ORIGINAL ARTICLE

Exome Sequencing and the Management of Neurometabolic Disorders

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ABSTRACT

BACKGROUND

Whole-exome sequencing has transformed gene discovery and diagnosis in rare diseases. Translation into disease-modifying treatments is challenging, particularly for intellectual developmental disorder. However, the exception is inborn errors of metabolism, since many of these disorders are responsive to therapy that targets pathophysiological features at the molecular or cellular level.

METHODS

To uncover the genetic basis of potentially treatable inborn errors of metabolism, we combined deep clinical phenotyping (the comprehensive characterization of the discrete components of a patient's clinical and biochemical phenotype) with whole-exome sequencing analysis through a semiautomated bioinformatics pipeline in consecutively enrolled patients with intellectual developmental disorder and unexplained metabolic phenotypes.

RESULTS

We performed whole-exome sequencing on samples obtained from 47 probands. Of these patients, 6 were excluded, including 1 who withdrew from the study. The remaining 41 probands had been born to predominantly nonconsanguineous parents of European descent. In 37 probands, we identified variants in 2 genes newly implicated in disease, 9 candidate genes, 22 known genes with newly identified phenotypes, and 9 genes with expected phenotypes; in most of the genes, the variants were classified as either pathogenic or probably pathogenic. Complex phenotypes of patients in five families were explained by coexisting monogenic conditions. We obtained a diagnosis in 28 of 41 probands (68%) who were evaluated. A test of a targeted intervention was performed in 18 patients (44%).

CONCLUSIONS

Deep phenotyping and whole-exome sequencing in 41 probands with intellectual developmental disorder and unexplained metabolic abnormalities led to a diagnosis in 68%, the identification of 11 candidate genes newly implicated in neurometabolic disease, and a change in treatment beyond genetic counseling in 44%. (Funded by BC Children's Hospital Foundation and others.)

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Next-generation sequencing has revolutionized the discovery of genes in which variants cause rare mendelian diseases.[1] A diagnostic yield of 16% (with most variants classified as de novo mutations) has been documented for whole-exome sequencing among patients with unexplained intellectual developmental disorder,[2,3] a condition that affects an estimated 3% of the population worldwide.[4] Along with coexisting illnesses that include epilepsy, psychiatric or behavioral disturbances, movement disorders, sensory deficits, and other organ dysfunction, intellectual developmental disorder poses a substantial emotional, functional, and economic burden.[5] Copy-number variants, methylation abnormalities, and single-gene defects are known to cause intellectual developmental disorder.[2,3,6,7]

Diagnosis is essential for accurate genetic counseling, informed decision making by families and physicians, and access to appropriate medical support and services in the community but does not often translate into disease-modifying treatments. The exception is inborn errors of metabolism, the largest group of genetic intellectual developmental disorders that are amenable to causal therapy — in other words, interventions that directly target pathogenesis at the cellular and molecular level.[8] For example, the discovery that pyridoxine-dependent epilepsy is caused by variants in *ALDH7A1*, which encodes an enzyme that catabolizes lysine, led to the implementation of a lysine-restricted diet and arginine supplementation, which improved neurodevelopmental outcomes.[9] Approximately 90 treatable inborn errors of metabolism are known to cause intellectual developmental disorder,[8,10,11] and it seems likely that more such neurometabolic disorders remain to be discovered. We therefore sequenced the exomes of consecutive patients with intellectual developmental disorder that had an unexplained metabolic phenotype and then used a semiautomated bioinformatics pipeline and a multidisciplinary approach to identify causal variants.

Methods

Patients

Patients of all ages were consecutively enrolled if they had confirmed or potential intellectual developmental disorder (i.e., the presence of toxic metabolites that are known to cause brain damage in the neonatal period) along with a metabolic phenotype of unknown cause after comprehensive clinical phenotyping with extensive previous metabolic or genetic testing. A metabolic phenotype was defined as one or more of the following: a pattern of abnormal metabolites in urine, blood, or cerebrospinal fluid; abnormal results on functional studies at a biochemical or cellular level (e.g., a deficiency in the mitochondrial-respiratory-chain complex); or abnormalities on clinical history (e.g., developmental or cognitive regression), physical examination (e.g., organomegaly), neuroimaging or physiological analysis (e.g., leukodystrophy), or pathological analysis (e.g., storage vacuoles) suggestive of a neurometabolic disorder.

