Apparent Mineralocorticoid Excess Syndrome in a Brazilian Boy Caused by the Homozygous Missense Mutation p.R186C in the *HSD11B2* Gene

clinical case report

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ABSTRACT

The apparent mineralocorticoid excess syndrome (AME) is a rare autosomal recessive disorder due to the deficiency of 11β-hydroxysteroid dehydrogenase type 2 enzyme (11beta-HSD2). The 11beta-HSD2 enzyme, encoded by *HSD11B2* gene, metabolizes active cortisol in cortisone. Mutations on *HSD11B2* gene affect the enzyme activity by leading to an excess of cortisol, which causes its inappropriate access to mineralocorticoid receptor. Therefore, cortisol will bind mineralocorticoid receptor. The human *HSD11B2* gene maps to chromosome 16q22 and consists of five exons encoding a protein of 405 amino acids. We present here clinical and molecular studies on a Brazilian boy who was born pre-term after an oligodramnious pregnancy. He was diagnosed as having AME at the age of 26 months. His parents are second cousins. Molecular characterization of the *HSD11B2* gene revealed the homozygous mutation p.R186C. The patient described here is the second case of *HDS11B2* gene mutation reported in Brazilian patients with AME. **(Arq Bras Endocrinol Metab 2008; 52/8:1277-1281)**

Keywords: Apparent mineralocorticoid excess; *HSD11B2* gene; Hypertension; Deficiency of 11β-hydroxysteroid dehydrogenase type 2; Mutations

RESUMO

Síndrome de Excesso Aparente de Mineralocorticóide em um Menino Brasileiro Causada pela Mutação p.R186C em Homozigose no Gene *HSD11B2*.

A síndrome de excesso aparente de mineralocorticóide (AME) é uma doença autossômica recessiva rara devido à deficiência da enzima 11β-hidroxiesteróide desidrogenase tipo 2 (11beta-HSD2). A enzima 11beta-HSD2 metaboliza o cortisol ativo a cortisona. As mutações no gene *HSD11B2*, que codifica a enzima, afetam sua atividade levando a um excesso de cortisol, que terá acesso inapropriado ao receptor de mineralocorticóide, competindo com a ligação da aldosterona. O gene *HDS11B2* humano está localizado no cromossomo 16q22 e é formado por 5 éxons que codificam uma proteína de 405 aminoácidos. Este relato apresenta os estudos clínicos e moleculares de um paciente brasileiro do sexo masculino que nasceu prematuro depois de uma gestação sob oligodrâmnio. Recebeu o diagnóstico de AME com 26 meses de idade. Seus pais são primos em segundo grau. A caracterização molecular do gene *HSD11B2* revelou a mutação p.R186C em homozigose. O paciente descrito é o segundo caso relatado de brasileiro com mutação no gene *HSD11B2*. **(Arq Bras Endocrinol Metab 2008; 52/8:1277-1281)**

Descritores: Excesso aparente de mineralocorticóide; Gene *HDS11B2*; Hipertensão; Deficiência da11β-hidroxiesteróide desidrogenase tipo 2; Mutações

INTRODUCTION

Apparent mineralocorticoid excess (AME; OMIM # 207765) is a rare autosomal recessive disorder, which consists in an inherited form of hyperten-

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sion caused by the deficiency of 11β-hydroxysteroid dehydrogenase type 2 (11beta-HSD2) (1-3). This enzyme is highly expressed in mineralocorticoid target tissues and functions unidirectionally to convert cortisol to cortisone, an inactive form (4). Although cortisol is a less potent mineralocorticoid than aldosterone, the main mineralocorticoid produced in the adrenal gland, both aldosterone and cortisol bind to the mineralocorticoid receptor (MR) with equal affinity (5), whereas cortisone does not. Therefore, 11beta-HSD2 enzyme protects normal subjects from cortisol intoxication by converting it into cortisone and providing a mechanism that ensures the proper occupation of MR by aldosterone (6). As cortisol is secreted in concentrations much higher than aldosterone, the deficiency of 11beta-HSD2 leads to an inappropriate inactivation of cortisol, which in excess, acts as a mineralocorticoid. This cortisol excess stimulates the MR causing intense sodium retention, hypokalemia and hypertension (1-3).

Two isoforms of 11beta-HSD have been identified and characterized (7). The type 1 isoform, which is expressed in several human tissues, is a NADP-dependent enzyme with reductase activity (8, 9). The type 2 isoform, which is only 20-26% identical to type 1, is NAD-dependent and presents dehydrogenase activity (10). The human *HSD11B2* gene, encoding the 11beta-HSD2 enzyme, is located on chromosome 16q22, consists of 5 exons spanning about 6.2 Kb, and encodes a protein of 405 amino acids (11).

AME is a result of mutations in *HSD11B2* gene (12-15). In general, the disease is present in early childhood, with a severe phenotype including low birth weight, failure to thrive, hypokalemic metabolic alkalosis, and high mortality rate in untreated patients (2, 16). However, milder phenotypes have been described in adults with clinical features such as hypertension without electrolyte abnormalities (17-20). Milder phenotypes are associated with mutations causing only partial inactivation of HSD11B2 (21).

