Utility of trio-based exome sequencing in the elucidation of the genetic basis of isolated syndromic intellectual disability: illustrative cases

Introduction: Exome sequencing is recognized as a powerful tool for identifying the genetic cause of intellectual disability (ID). It is uncertain, however, whether only the exome of the proband should be sequenced or if the sequencing of parental genomes is also required, and the resulting increase in diagnostic yield justifies the increase in costs.

Patients and methods: We sequenced the exomes of eight individuals with sporadic syndromic ID and their parents.

Results and discussion: Likely pathogenic variants were detected in eight candidate genes, namely homozygous or compound heterozygous variants in three autosomal genes (ADAMTSL2, NALCN, VPS13B), one in an X-linked gene (MID1), and de novo heterozygous variants in four autosomal genes (RYR2, GABBR2, CDK13, DDX3X). Two patients harbored rare variants in two or more candidate genes, while in three other patients no candidate was identified. In five probands (62%), the detected variants explained their clinical findings. The causative recessive variants would have led to diagnosis even without parental exome sequencing, but for the heterozygous dominant ones, the exome trio-based approach was fundamental in the identification of the de novo likely pathogenic variants.

Keywords: exome, intellectual disability, next-generation sequencing

Introduction

Intellectual disability (ID) is a complex and heterogeneous clinical condition that affects 1%–2% of the general population, and can result from genetic or environmental factors, or a combination of both. However, most severe forms of ID have a single genetic basis, ranging from chromosomal alterations to point mutations.1–3

About 700 genes have already been associated with ID;4 however, a clear genetic explanation for the phenotype of many patients remains unknown. The implementation of whole exome sequencing (WES) in the last decade increased the identification yield of new mutations and genes associated with various diseases, and led to the demonstration that de novo mutations are a frequent cause of ID.5 WES has also successfully identified autosomal recessive6,7 and X-linked8,9 causative mutations in ID cohorts.

In non-familial cases, the situation is complicated by the lack of information on the type of inheritance underlying the phenotype. To date, studies of sporadic cases that have been performed using WES to elucidate the causes of ID have led to the diagnosis of 15%–30% of the patients.10–12

Through the exome sequencing of probands and their unaffected parents (trio analysis), this work aimed at identifying variants, which could explain ID in sporadic
cases, and evaluating the utility of trio-based exome sequencing in the identification of pathogenic variants.

**Patients and methods**

The patients were referred to the Genetic Counseling Service of the Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo. The study was approved by the Ethics Committee of the institution. Written informed consent was obtained from the parents of all patients.

The patients had ID ranging from moderate to severe and other associated clinical signs. Table 1 summarizes the main clinical findings of the patients in the cohort.

Genomic DNA from peripheral blood samples was extracted, according to standard procedures. Fragile-X syndrome (AmpliDEX® FMR1; Asuragen, Austin, TX, USA) and genomic imbalances (180K platform; Agilent Technologies, Santa Clara, CA, USA, or 850K platform; Illumina, San Diego, CA, USA) had previously been excluded in these families.

Genomic libraries were constructed using the SureSelect XT or SureSelect QXT kit V6 (Agilent SureSelect Whole Exome Enrichment kit), according to the manufacturer's instructions, with 100× coverage and 90% of the targets covered at 20×; sequencing was performed on the Hiseq 2500 sequencer from Illumina. The quality of the sequencing was verified through the FastQC program (Babraham Institute). The raw reads were aligned to the reference genome (GRCh37/hg19), using the Burrows–Wheeler Aligner,13 and indel realignment, base quality score recalibration, base alignment quality scoring, and variant calling. Filtering and prioritization were conducted using VarSeq® software (Golden Helix, Bozeman, MT, USA) and variant effect predictor. After coding, non-synonymous variants fitting the models of dominant de novo or recessive homozygote/compound heterozygote/hemizygote were filtered per frequency (1%) against the databases: NHLBI ESP6500SI-V2 exomes variant frequencies, ClinVar,15 dbSNP138, 1000 Genome Project,16 ExAC Browser,17 and ABRAOM.18

After variant filtering, in silico prediction of pathogenicity of variants was performed using five prediction algorithms, namely SIFT,19 PolyPhen-2,20 Mutation Taster,21 Mutation Assessor, and FATHMM.22 The VarElect online tool was used to prioritize variants according to the phenotype. The OMIM database and scientific literature were used to compare the expected phenotypes with the clinical features of the patients.

Potentially pathogenic variants in the proband were validated by Sanger sequencing also performed to analyze the presence or absence of the same variants in their parents. Variants were classified according to the ACMG guideline.23

**Results and discussion**

We sequenced the exomes (WES) of eight patients with idiopathic syndromic ID and their parents (trios). Rare variants in eight genes were detected in five patients: a homozygous variant in ADAMTSL2; compound heterozygous variants in NALCN and VPS13B; a variant in the X-linked gene MID1; and four heterozygous de novo variants in the autosomal genes RYR2, GABBR2, DDX3X, and CDK13. Table 2 summarizes the WES findings; the variants in bold were considered as causative of the clinical phenotypes. The likely pathogenic variants found in this study were missense, except for those in VPS13B (stop gain), and the clinical impact could only be estimated, even with the help of prediction algorithms. Figure 1 illustrates the results, showing the de novo variant

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<th>Table 1 Main clinical findings of the patients in the cohort</th>
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Trio-based exome sequencing detected in \textit{GABBR2} and the maternally inherited variant in \textit{MID1}.