The study was approved by the ethics committee of the Faculty of Medicine at the University of British Columbia. Each patient or designated guardian provided written informed consent for participation in the study and publication of the results. During the informed-consent process, investigators explained the risks and benefits of research-based whole-exome sequencing analysis to patients and their families, and an option for disclosure of medically actionable incidental findings was provided.

Sequencing and Bioinformatics Analysis

We isolated genomic DNA, using standard techniques, from either peripheral blood or saliva obtained from the proband and from both parents and all affected and unaffected siblings (if available). We performed whole-exome sequencing analysis on samples obtained from the probands, the parents, and any affected siblings using either the SureSelect targeted capture kit (Agilent) on the Illumina HiSeq 2000 sequencer or the Ion AmpliSeq Exome Kit and Ion Proton System (ThermoFisher).

We developed and applied a semiautomated gene-discovery pipeline, which involves manual inspection of data quality and collaborative interactions between clinicians and bioinformaticians (Fig. 1). (Details are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.) After the completion of whole-exome sequencing, we provided a clinician-referral form containing data on phenotype, fam-

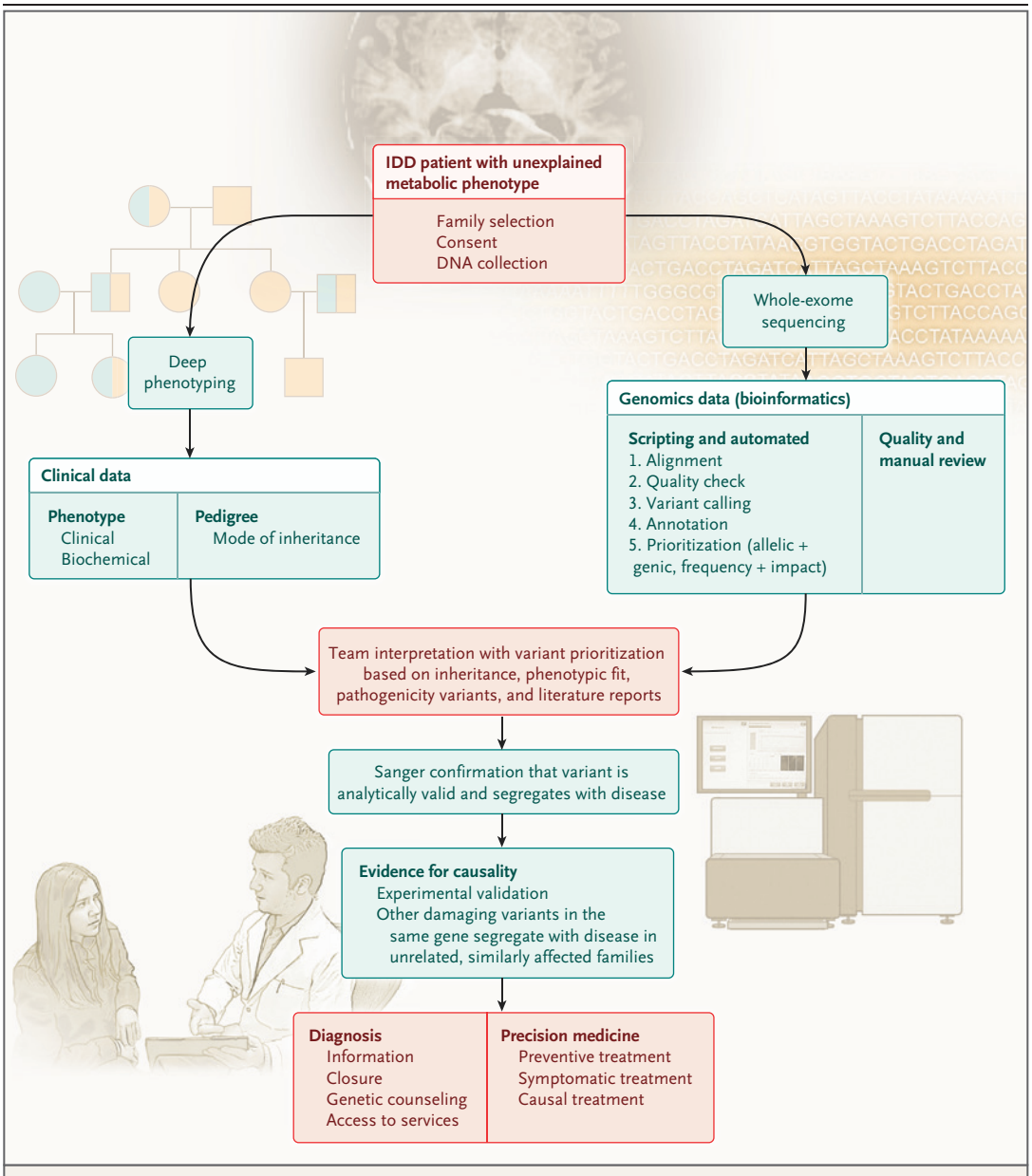

Figure 1. Flow Chart Showing Gene-Discovery Approach with the Use of Collaborative Phenomics and Semiautomated Genomics.

This research process of uncovering the genetic basis of potentially treatable neurometabolic conditions in patients with an intellectual developmental disorder (IDD) involves a combination of deep clinical phenotyping (the comprehensive characterization of the discrete components of a patient's clinical and biochemical phenotype), whole-exome sequencing analysis, team interpretation, validation, and translation to the clinical setting. Whole-exome sequencing analysis is performed on DNA samples obtained from the proband and on available samples from parents and any affected siblings. The semiautomated gene-discovery pipeline involves manual inspection of data quality and collaborative interactions among clinicians, laboratory scientists, and bioinformaticians for interpretation, candidate-gene selection, and subsequent experimental and clinical validations. The benefits of a diagnosis for patients and their families are shown; in some cases, the diagnosis enables a targeted treatment strategy. Details are provided in the Supplementary Appendix.

