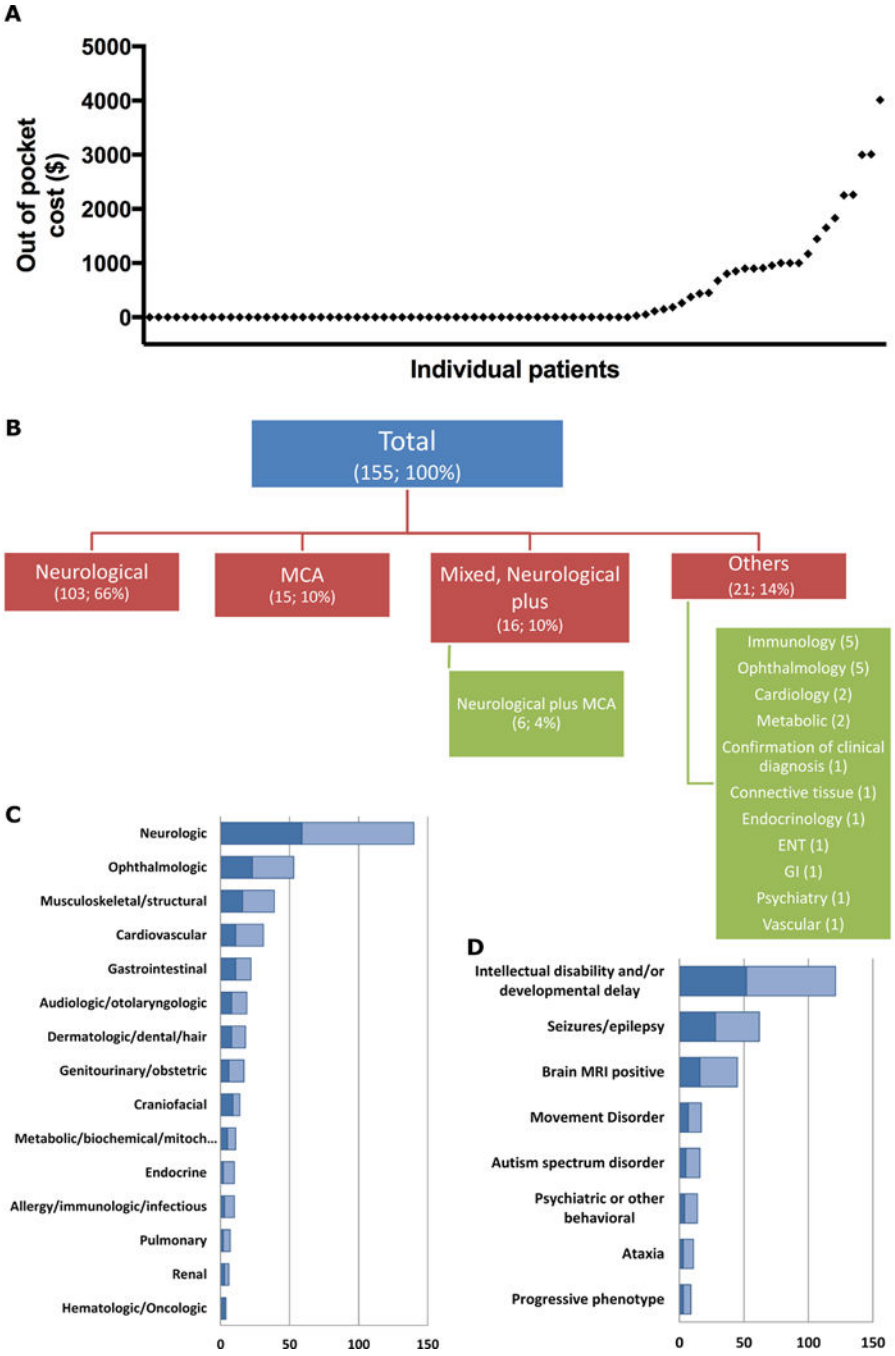

Figure 1. Cost and phenotypic characterization of the cohort

A) Scatter plot of the out-of-pocket cost in ascending order. B) Each case was assigned a phenotype-based, single primary indication for performing ES. The number and percentage of cases are shown in parenthesis. MCA: Multiple congenital anomalies. C) Each phenotypic feature of the probands was assigned to an organ system, and the total count of cases is displayed. D) The frequency and distribution of the neurodevelopmental phenotypes in the cohort. The darker portion of the bar in C and D indicates the proportion of cases that achieved a definitive diagnosis.

