ORIGINAL ARTICLE

Diagnostic Exome Sequencing in Persons with Severe Intellectual Disability

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ABSTRACT

BACKGROUND

The causes of intellectual disability remain largely unknown because of extensive clinical and genetic heterogeneity.

METHODS

We evaluated patients with intellectual disability to exclude known causes of the disorder. We then sequenced the coding regions of more than 21,000 genes obtained from 100 patients with an IQ below 50 and their unaffected parents. A data-analysis procedure was developed to identify and classify de novo, autosomal recessive, and X-linked mutations. In addition, we used high-throughput resequencing to confirm new candidate genes in 765 persons with intellectual disability (a confirmation series). All mutations were evaluated by molecular geneticists and clinicians in the context of the patients' clinical presentation.

RESULTS

We identified 79 de novo mutations in 53 of 100 patients. A total of 10 de novo mutations and 3 X-linked (maternally inherited) mutations that had been previously predicted to compromise the function of known intellectual-disability genes were found in 13 patients. Potentially causative de novo mutations in novel candidate genes were detected in 22 patients. Additional de novo mutations in 3 of these candidate genes were identified in patients with similar phenotypes in the confirmation series, providing support for mutations in these genes as the cause of intellectual disability. We detected no causative autosomal recessive inherited mutations in the discovery series. Thus, the total diagnostic yield was 16%, mostly involving de novo mutations.

CONCLUSIONS

De novo mutations represent an important cause of intellectual disability; exome sequencing was used as an effective diagnostic strategy for their detection. (Funded by the European Union and others.)

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Severe intellectual disability, which is also referred to as cognitive impairment or mental retardation, affects approximately 0.5% of the population in Western countries[1,2] and represents an important health burden. A clinical diagnosis of severe intellectual disability is generally based on an IQ of less than 50 and substantial limitations in activities of daily living. In early childhood, the diagnosis is based on substantial developmental delays, including motor, cognitive, and speech delays. Children with different nonsyndromic forms of intellectual disability are clinically indistinguishable.

Intellectual disability can be caused by nongenetic factors, such as infections and perinatal asphyxia. In developed countries, most severe forms of intellectual disability are thought to have a genetic cause,[2] but the cause remains elusive in 55 to 60% of patients.[3,4] An understanding of the genetic cause of intellectual disability can benefit patients and their families, because a diagnosis may provide information on the prognosis, precludes further unnecessary invasive testing, and may lead to appropriate therapy. Moreover, a diagnosis often facilitates access to appropriate medical and supportive care.[5-8] Family members may benefit from knowledge of the risk of recurrence, reproductive counseling, and possible prenatal diagnosis.

We[9] and others[10] have reported evidence supporting the hypothesis that rare de novo point mutations can be a major cause of severe intellectual disability. Recent studies have indicated that there are more de novo mutations in persons with intellectual disability than in healthy controls, highlighting the clinical importance of these mutations.[11-15] That intellectual disability is often sporadic, without obvious environmental or familial factors, provides additional support for the hypothesis that a large proportion of cases of intellectual disability are caused by de novo mutations. It has been estimated that mutations in more than 1000 different genes may cause intellectual disability.[16] Because of this large aggregate target, rare de novo mutations in these genes may collectively compensate for the very low rate of reproduction among patients with intellectual disability, keeping the incidence of the disorder in the general population stable.[15]

In the absence of diagnostic clues from the clinical phenotype, unbiased genomewide approaches are required to detect genetic mutations causing intellectual disability.[9,17,18] We have therefore evaluated the role of de novo as well as X-linked and autosomal recessive inherited mutations in a series of 100 patients with unexplained intellectual disability (defined as an IQ of <50), using a family-based exome-sequencing approach in a clinical diagnostic setting. Previous extensive clinical and genetic evaluation of these patients had not led to an etiologic diagnosis. Thus, this series of patients represents the end point of current diagnostic strategies, with all conventional genetic resources exhausted, which is the typical scenario for patients with severe intellectual disability.[19]

METHODS

PATIENTS

We enrolled 100 patients (53 females and 47 males) with unexplained severe intellectual disability and their unaffected parents (trios). This series is broadly representative of patients with severe intellectual disability who are referred to our tertiary care clinic (see Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). All patients were evaluated by a clinical geneticist. Detailed clinical phenotypes of the 100 patients are provided in the section on the clinical descriptions of patients in the Supplementary Appendix and are summarized in Table 1.

