Original Investigation

Molecular Findings Among Patients Referred for Clinical Whole-Exome Sequencing

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IMPORTANCE Clinical whole-exome sequencing is increasingly used for diagnostic evaluation of patients with suspected genetic disorders.

OBJECTIVE To perform clinical whole-exome sequencing and report (1) the rate of molecular diagnosis among phenotypic groups, (2) the spectrum of genetic alterations contributing to disease, and (3) the prevalence of medically actionable incidental findings such as *FBN1* mutations causing Marfan syndrome.

DESIGN, SETTING, AND PATIENTS Observational study of 2000 consecutive patients with clinical whole-exome sequencing analyzed between June 2012 and August 2014. Whole-exome sequencing tests were performed at a clinical genetics laboratory in the United States. Results were reported by clinical molecular geneticists certified by the American Board of Medical Genetics and Genomics. Tests were ordered by the patient's physician. The patients were primarily pediatric (1756 [88%]; mean age, 6 years; 888 females [44%], 1101 males [55%], and 11 fetuses [1% gender unknown]), demonstrating diverse clinical manifestations most often including nervous system dysfunction such as developmental delay.

MAIN OUTCOMES AND MEASURES Whole-exome sequencing diagnosis rate overall and by phenotypic category, mode of inheritance, spectrum of genetic events, and reporting of incidental findings.

RESULTS A molecular diagnosis was reported for 504 patients (25.2%) with 58% of the diagnostic mutations not previously reported. Molecular diagnosis rates for each phenotypic category were 143/526 (27.2%; 95% CI, 23.5%-31.2%) for the neurological group, 282/1147 (24.6%; 95% CI, 22.1%-27.2%) for the neurological plus other organ systems group, 30/83 (36.1%; 95% CI, 26.1%-47.5%) for the specific neurological group, and 49/244 (20.1%; 95% CI, 15.6%-25.8%) for the nonneurological group. The Mendelian disease patterns of the 527 molecular diagnoses included 280 (53.1%) autosomal dominant, 181 (34.3%) autosomal recessive (including 5 with uniparental disomy), 65 (12.3%) X-linked, and 1 (0.2%) mitochondrial. Of 504 patients with a molecular diagnosis, 23 (4.6%) had blended phenotypes resulting from 2 single gene defects. About 30% of the positive cases harbored mutations in disease genes reported since 2011. There were 95 medically actionable incidental findings in genes unrelated to the phenotype but with immediate implications for management in 92 patients (4.6%), including 59 patients (3%) with mutations in genes recommended for reporting by the American College of Medical Genetics and Genomics.

CONCLUSIONS AND RELEVANCE Whole-exome sequencing provided a potential molecular diagnosis for 25% of a large cohort of patients referred for evaluation of suspected genetic conditions, including detection of rare genetic events and new mutations contributing to disease. The yield of whole-exome sequencing may offer advantages over traditional molecular diagnostic approaches in certain patients.

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We previously reported a molecular diagnosis rate of 25% for the first 250 patients without prior diagnosis who were referred to our diagnostic laboratory for whole-exome sequencing.[1] Whole-exome sequencing analyzes the exons or coding regions of thousands of genes simultaneously using next-generation sequencing techniques. By sequencing the exome of a patient and comparing it with a normal reference sequence, variations in an individual's DNA sequence can be identified and related back to the individual's medical concerns in an effort to discover the cause of the medical disorder. The overall molecular diagnostic rate was higher than several other comparable genetic tests, including chromosome studies (5%-10%)[2,3] and chromosomal microarray analysis (15%-20%).[4] Notably, in 4 separate cases, molecular findings were reported for 2 Mendelian disorders in the same patient, with clinical features characteristic of the 2 different Mendelian disorders. Secondary (incidental) findings were also observed at a low rate.[1,5-7]

The clinical application of molecular diagnoses by whole-exome sequencing was demonstrated in our pilot study[1]; however, fundamental questions remained unanswered. The robustness of the 25% frequency rate for attaining a molecular diagnosis, the contribution of rare variants, modes of inheritance in the patient population, and the precise rate at which rare genetic events such as mosaicism, multiple loci with contributing mutations, and new mutations contribute to disease remained to be established. Refinement of the coupling between clinical data and molecular interpretation is of particular interest because current methods include considerable expert human involvement and are not readily scalable without further automation. Knowledge of pathogenic variation in an ever-increasing number of Mendelian disease genes is growing,[8] as well as an increasing understanding of tolerated loss of function mutations in healthy controls.[9] This study reports findings from clinical whole-exome sequencing evaluations for 2000 consecutive patients.

Methods

Clinical Samples

There were 2000 consecutive, unrelated patient cases in this study who were referred from physicians starting in June 2012 through November 2013 for clinical whole-exome sequencing at the Whole Genome Laboratory of Baylor College of Medicine. The laboratory has been certified by both the College of American Pathologists and the US Centers for Disease Control and Prevention Clinical Laboratory Improvement Amendments of 1988. A request for whole-exome sequencing testing was made solely at the discretion of the referring physician with no inclusion or exclusion criteria and no filtering by the laboratory.[10] The only reason for the laboratory to decline testing was for financial reasons (eg, denial of coverage by insurance). Representative clinical cases are presented in **Table 1** as examples of prior diagnostic evaluations for patients referred for whole-exome sequencing. These examples were selected based on verification of completeness of prior laboratory testing and for demonstration of possible outcomes of whole-exome sequencing (total cost for laboratory testing for case No. 218 appears in eTable 1 in the Supplement). The initial 250 cases previously reported were excluded.[1] Requisition and consent forms are available at https://www.bcm.edu/geneticlabs/.