ily history with pedigree, ancestry, and previous results of diagnostic testing for variant interpretation. We classified the pathogenicity of the variants according to recent standards and guidelines of the American College of Medical Genetics and Genomics (ACMG).[12] Variant genes were described as newly identified (novel), candidate, or known to cause disease.[2] Genes that were described as newly identified had not been implicated in human disease previously and harbored distinct damaging variants in at least two affected patients with striking phenotypic overlap from unrelated families. When such a variant was observed in only a single family, it was described as a candidate gene.

RESULTS

PATIENTS

From October 2012 through January 2015, we recruited and completed whole-exome sequencing on samples obtained from 47 probands who met the eligibility criteria. All the patients were referred by local medical specialists in Vancouver, Canada, except for 3 patients, who were referred by clinicians in Greece, the United Kingdom, and Saudi Arabia. Negative results on whole-exome sequencing that ruled out a monogenic cause and details regarding the clinical course at the time of the study helped to confirm previously suspected diagnoses of teratogen exposure (in 1 patient), congenital infection (in 1 patient), and an autoimmune disorder (in 2 patients). In one patient, a chromosomal copy-number variant that was classified as having unknown significance before the study was determined to be pathogenic on the basis of a new entry in the ClinVar database (www.ncbi.nlm.nih.gov/clinvar/), which describes the same copy-number variant in an unrelated person with a similar phenotype. None of the remaining patients were identified as having a copy-number variant (either pathogenic or of unknown significance). In addition, one family, for whom whole-exome sequencing identified a de novo pathogenic variant in a known syndromic gene associated with intellectual developmental disorder, withdrew from the study.

Of the 41 remaining probands, 37 (90%) were children. The age at the time of enrollment ranged from 8 months to 31 years (median, 5.9 years); 15 of the probands were female (37%), and 26 were male (63%). Among these patients with intellectual developmental disorder, 19 had mild disease, 14 had moderate disease, and 8 had profound disease, with a spectrum of additional clinical and biochemical manifestations (Table 1). A total of 26 of the probands (63%) were of European descent and had been born to nonconsanguineous parents without a family history of intellectual developmental disorder. In all the probands, diagnostic investigations for intellectual developmental disorder had been initiated during early childhood (in the 1990s through 2014) with regular follow-up for additional testing. All the patients had undergone biochemical testing according to a published diagnostic algorithm for treatable intellectual developmental disorder[10] and a combination of clinical genetics testing (including mitochondrial DNA sequencing) without receiving a diagnosis. Nine probands had affected siblings who also underwent whole-exome sequencing analyses. A summary of the results for all 50 patients (41 probands and 9 siblings) is provided in Table S1 in the Supplementary Appendix.