Before enrollment, the patients had undergone an extensive diagnostic workup, including genomic profiling (performed with the use of the 250K Affymetrix SNP array) and targeted gene tests, with metabolic screening whenever indicated, but these evaluations had not led to an etiologic diagnosis. The study was approved by the ethics committee at the Radboud University Nijmegen Medical Center. The parents of all patients in the study provided written informed consent.

DETECTION OF MUTATIONS

Genomic DNA was isolated from blood with the use of a QIAamp DNA Mini Kit (Qiagen). Exomes were enriched with the use of a SOLiD-Optimized SureSelect Human Exome Kit (Agilent version 2, 50Mb), followed by SOLiD 4 System sequencing (Life Technologies). After sequencing the exomes of each trio, we selected candidate de novo mutations by excluding variants inherited from

either parent and selected candidate recessive and X-linked mutations by segregation analysis (Fig. S1 in the Supplementary Appendix). Candidate de novo mutations were validated by conventional Sanger sequencing methods in DNA samples obtained from the patients and their parents (see the section on validation of de novo mutations in the Supplementary Appendix).

TESTING OF CANDIDATE GENES

We reanalyzed all candidate genes that were identified in this study for the presence of possible de novo mutations in previously generated exome data obtained from 10 patients with severe intellectual disability.[9] In addition, we resequenced five candidate genes associated with intellectual disability (*DYNC1H1*, *KIF5C*, *ASH1L*, *GATAD2B*, and *CTNNB1*) using array-based enrichment on pooled DNA samples from a second series of 765 patients with intellectual disability. These samples were selected from our in-house collection of 5621 samples from patients with undiagnosed intellectual disability (see the section on patient selection in the Supplementary Appendix). The parents of these patients had previously provided written informed consent. All patients had been evaluated by a clinical geneticist to rule out known causes of intellectual disability, and genomic array analysis had not revealed causal copy-number variants. Detected variants were annotated and prioritized according to their presumed relevance to disease. Variants fulfilling prioritization criteria were validated by means of conventional Sanger sequencing (see the section on recurrence screening in the Supplementary Appendix).

INTERPRETATION OF CONFIRMED MUTATIONS

We classified the mutations on the basis of the existing guidelines for evaluation of the pathogenicity of variants[20,21] (Fig. 1, and the section on clinical interpretation of mutations in the Supplementary Appendix). These guidelines call for the evaluation of seven factors: the function of the affected gene, the effect of the mutation on the codon (i.e., stop, frameshift, or missense mutation), in silico prediction of the functional effect at the amino acid level, evolutionary conservation, brain-expression patterns, analysis of gene-ontology (GO) terms, and the use of animal models, if available (see the section on mutational effect and functional relevance in the Supplementary Appendix).

Table 1. Clinical Characteristics of 100 Patients with Intellectual Disability of Unknown Cause.

