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CASE REPORT

Case Report: Application of whole exome sequencing for accurate diagnosis of rare syndromes of mineralocorticoid excess [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Syndromes of mineralocorticoid excess (SME) are closely related clinical manifestations occurring within a specific set of diseases. Overlapping clinical manifestations of such syndromes often create a dilemma in accurate diagnosis, which is crucial for disease surveillance and management especially in rare genetic disorders. Here we demonstrate the use of whole exome sequencing (WES) for accurate diagnosis of rare SME and report that p.R337C variation in the *HSD11B2* gene causes progressive apparent mineralocorticoid excess (AME) syndrome in a South Indian family of Mappila origin.

Keywords

whole exome sequencing , mineralocorticoid excess

Open Peer Review

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2. **Shitij Arora** , Albert Einstein College of Medicine, Bronx, USA

Any reports and responses or comments on the article can be found at the end of the article.

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Competing interests: No competing interests were disclosed.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Abbreviations

SME: Syndromes of mineralocorticoid excess; AME: Apparent Mineralocorticoid Excess; CAH:17 α -hydroxylase: Congenital adrenal hyperplasia due to 17 α -hydroxylase deficiency, CAH: 11 β -hydroxylase deficiency: Congenital adrenal hyperplasia due to 11 β -hydroxylase deficiency; CYP17A1: cytochrome P450, family 17, subfamily A, polypeptide 1; CYP11B1: cytochrome P450, family 11, subfamily B, polypeptide 1, HSD11B2: hydroxysteroid (11 β) dehydrogenase 2, *ENaC*: the epithelial sodium channel subunit genes, *WNK1*: WNK lysine deficient protein kinase 1, *WNK4*: WNK lysine deficient protein kinase 1; *KLHL3*: kelch-like family member 3, *CUL3*: cullin 3; *SPAK*: type III secretion system chaperone; *MCR/NR3C2*: Nuclear Receptor Subfamily 3, Group C, Member 2; WES: Whole exome sequencing.

Report

Syndromes of mineralocorticoid excess (SME) are a group of syndromes characterized by an abnormal activation of the amiloride-sensitive sodium channels in the distal tubules of the kidney resulting in an abnormal salt balance. The symptoms are characteristically an abnormality in salt balance (due to an over activation of the channels mediated through the mineralocorticoid receptor), water retention, hypokalemia, low renin levels and hypertension¹⁻³. Based on the clinical presentation, differential diagnosis would include Liddle syndrome, Geller syndrome, Gordon syndrome, Apparent Mineralocorticoid Excess (AME) and other milder variants such as CAH:17 α -hydroxylase and CAH:11 β -hydroxylase deficiency. Mutations in various genes including *CYP17A1*, *CYP11B1*, *HSD11B2*, *ENaC*, *WNK1*, *WNK4*, *KLHL3*, *CUL3*, *SPAK* and *MCR/NR3C2* may result in clinical features that resemble

SME¹⁻³. Due to the large diversity and overlapping clinical features, the accurate diagnosis is highly reliant on the genetic characterization. The need to screen a large number of variants and genes that could cause disease, using conventional approaches such as capillary sequencing is tedious, time consuming and often expensive. Whole exome sequencing (WES) has emerged as an alternative strategy in such clinical settings^{4,5}.

Five siblings of a third degree consanguineous family (Figure 1A) were evaluated at the Department of Nephrology, KMCT Medical College Hospital, Kerala, India. They were aged between 14 and 30 years. All but the youngest of the five siblings were hypertensive. On evaluation, four of the siblings had significant hypokalemia without significant acidosis or alkalosis but with ultrasound evidence of medullary nephrocalcinosis (Figure-1A). The youngest sibling, aged 14 years, did not have hypokalemia, acid base abnormality or renal dysfunction but exhibited overt medullary nephrocalcinosis on ultrasound. Two of the older siblings had concentric left ventricular hypertrophy and renal dysfunction with mild proteinuria. Plasma renin and aldosterone levels were evaluated in the two male siblings and were found to be low (Plasma renin activity 0.14 ng/ml/hr and Plasma Aldosterone 28 pg/ml (sitting upright position) in the elder brother and Plasma renin activity 0.1 ng/ml/hr and Plasma aldosterone 6.5 pg/ml in the younger sibling). Both of them required multiple antihypertensive drugs (Amlodipine 10 mg and Metoprolol Extended Release 50 mg daily) in addition to spironolactone (50 mg daily) for control of blood pressure while the others were well controlled on spironolactone (50 mg daily) alone. There was no past history of similar phenotypes in the parents. A provisional diagnosis of AME was made in

Figure 1. (A) Family pedigree marked with progressive phenotypes. (B) Secondary structure of HSD11B2 marked with major domains and the p.R337C amino acid position. (C) Capillary sequencing chromatogram representation of p.R337C variation in the family; arrow and asterisks marks depict the variation loci and affected individuals respectively.

view of the clinical picture and inheritance pattern. The 24-hour urinary cortisol to cortisone ratio estimation was not done due to unavailability of the test.