In this study, we report a boy with typical clinical features of AME with p.R186C homozygous mutation in the *HSD11B2* gene.

SUBJECTS AND METHODS

Blood specimens and clinical data of the patient and relatives were collected with approval by the appropriate institutional review board; signed informed consent was obtained.

Clinical data

A Brazilian male, son of a consanguineous marriage, was born pre-term (weight = 1,700 g; length = 40 cm), Apgar score 8-9, after an oligodramnious pregnancy. He had normal male genitalia with palpable testes. His development was normal up to the age of 5 months when he had bronchiolitis. Thereafter he presented failure to thrive. When he was 17 months old he was admitted in a hospital for pneumonia when he presented polyuria, polydipsia, hypokalemia (serum potassium, 1.9 mmol/L) and hypertension. He was first examined by us at the age of 26 months when he was referred to our hospital for evaluation because prednisone and chlorthalidone failed to control his labile hypertension. At this time his weigh (8,580 g) and height (76.8 cm) were both below the third percentile (zW = - 3.32 and zH = - 2.53). He also presented hypertension (130/90 mmHg). Laboratorial findings indicated hypokalemia (serum potassium, 2.5 mmol/L), alkalosis (pH = 7.51) and normal sodium (141 mmol/L), plasma renin activity (0.48 ng/ml/h, normal 0.15 to 2.33), aldosterone (26 pg/ml, normal 10 to 160 pg/mL), 11-deoxycortisol (2.0 ng/mL, normal < 8.0 ng/mL) and serum cortisol (9.9 µg/dL, normal 7.58 to 27 µg/dL,). On 24-hour urine he presented normal cortisol (31.9 µg/24hs, normal 20 to 90 µg/24hs) and hypercalciuria (18 mg/kg/day, normal < 4 mg/kg/day). Unfortunately, neither cortisol nor cortisone urinary metabolites could be evaluated.

Renal ultrasounds revealed the presence of bilateral nephrocalcinosis (not progressive) whereas cardiac echocardiography showed mild left ventricular hypertrophy when he was 5 years old.

He has been treated with diuretics, antihypertensive drugs, salt restriction and potassium supplementation and he responded well to the treatment. On follow-up, biochemical results indicated normal serum levels of both sodium and potassium, and blood pressure in the upper limit of normality. He also had an improvement of growth parameters. At the age of 11.1 years his weight was 32,300 g (Z = –0.61), height 140.4 cm (Z = –0.48), pubertal stage G2P3, his blood pressure was normal (110/60 mmHg). In the last consultation, his clinical features were stable upon treatment with KCl (3.30 g/day), Amiloride (12.5 mg/day), Captopril (100 mg/day), Hydrochlorothiazide (25 mg/day), Atenolol (18 mg/day) and Losartam Potassium (25 mg/day).

Molecular analysis

Genomic DNA was extracted from peripheral blood leukocytes by standard phenol/chloroform method (22). *HSD11B2* gene was amplified by PCR amplification of the entire coding region including exon-intron junctions and both 5'UTR and 3'UTR regions using synthetic oligonucleotides (Invitrogen, CA, USA) as primers (table 1), which were designed using Primer 3 open access software (http://primer3.sourceforge.net). The amplified fragments were directly sequenced using Big Dye TM Terminator Cycle Sequencing Kit V3.1 Ready Reaction (ABI PRISM / PE Biosystems, Foster City, CA, USA). The sequences obtained in an ABI377 Semi-Automated Sequencer (ABI PRISM / PE Biosystems) were compared to the normal sequence of the gene (NCBI # U27317).

RESULT

HSD11B2 sequence analysis on patient's DNA revealed a c.556C>T homozygous transition in codon 186 located in exon 3. This nucleotide substitution leads to the p.R186C missense mutation (Figure 1A). Further sequence analysis on parent's DNA samples showed heterozygosis for the mutation in both father and mother (Figure 1B).

DISCUSSION

In the present study we describe a boy diagnosed as having AME disease. Clinical and hormonal data supported the presence of a deficiency in the 11beta-HSD2 enzyme activity. Facing clinical and laboratorial featu-

Table 1. Primers used for PCR *HSD11B2* gene amplification.