Characteristic	No. of Patients
IQ	
<30	62
30 to 50	38
Sex	
Male	47
Female	53
Age group	
<10 yr	37
10–20 yr	41
>20 yr	22
No. of siblings	
0	12
1	47
2	36
3	1
4	2
Unknown	2
No. of major congenital anomalies	
0	62
1	31
2	7
3	0
Short stature	
Yes	24
No	76
Microcephaly	
Yes	30
No	70
Macrocephaly	
Yes	4
No	96
Epilepsy	
Yes	52
No	48
Abnormality on brain imaging	
Yes	30
No	40
Not assessed	30
Cardiac malformation	
Yes	2
No	98
Abnormality of the urogenitary system	
Yes	13
No	87

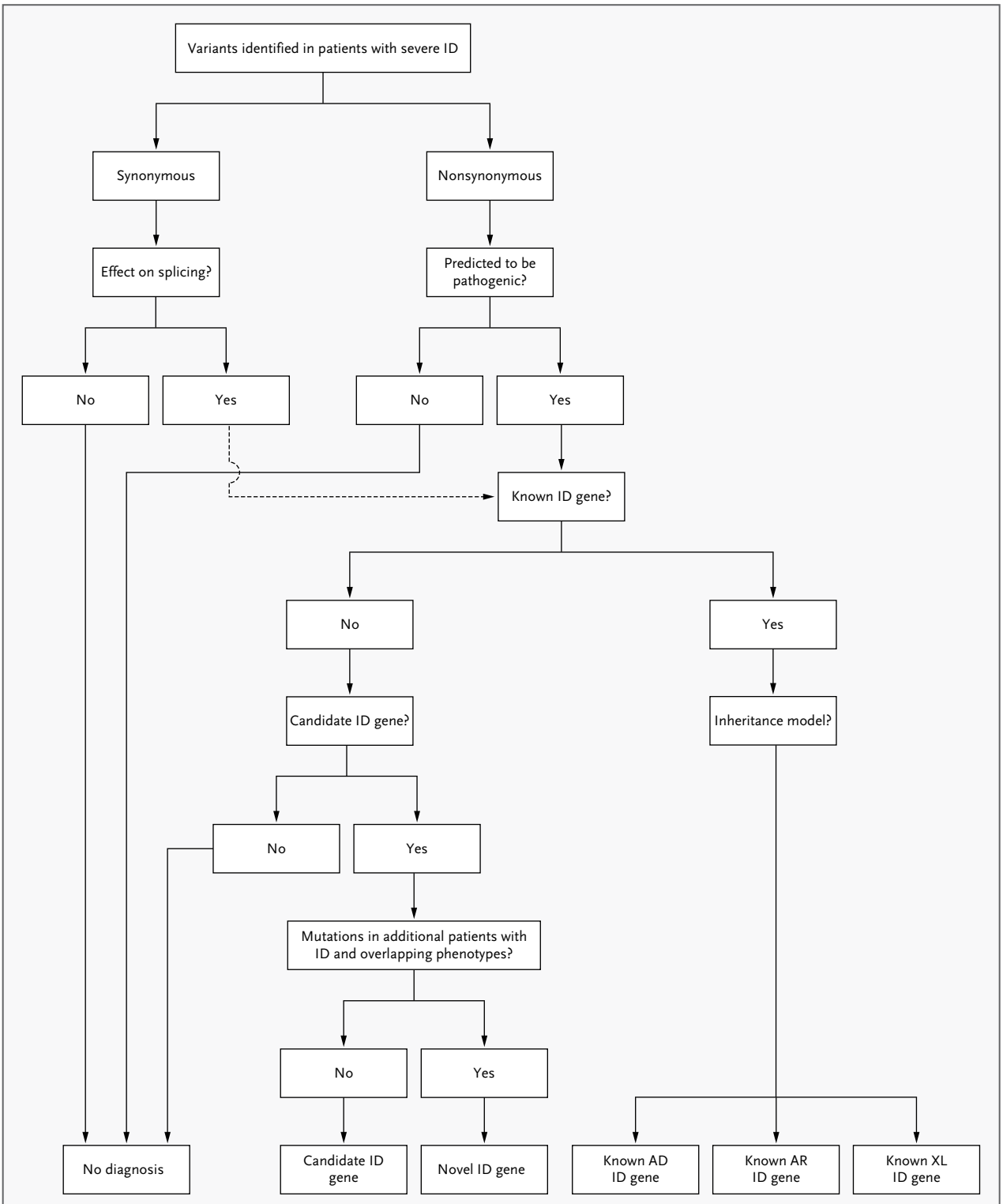

Figure 1. Classification of Variants Detected in Patients with Severe Intellectual Disability (ID).