DIAGNOSTIC YIELD

We established a genetic diagnosis in 28 of the 41 probands (68%), which included the identification of variants in 2 genes newly implicated in disease, 22 known genes with newly identified phenotypes, and 9 genes with expected phenotypes. In most of the genes, the variants were classified as either pathogenic or probably pathogenic. In 9 additional probands, we identified 9 candidate genes (Table 2). Our group generated experimental data providing evidence for the effect of variants on protein function for 14 of these genes (2 newly implicated genes, 6 candidate genes, and 6 known genes with newly identified phenotypes) (Section C in the Supplementary Appendix).

We performed whole-exome sequencing on samples obtained from the proband and from both parents in 28 of the cases (68%). In total, we identified 58 diagnostic variants in 42 genes; all except the previously reported somatic *KRAS* variant were germ-line variants.[13,14] Of the 58 variants, 24 (41%) were classified as pathogenic and 17 (29%) as probably pathogenic, according to recently published ACMG standards and guide-

lines (Table S2 in the Supplementary Appendix).[12] Most of these variants (78%) were not present in either our database (which consists of 350 individual exomes or genomes) or the database of single-nucleotide polymorphisms of the National Center for Biotechnology Information (dbSNP, version 138), whereas 13 variants were present but rare (average allele frequency, 0.006) in the dbSNP. Seven diagnostic variants (12%) had been previously identified as pathogenic (Table S3 in the Supplementary Appendix), a prevalence that is similar to that in a previous study.[15] As compared with variants in the Exome Aggregation Consortium (ExAC), a database of 61,486 unrelated persons, 31 of the 58 variants (53%) were newly identified and 27 (47%) were rare, with an average allele frequency of 0.004. The ExAC data set includes patients with mental illnesses, so the presence of a rare variant in this data set does not exclude the possibility that it is pathogenic.

Of the 58 variants, 51 (88%) were single-nucleotide variants; these included 43 missense variants (74%), 4 nonsense variants (7%), and 4 splice-site variants (7%); 4 in-frame omissions of conserved amino acids (7%) and 3 frameshift variants (5%) were also identified (Table 2). The mode of inheritance was recessive in 30 of the 42 genes (71%), including compound heterozygous variants in 16 genes (38%), homozygous variants in 8 genes (19%), and X-linked recessive variants in 6 genes (14%) (Table 3). Dominant variants were identified in 12 patients (29%), including 11 de novo variants (10 heterozygous and 1 mosaic) and a single familial autosomal dominant variant with variable penetrance. For the nine families with pathogenic variants in known human disease genes and expected phenotypes, pedigrees with electropherograms are provided in Figure S1 in the Supplementary Appendix.

EFFECT ON CLINICAL MANAGEMENT

Genetic diagnosis affected the clinical treatment of 18 probands (44%) in whom a pathogenic or probably pathogenic variant was identified (Table S4 in the Supplementary Appendix). These changes consisted of preventive measures, such as regular screening for cancer and avoidance of disease triggers in 4 probands (with a variant in *CBL*,[16] *SMAD4*, *MTO1*, or *PRSS1*); immune-modu-

Table 1. Clinical Characteristics of the 41 Probands.*

Characteristic	Proband
	no. (%)
Sex	
Male	26 (63)
Female	15 (37)
Age	
<19 yr	37 (90)
≥19 yr	4 (10)
Family structure	
Nonconsanguineous	
One affected child	30 (73)
Two affected children	7 (17)
Consanguineous	
One affected child	2 (5)
Two affected children	2 (5)
Population†	
White European	26 (63)
East Asian	3 (7)
West Asian	7 (17)
South Asian	3 (7)
Latino	2 (5)
Phenotype	
Intellectual developmental disorder	41 (100)
Unexplained metabolic phenotype	39 (95)
Abnormal neuroimaging	26 (63)
Abnormal muscle tone	18 (44)
Seizure	13 (32)
Abnormal movement	11 (27)
Epilepsy	11 (27)
Psychiatric symptoms	8 (20)
Dysmorphic features	8 (20)
Cardiac defect	7 (17)
Short stature	4 (10)
Immune dysfunction	4 (10)
Clinical, genetic, and biochemical analyses	
Chromosomal microarray analysis	36 (88)
Targeted gene sequencing	34 (83)
Mitochondrial DNA sequencing	18 (44)
Biochemical testing	41 (100)

* Percentages may not total 100 because of rounding.
† The population group was determined by self-report and a detailed family history.

