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A Novel Mutation in the *HSD11B2* Gene Causes Apparent Mineralocorticoid Excess in An Omani Kindred

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Key Words: Low-renin hypertension; molecular genetics, computational modeling, *in silico* mutation analysis

Abstract

Apparent mineralocorticoid excess (AME) is a rare autosomal recessive genetic disorder causing severe hypertension in childhood, due to a deficiency of 11β-hydroxysteroid dehydrogenase type 2 enzyme (11βHSD2), which is encoded by the *HSD11B2* gene. Without treatment, chronic hypertension leads to early development of end organ damage. Approximately 40 causative mutations in *HSD11B2* have been identified in ~100 AME patients worldwide. We have studied the clinical presentation, biochemical parameters, and molecular genetics in 6 patients from a consanguineous Omani family with AME. DNA sequence analysis of affected members of this family revealed homozygous c.799A>G mutations within exon 4 of the *HSD11B2* gene, corresponding to a p.T267A mutation of the 11βHSD2 enzyme. The structural change and predicted consequences owing to this mutation, p.T267A, have been modeled *in silico*. We conclude that this novel mutation is responsible for apparent mineralocorticoid excess in this family.

Introduction

Apparent mineralocorticoid excess (AME), arising from 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) deficiency, is a rare monogenic disorder causing severe hypertension in childhood. AME was first described hormonally in 1977 in a 3 year-old Native American girl with severe hypertension.[1] Prenatal and postnatal growth failure and juvenile hypertension are seen in the most severe phenotypes. As a result of chronic hypertension, end organ damage can occur, with the renal, neurological, cardiovascular, and ocular systems being the most sensitive to damage.

Clinical manifestations of AME mimic those of excessive mineralocorticoid activity, but without any elevation of known mineralocorticoids. The specificity of the mineralocorticoid receptor (MCR) function depends on the metabolic enzyme, 11βHSD2, rather than the receptor itself. MCR is non-selective and cannot distinguish between aldosterone, its natural ligand, and cortisol, a glucocorticoid.[2, 3] Cortisol is present in the circulation at concentrations approximately 1,000 times higher than aldosterone. To prevent the activation of MCR by cortisol, 11βHSD2 converts cortisol to the inactive metabolite, cortisone. Aldosterone is not metabolized by 11βHSD2 because it forms a C_{11}-C_{18} hemi-ketal group. In patients with 11βHSD2 deficiency, cortisol can bind to MCR and acts as a mineralocorticoid. This results in the clinical phenotype of elevated mineralocorticoid activity in the absence of mineralocorticoid excess. Thus, the disease was named apparent mineralocorticoid excess.

In 1998, we described the phenotype and genotype of the first 14 pediatric patients with AME, the largest cohort of this disease studied by molecular genetics.[4] Patients with AME have significantly lower birth weights compared to their unaffected sibs; they were also short and hypertensive for age. Variable organ damages in kidneys, retina, heart, or the central nervous system were found in all of the patients except one.[4] These patients display an elevated ratio of

urinary cortisol to cortisone metabolites, *i.e.* tetrahydrocortisol plus 5α-tetrahydrocortisol to tetrahydrocortisone [(THF + 5αTHF)/THE], which ranged from 6.7 to 33 compared to the normal ratio of 1.0. Of this cohort, three patients died of cardiac complications in adolescence, and two patients received renal transplantations as a result of kidney failure. Since then, approximately 100 patients with AME have been studied clinically and biochemically worldwide.

Early genetic diagnosis of AME allows for effective treatment with spironolactone to reduce hypertensive complications, electrolyte disturbances, and mortality.[4] The *HSD11B2* gene is located on the long arm of chromosome 16 (16q22) and is approximately 6 kb in length containing five exons. In 1995, the first mutation in the *HSD11B2* gene was discovered in a consanguineous Iranian family with three siblings suffering from AME.[5] To date, approximately 40 causative mutations in the *HSD11B2* gene have been identified.[6] Patients with AME carrying homozygous *HSD11B2* mutations are often found to be the offspring of consanguineous families.[4, 7] AME is more commonly the cause of hyporeninemic hypertension in certain ethnic groups, such as Native American and Omani populations.[4, 8, 9] Thus far, the largest cohort of Omani patients with AME studied with molecular genetics includes 9 affected children with five different mutations in the *HSD11B2* gene in four families.[9]

Here, we present the clinical and molecular genetic data of 6 patients affected with apparent mineralocorticoid excess in a consanguineous Omani family.