Variants in known autosomal recessive (AR) genes were considered to be diagnostically relevant only if biallelic, predicted pathogenic variants were detected. AD denotes autosomal dominant, and XL X-linked.

Mutations in genes with a known association with intellectual disability were considered to be a cause of intellectual disability when the mutations were predicted to be pathogenic by the majority of three prespecified in silico analyses (see the section on mutational effect in the Supplementary Appendix) and when they occurred in persons with phenotypes similar to those described in other persons with mutations in these genes.

Mutations were considered to affect candidate genes that had not previously been implicated in intellectual disability when the mutations were predicted to be pathogenic by the majority of three prespecified in silico analyses, showed a link to brain or embryonic development in a review of the literature, and met at least two of the following criteria: evolutionary conservation, brain-expression pattern, positive results on GO-term analysis, or implication on the basis of animal models (see the section on functional relevance in the Supplementary Appendix). If multiple patients were found to have a de novo mutation in such a candidate gene and their phenotypes showed striking overlap, the candidate gene was redefined as a novel intellectual-disability gene, and the mutations were reported as a cause of intellectual disability. All mutations in other candidate genes were reported as a possible cause of intellectual disability. For patients without causal de novo, X-linked, or biallelic inherited mutations, the diagnostic report stated that the genetic cause of intellectual disability was not identified.

RESULTS

EXOME SEQUENCING

The power of family-based exome sequencing to provide a genetic diagnosis was evaluated for 100 patients with unexplained intellectual disability. The median sequence coverage was 64, with an average of 87% of all targeted exons covered by at least 10 sequence reads (Table S1 and Fig. S2 in the Supplementary Appendix). We detected an average of 24,324 genetic variants per patient (Table S2 in the Supplementary Appendix). An automated prioritization scheme was applied to systematically identify candidate de novo mutations (Fig. S1 in the Supplementary Appendix), resulting in a total of 690 candidate de novo mutations (average number per patient, 7; range, 2 to 20) (Table S2 in the Supplementary Appendix).

An inherent challenge in family-based exome sequencing is the difficulty in distinguishing between a true de novo mutation and a sequencing error, since both appear to be a new allele in the patient. Therefore, we tested the veracity of all candidate de novo mutations using Sanger sequencing as an independent method and confirmed the presence of 79 de novo mutations in 53 patients (range per patient, 1 to 4) (Tables S3 and S4 and Fig. S3 and S4 in the Supplementary Appendix).

DESCRIPTION OF DE NOVO MUTATIONS

Of the 79 de novo mutations (affecting 77 genes), 16 were synonymous (Fig. 1). Of these mutations, none were predicted to alter splicing. Therefore, these mutations were classified as not causative for intellectual disability. The remaining 63 changes were nonsynonymous and included 15 mutations that were predicted to be severely disruptive (4 nonsense mutations, 2 canonical splice-site mutations, and 9 insertions or deletions) and 48 missense mutations (Table S3 in the Supplementary Appendix). On the basis of random mutation modeling,[22] our observation of 4 nonsense mutations (6.1%) exceeded expectations (3.3%) and thus supports an elevated level of such mutations in patients with intellectual disability (Table S5 in the Supplementary Appendix). Prediction of all 48 missense mutations with the use of Polymorphism Phenotyping, version 2 (PolyPhen2), showed a significant increase in mutations that were probably damaging ($P=0.03$) (Table S5 in the Supplementary Appendix). This finding suggested that a large portion of these missense mutations might have phenotypic consequences.