Table 2. Diagnostic Yield and Summary of Whole-Exome Sequencing Analysis in 41 Probands.

Variable	Value
Diagnosis by means of whole-exome sequencing — no. of probands	
Positive diagnosis (single contributing gene)	32
Positive diagnosis (two contributing genes)	5
No diagnosis	4
Extent of whole-exome sequencing — no. of probands	
Proband only	3
Proband and affected sibling	1
Proband and unaffected parent	1
Proband and both unaffected parents	28
Proband, affected sibling, and unaffected parents	5
Proband, unaffected sibling, and unaffected parents	3
Gene category — no. of genes (gene name)	
Not previously implicated in human disease*	11
Newly identified gene	2 (*NANS*, *CA5A*)
Candidate gene	9 (*ACACB*, *RBSN*, *GOT2*, *FAAH2*, *SENP1*, *SYTL2*, *RYR3*, *MFNG*, *NPL*)
Known gene with a new phenotype	22
Known gene with a known phenotype	9
Type of variant — no.	
All variants	58
Single-nucleotide variant	51
Missense	43
Nonsense	4
Splice-site	4
Insertion or deletion	7
In-frame	4
Frameshift	3

* The classification by de Ligt et al.[2] was used for genes that had not been implicated in human disease previously. Genes were described as newly identified (novel) if additional unrelated families with striking phenotypic overlap and variants affecting the same gene were identified. Otherwise, a candidate classification was used.

lating therapies, such as chemotherapy or stem-cell transplantation in 3 probands (with a variant in *SENP1*, *SYTL2*, or *KRAS*); more precise symptomatic treatment, such as supplementation with 5-hydroxytryptophan, levodopa, carbidopa, serine, or folinic acid supplementation in 5 probands (with a variant in *CNKSR2*, *SCN2A*, *ANO3*, *ATP2B3*, or *MECP2*); and treatments targeting the identified abnormality at a cellular or molecular level in 7 probands (with a variant in *CA5A*, *ACACB*, *GOT2*, *PCK1*, *NANS*, *MTO1*, or *QARS*). The diagnosis of MTO1 deficiency in a female sibling pair enabled both preventive and targeted therapy. Although it is possible that these changes in clinical treatment may have improved health outcomes in all cases, it must also be acknowledged that different degrees of stabilization or improvement in patients with intellectual developmental disorder (or related outcomes) can be expected in different groups.

ILLUSTRATIVE CASES

New and Candidate Causes of Intellectual Developmental Disorder and Therapy

We describe two newly identified forms of inborn errors of metabolism that are potentially amenable to dietary restriction, supplementation, or pharmacologic interventions (see Case Reports and Table S4 in the Supplementary Appendix). The first discovery was a homozygous missense variant in *CA5A* (Mendelian Inheritance in Man [MIM] number 114761), encoding mitochondrial carbonic anhydrase VA, which is pivotal to the function of mitochondrial enzymes.[17] Our group reported that a deficiency in this enzyme causes neonatal hyperammonemia, hyperlactatemia, and hypoglycemia.[17] Such a deficiency is amenable to treatment with carglumic acid and an emergency protocol, which can prevent irreversible neurologic sequelae. We added this condition to the two-tiered diagnostic algorithm and interactive tool at Treatable ID.org (freely available as native and Web applications at www.treatable-id.org) to support clinicians in early identification.[9,10]

The second newly identified inborn error of metabolism was caused by compound heterozygous variants in *NANS*, encoding N-acetylneuraminic acid phosphate synthase (MIM number 605202), in a 4-year-old presenting with epileptic encephalopathy and dysmorphic features. We detected increased levels of N-acetylated mannosamine, the substrate of NANS, in his urine, plasma, and cerebrospinal fluid. Other recessive *NANS* variants have been identified in a cohort of eight other patients with similar phenotypes in five unrelated families. Models of the mutated organisms recapitulated the phenotype, which is amenable to treatment with early supplementation of N-acetylneuraminic acid.