Peripheral blood, tissue, or extracted DNA samples were collected from patients or their parents and submitted with a requisition form, which included informed consent and patient clinical data as previously described.[1] Following pretest counseling for whole-exome sequencing, patients and parents/guardians were given options of not receiving specific categories of results (detailed later). The phenotypes of the 2000 patients were categorized into 4 groups at the time of whole-exome sequencing data analysis according to the clinical data provided by the referring physician (**Table 2** and eTable 2 in the Supplement).

The neurological group consisted of patients with findings confined to neurological or developmental systems (eg, developmental delay, intellectual disability, autism, speech delay). The neurological plus other organ systems group included findings listed for the neurological group plus at least 1 finding from another organ system, which could include renal, cardiac, gastrointestinal, pulmonary, or multiple congenital anomalies. The specific neurological group included more defined neurological signs and symptoms (eg, ataxia, movement disorder, spastic paraplegia) than the neurological group. The nonneurological group had findings from organ systems other than neurological. The 4 groups were developed by clinical geneticists and medical directors of the laboratory and assignments were made by the laboratory directors at the time of case review and before the results of whole-exome sequencing were known. For cases with complex, overlapping features, consultation with the medical director was performed.

This analysis of deidentified patient data and aggregate clinical genomics data was approved by the institutional review board at Baylor College of Medicine.

Whole-Exome Sequencing and Analyses

A previously described[1] whole-exome sequencing protocol, including library construction, exome capture by VCRome version 2.1,[11] and HiSeq next-generation sequencing and data analysis,[12] was developed by the Human Genome Sequencing Center at Baylor College of Medicine and adapted for the clinical test of whole-exome sequencing. Given our minimum levels of depth of coverage (20 ×) and minimum variant calling requirements, about 94.6% of all single-nucleotide variants (SNVs) and 88.2% of indels (insertions or deletions) could potentially be identified (**Box**). However, in practice, because the coverage is typically in excess of 20 ×, we can detect greater than 94.5% of all indels. Our interpretation and review process was facilitated by internal annotation databases, a central in-house tracking system of all cases, and automation.

Detailed information about the methods regarding mitochondrial genome sequencing, the single-nucleotide polymorphism (SNP) array, de novo mutation detection, and the statistical analysis appear in the eMethods in the Supplement.

Table 1. Descriptions of Prior Diagnostic Evaluations for Cases Referred for Whole-Exome Sequencing

Case No.[a]	Age, y	Sex	Phenotype	Prior Laboratory Testing[b]	Whole-Exome Sequencing Diagnosis[c]	Clinical Utility and Implications
218	11.8	F	Intellectual disability, facial dysmorphism, autistic features, epilepsy, hypotonia, ataxia	Biochemical: plasma amino acid, plasma acylcarnitine, urine organic acid, plasma creatine and guanidinoacetate, homocysteine panel, creatine kinase, lactate, thymidine, cerebrospinal fluid neurotransmitters Chromosomal microarray: yes Mitochondrial genome: NA Molecular testing of nuclear genes: *MECP2* sequencing and deletion or duplication, *FOXG1* and *CDKL5* sequencing, *UBE3A* sequencing, methylation studies for Angelman syndrome, trinucleotide expansion studies for fragile X and myotonic dystrophy	Gene: *DEAF1* Inheritance: autosomal dominant Disease: mental retardation, autosomal dominant 24 MIM No.: 615828	Truncating diagnosis odyssey: the patient had extensive prior workup without receiving a definitive diagnosis[d]
76	2.5	F	Developmental delay, seizures, bilateral optic nerve hypoplasia, horizontal nystagmus, hypoplasia of the corpus callosum, and periventricular leukomalacia	Biochemical: plasma amino acid, plasma acylcarnitine, urine organic acid Chromosomal microarray: yes Mitochondrial genome: common mutations and deletions screening Molecular testing of nuclear genes: *PAX6* sequencing and deletion or duplcations	Gene: *MYO5A* Inheritance: autosomal recessive Disease: Griscelli syndrome type 1 MIM No.: 214450	Anticipatory guidance for organ involvement or other symptoms: initially the pigment abnormality in the patient was not noted prior to ordering of whole-exome sequencing. Based on the whole-exome sequencing findings, hair shaft analysis was performed and pigmentary defect was detected.
406	<1	M	Delayed motor milestones, dysmorphic features, chronic secretory diarrhea (dependent on total parenteral nutrition [TPN]), failure to thrive, hypothyroidism, cholestasis (secondary to TPN), anemia, and alopecia	Molecular testing of nuclear genes: *FOXP3* gene sequencing negative Information on other tests: NA	Gene: *TTC37* Inheritance: autosomal recessive Disease: trichohepatoenteric syndrome type 1 MIM No.: 222470	Disease management: before the whole-exome sequencing was ordered, immunodysregulation polyendocrinopathy enteropathy X-linked syndrome was suspected. The whole-exome sequencing identified trichohepatoenteric syndrome and the results can be used to guide disease management.
150	Fetus	U	Multiple congenital anomalies	Prenatal ultrasound: abnormal Chromosomal microarray: yes	Gene: *ALG12* Inheritance: autosomal dominant Disease: congenital disorder of glycosylation type 1G MIM No.: 607143	Molecular diagnosis results used in reproductive planning: prenatal diagnosis was ordered for the subsequent pregnancy based on results of whole-exome sequencing
112	3.3	F	Static encephalopathy, developmental delay, hypotonia, and seizures	Biochemical: plasma amino acid, plasma acylcarnitine, urine organic acid, plasma creatine and guanidinoacetate, thyroid function studies, creatine kinase, lactate, aldolase, very long-chain fatty acids Chromosomal microarray: yes Mitochondrial genome: NA Molecular testing of nuclear genes: *ISCU* gene sequencing	Gene: *SLC2A1* Inheritance: autosomal dominant Disease: glucose transporter deficiency syndrome types 1 and 2 MIM Nos.: 606777 and 612126	Molecular diagnosis leading to specific treatment: based on the whole-exome sequencing findings, the patient was referred to epilepsy clinic to start ketogenic diet. Evaluations after 6 mo on the ketogenic diet: the patient discontinued levetiracetam, no seizure to date, more active and alert, no longer napped during the day, balance was improved as were fine motor skills and speech.