Whole blood was obtained from the parents and the affected members after informed consent. 50 ng of the isolated high quality DNA was used to prepare library and exome capture using Nextera Rapid Capture Expanded Exome kit and Sequencing was performed on Illumina HiSeq 2500 sequencer using v3 reagents (Illumina Inc, USA) to generate over 49.48 million paired end reads of 101bp. The variation finding, annotation and prioritisation were performed as previously described⁵ and revealed the presence of homozygous variation p.R337C in *HSD11B2* gene (Figure-1B) annotated to be pathogenic in ClinVar⁶ and predicted to be deleterious using PROVEAN⁷. Human cell line studies have demonstrated that p.R337C mutation leads to the low activity of *HSD11B2* and causes low-renin hypertension thus resulting in AME⁸. The variant was further validated using Sanger sequencing of the amplicons, confirming the diagnosis (Figure-1C).

AME is a rare heterogeneous low renin retention SME disorder that manifests with severe juvenile hypertension, hypokalemic alkalosis, low birth weight, failure to thrive, poor growth, and in many cases nephrocalcinosis caused by homozygous and compound heterozygous mutations in the *HSD11B2* gene. *HSD11B2* oxidises the steroid hormone cortisol to inactive cortisone and mutations in this gene result in a high circulating level of cortisol, and further illegitimate activation of the mineralocorticoid receptor, outcompeting aldosterone and causing activation of the downstream pathways and a clinical presentation of AME symptoms.

In summary, we used WES to characterize and diagnose a family with an extremely rare clinical presentation. p.R337C loci of *11HSDB2* is widely reported to be associated with AME across the world and has been reported in Zoroastrians from India and Iran (Compound heterozygous for p.R337H and Δp.Y338, age of onset from 8 months), in Persian (p.R337C, age of onset from 4 years) and in Japanese (p.R337H and Δp.Y338, age of onset from 2 years) populations^{9,10}. The age of onset for the disease manifestation due to p.R337C mutation has been previously described to be as early as 4 years. Our family, though having the p.R337C mutation, did not present with clinical symptoms in early childhood but exhibits the progressive AME phenotypes with increasing age (Figure 1A) with the older individuals presenting more severe clinical manifestations including renal impairment. Patients with identical homozygous mutations from different families have

been described to have varying degrees of severity in clinical and biochemical features^{4,9}. In fact, homozygous mutations in *HSD11B2* have been described where the patient has only mild low renin hypertension without other features of AME¹⁰. Early diagnosis and prompt institution of salt restriction and spironolactone in these patients can prevent secondary organ damage. Our report also serves to highlight the utility of WES as a tool for diagnosing rare genetic diseases even where biochemical characterization is unavailable.

Patient consent

Written informed consent for publication of these data were obtained from the patients.

Data availability

The raw whole exome sequence are available at the NCBI Sequence Read Archive (<http://www.ncbi.nlm.nih.gov/sra>), accession number SRR3546815.

Author contributions

Clinical workup: RN; WES data generation, analysis, and validation: SKV, RJ, AV, VD; supervision and mentorship: VS and SS; All authors contributed important intellectual contents during manuscript drafting and accepts accountability for the overall work.

Competing interests

No competing interests were disclosed.

Grant information

Authors acknowledge funding from the Council of Scientific and Industrial Research (CSIR), India through Grant No. BSC0212 (Wellness genomics project) granted to SS and VS.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgement

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Version 1

Reviewer Report 09 January 2017

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Shitij Arora

Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY, USA

A very interesting study. It highlights the increasing use of WES in diagnosing rare diseases. The authors confirm the findings of ES with Sangers and sequence both the parent and the proband. The manuscript needs editing and rearrangement of text. The paragraph on SME and AME needs/can probably be put together as the introduction. Minor grammar corrections.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 03 October 2016

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Parag Tamhankar

Genetic Research Center, National Institute For Research In Reproductive Health, Mumbai, Maharashtra, India

Please could you add an introduction about syndrome of apparent mineralocorticoid excess, for example;

Syndrome of apparent mineralocorticoid excess (MIM#218030) is a rare cause of juvenile hypertension occurring due to homozygous or compound heterozygous mutations in HSD11B2

gene (MIM*614232). The gene encodes an NAD⁺-dependent enzyme viz corticosteroid 11- β -dehydrogenase isozyme 2, which is expressed in aldosterone-selective epithelial tissues such as the kidney, colon, salivary and sweat glands. This enzyme is responsible for oxidizes the glucocorticoid cortisol to the inactive metabolite cortisone. This protective mechanism is necessary because cortisol circulates at 100-1000-fold higher concentrations than aldosterone, and binds with equal affinity to the mineralocorticoid receptor, thereby out-competing aldosterone in cells that do not produce HSD11B2. In patients with SME the enzyme deficiency allows mineralocorticoid receptors to be occupied by cortisol leading to hypertension.

