Genetic diagnosis and genotype-phenotype association in 122 Brazilian individuals with reduced biotinidase activity

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Introduction

The enzyme biotinidase (EC 3.5.1.12) plays a critical role in the absorption of biotin from dietary sources and in its recycling. Biotinidase deficiency (BD, OMIM: 253260) is an autosomal recessive inborn error of metabolism caused by pathogenic variants in the BTD gene that can lead to the deficiency of both biotin and in biotin-dependent mitochondrial carboxylases.

BD is diagnosed through the measurement of biotinidase activity in plasma or serum. Thus, individuals with reduced biotinidase activity can be classified as having profound BD (residual activity <10%) or partial BD (activity within 10-30%) or be heterozygous for pathogenic variants in the BTD gene. However, this biochemical testing is subject to interfering factors that can produce artificially low results, and the genetic analysis can help in the diagnosis, being essential to understand the relationship between biochemical phenotype and genotype.

BD was included in the Brazilian Neonatal Screening Program in 2012. Since then, our group has been studying the BTD gene, publishing the profile of 72 Brazilian individuals detected with low biotinidase levels.

Objective

The aim of this study was to provide an update of the genetic diagnosis for BD in Brazil, including the results by Borsatto et al. (20141 and 20172).
Methods

Multicenter, observational, cross-sectional study with a convenience sampling strategy. The inclusion criteria was at least one reduced qualitative biotinidase activity result. The entire analyzed population with the inclusion of the new samples (N=50) comprised 122 subjects aged 1 month to 18 years from three regions of Brazil (Figure 1).

Quantitative enzymatic activity values were available for 107/122 (current normal=7, heterozygous=60, partial BD=24, borderline heterozygous/partial BD=8, profound BD=5, and borderline heterozygous/normal=3).

Genomic DNA was extracted from whole blood samples or buccal epithelial cells using commercial kits, followed by PCR and Sanger sequencing of exons 2, 3, and 4 of the BTD gene. The reference sequence was NG_008019.1.

The pathogenicity of the novel variants was evaluated according to the American College of Medical Genetics and Genomics (ACMG) recommendations.

The observed phenotype was established using the highest value of the quantitative biotinidase activity, and the expected phenotype - according to the genotype -, was established only for recurrent variants with known impact and determined cis/trans configuration (Table 1).

![Figure 1. Map of South America highlighting Brazil and the number of participants according to their region of origin; participants biochemical BD classification according to their region of origin (grey boxes); and characteristics of the study sample (blue box).](image-url)

**Table 1. Genetic basis for BD**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Profound BD</th>
<th>Partial BD</th>
<th>Unaffected Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heterozygous</td>
<td>Heterozygous</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Mild pathogenic variant</td>
<td>Severe pathogenic variant</td>
<td>Mild pathogenic variant</td>
</tr>
<tr>
<td>2</td>
<td>Mild pathogenic variant</td>
<td>Mild pathogenic variant</td>
<td>Wild type or non-pathogenic variant</td>
</tr>
</tbody>
</table>

Severe pathogenic variant: <50% biotinidase activity; mild pathogenic variant: ~60% biotinidase activity; and wild type or non pathogenic variant: >60% biotinidase activity.
Results

The total cohort had 23 different pathogenic variants identified. The most frequent variants (>3.0%) were c.1330G>C p.(Asp444His), c.755A>G p.(Asp252Gly), c.1368A>C p.(Gln456His), and c.[511G>A;1330G>C] p.[(Ala171Thr);(Asp444His)], with frequency values of 44.1%, 4.2% 3.8%, and 3.4%, respectively. The Figure 2 shows the distribution of the most common pathogenic variants worldwide, according to the literature.

Three novel variants were found in addition to the six previously published: c.269T>A, c.1321G>A (both pathogenic), and c.1004C>T (likely pathogenic), as shown in Table 2.