We detected 12 de novo mutations in known intellectual-disability genes: 6 severely disruptive mutations and 6 missense mutations (Table 2, and Table S3 in the Supplementary Appendix). Three de novo mutations were detected in genes known to cause a recessive form of intellectual disability when mutated. These mutations would be considered causal for intellectual disability only if a second, inherited mutation that was predicted to be pathogenic was identified. We identified no such mutation in *ARFGEF2* or *TUSC3*. However, we did detect a rare paternally inherited, predicted pathogenic variant (c.6160G→A; p.Asp2054Asn) in *LRP2*. The results of analyses performed to determine whether the de novo event occurred on the maternal haplotype were

Table 2. Genes Affected by De Novo Mutations Associated with Intellectual Disability.

Type of Mutation	Known Genes	Novel Genes*	Candidate Genes
Missense	*ARFGEF2,† GRIN2A,‡ GRIN2B, TCF4, TUSC3†*	*DYNC1H1*	*ASH1L, CAMKIIG, COL4A3BP, EEF1A2, GRIA1, KIF5C, LRP1, MIB1, PHACTR1, PPP2R5D, PROX2, PSMA7, RAPGEF1, TANC2, TNPO2, TRIO‡*
Nonsense	*SCN2A*	*GATAD2B*	*PHIP, WAC*
Frameshift	*LRP2,§ PDHA1, SLC6A8, TUBA1A*	*CTNNB1*	*MTF1, ZMYM6*
Splice site	*SYNGAP1*		*MYT1L*

* Genes were defined as novel if there were additional de novo mutations in patients with phenotypic overlap. Details on de novo mutations are provided in Table S3 in the Supplementary Appendix.
† This autosomal recessive gene was found in a patient in whom no second mutation was detected.
‡ De novo mutations in this gene were found in two patients.
§ This autosomal recessive gene was found in a patient in whom a second rare, inherited mutation was detected.

inconclusive. Recessive *LRP2* mutations cause the Donnai–Barrow syndrome, and clinical reevaluation of Patient 81 confirmed this diagnosis (Table S6 and the Clinical Description of Patients section in the Supplementary Appendix).

We analyzed the remaining 51 de novo mutations and identified 25 mutations in 24 candidate genes (Table S3 in and the Supplementary Appendix). A patient with a de novo *DYNC1H1* mutation and intellectual disability has been described previously.[9] A comparison between that patient and Patient 54 in our study showed that they both had severe intellectual disability and a variable presentation of a neuronal migration defect[23] (Fig. S5 in the Supplementary Appendix).

ADDITIONAL PATIENTS WITH INTELLECTUAL DISABILITY

We reanalyzed previously generated exome data for 10 patients with undiagnosed severe intellectual disability[9] and resequenced five candidate genes associated with intellectual disability (*DYNC1H1*, *GATAD2B*, *ASH1L*, *KIF5C*, and *CTNNB1*) in a series of 765 persons with intellectual disability in order to identify additional de novo mutations (Tables S7 and S8 in the Supplementary Appendix).

In this confirmation series, we identified a second de novo mutation in the transcriptional repressor *GATAD2B*. The two de novo mutations that were observed in this gene, a nonsense p.Gln470* and a frameshift p.Asn195Lys*fs**30 mutation, both resulting in a stop codon (indicated by a star symbol), were predicted to be severely disruptive and to result in loss of function (Fig. S6 in the Supplementary Appendix). Both patients with these mutations presented with severe cognitive and motor delays and limited speech, and the two patients had similar facial features. One additional severely disruptive de novo mutation was detected in *CTNNB1* (Fig. S7 in the Supplementary Appendix). This mutation (p.Arg515*) and the de novo mutation detected on exome sequencing (p.Ser425Thrfs*11) were predicted to result in loss of function. A third patient carried a p.Gln309* mutation in *CTNNB1*. This mutation was not present in maternal DNA, and paternal DNA was unavailable. All three patients presented with severe intellectual disability, absent or very limited speech, microcephaly, and spasticity with a severely impaired ability to walk.