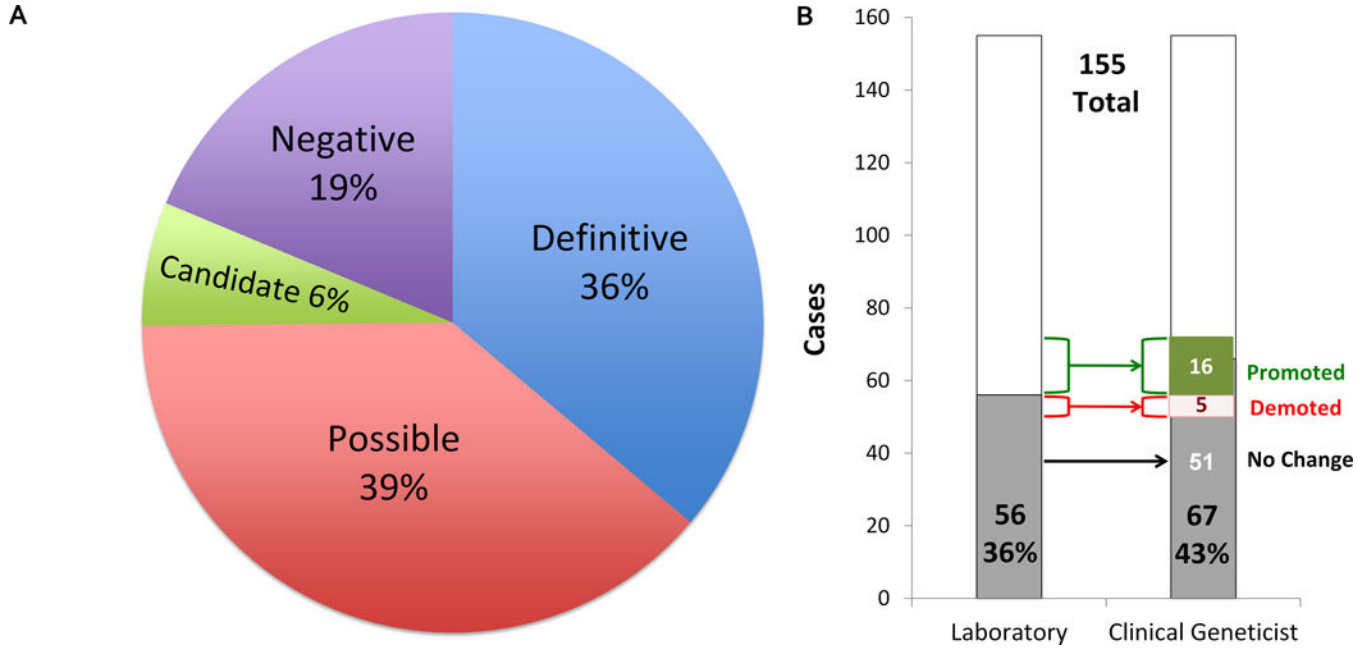

Figure 2. Characterization of case-level and clinical-level assertions

A) The relative percentages of each case-level classification as reported by the testing laboratory. B) The diagnostic rates according to case-level and clinical-level assertions are shown as the proportion of cases, in gray. The change in classification of cases is indicated, with 16 cases promoted and 5 demoted.

Table 1

Demographic Details of Cohort.