I believe that more details on clinical features should be provided, such as:

- clinical presentation and history. Draw three generation pedigree.
- detailed anthropometry, examination findings of the patients. For eg. was there any low birth weight, short stature, polydipsia, polyuria?
- what were the baseline blood pressure levels in the patients when hypertension was detected?
- was fundus examination done to detect retinopathy?
- were the parents hypertensive?
- give the detailed serum electrolyte levels, detailed 2ECHO report. Give normal ranges of plasma renin, aldosterone levels.
- clinical photos if relevant
- or uploading ultrasound kidney scans

Mutation: Please give the nucleotide change, exon number of the mutation identified in the patients with the correct Ensembl transcript ID. Please specify as the mechanism of loss of function of the mutation in patients with mutations at position p.335-339 is reduced protein stability due to rapid protein degradation at the proteasome, rather than reduced catalysis.

Grammar changes: I would begin the words aldosterone, amlodipine, metoprolol with lower case letters when in the midst of a sentence.

Typo: I think you should correct the following sentence - p.R337 loci of 11HSD2 to- "The p.R337 residue of 11- β -dehydrogenase isozyme 2 enzyme is a recognised mutation site. The mutation p.R337C has been previously identified in a family from Iran with three affected children."

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 30 Jul 2017

Vinod Scaria, CSIR Institute of Genomics and Integrative Biology (CSIR-IGIB), Delhi, India

Please could you add an introduction about syndrome of apparent mineralocorticoid excess, for example;

Syndrome of apparent mineralocorticoid excess (MIM#218030) is a rare cause of juvenile hypertension occurring due to homozygous or compound heterozygous mutations in HSD11B2 gene (MIM*614232). The gene encodes an NAD⁺-dependent enzyme viz corticosteroid 11- β -dehydrogenase isozyme 2, which is expressed in aldosterone-selective epithelial tissues such as the kidney, colon, salivary and sweat glands. This enzyme is responsible for oxidizes the glucocorticoid cortisol to the inactive metabolite cortisone. This protective mechanism is necessary because cortisol circulates at 100-1000-fold higher concentrations than aldosterone, and binds with equal affinity to the mineralocorticoid receptor, thereby out-competing aldosterone in cells that do not produce HSD11B2. In patients with SME the enzyme deficiency allows mineralocorticoid receptors to be occupied by cortisol leading to hypertension.

We thank reviewers for the comments. Clarifications are as follows:

Clarification: The suggested changes in introduction section are made.

1. Clinical presentation and history. Draw three generation pedigree.

Clarification 1: The suggestions have been incorporated. A two generation pedigree is provided. Information about the 3rd generation was not available.

2. detailed anthropometry, examination findings of the patients. For eg. was there any low birth weight, short stature, polydipsia, polyuria?

Clarification 2: There was no h/o low birth weight, polydipsia or polyuria. All developmental milestones were normally attained in all siblings.

3. what were the baseline blood pressure levels in the patients when hypertension was detected?

Clarification 3: Included in the case history section

4. was fundus examination done to detect retinopathy?

Clarification 4: No

5. were the parents hypertensive?

Clarification 5: No

6. give the detailed serum electrolyte levels, detailed 2ECHO report. Give normal ranges of plasma renin, aldosterone levels.

Clarification 6: Included in the case history section.

7. clinical photos if relevant

Clarification 7: None

8. or uploading ultrasound kidney scans

Clarification 8: The ultrasound scan is attached separately. It may not be of great quality, however shows medullary nephrocalcinosis (see this [link](#)).

9. Mutation: Please give the nucleotide change, exon number of the mutation

identified in the patients with the correct Ensembl transcript ID. Please specify as the mechanism of loss of function of the mutation in patients with mutations at position p.335-339 is reduced protein stability due to rapid protein degradation at the proteasome, rather than reduced catalysis.

Clarification 9: Suggestions have been incorporated.

10. Grammar changes: I would begin the words aldosterone, amlodipine, metoprolol with lower case letters when in the midst of a sentence.

Clarification 10: Suggestions have been incorporated.

11. Typo: I think you should correct the following sentence - p.R337 loci of 11HSD2 to- "The p.R337 residue of 11- β -dehydrogenase isozyme 2 enzyme is a recognised mutation site. The mutation p.R337C has been previously identified in a family from Iran with three affected children."

Clarification 11: Suggestions have been incorporated.

Competing Interests: No competing interests were disclosed.

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