Abbreviations: NA, not applicable; U, unknown.

[a] This Table presents representative cases; these case numbers correspond to eTable 4 in the Supplement, which includes the 504 molecularly diagnosed cases.

[b] Prior testing yielded negative results or was unrevealing.

[c] The MIM numbers are from the Online Mendelian Inheritance in Man.

[d] Prior workup cost information appears in eTable 1 in the Supplement.

Molecular Diagnosis

The whole-exome sequencing interpretations considered multiple sources of evidence, including the specific variant that was identified, the gene involved, and clinical case history. At the variant level, likely benign variants, including common variants and synonymous or intronic variants that were more than 5 bp from the exon boundaries, were electronically removed as previously described.[1] The filtered variant data were then interpreted via extensive literature and database review to consider potential relevance to disease phenotype, penetrance, segregation or inheritance, disease-causing mechanism, and potential pathogenicity of mutations according to the existing and proposed guidelines from the American College of Medical Genetics and Genomics (ACMG) and as previously described.[1,13,14]

Classification criteria for likely pathogenic and pathogenic variants are described in eTable 3 in the Supplement. Following the variant- and gene-level analyses, a whole-exome sequencing case was further evaluated in search of a molecular diagnosis. A whole-exome sequencing case was classified as molecularly diagnosed if pathogenic or likely pathogenic variants were detected in Mendelian disease genes that over-

Table 2. Patient Demographic Information and Molecular Diagnosis Rates

	Molecular Diagnosis Rates by Phenotype Groups									
	Neurological[a]		Neurological Plus Other Organ Systems		Specific Neurological[b]		Nonneurological		Overall	
	No./ Total	Rate, % (95% CI)	No./ Total	Rate, % (95% CI)	No./ Total	Rate, % (95% CI)	No./ Total	Rate, % (95% CI)	No./ Total	Rate, % (95% CI)
Age, y										
Fetus	0	0	5/7	71.4 (30.3-94.9)	1/1	100 (NA)	0/3	0 (NA)	6/11	54.5 (24.6-81.9)
<5	63/207	30.4 (24.3-37.3)	143/548	26.1 (22.6-30.0)	11/22	50.0 (30.7-69.3)	30/123	24.4 (17.3-33.1)	247/900	27.4 (24.6-30.4)
5-18	70/268	26.1 (21.1-31.9)	115/478	24.1 (20.3-28.2)	11/36	30.6 (16.9-48.3)	14/63	22.2 (13.1-34.8)	210/845	24.9 (22.0-27.9)
>18	10/51	19.6 (10.3-33.5)	19/114	16.7 (10.6-25.1)	7/24	29.2 (13.4-51.2)	5/55	9.1 (3.4-20.7)	41/244	16.8 (12.5-22.2)
Sex										
Male	70/285	24.6 (19.8-30.1)	134/653	20.5 (17.5-23.9)	15/46	32.6 (20.0-48.1)	22/117	18.8 (12.4-27.3)	241/1101	21.9 (19.5-24.5)
Female	73/241	30.3 (24.6-36.6)	143/487	29.4 (25.4-33.7)	14/36	38.9 (23.6-56.5)	27/124	21.8 (15.1-30.3)	257/888	28.9 (26.0-32.0)
Unknown (fetus)	0	0	5/7	71.4 (30.3-94.9)	1/1	100 (NA)	0/3	0 (NA)	6/11	54.5 (24.6-81.9)
Overall	143/526	27.2 (23.5-31.2)	282/1147	24.6 (22.1-27.2)	30/83	36.1 (26.1-47.5)	49/244	20.1 (15.6-25.8)	504/2000	25.2 (23.3-27.2)

Abbreviation: NA, data not applicable.

[a] Included developmental delay, speech delay, autism spectrum disorder, and intellectual disability. Neurological disorders are defined as conditions that affect development or function of the central nervous system, the peripheral nervous system, or both. Developmental delay is defined as delay of gross motor or fine motor development exceeding the upper limit of normal as described in the Denver Developmental Assessment. Intellectual disability is defined as subaverage general intellectual functioning that originates prior to 18 years of age and is associated with impairment in adaptive behavior. Although IQ measurement is not required for the diagnosis, it is generally accepted that intellectual disability is associated with an IQ of less than 70.

[b] Included patients with a specific neurological problem such as ataxia or seizures.

lapped with described phenotypes of the patients, and for recessive disorders if the variants were on both alleles of the same gene (ie, biallelic).