Gender	
Male	87 (56%)
Female	68 (44%)
Ethnicity	
Caucasian	130 (84%)
Mixed	14 (9%)
African-American	8 (5%)
Hispanic	3 (2%)
Patient location	
Outpatient	133 (86%)
Inpatient	22 (14%)
Insurance (133 cases)	
Private	90 (68%)
Medicaid	43 (32%)
Dysmorphism (154 cases)	
Yes	73 (47%)
Mild	17 (11%)
No	64 (42%)
OFC	
Normal	93 (61%)
< −1.88 SD	42 (28%)
> +1.88 SD	17 (11%)
Height	
Normal	99 (64%)
< 5^{th} centile	50 (32%)
> 95^{th} centile	6 (4%)
Weight	
Normal	106 (68%)
< 5^{th} centile	36 (23%)
> 95^{th} centile	13 (8%)
Consanguinity	6 (3.9%)
Average age at ES (range)	6 years (3 days–33 years)
Average turnaround time in months (range)	4.7 (1.3–7.9)

Table 2

Description of Definitively Diagnosed Cases.

Case Number	Gene	Variant(s)	De Novo	Inherited	Unknown Inheritance	Sequencing Company Classification(s)	Medical Geneticist's Interpretation	Testing Laboratory[a]	Disease Mode of Inheritance[b]	Phenotype MIM Number	Final clinical diagnosis
WES152	*ADAR*	c.577C>G, p.P193A; c.3020-3C>G, IVS11-3C>G		X, X		Definitive	Concordant	G	AR	615010	Aicardi-Goutieres syndrome
WES111	*AHDC1*	c.2373_2374delTG, p.C791WfsX57			X	Definitive	Concordant	G	AD	615829	Xia-Gibbs syndrome
WES056	*ANKRD11*	c.6159_6162delGGCT, p.A2054PfsX32	X			Definitive	Concordant	G	AD	148050	KBG syndrome
WES066	*ARID1B*	c.3644delC, p.1215QfsX9			X	Definitive	Concordant	G	AD	135900	Coffin-Siris syndrome 1
WES030	*ARID1B / FGFR3*	c.2281G>A, p.G761S / c.445+(2_5)delTAGG, IVS4+(2_5)delTAGG		X (FGFR3)	X (ARID1B)	Possible / Possible	Promoted / Promoted	G	AD / AD	135900 / 149730	Coffin-Siris syndrome 1/ LADD syndrome
WES126	*ATM*	c.3993+1G>A, IVS26+1G>A; c.5763-1050A>G, IVS39-1050A>G		X, X		Possible	Promoted	G	AR	208900	Ataxia telangectasia
WES028	*ATP2B3 / LAMA1*	c.1445G>A, p.R482H / c.6074C>T, p.T2025M; c.1741C>T, p.R2381C		X / X, X		Possible / Possible	Promoted / Promoted	G	XL / AR	302500 / 615960	ATP2B3-related disorder / LAMA1-related disorder
WES076	*CHD7*	c.8279delA, p.N2760IfsX39	X			Definitive	Concordant	G	AD	214800	CHARGE syndrome
WES086	*COL1A1*	c.652G>T, p.G218C	X			Definitive	Concordant	G	AD	166200	Osteogenesis imperfecta type 1
WES085	*COL4A1*	c.2291G>A, p.G764D	X			Definitive	Concordant	G	AD	607595	COL4A1-related disorders
WES121	*COQ4*	c.245T>A, p.L82Q; c.473G>A, p.R158Q		X, X		Candidate	Promoted	G	AR	616276	COQ4-related disorder
