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Case Report

Reporting a Novel Homozygous Variant in the *HSD11B2* Gene: Reclassifying the Variant Using Sherloc Refinement: A Case Report Study

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Abstract

Background and Aim: Apparent mineralocorticoid excess (AME) is an autosomal recessive disorder resulting from a deficiency of 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) caused by mutations in the *HSD11B2* gene. The mutated gene affects the enzyme activity which results in the rising of cortisol that can be associated with hypokalemia, severe low-renin mineralocorticoid, hypertension, and sodium retention. Few genetic variants, almost 40, have been reported in this gene and more genetic studies are necessary. In this study, we aim to investigate an Iranian patient suspected of being affected by AME.

Methods: A 2.5-year-old girl from consanguineous parents was referred to Ali Asghar Children's Hospital. She was born prematurely with a birth weight of 2.20 kg. Her chief complaint was fever, failure to thrive, polydipsia and polyuria. The initial diagnosis was cystic fibrosis (CF), but the results of the sweat test were normal. Other differential diagnoses were apparent mineralocorticoid excess syndrome type 2, Liddle syndrome, and Bartter syndrome type2. Biochemical tests performed on the patient's free urine showed a high ratio, almost 12, of cortisol to cortisone. Whole exome sequencing (WES) was performed to find out the causative gene.

Conclusion: WES showed a novel homozygous variant in the *11βHSD2* gene. According to the American College of Medical Genetics and Genomics (ACMG) guideline, it was a vindicated uncertain significance (VUS), but using Sherloc refinement suggested that this transversion mutation is most likely to be pathogenic.

Keywords: Apparent mineralocorticoid excess; hsd11b2 variant; AME; Case Report.

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Introduction

Apparent mineral corticoid excess (AME, OMIM #218030) is a rare congenital genetic disorder in the steroid hormone biosynthesis pathway with an autosomal recessive inheritance pattern. This disease is caused by a mutation in the 11-beta-hydroxysteroid dehydrogenase type II (HSD11B2) gene which is located on chromosome16q22.1.

HSD11B2 gene encodes an NAD^+ dependent dehydrogenase enzyme that metabolizes cortisol into cortisone. Mutation in this gene inhibits converting cortisol which is an active metabolite to cortisone, the inactive metabolite (1). Cortisol in high concentration tends to bind to the mineralocorticoid receptor in mineralocorticoid target tissues such as the kidney, colon, and small intestine which results in sodium retention, hypokalemia, hypertension, low renin, hypoaldosteronism and ratios of free cortisol (UFF) to cortisone (UFE) is high (2).

HSD11B2 gene is also known as *AME, HSD11K, AME1, HSD2,* and *SDR9C3*. The gene has 6431bp and contains 5 exons encoding a protein with 405 amino acids and a molecular weight of 45kDa (3). Sequencing alignment also represents that the *HSD11B2* gene has 35% similarity with the *HSD17B2* gene and 14% with the *HSD11B1* gene (4).

The critical region of the protein resides in the C-terminus of 11β-HSD2 (residues 335-339) with a cluster of basic and alcoholic residues that plays an essential role in protein stability that mutation in this region reduces the half-life of the enzyme (5).

Up to now, almost 40 variants have been reported in the *HSD11B2* gene in which most of which are missense and take place in exons 3, 4 and 5 (6, 7).

Case Presentation

The proband was a 2.5-year-old girl from consanguineous parents who were referred to Ali Asghar children's hospital (Figure 1). At the first visit at the age of 6 months, she had a fever, failure to thrive, polydipsia and polyuria. She was born prematurely with h birth weight of 2.20 kg. The initial diagnosis was cystic fibrosis, but the results of the sweat test, which measures the amount of chloride, were normal. Biochemical tests performed on the patients' free urine showed a high ratio (about 12) of cortisol to cortisone (the normal (UFF) / (UFE) ratio is almost 0.5 ± 0.05 but in affected individuals, it is more than 5) (8). Liddle syndrome 1, Bartter syndrome 2, and AME were also suggested as differential diagnosis. She was Canada's date to do whole exome sequencing (WES).