Whole-Exome Sequencing Reporting

The format for reporting of whole-exome sequencing data used the 2-tier strategy as described.[1] In brief, the tier 1 (focused) report included the following 6 variant reporting categories: (1) deleterious mutations (also known as pathogenic variants) related to the disease phenotype; (2) variants of unknown clinical significance related to the disease phenotype; (3) medically actionable mutations in genes with potential therapies or established surveillance protocols, including but not limited to the 56 genes recommended by ACMG for medically actionable incidental findings[6]; (4) autosomal recessive carrier status for genes from the ACMG-recommended population screening panel[15]; (5) a limited number of pharmacogenetic variants[1]; (6) clinically relevant pathogenic mutations in the mitochondrial genome, which is a new category not included in our prior study,[1] including deleterious point mutations and large structural rearrangements in the homoplasmic state or in greater than 20% of the heteroplasmic state. Variant reporting categories 4 and 5 include secondary findings that the patients and parents may opt out of receiving. Following the publication of the ACMG guidelines for medically actionable incidental finding genes,[6] the consent form was updated to include an opt-out for non-ACMG incidental findings; this option was available for samples received on or after September 2013.

Tier 2 reporting included deleterious mutations or variants of unknown clinical significance unrelated to the disease phenotype, and predicted deleterious mutations such as nonsense or splice site mutations in nondisease genes.[1] This information may become clinically relevant as new disease-gene relationships become reported in the literature (eg, *ARID1B*).[1,16]

Results

Demographics of Clinical Cases

The 2000 consecutive cases submitted to the clinical laboratory for whole-exome sequencing testing were primarily pediatric patients. There were 900 children younger than 5 years (45.0%), 845 children and adolescents from 5 to 18 years of age (42.2%), 244 adults older than 18 years (12.2%), and 11 fetal samples from terminated pregnancies (0.6%) (Table 2). The majority of the patients had neurological disorders or developmental delay (87.8%; neurological, neurological plus other organ systems, and specific neurological groups), and only 12.2% of patients had nonneurological disorders (nonneurological group). The clinical presentations of the 2000 patients in terms of most frequent presenting sign or symptom appear in eTable 2 in the Supplement.

Of the 2000 patients, 128 (6.4%) and 154 (7.7%) parents declined reporting for recessive disorders and pharmacogenetic variants, respectively. Of the 190 patients given the opt-out for non-ACMG incidental gene findings,[6] 2 (1.1%) opted out of this

Box. Glossary of Terms

Absence of Heterozygosity
A stretch of the human genome in which there is no evidence of heterozygous (2 different) variant alleles, only apparently homozygous (the same) variant allele. This may result from a deletion on 1 allele, consanguinity, or uniparental disomy (see below).

Copy Number Variation
Gain or loss of large fragments of DNA in the genome.

Depth of Coverage
The number of times uniquely aligned sequence reads cover an exome target nucleotide generated during the next-generation sequencing process.

Medically Actionable Incidental Finding
This term has been used in a variety of clinical and research contexts to indicate unexpected positive findings. Other terms have been used to describe these findings, particularly when they are actively sought (rather than being unexpectedly discovered). We used incidental findings in this article to indicate the results of a deliberate search for pathogenic or likely pathogenic alterations in genes that are not apparently relevant to a diagnostic indication for which the sequencing test was ordered.[6]

Molecular Diagnosis
Testing designed to confirm or exclude a known or suspected genetic disorder in a symptomatic individual or, prenatally, in a fetus at risk for a certain genetic condition.[35]

Uniparental Disomy
The situation in which both members of a chromosome pair or segments of a chromosome pair are inherited from 1 parent and neither is inherited from the other parent; uniparental disomy can result in an abnormal phenotype in some cases.[35] Uniparental disomy can occur as a random event during the formation of egg or sperm cells or may happen in early fetal development. It can also occur during trisomy rescue or monosomy rescue. Uniparental disomy can cause autosomal recessive disease gene mutations to be homozygous in a patient (often referred as unmasking the autosomal recessive mutation) because the patient inherits 2 copies of the chromosome with the mutation from 1 parent, conveying a form of non-Mendelian inheritance and leading to the recessive disease phenotype observed in the patient.

additional reporting. Overall, 1808 families (90.4%) requested all aspects of the focused report (tier 1 with the 6 variant reporting categories). In addition, the expanded report (tier 2, which included deleterious mutations or variants of unknown clinical significance unrelated to the disease phenotype) was ordered by physicians for 524 patients (26.2%).

Variants Analyzed

Approximately 200 000 to 400 000 variants were identified in each patient. After removing low-quality variants, approximately 1 750 800 variants were analyzed for the 2000 samples (average of about 875 variants per sample), including about 52 000 deleterious mutations (3.0%), 153 230 variants of unknown clinical significance (8.8%), and 1 545 000 benign variants (88.3%). Review time spent on variant classification is facilitated by accumulated curated information on the pathogenicity, familial study results, and frequency at the variant level. For example, checking inheritance patterns for genes and related genetic disorders has been shortened from approximately 6 hours at the launch of whole-exome sequencing testing on October 2011 to approximately 0.5 hours per case at present. Overall, reporting time per case review is approximately 7 hours, which is an improvement from approximately 18 hours during the initial implementation period.

Molecular Diagnoses

Molecular diagnoses were reported for 504 patients (25.2% [95% CI, 23.3%-27.2%]; Table 2 and eTables 4 and 5 in the Supplement), which is a molecular diagnostic yield similar to our initial study.[1] We divided the 2000 patients into 4 groups based on the phenotypes provided. The rates for molecular diagnosis varied with clinical presentation. The lowest yield was for patients in the nonneurological group (20.1%) and the highest was for the specific neurological group (36.1%) (Table 2).