WES038	*CTBP1*	c.991C>T, p.R331W	X			Candidate	Promoted	G	AD	None yet	CTBP1-related disorder
WES050	*CYB5R3*	c.250C>T, p.R84X		X[c]		Possible	Promoted	G	AR	250800	Methemoglobinemia type II
WES109	*CYP11A1*	c.1078C>T, p.R360W		X[c]		Definitive	Concordant	G	AR	613743	CYP11A1-related adrenal insufficiency with sex reversal
WES131	*DNMT3A*	c.2645G>A, p.R882H	X			Possible	Promoted	G	AD	615879	Tatton-Brown-Rahman syndrome
WES128	*DYRK1A / STK11*	c.889_893dupAGGTT, p.F298LfsX40; c.665-9_665-5delCTCTT, IVS5-9_IVS5-5delCTCTT	X, X			Definitive	Concordant	G	AD / AD	614104 / 175200	DYRK1A-related intellectual disability / Peutz-Jeghers syndrome
WES047	*EIF2B5*	c.318A>T, p.L106F; c.799C>T, p.Q267X	X	X		Definitive	Concordant	G	AR	603896	Vanishing white matter disease
WES117	*FGD1*	c.563_570delTGCCTGCC, p.L188RfsX26	X			Definitive	Concordant	G	XL	305400	Aarskog syndrome
WES134	*FGFR1*	c.2152C>G, p.R718G	X			Definitive	Concordant	G	AD	615465	Hartsfield syndrome
WES020	*FHL1*	c.799delC, p.H267Tfs*23		X		Definitive	Concordant	A	XL	300696	Emery-Dreifuss muscular dystophy type 6
WES104	*FLG*	c.1501C>T, p.R501X		X		Definitive	Concordant	G	AD	146700	Ichthyosis vulgaris
WES051	*FOXG1*	c.700T>C, p.S234P	X			Definitive	Concordant	G	AD	613454	FOXG1-related disorder, Rett-like
WES049	*GABRA1*	c.643C>G, p.L215V	X			Definitive	Concordant	G	AD	615744	GABRA1-related disorder
WES052	*GABRB2*	c.909G>T, p.K303N	X			Candidate	Promoted	G	AD	None yet	GABRB2-related disorder
WES062	*GABRB2*	c.863T>G, p.I288S	X			Definitive	Concordant	G	AD	None yet	GABRB2-related disorder
WES105	*GABRB2*	c.845T>C, p.V282A	X			Definitive	Concordant	G	AD	None yet	GABRB2-related disorder
WES070	*GALNS / SUFU*	c.1485C>G, p.N495K; c.539T>C, p.V180A / c.794_808del15, p.N265_V269del		X / X	X	Possible / Possible	Promoted / Promoted	B	AR / AD	253000 / 109400	Morquio syndrome / Gorlin syndrome
WES140	*GNAO1*	c.833_835delAGA, p.K278del	X			Definitive	Concordant	G	AD	615473	GNAO1-related disorder (Early infantile epileptic encephalopathy 17)
WES019	*GRIN2B*	c.1916C>T, p.A639V	X			Possible	Promoted	A	AD	616139	GRIN2B-related disorder (Early infantile epileptic encephalopathy 27)
WES039	*HNRNPK*	c.1008+1G>A, IVS12+1G>A	X			Definitive	Concordant	G	AD	616580	Au-Kline syndrome