Table 2. Novel BTD variants identified in Brazilian individuals with reduced biotinidase activity.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Protein</th>
<th>Exon</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Observed phenotype</th>
<th>Classification</th>
<th>ACMGG</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.1197&gt;C</td>
<td>p.(Leu40Pro)</td>
<td>2</td>
<td>c.1197&gt;C</td>
<td>c.1330G&gt;C</td>
<td>Partial</td>
<td>P (S)</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>c.4796&gt;G</td>
<td>p.(Cys1593Phe)</td>
<td>4</td>
<td>c.4796&gt;G</td>
<td>c.1330G&gt;C</td>
<td>Partial/ heterozygous</td>
<td>P (S/M)</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>c.962G&gt;A</td>
<td>p.(Trp314*)</td>
<td>4</td>
<td>c.962G&gt;A;</td>
<td>c.1413T&gt;C</td>
<td>Normal</td>
<td>P (S)</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>c.517T&gt;C</td>
<td>p.(Leu44Pro)</td>
<td>2</td>
<td>c.517T&gt;C</td>
<td>c.1413T&gt;C</td>
<td>Normal</td>
<td>B (LP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.466A&gt;G</td>
<td>p.(Asn469Ser)</td>
<td>4</td>
<td>c.466A&gt;G</td>
<td>c.1330G&gt;C;</td>
<td>Partial</td>
<td>P (M)</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>c.269T&gt;A</td>
<td>p.(Leu90His)</td>
<td>2</td>
<td>c.269T&gt;A</td>
<td>Normal</td>
<td>Heterozygote</td>
<td>P (S)</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>c.1004C&gt;T</td>
<td>p.(Ala335Val)</td>
<td>4</td>
<td>c.1004C&gt;T</td>
<td>c.1330G&gt;C</td>
<td>Partial</td>
<td>P (S)</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>c.3216G&gt;A</td>
<td>p.(Gly441Arg)</td>
<td>4</td>
<td>c.3216G&gt;A</td>
<td>Normal</td>
<td>Heterozygote</td>
<td>P (S)</td>
<td>P</td>
<td></td>
</tr>
</tbody>
</table>

P= pathogenic; B= benign; LP= likely pathogenic; S= severe; M= mild; ACMGG= American College of Medical Genetics and Genomics.
Results

The biotinidase deficiency classification according to the genotype was available for 105/122 (86.1%). These were identified as heterozygous N = 44 (41.9%); partial BD N = 28 (26.7%); profound BD N = 6 (5.7%); and undetermined N = 17 (16.1%).

The genotype-based classification matched the biochemical phenotype in 62/90 (68.9%) cases (Figures 3 and 4).

Figure 3. BD classification according to the phenotype (N=107) and the genotype (N=105). The comparison, available for 90 cases, was consistent for 62 (68.9%) of them.

Figure 4. Proportion of genotypes identified in individuals with enzymatic activity corresponding to: profound DB (A), partial DB (B), heterozygous (C), borderline partial/heterozygous (D), borderline heterozygous/normal (E), and current normal (F).
Conclusions

The Brazilian neonatal screening program is effective to detect total and partial BD patients, however seems to be detecting very often heterozygous individuals, which can lead to unnecessary distress for the patients and families. This highlights the importance of considering the modification of the cut-off point for the enzymatic test. Profound BD in the country is rare. However, the fact that this is a convenience sample strategy-based study has to be considered. Moreover, the quantitative biotinidase activity is not always successful in differentiating between the disease severities.

The Brazilian population is one of the most ethnically heterogeneous in the world, as the result of five centuries of miscegenation among indigenous, European, and African populations. The findings of this study suggest that the Brazilian mutational profile of BTD is more heterogeneous than in other countries.

Although the genotype-phenotype association is not always consistent (here, about 70%), genetic analysis is useful for clarifying borderline and consecutive discordant biochemical results, as well as for genetic counseling purposes, and for the identification of novel BTD variants.

References