Mendelian Patterns Observed

The presumed modes of inheritance of the molecular diagnoses included 280 (53.1%) autosomal dominant, 181 (34.3%) autosomal recessive, 65 (12.3%) X-linked, and 1 (0.2%) mitochondrial (**Table 3**). Of the 280 autosomal dominant conditions diagnosed, 208 (74.3%) arose as a result of de novo mutations, 32 (11.4%) were inherited, and 40 (14.3%) were undetermined due to lack of parental samples. Of the 65 X-linked disorders, 34 (52.3%) occurred in males and 31 (47.7%) in females; 40 (61.5%) X-linked alleles resulted from de novo mutations, including 17 (42.5%) in males and 23 in females (57.5%). Among the 181 autosomal recessive disorders, 108 (59.7%) demonstrated compound heterozygosity of 2 distinct mutations and 73 (40.3%) had apparently homozygous mutations, including 5 patients with uniparental disomy.

Notably, among the cases with de novo mutations in disease genes, mosaicism of the mutant allele was seen in 5 probands (3 with autosomal dominant and 2 with X-linked disorders) (Table 3 and eTable 6 in the Supplement), suggesting the mutation occurred after fertilization. In 4 of the 5 patients, the ratio of mutant allele fraction is low, ranging from 10% to 20%, whereas in the fifth patient the mutant allele was predominant with a mutant allele fraction of 76%, as seen by both whole-exome sequencing calls and Sanger sequencing. This could result from lymphocytes reverting back to the wild-type sequence in a subset of cells. In addition, mosaicism in the parental samples of 2 inherited cases was detected (eTable 6 in the Supplement).

Rare Variants Account for the Majority of Mutant Alleles

A total of 708 presumptive causative variant alleles were identified from the 504 positive cases. The majority of the disease-associated variants are novel (409/708; 57.8%) as defined by neither being previously reported in public mutation databases nor in patient case reports described in the literature at the time of clinical sign out. There were 237 alleles previously reported (33.5%) in patients described in the literature and 62 heterozygous variants in recessive genes were not previously reported (8.8%) in patients but seen in controls predicting car-

rier status at very low frequencies. There is a wide spectrum of mutant alleles among the disease-associated changes, including 346 missense, 149 frameshift, 134 nonsense, 57 splice, 8 in-frame deletions or duplications, 6 large deletions, 5 start codon defects, 1 stop loss (loss of stop codon), 1 promoter region, and 1 mitochondrial DNA mutation (eTable 4 in the Supplement).

Of 6 probands with large deletion mutations, 2 had large deletions encompassing the Prader-Willi/Angelman region on chromosome 15 as identified by chromosome SNP array. The other 4 patients harbored a point mutation or SNV on 1 allele, opposite a large deletion copy number variant on the other allele as identified by chromosome SNP array or chromosomal microarray studies (eFigure 1 in the Supplement).[17]

Recurrent Molecular Diagnoses

The majority of the diagnosed cases (282/504; 56.0%) had mutations in a gene found at least twice in the series (eTable 5 in the Supplement). Approximately 30% of the molecular diagnoses occurred in disease genes that were only recently described in the literature (2011 or later; eFigure 2 in the Supplement). Sixty-five of the 504 molecular diagnoses (12.9%) (eTable 5 in the Supplement) were in genes not available at the time the whole-exome sequencing test was ordered as either a single gene or sequencing panel clinical test as described in the Genetic Testing Registry (http://www.ncbi.nlm.nih.gov/gtr/) or other sources.

Variants at 2 Genetic Loci in 1 Personal Genome Potentially Related to the Phenotype

In this series, 23 patients (4.6% of those with diagnoses and 1.4% of all patients) had mutations at 2 distinct disease loci that were related to the phenotype (Table 3 and eTable 7 in the Supplement). As previously reported,[1] multiple molecular events in 1 patient leading to blended and often complicated phenotypes remains an appreciable cause of disease.

Uniparental Disomy Resulting in Apparently Homozygous Recessive Disease Alleles

In 5 cases, uniparental disomy of a region was indicated by chromosome SNP array data, 2 involving chromosome 2 and 1 each involving chromosomes 3, 9, and 22. Uniparental disomy of chromosomes 2, 3, 9, and 22 can be seen in healthy controls and there is no evidence for imprinted gene expression leading to a clinical phenotype associated with uniparental disomy of those chromsomes.[18] However, in our patients, uniparental disomy caused autosomal recessive disease gene mutations to be homozygous in the proband because the child inherits 2 copies of the chromosome with the mutation from 1 parent, conveying a form of non-Mendelian inheritance and leading to the recessive disease phenotype observed in the patient (Table 3 and eTable 8 and eFigure 3 in the Supplement).

Medically Actionable Incidental Findings

In the 2000 cases, 95 medically actionable incidental findings were reported in 92 patients (4.6%). Three patients had more than 1 such finding. In 59 patients (3%), the incidental findings occurred in genes included in the ACMG list of 56 genes recommended to be disclosed.[6] The remaining 33 patients (1.7%) had mutations in genes reported based on our local criteria for reporting of medically actionable results (**Table 4**). Of the non-ACMG findings, 6 were cases of glucose-6-phosphate dehydrogenase deficiency (X-linked) and 5 were cases carrying mitochondrial DNA mutations associated with an increased risk of aminoglycoside-induced nonsyndromic hearing loss. We report these 2 disorders given the current recommendations for mutation carriers to avoid exposure to specific agents. Similarly, the incidental finding of Fabry disease in 1 young male patient has direct clinical benefit to the patient and family because of the clinical availability of enzyme therapy.[19]

Our protocol returns medically actionable results for the proband but does not automatically report the results for parents. Testing of parents for the medically actionable finding can be ordered free of charge after disclosure of the proband's results. To date, of the 92 patients with incidental findings, 33 parents from 19 families have requested results.