Patients 4 and 15 had de novo missense mutations in *TRIO*: p.Asp1368Val and p.Thr2563Met, respectively. *TRIO* encodes a protein that acts in several signaling pathways that control cell proliferation.[24] These patients were not similar in any clinical respect other than intellectual disability (see the section on clinical descriptions in the Supplementary Appendix). Both patients also carried a mutation in a known intellectual-disability gene: *PDHA1* in Patient 4 and *TCF4* in Patient 15. Their phenotypes overlapped markedly with those of other patients with mutations in *PDHA1* and *TCF4* (Table S6 in the Supplementary Appendix), indicating that these mutations are the most likely cause of intellectual disability, although the mutations in *TRIO* may also play a part.

INHERITED MUTATIONS IN AUTOSOMAL RECESSIVE AND X-LINKED GENES

We detected 14 X-linked inherited mutations in 12 male patients (Table S9 in the Supplementary Appendix). Three of these mutations were located in known X-linked intellectual-disability

genes (one in *PDHA1* and two in *ARHGEF9*). These mutations were predicted to be pathogenic, and the phenotypes that were observed in the patients were consistent with previous reports of affected persons carrying mutations in these genes (Table S6 in the Supplementary Appendix). In 10 male patients, we also detected 11 X-linked inherited mutations in 11 genes that had not previously been associated with intellectual disability. Of these genes, *TRPC5* was classified as possibly causal. The analysis for autosomal recessive causes of intellectual disability revealed biallelic inherited mutations in 9 genes, including 2 genes (*PCNT* and *VPS13B*) that had previously been associated with an autosomal recessive form of intellectual disability. None of these mutations had been classified as a possible cause of intellectual disability (Table S9 in the Supplementary Appendix).

Table 3. Diagnostic Yield of Exome Sequencing in the Patients.

Positive Diagnosis	No. of Patients
All mutations	16
De novo mutations	13
Autosomal dominant	10*
X-linked	2
Autosomal recessive	1†
Inherited mutations	3
X-linked	3
Autosomal recessive	0

* Seven patients had mutations in autosomal dominant genes that had previously been associated with intellectual disability, and three patients had mutations in novel autosomal dominant genes.

† This patient had one de novo mutation and a second inherited, predicted pathogenic mutation.

FAMILY-BASED EXOME SEQUENCING

Conclusive genetic diagnoses were obtained for 10 patients with de novo mutations in known intellectual-disability genes and for 3 male patients with severely disruptive, maternally inherited mutations in known X-linked intellectual-disability genes (Tables S3 and S9 in the Supplementary Appendix). The phenotypes of these patients fit well with previously reported phenotypes caused by mutations in these genes (Table S6 and the section on clinical descriptions in the Supplementary Appendix). No diagnostically relevant, inherited autosomal recessive mutations were identified. Thus, a diagnostic yield of 13% was obtained from mutations in known intellectual-disability genes (Table S10 in the Supplementary Appendix).

Our study identified 24 novel candidate genes affected by de novo mutations. A pathogenic role for 3 of these genes was substantiated by the identification of additional patients with intellectual disability and severely disruptive mutations. In each case, there was striking phenotypic overlap observed among the patients with mutations in the same gene. We therefore conclude that *DYNC1H1*, *GATAD2B*, and *CTNNB1* are novel intellectual-disability genes, which raises the diagnostic yield of exome sequencing to 16% (Tables 2 and 3).