Materials and Methods

Clinical Evaluation

Clinical data was collected retrospectively through chart reviews. Patients with AME were evaluated and managed by pediatric endocrinologists at the Royal Hospital in Oman.

Informed consents were obtained from patients or parents, when appropriate, for genetic testing.

Laboratory evaluation

A morning sample of blood was drawn for the measurement of plasma renin activity and aldosterone. Serum potassium and bicarbonate were also measured. Mass spectrometry of 24-h urinary steroid quantification was performed to demonstrate an elevated (THF + 5αTHF)/THE ratio (normal range: 0.66–2.44; AME: 6.7–73.8) on subjects who have not been previously studied. Urinary steroid metabolites were measured by assays described by Shackleton *et al.*[10]

Molecular genetic analysis

Sequencing of the *HSD11B2* gene was performed in our laboratory at the Icahn School of Medicine at Mount Sinai to detect mutations, as previously described.[4]

Computational prediction of the consequences of the p.T267A Mutation

To assess the severity of the mutation detected in this family, we utilized four bioinformatics tools, namely Protein Variation Effect Analyzer (PROVEAN, http://provean.jcvi.org/index.php)[11], Sorting Intolerant From Tolerant (SIFT, http://sift.jcvi.org/)[12], Polymorphism Phenotyping (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/)[13], and MutPred (http://mutpred.mutdb.org/)[14] to predict whether this missense mutation has a deleterious effect on protein function.

In silico model of 11βHSD2

We have constructed a computational model of 11βHSD2 to provide structural explanations for the clinical manifestations arising from each of the known mutations in

HSD11B2. Since the crystal structure for 11βHSD2 is not available, the structure of the human estradiol 17β-dehydrogenase 2 (HSD17B2) (PDB id 1IOL, IJTV, 1FDV), which covers 84% of the HSD11B2 sequence, was used as templates to construct a model of 11βHSD2. Models of human HSD11B2 were generated using the Internal Coordinate Mechanics method implemented in the Molsoft ICM software,[15] and stereochemical properties were evaluated using the program PROCHECK version 3.5.4.[16] The final model was chosen based on low-energy function and a low Cα root-mean-square-distance (rmsd) overlap between the HSD17B2 and the human HSD11B2 model. The model was constructed with the spatial position of heme and ligands retained in their respective binding sites. This was based on the assumption that the ligands and cofactor interact in a similar manner in HSD11B2 as in the HSD17B2 crystal structure; this comparison replicated all conserved interactions from the template into the model. Several rounds of energy minimization were carried out to obtain a final low-energy conformation with no steric clashes between side chains. This methodology has previously been used to correlate structural changes of 21-hydroxylase owing to mutations in CYP21A2 causing congenital adrenal hyperplasia.[17]

Results

Six patients affected with AME were identified in an Omani family. These patients are delineated on the pedigree in Figure 1A as V-3, V-7, V-12, V-13, V-14, and V-15. Table 1 shows baseline clinical and biochemical characteristics of these patients as well as results of follow up studies.

Patient V-3

Patient V-3 is the product of a consanguineous marriage (Figure 1A) of IV-1 and IV-2. He was born at full term by spontaneous vaginal delivery at a birth weight of 2 kg. At 10 months of age, he was noted to have polyuria and polydipsia. His baseline blood pressure was

elevated at 146/80 mm Hg (90th percentile for age and sex is 102/53). The initial laboratory evaluation showed hypokalemic. Upon evaluation by the pediatric endocrinologist, the patient was diagnosed with hyporeninemic hypertension (plasma renin activity of 0.9 ng/ml/hr) associated with a low serum aldosterone concentration of 1.1 ng/dL. He is currently treated with Spironolactone 25mg QID, Amlodipine 7.5mg OD, Amiloride Hydrochloride 5mg OD, Atenolol 12.5mg BID, and potassium chloride supplementation. Blood pressure at his most recent follow-up was 130/79 (90th percentile for age and sex is 119/77). His last echocardiogram showed aortic root dilation, and renal ultrasound showed nephrocalcinosis.