As shown in Table 2, Patient 1 had rare variants of uncertain significance (VUS) in three different genes: a missense variant in \textit{RYR2}, a gene associated with dominant arrhythmogenic right ventricular dysplasia or ventricular tachycardia, neither of the conditions documented in the patient; a homozygous missense variant in \textit{ADAMTSL2}, whose mutations are known to cause recessive Ehlers–Danlos syndrome, not compatible with the patient’s phenotype; and a maternally inherited missense variant in the \textit{MID1} gene, associated with Opitz G/BBB syndrome, which could explain the cognitive impairment, cardiac defects, and cryptorchidism exhibited by the patient. The variant in \textit{MID1} was predicted as damaging (SIFT – http://sift.jcvi.org/) or probably damaging (PolyPhen-2 – http://genetics.bwh.harvard.edu/pph2/), but the \textit{RYR2} variant was also considered probably damaging, and diagnosis of Opitz G/BBB syndrome mostly relied on the fact that the \textit{MID1} variant could explain the phenotype.

Patients 4, 6, and 8 carried de novo variants. The variants in \textit{GABBR2} and in \textit{CDK13} had not been described in the searched databases (GnomAD, dbSNP, and ClinVar) and are therefore novel, while the variant in \textit{DDX3X} had already been described in ClinVar and classified as probably pathogenic.

Patient 4 harbored VUS in \textit{GABBR2}. Variants in this gene have been described recently, and the associated phenotype has not been consolidated in OMIM, but they emerge as important contributors for epileptic encephalopathies,\textsuperscript{24–27} in accordance with our patient clinical phenotype.

Patient 6 carried a known pathogenic variant in \textit{DDX3X}; the disorder associated with this gene, x-linked mental retardation, is clinically variable and includes other symptoms in addition to cognitive impairment, such as hearing loss, which is present in this patient. On the other hand, polycystic kidney disease, exhibited by the patient, has not been reported in \textit{DDX3X} mutation carriers. Although mutations in \textit{DDX3X} have only recently been reported, it is estimated that they are responsible for 1%–3% of idiopathic ID in females.\textsuperscript{28}

\textbf{Figure 1} Example of pathogenic mutations identified in the cohort.

\textbf{Notes:} (A) Image of the binary alignment map files showing the forward and reverse reads of a segment of the \textit{GABBR2} gene; the mutation from C to T in heterozygosity in the proband, not present in his parents, can be seen in dark blue. Underneath, Sanger sequencing validation of the C/T substitution. (B) Image of the BAM files showing the forward and reverse reads of a segment of the \textit{MID1} gene; the mutated allele, in heterozygosity in the mother and hemizygosity in the proband, is seen in dark blue. Underneath, Sanger sequencing validation of the C/T substitution.
Table 2 The candidate mutations identified in five of the patients of the cohort

| Patients | Gene          | Mutation Position | Amino acid exchange | ClinVar | dbSNP | Effect | OMIM phenotype | Position | Exon | Inheritance | Family segregation | Posteriorly rotated ears | Broad nasal bridge | Low-set, inverse epicanthal folds | Hypertelorism, telecanthus | Neocortical malformations | without congenital heart disease and seizures |
|----------|---------------|--------------------|---------------------|---------|-------|--------|-----------------|----------|-----|-------------|----------------------|--------------------------|----------------------|--------------------------|--------------------------|----------------------------------|
| 1        | RYR2          | G/A 1:237957254    | p.Asp4624Asn         | Missense| 95    | Dominant| 609996, 604772  | 300000   | 2   | Homozygous, de novo | 600996, 604772          | -                        | -                     | -                        | -                        | Medical interest                |
| 2        | MID1          | C/T X:10535359     | p.Val77Ile           | Missense| 95    | Dominant| 300000          | 95       | 9 | X-linked       | 300000          | p.Val77Ile               | -                     | -                        | -                        | -                        | Medical interest                |
| 3        | MID1          | C/T X:10535359     | p.Val364Ile          | Missense| 95    | Dominant| 300000          | 95       | 9 | X-linked       | 300000          | p.Val364Ile               | -                     | -                        | -                        | -                        | Medical interest                |
| 4        | MID1          | C/T X:10535359     | p.Val1528Ile         | Missense| 95    | Dominant| 300000          | 95       | 9 | X-linked       | 300000          | p.Val1528Ile               | -                     | -                        | -                        | -                        | Medical interest                |
| 5        | MID1          | C/T X:10535359     | p.Tyr1400Phe         | Missense| 95    | Dominant| 300000          | 95       | 9 | X-linked       | 300000          | p.Tyr1400Phe               | -                     | -                        | -                        | -                        | Medical interest                |
| 6        | MID1          | C/T X:10535359     | p.V663F             | Missense| 95    | Dominant| 300000          | 95       | 9 | X-linked       | 300000          | p.V663F                  | -                     | -                        | -                        | -                        | Medical interest                |
| 7        | MID1          | C/T X:10535359     | p.Tyr1400Phe         | Missense| 95    | Dominant| 300000          | 95       | 9 | X-linked       | 300000          | p.Tyr1400Phe               | -                     | -                        | -                        | -                        | Medical interest                |
| 8        | MID1          | C/T X:10535359     | p.V663F             | Missense| 95    | Dominant| 300000          | 95       | 9 | X-linked       | 300000          | p.V663F                  | -                     | -                        | -                        | -                        | Medical interest                |

Note: Variants in bold are considered to be causative of the phenotype. Abbreviations: Alt, alternate nucleotide; LoF, loss of function; Orig, Online Mendelian inheritance in Man. Red, reference nucleotide.
the three patients with dominant disorders, parental exome sequencing was instrumental to reach the conclusion that the de novo variants were likely pathogenic. A recent publication from the Deciphering Developmental Disorders Study on the exomes of 4,293 families reported damaging de novo mutations in 42% of the cohort. These results clearly show that parental exome sequencing is fundamental for efficient diagnosis in isolated cases.

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Disclosure

The authors report no conflicts of interest in this work.

References