DISCUSSION

Mutations in more than 400 genes have been linked to intellectual disability, but most of these mutations have a very low prevalence and their phenotypes are often indistinguishable. This argues for an unbiased diagnostic approach, especially since these 400 genes may represent less than half of all intellectual-disability genes. We implemented family-based diagnostic exome sequencing for patients with severe, unexplained intellectual disability. Exome sequencing is a procedure that is highly amenable to automation. Variants with potential clinical consequences can easily be validated with the use of Sanger sequencing as an independent method. We did not identify any major hurdles in the laboratory workflow in this study, which allowed for smooth integration of this process into diagnostics. De novo mutations were present in 53% of the patients and provided a conclusive genetic diagnosis in at least 13%, with an additional 3% of X-linked inherited mutations in known intellectual-disability genes. This diagnostic yield is similar to that of current chromosomal analyses based on genomic arrays, and the two approaches are complementary.[4,25-27] We expect that the diagnostic rate of exome sequencing will increase with the identification of additional patients who have mutations in the novel candidate genes reported here.

The identification of causal mutations in known intellectual-disability genes in 16 of 100 patients provides clinically useful information for clinicians and for patients and their families, since much is known about the prognoses associated with these mutations. The identification of the underlying

genetic cause may also lead to specific treatment options or dietary advice. As an example, a ketogenic diet was recommended for our patients with a mutation in *PDHA1*.[28] In addition, a specific antiepileptic treatment, with the avoidance of sodium-channel blockers, was suggested for our patient with a de novo *SCN2A* mutation, since this therapy leads to better seizure control and improvement in cognitive functioning and quality of life in patients with *SCN1A* mutations.[29]

Our studies suggest that several of the new candidate genes that we identified may be confirmed as having recurrent mutations in patients with intellectual disability. We already identified additional de novo mutations in three of five genes (*DYNC1H1*, *GATAD2B*, and *CTNNB1*) that were sequenced in a second set of affected persons, and detailed clinical analysis of these patients provided definitive evidence that these three genes cause intellectual disability when mutated. The identification of recurrently mutated genes in combination with detailed clinical phenotyping may reveal novel intellectual-disability genes and identify clinical subtypes of intellectual disability that may require specific approaches to clinical management. We observed evidence of autosomal recessive disease in only 1 affected patient, who carried a de novo mutation and a rare inherited mutation in *LRP2*. The apparent absence of pathogenic mutations in autosomal recessive intellectual-disability genes in our series suggests that this form of intellectual disability is rare in patients with isolated intellectual disability from nonconsanguineous parents. An analysis of referrals for intellectual disability to our tertiary care center showed that approximately 90% of patients have sporadic intellectual disability and nonconsanguineous parents (see the Supplementary Appendix). X-linked forms of intellectual disability were diagnosed in 5 of 100 patients, and in 2 of these 5 patients, the mutation occurred de novo. Mutations outside the coding regions, as well as mosaic, digenic, or oligogenic causes of intellectual disability, remain to be defined.

Unbiased diagnostic approaches such as exome sequencing may also reveal clinically relevant mutations that are not related to the disease under investigation. An independent expert panel determined the clinical relevance of such incidental findings. Before study enrollment, all families were counseled about this possibility and consented to being informed if the findings were deemed to be relevant by this panel. No families objected to being informed about incidental findings. In this study, we encountered one incidental finding, a de novo c.517C→T (p.Tyr173His) change in *RB1*. Mutations in this gene are associated with retinoblastoma (Online Mendelian Inheritance in Man [OMIM] number, 180200), an embryonic malignant neoplasm of retinal origin that is associated with a low risk of osteosarcoma.[30] The expert panel considered the risk of retinoblastoma to be negligible for this patient, since he had reached the age of 8 years, but decided that it was important to inform the parents of the small chance that a sudden, painful swelling of the limbs could be caused by an osteosarcoma and that they should consult an oncologist at the first sign of such swelling. No further incidental findings were encountered.

In conclusion, our study shows that exome sequencing can be used as a diagnostic procedure for patients with severe intellectual disability of unknown cause. The diagnostic yield, which was 16% in our series, may increase with improvement in methods and the identification of additional genes associated with intellectual disability.

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