Case Number	Gene	Variant(s)	De Novo	Inherited	Unknown Inheritance	Sequencing Company Classification(s)	Medical Geneticist's Interpretation	Testing Laboratory[a]	Disease Mode of Inheritance[b]	Phenotype MIM Number	Final clinical diagnosis
WES120	*KAT6B*	c.2184T>G, p.Y728X			X	Definitive	Concordant	G	AD	603736	KAT6B-related disorder
WES095	*KCNB1*	c.629C>T, p.T210M			X	Definitive	Concordant	G	AD	616056	KCNB1-related disorder (Early infantile epileptic encephalopathy 26)
WES071	*KCNQ2*	c.740C>T, p.S247L	X			Definitive	Concordant	G	AD	613720	KCNQ2-related disorder (Early infantile epileptic encephalopathy 7)
WES107	*KCNT1*	c.1193G>A, p.R398Q	X			Definitive	Concordant	G	AD	614959	KCNT1-related disorder (Early infantile epileptic encephalopathy 14)
WES114	*KMT2A*	c.7419delT, p.P2474LfsX35	X			Definitive	Concordant	G	AD	605130	Wiedemann-Steiner syndrome
WES029	*KMT2D*	c.12039_12046delAGCCCTGG, p.A4014SfsX23			X	Definitive	Concordant	G	AD	147920	Kabuki syndrome
WES153	*NBAS*	c.688dupT, p.S230QfsX4; c.2524G>T, p.V842F		X, X		Definitive	Concordant	G	AR	616483	Infantile liver failure syndrome 2
WES037	*NGLY1*	c.347C>G, p.S116X; c.881+5G>T, IVS5+5G>T		X, X		Definitive	Concordant	G	AR	615273	NGLY1-related congenital disorder of deglycosylation
WES096	*NGLY1*	c.953T>C, p.L318P; c.1169G>C, p.R390P		X, X		Definitive	Concordant	G	AR	615273	NGLY1-related congenital disorder of deglycosylation
WES007	*OCA2*	c.1327G>A, p.V443I		X		Definitive	Concordant	B	AR	203200	Oculocutaneous Albinism, type II
WES155	*OPHN1*	c.155-2A>C, IVS2-2A>C		X		Definitive	Concordant	G	XL	300486	OPHN1-related disorder
WES065	*PANK2*	c.1561G>A, p.G521R; c.1264T>C, p.C422R	X	X		Definitive	Concordant	G	AR	234200	PANK2-related disorder (Neurodegeneration with Brain Iron Accumulation)
WES129	*PGAP1*	c.1546_1549delGTCA, p.V516KfsX4; c.1077T>G, p.Y359X		X, X		Possible	Promoted	G	AR	615802	PGAP-related disorder
WES025	*PHF6*	c.915_916delTGinsAA, p.C305X	X			Definitive	Concordant	A	XL	301900	Borjeson-Forssman-Lehmann syndrome
WES059	*PHGDH*	c.1538C>T, p.S513F; c.1078+1G>A, IVS9+1G>A		X, X		Definitive	Concordant	G	AR	601815	Phosphoglycerate dehydrogenase deficiency
WES089	*PIK3CD*	c.3061G>A, p.E1021K	X			Definitive	Concordant	G	AD	615513	Primary immunodeficiency 14
WES040	*PKHD1*	c.930delC, p.T311LfsX8; c.5134G>A, p.G1712R		X, X		Definitive	Concordant	G	AR	263200	Autosomal recessive polycystic kidney disease
WES087	*PLA2G6*	c.1613G>A, p.R538H; c.319dupC, p.L107PfsX10		X, X		Definitive	Concordant	G	AR	256600	Infantile neuronal axonal dystrophy type 1
WES148	*POLR3B*	c.2570+5G>A, IVS22+5G>A; c.3317T>C, p.I1106T		X, X		Possible	Promoted	G	AR	614381	Hypomyelinating leukodystrophy type 8
WES077	*PTPN11*	c.922A>G, p.N308D	X			Definitive	Concordant	G	AD	163950	Noonan syndrome
WES154	*PTPN11*	c.836A>G, p.Y279C	X			Definitive	Concordant	G	AD	151100	Noonan syndrome with multiple lentigines
WES074	*SCN1A*	c.677C>T, p.T226M	X			Definitive	Concordant	G	AD	604403	SCN1A-related epilepsy disorder
WES013	*SCYL1*	c.1039C>T, p.Q347*		X[c]		Possible	Promoted	A	AR	616719	SCYL1-related disorder
WES113	*SLC16A2*	c.623_624delGCinsAA, p.G208E		X		Definitive	Concordant	G	XL	300523	Allan-Herndon-Dudley syndrome
WES122	*SNX27*	c.510C>G, p.Y170X; c.1295G>A, p.C432Y		X, X		Candidate	Promoted	G	AR	None yet	SNX27-related disorder
WES009	*STXBP1*	c.875G>T, p.R292L	X			Definitive	Concordant	A	AD	612164	STXBP1-related disorder (Early infantile epileptic encephalopathy 4)
WES147	*TBC1D24*	c.1008delT, p.H336QfsX12; c.680G>T, p.R227L		X, X		Definitive	Concordant	G	AR	220500	DOORS syndrome (deafness, onychodystrophy, osteodystrophy, mental retardation and seizures)
WES082	*TBX1*	c.1392_1403del12, p.A473_A476del		X		Definitive	Concordant	G	AD	188400	TBX-1-related DiGeorge syndrome
WES118	*TBX3*	c.1090G>T, p.E364X	X			Definitive	Concordant	G	AD	181450	Ulnar-mammary syndrome
WES060	*TCF12 / EFTUD2*	c.1319delA, p.N440TfsX79 / c.270A>G, p.T90T	X / X			Definitive / Definitive	Concordant / Concordant	G	AD / AD	615314 / 610536	Craniosnostosis type 3 / Mandibulofacial dysostosis Guion-Almeida type
WES079	*TELO2*	c.1100G>T, p.C367F; c.2296G>A, p.V766M		X, X		Candidate	Promoted	G	AR	616954	TELO2-related disorder
WES132	*TNNT2*	c.833G>A, p.R278H		X		Definitive	Concordant	G	AD	115195	TNNT2-related disorder
WES017	*TRMU*	c.718C>T, p.R240X		X[c]		Definitive	Concordant	G	AR	613070	Combined Respiratory Chain Deficiency (Infantile Liver Failure)