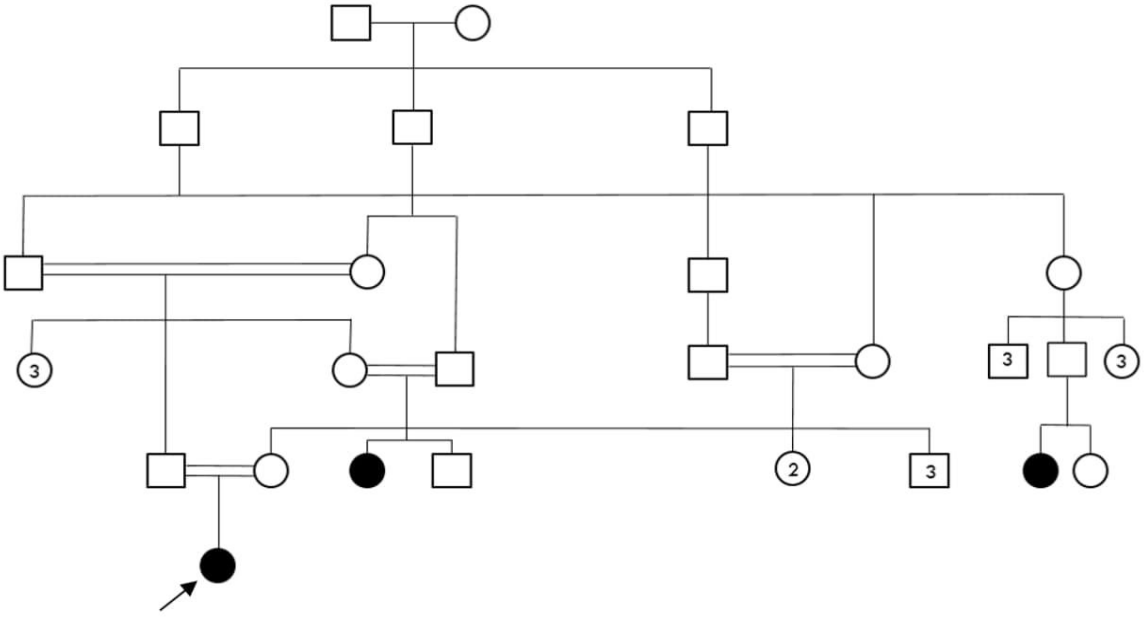

Figure 1. Pedigree of the affected child, which shows multiple consanguineous marriages in her family. Her parents are a first cousins or first cousins once removed. There are other people who have similar symptoms but they were reluctant to cooperate.

Methods

A whole blood sample was taken from the patient and her parents. Written informed consent was obtained from the parents before sampling.

The blood sample was sent to Centogen, Germany for the WES test. Nextera Rapid Capture Exome kit was used and WES was implemented by the Illumina HISeq 2500 systems sequencing platform with an average depth of 100X. Sanger sequencing in the proband and his parents has been done to confirm the identified variants.

The quality of the readings was checked by the fastQC program (9). Reads were aligned to the human genome version hg19 by CLC genomic workbench (version 21.0.3) and local realignment, refined read mapping, removal of duplicate mapped read, and basic variant calling also was done by this program (10). VCF file was exported and variants were annotated with the wANNOVAR online tool (https://wannovar.wglab.org/). Variants in the target gene were evaluated to find rare variants that could potentially cause the disease. All variants' pathogenicity was interpreted according to the joint consensus recommendation of the ACMG guideline for the interpretation of sequence variants (11). WES revealed a rare homozygous variant of c.759 G>C in the exon 4 of the *HSD11B2* gene, a missense mutation

that changes the amino acid tryptophan to cysteine (p.Trp253Cys). Both parents were heterozygous for the variant and the proband was homozygous (Figure 2).

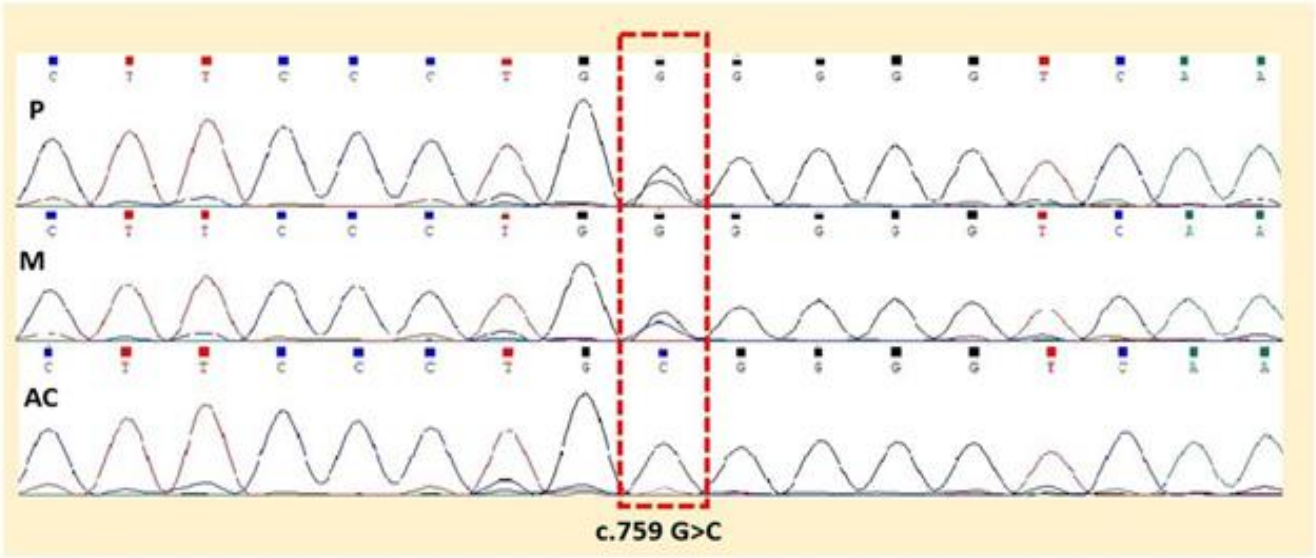

Figure 2. Confirmation of mutation in parents and child by sanger sequencing; parents (P, M) are heterozygous for the variant but the proband (AC) is homozygous.