Patient V-7

Patient V-7 was identified on screening at 12 months owing to a family history of AME in his paternal cousins, V-12 and V-13. She was born full term at a birth weight of 2.9 kg to consanguineous parents, IV-5 and IV-6. Her blood pressure at 12 months of age was elevated at 125/74 mmHg and she was found to be hypokalemic. Endocrinological evaluation revealed an undetectable plasma renin activity and an undetectable serum aldosterone concentration. The major urinary metabolites of cortisol (THF) and cortisone (THE) were measured in this patient. The ratio of tetrahydrocortisol (THF) plus 5αTHF/tetrahydrocortisone (THE) ratio was significantly elevated at 31.8. She is currently treated with Spironolactone 25mg TID, Amiloride Hydrochloride 10mg BID, Valsartan 15mg OD, and potassium chloride supplementation. At follow up, aortic root dilatation and left ventricular dilation were seen on echocardiogram. Renal ultrasound showed nephrocalcinosis.

Patient V-12, V-13, V-14 and V-15

Patient V-12 presented with weakness at 5 years of age. Her blood pressure on examination at 5 years old was 140/58 mm/Hg (90th percentile for age and sex is 109/69). Laboratory evaluation showed mild hypokalemia with low plasma renin activity and low serum

aldosterone concentration. She is currently treated with Spironolactone 125mg per day, Hydrochlorothiazide 12.5mg OD and potassium chloride supplementation. Hypertensive cardiac changes were not noted on echocardiogram at follow up, but nephrocalcinosis is present.

Patients V-13, V-14 and V-15 are siblings of V-12 diagnosed at 1 year, 4 years, and 4 years respectively. These patients' blood pressures at diagnosis were above the 90th percentile for age and sex. Potassium concentrations ranged from 2.2 to 2.9 mEq/L. Plasma renin activity was undetectable in the 3 siblings and serum aldosterone concentrations were undetectable in V-14 and V-15. Table 1 shows follow up data for the siblings.

Molecular genetic analysis

Sanger sequencing of the *HSD11B2* gene revealed homozygous c.799A>G mutations in all 6 affected patients (V-3, V-7, V-12, V-13, V-14 and V-15) (Figure 1B and 1C). This missense mutation is located in exon 4, causing a change in the amino acid residue at position 267 from threonine to alanine (p.T267A) (Figure 1D).

In silico mutation analysis

In silico mutation analyses were performed using PROVEAN, SIFT, PolyPhen-2 and MutPred. These programs rely on sequence homology and/or structure-function information from annotated UniProt entries to predict functional consequences of changes in protein sequences. All four software predicted a damaging effect of the p.T267A mutation on HSD11B2 function (Table 2). That p.T267 is conserved in the vertebrate HSD11B2 protein further suggests an important role of this residue at this position (Figure 1E).

Structural modeling

The humanized 11βHSD2 model exhibits the characteristic conserved NAD-binding Rossmann Fold motif. Structural elements that enclose the NAD binding site are most conserved. p.T267, along with 18 other amino acid residues, lie within 5.0Å of the NAD binding site. The hydroxyl group side chain of p.T267 forms a hydrogen bond with the amide nitrogen present in the nicotinamide component of NAD (Figure 1F). This interaction helps the positioning of NAD in the coenzyme-binding pocket. A mutation to alanine results in the loss of the hydrogen bond, resulting in misalignment of NAD in the coenzyme-binding site (Figure 1G).

Discussion

In this report, we have identified a novel homozygous mutation, p.T267A, in the *HSD11B2* gene in six members affected with apparent mineralocorticoid excess of an Omani family. Of the ~40 mutations identified in the *HSD11B2* gene, 6 mutations have been previously reported in the Omani population.[4, 9] The majority of these patients presented with symptoms of the severe form of AME, which include low birth weight, failure to thrive, hypokalemic metabolic alkalosis and significant hypertension compared to the 90th percentile for age and gender.

AME owing to homozygous mutations is frequently reported in consanguineous families.[4, 5] In a cohort of 14 AME patients, homozygosity of mutations in *HSD11B2* was found in 13 patients from 10 different families, very likely the result of endogamy or consanguinity.[4] Similarly, patients in the family reported here showed the same pattern.

