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ORIGINAL ARTICLE

Whole-exome sequencing as a diagnostic tool for distal renal tubular acidosis☆

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Abstract
Objective: Distal renal tubular acidosis (dRTA) is characterized by metabolic acidosis due to impaired renal acid excretion. The aim of this study was to demonstrate the genetic diagnosis of four children with dRTA through use of whole-exome sequencing.
Methods: Two unrelated families were selected; a total of four children with dRTA and their parents, in order to perform whole-exome sequencing. Hearing was preserved in both children from the first family, but not in the second, wherein a twin pair had severe deafness. Whole-exome sequencing was performed in two pooled samples and findings were confirmed with Sanger sequencing method.
Results: Two mutations were identified in the ATP6V0A4 and ATP6V1B1 genes. In the first family, a novel mutation in the exon 13 of the ATP6V0A4 gene with a single nucleotide change GAC → TAC (c.1232G>T) was found, which caused a substitution of aspartic acid to tyrosine in position 411. In the second family, a homozygous recurrent mutation with one base-pair insertion (c.1149_1155insC) in exon 12 of the ATP6V1B1 gene was detected.
Conclusion: These results confirm the value of whole-exome sequencing for the study of rare and complex genetic nephropathies, allowing the identification of novel and recurrent mutations. Furthermore, for the first time the application of this molecular method in renal tubular diseases has been clearly demonstrated.

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PALAVRAS-CHAVE
ATP6V0A4;
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Genética;
Sequenciamento total do exoma

Sequenciamento total do exoma como ferramenta de diagnóstico de acidose tubular renal distal

Resumo

Objetivo: A acidose tubular renal distal (ATRd) é caracterizada por acidose metabólica devido a excreção renal de ácido prejudicada. O objetivo deste artigo é apresentar o diagnóstico genético de quatro crianças com ATRd utilizando o sequenciamento total do exoma.
Métodos: Selecionamos duas famílias não relacionadas, totalizando quatro crianças com ATRd e seus pais, para realizar o sequenciamento total do exoma. A audição foi preservada em ambas as crianças da família um, porém em nenhuma criança da família dois, na qual um par de gêmeas teve perda auditiva severa. Realizamos o sequenciamento total do exoma em dois conjuntos de amostras e confirmamos os achados com o método de Sequenciamento de Sanger.
Resultados: Duas mutações foram identificadas nos genes ATP6V0A4 e ATP6V1B1. Na família um, detectamos uma nova mutação no éxon 13 do gene ATP6V0A4 com uma alteração em um nucleotídeo único GAC → TAC (c.1232G>T) que causou substituição de ácido aspártico por tirosina na posição 411. Na família dois, detectamos uma mutação recorrente do homozigoto com inserção de um par de bases (c.1149_1155insC) no éxon 12 do gene ATP6V1B1.
Conclusão: Nossos resultados confirmam o valor do sequenciamento total do exoma para o estudo de nefropatias genéticas complexas, permitindo a identificação de mutações novas e recorrentes. Adicionalmente, demonstramos claramente pela primeira vez a aplicação desse método molecular em doenças tubulares renais.

Introduction

Distal renal tubular acidosis (dRTA) is a rare and complex renal disease due to a defect in the excretion of acid load (H^+ and ammonium ions) in alpha-intercalated cells of the collecting duct. The acid load accumulation in the distal nephron results in consumption and reduction of the bicarbonate/CO_2 buffer in blood.[1] The main clinical features of dRTA are vomiting, diarrhea, and/or constipation, loss of appetite, polydipsia, and polyuria. Chronic acidosis and secondary alterations such as vomiting, polyuria, and dehydration affect growth, leading to failure to thrive. Ultrasound studies can show nephrocalcinosis and/or nephrolithiasis.[2] In general, dRTA has good prognosis if it is diagnosed at an early age and alkaline treatment is continued. Untreated, dRTA causes growth retardation and rickets in children and osteomalacia in adults. Deterioration of renal function can occur over the years.[3]

Distal RTA can be transmitted as either an autosomal dominant or an autosomal recessive trait.[4] The autosomal dominant phenotype typically courses mildly in adolescence or adulthood;[4] one parent suffers from and is the carrier of the disease, or it is due to *de novo* mutation. Mutations in the *SLC4A1* gene in families with autosomal dominant dRTA have been identified.[2,5,6] The symptoms in the autosomal recessive phenotype predominantly appear at infancy or early childhood, in which growth retardation is very common. This variant can occur with or without deafness, and parents are not affected.[2] Autosomal recessive dRTA is associated with mutations in any of the following genes: *SLC4A1*,[7] *ATP6V0A4*, and *ATP6V1B1*.[2,8] Individuals without hearing defects usually carry mutations in the *ATP6V0A4* gene, while those with deafness have *ATP6V1B1* gene mutations. In approximately 20% of the patients with dRTA, no mutations were found in any of these related genes.[3] Indeed, there are dRTA patients with deafness without *ATP6V1B1* gene mutations, and others with normal hearing who do not have *ATP6V0A4* gene mutations.[3] These findings suggest that other transporters or channels might cause dRTA. In terms of complexity, it is known that some patients with mutations in the *ATP6V0A4* gene develop deafness only in the second decade of life. Thus, there remains much to be elucidated in terms of phenotype–genotype correlations.[8–10] So far, more than 20 mutations in *ATP6V0A4* are already known.

Whole-exome sequencing provides coverage of more than 95% of the exons, which contain 85% of disease-causing mutations in Mendelian disorders and many disease-predisposing single nucleotide polymorphisms (SNPs) throughout the genome.[11,12] Whole-exome sequencing is worthwhile to evaluate the disease pathogenesis and to recognize new pathogenic genes or mutations associated to disorder, especially in Mendelian disorders.[11,12] In this regard, the present study aimed to evaluate the usefulness of whole-exome sequencing for genetic diagnosis of dRTA.

Patients and methods

Subjects and clinical assessment

Four children with confirmed dRTA from two unrelated families were selected for this study. All patients were followed up at the Pediatric Nephrology Unit of the Federal University of Minas Gerais (UFMG), Brazil. The first family (Family 1) consisted of two affected siblings, a girl and a boy, with dRTA but without deafness, and their unaffected parents. The second family (Family 2) had a monozygotic twin pair