Based on the ACMG guideline for sequence interpretation, this variant was VUS but according to the Sherloc's scores (a comprehensive refinement of the ACMG – AMP variant classification criteria), it was likely pathogenic with a 4.5p score. The deleterious Annotation of genetic variants using Neural Networks (DANN) score for this variant was 0.9923, Genomic Evolutionary Rate Profiling (GERP) was 5.2899, Combined Annotation Dependent Depletion (CADD) was 28.7, SIFT and PolyPhen-2 predicted this variant as a damaging one, and probably damaging (score of 1.000) respectively (12-17).

HOPE represented that based on conservation scores; this mutation is located near a highly conserved position and the wild-type and mutant amino acids differ in size, the mutant residue is smaller, which might lead to loss of external interactions (18). MutaionTaster and Functional Analysis through Hidden Markov Models (FATHMM) also predicted it to be pathogenic (19, 20).

Discussion and Conclusion

Apparent mineralocorticoid excess is a rare potentially fatal genetic disorder causing severe hypertension in children, pre-and postnatal growth failure, hypokalemia, and low to undetectable levels of aldosterone and renin.

In 1995, a mutation in the *HSD11B2* gene in a consanguineous Iranian family with AME was reported (C to T transition in the first nucleotide of codon 337) (21). In 1998, the first patient with a mild form of AME that revealed a homozygous C to T transition in the second nucleotide of codon 227 (CCG to CTG) was reported which resulted in an HSD11B2 protein with attenuated activity. The patient came from an inbred Mennonite family (22).

Today, with the advancement of high throughput sequencing methods like NGS, it has become easier to study disease-causing mutations which we used in this study.

In the present study, we found the novel mutation of c.759 G>C in the *HSD11B2* gene. We utilized different in silico tools to interpret the effect of this variant on the protein structure and function. DANN is a pathogenicity scoring methodology that is based on deep neural networks and the value range is 0 to 1 this means that variants that get a 1 score, are predicted to be the most damaging (12). Genomic Evolutionary Rate Profiling or GERP is a conservation score that is scored based on the stability, distribution, and intensity of constraint in mammalian genomic sequence. It ranges from -12.3 to 6.17, with 6.17 being the most conserved (14). DANN and GERP scores for this variant were 0.9923 and 5.2899 respectively which indicates the damaging effect of this variant.

Mutation Significance Cutoffs (MSC) of human genes represents the lowest expected clinically/biologically relevant CADD cutoff value for a specific gene. Based on calculating the CADD score (by 95% confidence interval), for the *HSD11B2* gene, this gene has 8.589MSC-CADD_Score and this variant has 28.7 CADD_Score, that shows this variant is most likely leading to a deleterious effect (23, 24). As it was mentioned before, other in-silico tools like mutation taster, SIFT, PolyPhen-2and FATHM predicted this variant to have deleterious effects on the protein.

The Sherloc criteria clarified the ACMG guidelines to comprehensively evaluate variants' pathogenicity based on both functional and clinical evidence. The point score threshold for likely pathogenic is four (4P) and for pathogenic variants is five points (5P). Since this variant has not been reported in the general population and ExAC project, it gets 1.5 points which raises the possibility of pathogenicity; because of clinical observation and functional experiments (biochemical and molecular tests), it gets 2.5 points and according to the variant effect prediction tools, it is awarded 1.5 points towards pathogenicity.

According to the ACMG guideline, this variant is vindicated to be VUS, but getting 4.5 points from Sherlock score indicated it as a likely pathogenic variant.

More severe mutations result in reduced enzyme expression, earlier onset, and more severe symptoms but the low prevalence of AME, fewer than 100 cases, and the relatively small number of mutations have been reported in this gene, have together resulted in difficulty in evaluating genotype-phenotype correlation (25-27).

In this study a novel mutation, c.759 G>C in the exon 4 in this gene was revealed, that based on clinical observation and functional experiments this variant most likely causes the mentioned symptom. Consultation with her family also represents that; there are two other people in their family that have similar symptoms. It has been better if they had been examined as well but they did not tend to contribute to this study (Figure 1).

This study also showed that sometimes ACMG guidelines together with Sherlock scoring can predict the pathogenicity of a variant.

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Conflict of Interest

There is no conflict of interest among authors.

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Not declared.

Ethics

Written informed consent was obtained from the parents of the patient.

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