Updated Summary Analysis

We have performed a summary analysis of unselected, unrelated cases completed and reported from the close of the current 2000 case cohort (November 2013) through August 30, 2014,

Table 3. Selected Contributing Genetic Events in Whole-Exome Sequencing Cases With Molecular Diagnoses

Mode of Inheritance	No. of Cases[a]
Autosomal dominant (n = 280)[b]	
De novo	208
Imprinting	6
Mosaicism	3
Mosaicism in a parent	2
Autosomal recessive (n = 181)[b]	
Compound heterozygous single-nucleotide variants (SNVs)	104
Compound heterozygous SNV and copy number variant	4
Homozygous variants (parents studied)	59
Apparently homozygous variants (parents not studied)	9
Homozygous variants caused by uniparental disomy	5
X-linked (n = 65)[b]	
De novo	40
Mosaicism	2
Mitochondrial disorder (n = 1)[b]	
De novo	1
Two diagnoses (n = 23)[b,c]	
Autosomal dominant + autosomal dominant	7
Autosomal dominant + autosomal recessive	8
Autosomal dominant +X-linked	4
Autosomal recessive + autosomal recessive	3
Autosomal recessive +X-linked	1

[a] Additional information appears in eTable 4 and eTable 6 in the Supplement.

[b] Each category contains events (eg, de novo, mosaic, etc) that are not mutually exclusive (ie, a mosaic finding is generally also de novo); therefore, the individual events will not sum to the total for each category.

[c] Additional information appears in eTable 7 in the Supplement.

Table 4. Medically Actionable Incidental Findings

Disease	Inheritance	Gene (MIM No.)[a]	No. of Patients With Incidental Findings
Familial breast-ovarian cancer type 2, susceptibility to male breast cancer, Fanconi anemia complementation group D1, pancreatic cancer, prostate cancer, and Wilms tumor	AD/AR	*BRCA2*[b] (600185)	9
Familial breast-ovarian cancer type 1 and susceptibility to pancreatic cancer type 4	AD	*BRCA1*[b] (113705)	5[c,d]
Arrhythmogenic right ventricular dysplasia type 11	AD	*DSC2*[b] (125645)	5
Familial atrial fibrillation type 3, Jervell and Lange-Nielsen syndrome, long QT syndrome type 1, and short QT syndrome type 2	AD/AR	*KCNQ1*[b] (607542)	5
Familial atrial fibrillation type 10, Brugada syndrome type 1, dilated cardiomyopathy type 1E, nonprogressive heart block, long QT syndrome type 3, sick sinus syndrome type 1, and familial ventricular fibrillation type 1	AD/AR	*SCN5A*[b] (600163)	5
Marfan syndrome; mitral valve prolapse, aortic enlargement, skin and skeletal findings syndrome; familial ectopia lentis; aortic aneurysm, ascending, and dissection; and stiff skin syndrome	AD	*FBN1*[b] (134797)	4
Familial hypertrophic cardiomyopathy type 10	AD	*MYL2*[b] (160781)	4
Hereditary nonpolyposis colorectal cancer type 4 and mismatch repair cancer syndrome	AD	*PMS2*[b] (600259)	4
Arrhythmogenic right ventricular dysplasia type 9	AD	*PKP2*[b] (602861)	3
Arrhythmogenic right ventricular dysplasia type 10 and dilated cardiomyopathy type 1BB	AD	*DSG2*[b] (125671)	2
Familial hypercholesterolemia	AD	*LDLR*[b] (606945)	2
von Hippel-Lindau syndrome, pheochromocytoma, and familial erythrocytosis type 2	AD	*VHL*[b] (608537)	2
Hypercholesterolemia due to ligand-defective apolipoprotein B	AD	*APOB*[b] (107730)	1
Arrhythmogenic right ventricular dysplasia type 8, dilated cardiomyopathy, epidermolysis bullosa, keratosis palmoplantaris striata type II, and skin fragility-woolly hair syndrome	AD/AR	*DSP*[b] (125647)	1
Fabry disease	X-linked	*GLA*[b] (300644)	1
Hereditary nonpolyposis colorectal cancer type 5, familial endometrial cancer, and mismatch repair cancer syndrome	AD	*MSH6*[b] (600678)	1
Familial hypertrophic cardiomyopathy type 4	AD/AR	*MYBPC3*[b] (600958)	1
Familial thoracic aortic aneurysm type 4	AD	*MYH11*[b] (160745)	1
Familial hypercholesterolemia type 3	AD	*PCSK9*[b] (607786)	1
Medullary thyroid carcinoma, multiple endocrine neoplasia types IIA and IIB, and congenital central hypoventilation syndrome	AD	*RET*[b] (164761)	1
Familial hypertrophic cardiomyopathy type 7, dilated cardiomyopathy types 1FF and 2A, and familial restrictive cardiomyopathy	AD/AR	*TNNI3*[b] (191044)	1
Cardiomyopathy	AD	*TNNT2*[b] (191045)	1
Favism and hemolytic anemia due to glucose-6-phosphate dehydrogenase deficiency	X-linked	*G6PD*[e] (305900)	7[c,f]
Aminoglycoside-induced nonsyndromic hearing loss	Mitochondrial	*MTRNR1*[e] (561000)	5
Brugada syndrome type 2	AD	*GPD1L*[e] (611778)	3
Familial atrial fibrillation type 12, dilated cardiomyopathy type 10, and hypertrichotic osteochondrodysplasia	AD	*ABCC9*[e] (601439)	2
Long QT syndrome type 4	AD	*ANK2*[e] (106410)	2
Familial atrial fibrillation type 7	AD	*KCNA5*[e] (176267)	2
Polycystic kidney disease type 2	AD	*PKD2*[e] (173910)	2
ANKRD1-related dilated cardiomyopathy and dilated cardiomyopathy	AD	*ANKRD1*[e] (609599)	1
Familial diffuse gastric cancer with or without cleft lip, palate, or both	AD	*CDH1*[e] (192090)	1
Dominant and recessive myotonia congenita and recessive myotonia levior	AD/AR	*CLCN1*[e] (118425)	1
Retinitis pigmentosa type 25	AR	*EYS*[e] (612424)	1[g]
Autosomal dominant factor XI deficiency	AD/AR	*F11*[e] (264900)	1
Brugada syndrome type 6	AD	*KCNE3*[e] (604433)	1
Restrictive cardiomyopathy	AD	*MYPN*[e] (608517)	1
Fanconi anemia complementation group N, susceptibility to breast cancer, and susceptibility to pancreatic cancer type 3	AR/AD	*PALB2*[e] (610355)	1
Susceptibility to familial breast-ovarian cancer type 4	AD	*RAD51D*[e] (602954)	1
Progressive, familial heart block type IB	AD	*TRPM4*[e] (606936)	1
Cardiomyopathy, muscular dystrophy, and early-onset myopathy with fatal cardiomyopathy	AD/AR	*TTN*[e] (188840)	1[g]
von Willebrand disease types 1, 2A, 2B, 2M, 2N, and 3	AD/AR	*VWF*[e] (613160)	1