In addition, we identified two candidate genes

Table 3. Inheritance Patterns of the 42 Genes Identified in the Study.

Inheritance	No. of Genes Affected	Gene ID
Autosomal recessive		
Compound heterozygous	16	*ACACB*, *RMND1*, *QARS*, *MTO1*, *RYR3*, *H6PD*, *MFNG*, *SCN4A*, *NDST1*, *ANO3*, *NPL*, *NANS*, *TMEM67*, *SYTL2*, *GOT2*, *MAT1A*
Homozygous	8	*CA5A*, *RBSN*, *AIMP1*, *GALC*, *GJB2*, *PCK1*, *SENP1*, *OSMR*
X-linked		
Recessive	6	*CNKSR2*, *PIGA*, *FAAH2*, *MED12*, *PLP1*, *ATP2B3*
Dominant de novo heterozygous	1	*MECP2*
Autosomal dominant		
De novo heterozygous	9	*SCN2A*, *CBL*, *DYRK1A*, *SMAD4*, *KMT2A*, *KCNQ2*, *EHMT1*, *PACS1*, *PUF60*
De novo mosaic	1	*KRAS*
Inherited	1	*PRSS1*

that led us to conclude that the phenotypes for intellectual developmental disorder were potentially treatable. In a 6-year-old boy with acquired microcephaly, severe seizure disorder, spasticity, sleep disturbances, abdominal spasms, and low levels of serine in plasma and cerebrospinal fluid, we identified compound heterozygous variants in *GOT2* (MIM number 138150), encoding mitochondrial glutamate oxaloacetate transaminase.[18] After receiving oral serine and pyridoxine supplements, the patient showed improved head growth, psychomotor development, and seizure control. Finally, we are evaluating whether a deficiency in acetyl-coenzyme A carboxylase beta associated with compound heterozygous variants in *ACACB* (MIM number 601557) is a potentially treatable inborn error of metabolism.

Expansion of the Phenotypic Spectrum

We identified variants in 22 genes that have previously been reported to cause monogenic conditions[19-25] (Table S4 in the Supplementary Appendix). In all these conditions, we observed previously unreported clinical symptoms.

The delineation of phenotype can point to new treatment targets. An example from this study was an 8-year-old boy with intellectual developmental disorder, autism, movement disorder, intractable epileptic encephalopathy, and persistently abnormal neurotransmitter profiles (low levels of homovanillic acid, 5-hydroxyindoleacetic acid, and neopterin in cerebrospinal fluid) in whom we identified a de novo pathogenic splice-site variant. This mutation resulted in the deletion of exon 14 in *SCN2A*,[24] encoding voltage-gated sodium channel type II. Voltage-gated sodium channels are heteromeric complexes that generate and propagate action potentials. The known phenotype of SCN2A deficiency varies and includes benign forms of epilepsy, severe epileptic encephalopathy, autism, and intellectual developmental disorder without seizures, as well as rare cases of dystonia, hypotonia, and hypersomnia.[26] Neurotransmitter deficiencies have not been described in this disorder previously. We hypothesized that this channelopathy causes abnormal synaptic secretion and uptake of monoamine metabolites through impaired vesicular release and imbalance in electrochemical ion gradients, which in turn aggravate the seizures. Treatment with oral 5-hydroxytryptophan, levodopa, carbidopa, and a dopa agonist normalized the child's levels of neurotransmitters in the cerebrospinal fluid and was associated with improvements in attention, communication, and seizure control.

Combined Phenotypes from Two Coexisting Monogenic Defects

Multiple genetic events leading to complex phenotypes may be mistaken for new disorders or newly identified phenotypes of a known disorder. This reminds us that a layer of unbiased and systematic interpretation of data from next-

generation sequencing is necessary in any clinical pipeline. Recent reports regarding next-generation sequencing support the hypothesis that blended phenotypes are an appreciable cause of disease.[1,27] This concept was shown in our study group, in which 5 of 37 probands (14%) for whom diagnoses had been established harbored variants at two distinct disease loci associated with the phenotype (Table S5 in the Supplementary Appendix). For instance, in a 19-year-old male patient who was born to nonconsanguineous Filipino parents and who had progressive dilated cardiomyopathy, sensorineural hearing loss, and unexplained sialic aciduria, whole-exome sequencing revealed compound heterozygous damaging missense variants in *NPL* (MIM number 611412) encoding N-acetylneuraminate pyruvate lyase (which controls the final step of sialic acid metabolism) and a known homozygous missense variant in *GJB2* (connexin 26) reported to cause deafness.