Case Number	Gene	Variant(s)	De Novo	Inherited	Unknown Inheritance	Sequencing Company Classification(s)	Medical Geneticist's Interpretation	Testing Laboratory[a]	Disease Mode of Inheritance[b]	Phenotype MIM Number	Final clinical diagnosis
WES130	*TUBA1A*	c.1168C>T, p.R390C	X			Definitive	Concordant	G	AD	611603	Lissencephaly type 3
WES084	*UBE3A*	c.2563_2566dupCTTA, p.K856TfsX2	X			Definitive	Concordant	G	AD	105830	UBE3A-related disorder
WES015	*UBE3B*	c.2990G>C, p.R997P		X[c]		Possible	Promoted	A	AR	244450	Blepharophimosis-Ptosis-Intellectual disability syndrome (Kaufman oculocerebrofacial syndrome)
WES057	*WAC*	c.1721G>A, p.W574X		X		Definitive	Concordant	G	AD	616708	DeSanto-Shinawi syndrome

[a] G: GeneDx, B: Baylor Genetics, C: Ambry Genetics.

[b] AR: autosomal recessive; AD: autosomal dominant; XL: X-linked.

[c] Homozygous variant.

Table 3

Reasons for Changing the Diagnosis.

Case Number	Gene(s)	Variant(s)	Testing Laboratory	Laboratory Case-Level Classification	Clinical Geneticist Clinical-Level Classification	Reason
Cases That Were Demoted by the Clinical Geneticist						
WES002	*HEXA / VPS13B*	c.1073+1G>AIVS9+1G>A / c. 11256_11290+10del, IVS58+10delC	B	Definitive	Unlikely	Hexosaminidase A activity was normal and clinical phenotype is not consistent with Tay Sachs / Lack of a second mutation in *VSP13B* and phenotype is not consistent with Cohen syndrome
WES003	*PANK2*	c.1561G>A, p.G521R	B	Definitive	Unlikely	Brain MRI and clinical course are not consistent with *PANK2*-related phenotype
WES069	*UPB1 / GAMT*	c.917-1G>A, IVS8-1G>A / c. 327G>A, p.K109K	B	Definitive	Unlikely	Negative biochemical studies for creatine deficiency syndromes and pyrimidine metabolism defects
WES090	*DPYD*	c.1905+1G>A, IVS14+1G>A; c. 1679T>G, p.I560S	G	Definitive	Possible	Biochemical studies were consistent but clinical phenotype did not fit with the phenotype of dihydropyrimidine dehydrogenase deficiency
WES091	*DMD*	Deletion of exons 45-51	G	Definitive	Possible	The neurological and cardiac phenotypes, normal muscle histopathological findings, and normal CK are not consistent with the expected clinical findings of this in-frame *DMD* deletion
Cases That Were Promoted by the Clinical Geneticist						
WES013	*SCYL1*	c.1039C>T, p.Q347*	A	Possible	Definitive	Clinical phenotype of the patient matched a newly described syndrome 2 years after initial analysis
WES015	*UBE3B*	c.2990G>C, p.R997P	A	Possible	Definitive	Facial features and clinical phenotype of the patient matched published syndrome
WES019	*GRIN2B*	c.1916C>T, p.A639V	A	Possible	Definitive	Clinical phenotype of the patient matched neurological findings reported in patients with *GRIN2B* mutations
WES028	*ATP2B3 / LAMA1*	c.1445G>A, p.R482H / c. 6074C>T, p.T2025M; c. 1741C>T, p.R2381C	G	Possible	Definitive	*In vitro* functional studies showed impaired PMCA3 pump function and data supported a synergistic effect with *LAMA1* mutations[17]