Abbreviations: AD, autosomal dominant; AR, autosomal recessive.

[a] The MIM numbers are from Online Mendelian Inheritance in Man.

[b] These genes are on the actionable genes list recommended by the American College of Medical Genetics and Genomics (ACMG).[6]

[c] One patient had more than 1 finding.

[d] Biallelic pathogenic variants in *BRCA1* detected in 1 proband.

[e] These genes are not on the ACMG-recommended actionable genes list.[6]

[f] Biallelic pathogenic variants detected in 1 female proband.

[g] Homozygous variant in *EYS* and heterozygous variant in *TTN* detected in 1 proband.

bringing the total number of cases included in this report to 3386 cases. The overall molecular diagnostic rate for the total cases remains unchanged at 25% (830 molecular diagnosis of 3386 total cases).

Of the additional 1386 patients, the sex distributions were 639 females (46.1%), 740 males (53.4%), and 7 fetuses (0.5%). In addition, 553 were younger than 5 years (39.9%), 676 were 5 to 18 years of age (48.8%), and 150 were older than 18 years (10.8%).

It should be noted that the most recent 457 of these cases were analyzed using an updated capture reagent designed to improve sequence coverage of the exome.[20] A subanalysis of these 457 cases demonstrates a 24% diagnostic rate, which is not significantly different from the main cohort.

Discussion

Data from clinical whole-exome sequencing for 2000 sequentially referred patients allow further insight into both the application of whole-exome sequencing to medical practice and the genomic architecture of Mendelian disease. A molecular diagnosis rate of 25% was observed in our pilot study[1] of 250 cases and has remained consistent in this larger series of predominantly pediatric patients with diverse clinical presentations most notable for intellectual disability and neurological phenotypes. Of the 2000 whole-exome sequencing samples, the molecular diagnosis rate was highest for children with specific neurological findings (36.1%). This category is heterogeneous but was generally characterized by patients with more specific clinical presentations, perhaps facilitating correlations between genotype and phenotype.

Clinical exomes identified a broad range of inheritance patterns and molecular mechanisms for disease. Of patients diagnosed with an autosomal dominant disorder and with parental samples submitted, about 87% resulted from de novo mutations. This finding provides a cautionary note to the application of carrier testing to reduce the burden of genetic disease and demonstrates the need for detecting de novo events prenatally.

We observed an equivalent number of male and female patients diagnosed with X-linked disorders. The X-linked diagnoses in females were in genes known to affect mainly females (4 cases of *MECP2*, 2 cases of *CDKL5*) or males and females equally (eg, *KDM6A, SMC1A, PDHA1*)[21-24] or were associated with specific phenotypes seen in females (eg, *DCX* mutation associated with band heterotopia in females vs classic lissencephaly in males). Patients with apparently homozygous mutations causing autosomal recessive conditions were found to result from several molecular mechanisms, including 59 cases inheriting the same rare disease allele from each parent, 5 cases in which uniparental disomy caused homozygosity for a SNV allele, and 4 cases of compound heterozygosity for a point mutation and large deletion copy number variant in the same gene. Autosomal recessive disorders accounted for 34.3% (n = 181) of the molecular diagnoses, in contrast to a previous report of 100 patients with intellectual disability, in which only 1 of 16 patients with probable molecular diagnoses had an autosomal recessive disorder.[25] Excluding the uniparental disomy cases, 68 of our patients were apparently homozygous for the same rare allele of which half (n = 34) were in patients known to have consanguineous parents. The extent of absence of heterozygosity in the remaining patients suggested that an additional 9 had shared ancestry. Overall, homozygous mutations identical by descent may account for 8.5% (43 of 504) of the total positive cases, indicating that consanguinity may play a role in the higher percentage of autosomal recessive disorders observed in our diagnosed patients.