Medically Actionable Incidental Findings

In the families of the 41 probands, we identified only one medically actionable incidental finding in *CFTR* (MIM number 602421). Both alleles (rs78655421 and rs121908745) had been previously reported to be pathogenic. These alleles were found to be in trans on Sanger sequencing. The clinical phenotype did not include symptoms of cystic fibrosis. The family chose not to be informed of incidental findings, so we did not disclose this result to them.

DISCUSSION

Our approach, which involved phenotyping and whole-exome sequencing of samples obtained from 41 consecutively enrolled probands with intellectual developmental disorder who had an unexplained metabolic phenotype, provided a diagnostic yield of 68%, including variants in two newly implicated genes. We also identified nine candidate genes. Although the study was designed to evaluate the numerical yield of whole-exome sequencing in probands, affected siblings also benefited from a diagnosis in nine families (Table S1 in the Supplementary Appendix). Studies to validate or rule out causality of the candidate genes are ongoing. We have provided information on variants that were possibly pathogenic in these genes using more stringent weighting of available genetic evidence according to ACMG guidelines[12] and taking into account existing experimental data (Tables S2 and S4 and the Experimental Data section in the Supplementary Appendix). Our diagnostic rate exceeds that of most published studies applying next-generation sequencing in rare diseases.[2,3,15,28-31]

In one of four families with negative results on whole-exome sequencing, subsequent whole-genome sequencing identified a causal homozygous variant in *CSTB* (in a region of low coverage on whole-exome sequencing) in two siblings with neurodegenerative epilepsy who had a response to carbidopa–levodopa and 5-hydroxytryptophan. In another family, whole-exome sequencing of samples obtained from parents established a pathogenic de novo mutation (*MYLK*); previously, only the proband had undergone whole-exome sequencing analysis (see the Discussion section in the Supplementary Appendix). However, the most important outcome of our genomics study was the effect of diagnosis by means of whole-exome sequencing on the clinical treatment of 44% of the probands who were analyzed.

Knowledge of the precise genetic or biochemical defect in a metabolic pathway provides the opportunity to modify disease by means of nutritional manipulation, which does not require the expensive and time-consuming preclinical development that is typical in drug manufacturing. This advantage is illustrated by the discovery of GOT2 deficiency in a child with severe neurologic symptoms that was amenable to oral serine and pyridoxine supplements (the product and cofactor, respectively, of GOT2), both of which are affordable and have been deemed to be safe for other inborn errors of metabolism.[9,32] However, the diagnosis of NANS deficiency in another patient underscores a challenge presented by very rare diseases: how to test new treatments and obtain evidence of effect or lack thereof with a small number of patients.

The relatively high diagnostic yield that we report here may stem from the inclusion criterion of a metabolic abnormality, the prevalence of recessive conditions in metabolic disorders, or the close consultation with clinical specialists in our bioinformatics pipeline. We observed a higher portion of patients (14%) with variants at two distinct disease loci (leading to blended

phenotypes) than has been observed in other studies,[1,27] perhaps because we specified the inclusion of two phenotypes (metabolic and intellectual developmental disorder) in the patient-selection criteria.

Translational genomics requires collaborations among patients, their families, subspecialist clinicians for careful phenotyping, bioinformaticians for accurate data analysis, and basic scientists engaged in specific research involving genes, pathways, or model organisms.[33] Difficult decisions with respect to invasive and costly procedures such as hematopoietic stem-cell transplantation or chemotherapy (e.g., in a patient with SYTL2 deficiency[34]) are facilitated and supported by a genetic diagnosis. Outcome reports of such cases in the literature may help to guide other clinicians who are facing similar decisions. Data sharing and open communication are key to maximizing the diagnostic potential of next-generation sequencing and its clinical benefit in rare diseases.

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APPENDIX

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