	Primers	Sequences (5´---3´)	Tm (°C)	Size (pb)
5´-UTR	HSD11B2-5´-F	GGGGCTCTTCATAAGCTCG	60	255
	HSD11B2-5´-R	CCAGACGCAGGTCTGAGC		
Exon 1	HSD11B2-1F	TAGAAGCTCTCTCTCCCCGC	61	398
	HSD11B2-1R	CCTGTTGAGTGTCCAGTCCC		
Exon 2	HSD11B2-2F	TGGTGATTCTGGGGTTGTCT	60	394
	HSD11B2-2R	CACAGAGCAGAGGAGGGAAG		
Exon 3	HSD11B2-3F	GACACGGGGACTGGAAGTT	60	288
	HSD11B2-3R	GGCTCCTTTTTGCTCCAGT		
Exon 4	HSD11B2-4F	ACTGGAGCAAAAAGGAGCC	60	295
	HSD11B2-4R	CTGCCCCCATAAGACCATT		
Exon 5I	HSD11B2-5IF	CGCGGGTTAAACAGTCCTAA	61	300
	HSD11B2-5IR	CCCTGGCCGGGGTAATAG		
Exon 5II	HSD11B2-5IIF	ATGCCATCACAGATGCGCT	62	292
	HSD11B2-5IIR	GCCTCCTGTGCTGCAGTG		
3´-UTR - I	HSD11B2-3´-IF	AGGACCCAAACCTGAGCC	60	310
	HSD11B2-3´-IR	GTTCTCCAAGCTGCAGGGTA		
3´-UTR - II	HSD11B2-3´-IIF	CACTGTTTCATGAGCCCAAA	60	405
	HSD11B2-3´-IIR	CACACTGTGTCACTCAGCCA		

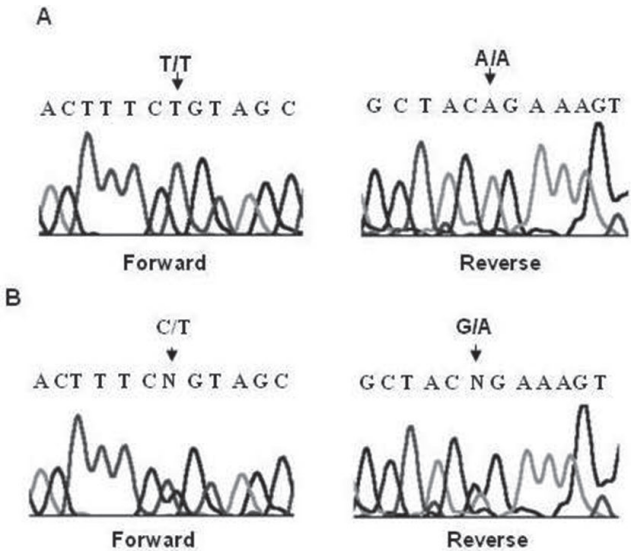

Figure 1. *HSD11B2* gene sequencing electropherograms showing part of exon 3. The CGT > TGT nucleotide change is denoted. This change causes the missense mutation R186C observed in homozygosis for patient's DNA (A) in the sequencing with both forward and reverse primers. The heterozygous status was verified for his parents (B).

res, the etiologies raised were: AME and Liddle Syndrome. Several clinical characteristics, such as low birth weight, failure to thrive, hypercalciuria and also parent's consanguinity favored AME diagnosis and led us to search for *HSD11B2* gene mutations.

Molecular investigation identified the p.R186C homozygous mutation in the *HSD11B2* gene. This mutation was previously described by Wilson and cols. (23). The aminoacid residue R186 is conserved across several species. In addition, computer analysis of the predicted protein structure revealed that p.R186C substitution yields a protein with a new P-sheet from residues 189 to 195 and an increased hydrophobicity from residues 180 to 191 (24). *In vitro* enzymatic activity assays showed complete inactivation of 11beta-HSD2 enzyme due to p.R186C mutation (24).

To date, over 30 different mutations on *HSD11B2* gene have been reported worldwide and in many ethnic groups, including Caucasians, Africans, Asians, and American Indians (25-28). Consanguinity, endogamy or a founder effect for AME have been considered in several families, especially those from ethnics in whom recurrence of certain *HSD11B2* mutations is observed (25). The Brazilian family described here is formed by a consanguineous marriage and both father and mother are p.R186C carriers. Since this mutation was previously described segregating in an African-American family (20), a founder effect should be considered. However, in the present case it is difficult to explain the mutation recurrence by considering a founder effect of an African-derived disease-causing allele without analyzing molecular markers, which could give an estimative of African ancestry index for this family as defined by Parra and cols. (29). Therefore, we cannot discard the possibility of this mutated allele being an African-originated allele.

Conversely, the C>T transition at codon 186 (**C**GT>**T**GT) is a typical CpG-consequence mutation. This dinucleotide is considered to be prompt to undergo mutations through spontaneous deamination of cytidine to uracil (30, 31). Therefore, the occurrence of the p.R186C mutation in two unrelated families could suggest that the codon R186 is a hot spot for mutations in *HSD11B2* gene, like other mutations located in exons 3 to 5 (21, 32). However, the number of patients bearing the mutation is low, therefore there are no significant evidences for this possibility unless more patients are studied.

In general there are few cases of AME reported worldwide, since this is a very rare autosomal recessive disorder (25, 27). The boy described in the present paper was clinically diagnosed with AME and molecular findings confirmed the phenotype. This is the second case of AME with a deleterious *HDS11B2* gene mutation reported in Brazilian patients (33).

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