Due to the change in sequencing technology in which each base in the exome is sequenced hundreds of times, whole-exome sequencing allows detection of patients who only carry the mutation in a small percentage of their cells (low-level mosaicism) and enables an improved estimate of the fraction of mutant cells.[26-29] Five of the 504 diagnosed patients (1%) demonstrated mosaicism for a mutant allele in genes with phenotypic overlap with the patient's presentation.

Approximately 30% of positive cases reported herein harbored presumptive causative mutations in disease genes discovered since 2011, reflecting the benefits of an accelerating pace of disease gene discovery. Whole-exome sequencing testing is a platform suitable for timely incorporation of new disease genes because it interrogates entire coding regions, making it possible to automate the updating of disease gene annotation for clinical reporting, even after the initial analysis is completed. Of the 65 positive cases that would not have been diagnosed by other molecular methods at the time the test was ordered, 13 were identified by reanalysis after the initial whole-exome sequencing report (eTable 5 in the Supplement). It is therefore likely that a significant proportion of undiagnosed cases harbor mutations in still yet to be discovered disease genes. In addition, new capture reagents targeted at poorly covered exome regions are being developed to improve the sequencing of known disease genes not well interrogated in the current assay to further improve molecular diagnosis yield.[20] Two molecular diagnoses were found within the individual personal genomes of 4.6% of the molecularly diagnosed cases. These cases highlight oligogenic models of disease etiology and reflect that simple Mendelian gene effects can compound to yield complex genetic profiles.[30]

There has been great attention to the reporting of incidental findings since the ACMG guidelines were published.[31-34] We have found a stable rate of approximately 3% of patients with mutations reported in the genes on the ACMG list. We identified and reported medically actionable findings in a total of 4.6% of cases when including other loci that by expert opinion of our clinical and diagnostic team are considered to be medically indicated, which is comparable with other studies.[7] Further studies are needed to analyze the clinical utility of this information as at-risk presymptomatic individuals (and their family members) are identified and potentially entered into screening protocols. Debate continues regarding the definition of medically actionable findings and the threshold for reporting.

The limitations of whole-exome sequencing as a diagnostic modality relate to incomplete coverage of exonic regions

and evolving knowledge of variant interpretation. The molecular diagnostic rate of 25% may be an underascertainment due to current technical limitations of exome sequencing: (1) to provide 100% coverage of the coding regions due to sequence architecture (eg, high G + C content) and (2) the ability to detect copy number variants. The interpretation of variants as pathogenic, nonpathogenic, or of uncertain significance is based on current information in the literature and databases such as ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) and may change as understanding of the genome evolves. Additional data from family studies or further feedback from referring physicians may also help establish more diagnoses. Limitations to knowledge of the clinical utility of whole-exome sequencing relate to incomplete information on patient outcomes. For the 25% of cases that received a molecular diagnosis, this information ended the diagnostic odyssey, provided more informed medical management, and allowed for precise determination of reproductive risks; however, relatively few cases resulted in specific treatment to reverse the condition. Our specific study is limited by the setting in a clinical diagnostic laboratory, which reflects the real-world diagnostic context, but does not allow for collection of complete medical histories, medical records, or prior testing.

In terms of adverse experiences in the reporting of whole-exome sequencing, there were 5 cases of suspected nonpaternity among the approximately 3000 cases in which whole-exome sequencing was performed. These were uncovered during our validation process of confirming variants identified in the proband in parental samples. Misidentified parentage is a well-described risk of genetic testing and is stated as such in our consent documents. Approximately 5% of cases received a medically actionable diagnosis that was unrelated to the indication for testing. There may indeed be cases in which disclosure of these results has brought anxiety and perhaps increased medical costs in terms of testing and evaluation of other family members; however, this is best addressed in studies of the ethical implications of genome-wide molecular diagnostic approaches.

Conclusions

Whole-exome sequencing provided a potential molecular diagnosis for 25% of a large cohort of patients referred for evaluation of suspected genetic conditions, including detection of a number of rare genetic events and new mutations, contributing to disease. The observed flexibility and yield of whole-exome sequencing suggest that whole-exome sequencing may offer advantages over traditional molecular diagnostic approaches in certain patients.

ARTICLE INFORMATION

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Acquisition, analysis, or interpretation of data: All authors.
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Conflict of Interest Disclosures: The authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. The Department of Molecular and Human Genetics at Baylor College of Medicine derives revenue from the clinical exome sequencing offered in the Medical Genetics Laboratory and Whole Genome Laboratory and the authors who are faculty members are indicated in the affiliation section. Dr Willis reported being currently employed by LabCorp, which performs commercial genetic testing. Dr Reid reported that being currently employed at Regeneron and owning stock in that company. Dr Bainbridge reported being the CEO of Codified Genomics. Dr Lupski reported owning stock in 23andMe and Ion Torrent Systems; and being a co-inventor on multiple European and US patents related to molecular diagnostics for inherited neuropathies, eye diseases, and bacterial genomic fingerprinting. No other disclosures were reported.

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