Case Number	Gene(s)	Variant(s)	Testing Laboratory	Laboratory Case-Level Classification	Clinical Geneticist Clinical-Level Classification	Reason
WES030	*ARID1B / FGFR3*	c.2281G>A, p.G761S / c.445+(2_5)delTAGG, IVS4+(2_5)delTAGG	G	Possible	Definitive	The blended phenotype in the patient matched published syndromes related to these genes
WES038	*CTBP1*	c.991C>T, p.R331W	G	Candidate	Definitive	The patient was one of 4 patients described with a new genetic syndrome[15]
WES050	*CYB5R3*	c.250C>T, p.R84X	G	Possible	Definitive	Follow up measurement of NADH to cytochrome b5 activity and methemoglobin level in blood were consistent with CYB5R3 deficiency
WES052	*GABRB2*	c.909G>T, p.K303N	G	Candidate	Definitive	Subsequent publication of new syndrome in other patients[12]; the patient is part of an ongoing study on a series of patients to define the phenotype
WES070	*GALNS / SUFU*	c.1485C>G, p.N495K; c.539T>C, p.V180A / c.794_808del15, p.N265_V269del	G	Possible	Definitive	Clinical phenotype of the patient matched the two published syndromes
WES079	*TELO2*	c.1100G>T, p.C367F; c.2296G>A, p.V766M	G	Candidate	Definitive	The patient was 1 of 6 patients described with a new genetic syndrome[16]
WES121	*COQ4*	c.245T>A, p.L82Q; c.473G>A, p.R158Q	G	Candidate	Definitive	The patient was 1 of 4 patients described with a new CoQ10 deficiency syndrome[13]
WES122	*SNX27*	c.510C>G, p.Y170X; c.1295G>A, p.C432Y	G	Candidate	Definitive	Brain MRI and neurological phenotype were consistent with newly described syndrome[11]
WES126	*ATM*	c.3993+1G>A, IVS26+1G>A; c.5763-1050A>G, IVS39-1050A>G	G	Possible	Definitive	Re-sequencing of *ATM* detected a second mutation; elevated AFP and neurological findings matched the diagnosis
WES129	*PGAP1*	c.1546_1549delGTCA, p.V516KfsX4; c.1077T>G, p.Y359X	G	Possible	Definitive	Clinical and neurological phenotype of the patient matched published syndrome
WES131	*DNMT3A*	c.2645G>A, p.R882H	G	Possible	Definitive	Clinical phenotype of the patient was consistent with a newly described syndrome[18]
WES148	*POLR3B*	c.2570+5G>A, IVS22+5G>A; c.3317T>C, p.I1106T	G	Possible	Definitive	Brain MRI and clinical phenotype of the patient matched published syndrome

G: GeneDx, B: Baylor Genetics, A: Ambry Genetics.