Our patients' presentations fall in the severe end of the disease spectrum of AME. Their birth weights ranged from 1.9 - 2.9 kg with 3 of 6 patients born small for gestational age. Five of the patients presented at a very young age (0.83 – 5 years) with clinical symptoms; patient V-7 was asymptomatic at diagnosis and screened owing to family history. At the time of diagnosis,

elevated blood pressures compared to the 90th percentile for age and gender, low serum potassium concentrations and hyporeninemic hypoaldosteronism were seen in all patients.

Mutation of residues that forms the substrate- or cofactor-binding pocket causes total elimination of enzyme activity.[18] p.T267 resides in the NAD-binding site of the 11βHSD2 enzyme. Its mutation to alanine results in the misalignment of NAD in the coenzyme-binding site. Furthermore, our *in silico* analysis predicts a potential reduction of 11βHSD2 enzyme activity with the p.T267A mutation.

This is the first report of the p.T267A mutation. Clinical data show that this mutation causes the severe form of apparent mineralocorticoid excess and is supported by structural modeling and *in silico* studies. Further *in vitro* expression studies will determine the enzymatic activity of this 11βHSD2 mutant to confirm the severity of the disease.

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Legends to Figure

Figure 1

A Novel p.T267A Mutation in the *HSD11B2* Gene Causes Apparent Mineralocorticoid Excess in An Omani Kindred. Pedigree of Omani family affected with AME owing to the p.T267A mutation in the *HSD11B2* gene (**A**). Consanguineous marriages are shown in double lines. Roman numerals on the left denote generation. Solid square and circle: affected male and female. Half solid square and circle: heterozygous male and female. Half grey square and circle: presumed heterozygous male and female. Open square and circle: clinically unaffected male and female. Those in generations I, II, and III are deceased. Sequencing electropherograms of normal individuals (**B**) and patients carrying the c.799A>G mutation in the homozygous state (**C**). c.799A>G in exon 4 of the *HSD11B2* gene results in the T267A mutation (**D**). Sequence analysis of HSD11B2 shows that p.T267 (red) is conserved in all vertebrates from human to fish (**E**). The 11βHSD2 structural model shows that the hydroxyl group side chain of T267 is responsible for anchoring the nicotinamide (NAD) group of the coenzyme in correct position via a direct hydrogen bond (**F**). A mutation to alanine results in a loss of that hydrogen bond and, in turn, misalignment of NAD (**G**).

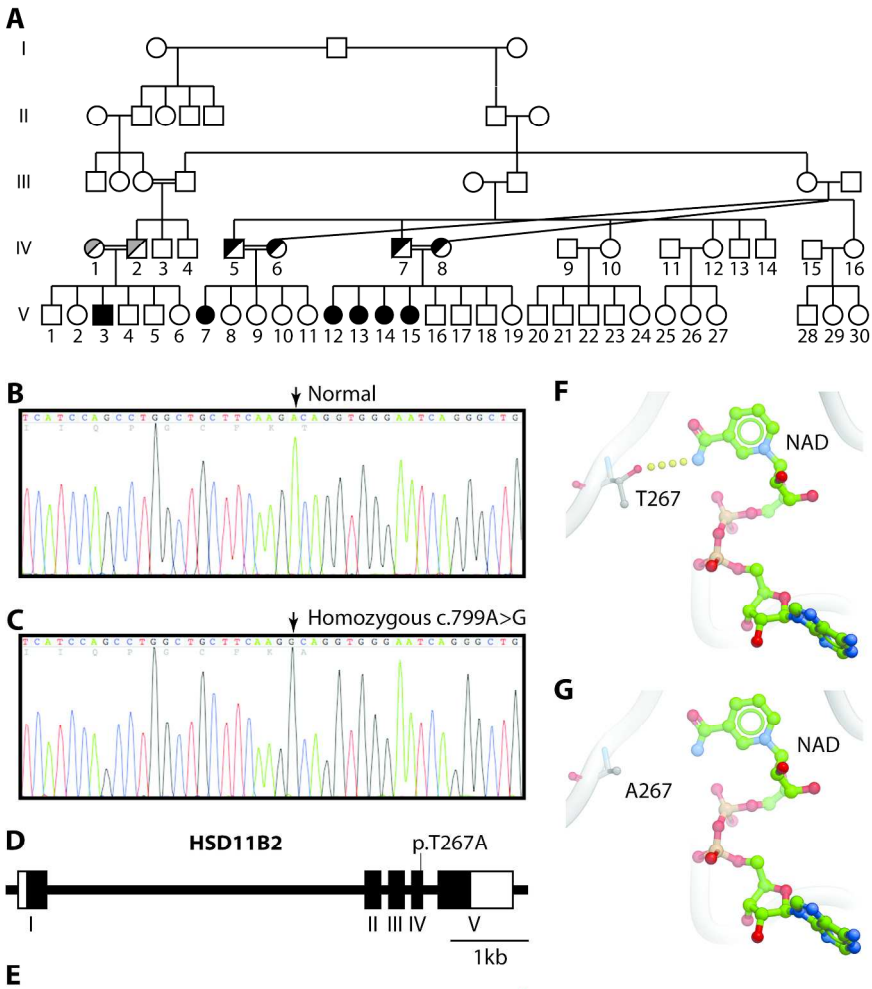

Figure 1
227x314mm (300 x 300 DPI)

Table 1: Clinical and Biochemical Features of 6 AME Patients at Diagnosis and Follow Up

Patient	V-3	V-7	V-12	V-13	V-14	V-15
Baseline characteristics at diagnosis						
Sex	M	F	F	F	F	F
Age at diagnosis (years)	0.83	1	5	1	4	4
Birth wt (kg)	2	2.9	1.9	2.9	2.6	2.3
Presenting symptoms	Polyuria, polydipsia, failure to thrive	BP screening owing to family history	Weakness	Renal calculi	Lethargy	Headaches
BP (mmHg)	146/80	125/74	140/58	120/80	135/87	184/108
Ref. BP (90th %ile for age and sex)	102/53	102/55	109/69	102/55	107/67	107/67
Serum K (mEq/L)	2.1	2.6	3.1	2.8	2.9	2.2
CO_2 (mEq/L)	25	31	25	27	25	24
PRA (ng/mL/h)	0.9	<0.2	0.2	<0.2	<0.2	<0.2
Aldosterone (ng/dL)	1.1	<1.1	2.5	2.5	<1.1	<1.1
Urinary (THF+5αTHF)/THE ratio		31.8		6.1	27	5.9
Characteristics at follow up						
Current age (years)	10	10	17	13	10	6
BP at follow up	130/79	132/88	134/79	134/64	120/84	129/77
Ref. BP (90th %ile for age and sex)	119/77	118/76	127/81	123/79	118/76	110/71
Serum K (mEq/L)	3.5	4.8	3.3	3.5	3.4	3.1
CO_2 (mEq/L)	27	25	21	28	24	26
Medical Therapy						
Spironolactone	75 mg/day	75 mg/day	125 mg/day	75 mg/day	125 mg/day	75 mg/day
Potassium chloride	√	√	√	√	√	√
Amiloride Hydrochloride	5 mg/day	20 mg/day	—	—	—	—
Hydrochlorothiazide	—	—	12.5 mg/day	25 mg/day	12.5 mg/day	25mg/day
Amlodipine	7.5 mg/day	—	—	—	—	—
Atenolol	25 mg/day	—	—	—	—	—
Valsartan	—	15 mg/day	—	—	—	—
Cardiac Complications	Aortic root dilatation	Aortic root dilatation, left ventricular dilation	None	Aortic root dilatation, left ventricular hypertrophy	Aortic root dilatation, left ventricular hypertrophy	None
Renal Complications	Nephrocalcinosis	Nephrocalcinosis	Nephrocalcinosis	None	Nephrocalcinosis	Nephrocalcinosis

Normal range – serum potassium (K): 3.5-4.5 mEq/L; serum bicarbonate (CO_2): < 25 mEq/L; urinary (THF+5αTHF)/THE ratio: 1.

Table 2: *In Silico* Prediction of the Consequence of the p.T267A mutation

Prediction software	Score	Consequence of Mutation
PROVEAN	-4.681	Deleterious
SIFT	0	Damaging
PolyPhen-2	1.000	Probably Damaging
MutPred	0.818	Deleterious – Loss of methylation at K266 (P = 0.0781) – Gain of molecular recognition feature (MoRF) binding (P = 0.1295) – Gain of loop (P = 0.2045) – Gain of catalytic residue at T267 (P = 0.2693) – Gain of ubiquitination at K266 (P = 0.2872)