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A novel homozygous stop gain mutation in *SLC12A3* gene cause Gitelman syndrome in Saudi consanguineous family

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ABSTRACT. Gitelman syndrome (GS) is a genetic disorder that affects kidney and causes an imbalance of charged atoms (ions) in the body, including ions of potassium, magnesium, and calcium. GS is characterized by hypokalemia and metabolic alkalosis. GS is a rare autosomal recessive renal tubulopathy disease caused by loss-of-function mutations in the *SLC12A3* gene. Objective of the present study is to find the genetic causes involved in a consanguineous Saudi family. Whole exome sequencing was done to find out the genetic mutation in the affected members of the family and the putative mutation was further validated by subsequent Sanger sequencing in other member of the family. We identified a novel homozygous mutation c.2686C>T as a result in p.Arg896* stop gained in exon 23 of *SLC12A3* gene. The mutation was ruled out in 100 unrelated healthy controls. A novel homozygous stop gain mutation detected in this study has not yet been reported as pathogenic in literature or variant databases. Functional prediction of this mutation was performed using various online *in silico* prediction tools and all predicted it be disease causing. In conclusion, the here detected homozygous *SLC12A3* mutation in the *SLC12A3* gene as a result a truncated protein produced with a premature stop gain would expected to lead to functional loss to cause GS in Saudi family. This study will add more knowledge about GS in Arab populations' database of genetic disorders and will help in the genetic counselling of the families in Saudi Arabia.

Key words: *SLC12A3* gene; Gitelman syndrome; Exome sequencing; Saudi family

INTRODUCTION

Gitelman syndrome (GS; OMIM # 263800) is also known as familial hypokalemia-hypomagnesemia, is a rare genetic disorder in which there is a specific defect in kidney function and is characterized mainly by metabolic alkalosis and hypokalemia (Wang et al., 2017). The phenotype was first described by Gitelman and his colleagues in 1966 (Gitelman et al., 1966). It is an autosomal recessive renal tubular disease, also known as familial hypokalemia-hypomagnesemia (Jie-wei et al., 2017). The chief biochemical features of this syndrome are hypokalemia, hypomagnesemia, low urinary calcium, and high aldosterone levels (Miao et al., 2016). Often in many cases it has a long asymptomatic period and frequently discovered incidentally after adolescence (Kim et al., 2016). The prevalence of GS is around 1 in 40000 Caucasians (Grillone et al., 2016). GS is caused by pathogenic mutation in the solute carrier family 12-member 3 (*SLC12A3*) gene that encodes thiazide-sensitive sodium-chloride cotransporter and to date more than 400 deleterious mutations have been identified in *SLC12A3* gene so far (Nozu et al., 2014). Most of the mutations identified are missense and nonsense mutations, but frameshift, intronic and splice-site mutations have also been described (Subasinghe et al., 2017). The functional loss of NCC channel leads to lower sodium re-absorption and subsequently secondary volume depletion and as a result the hypovolemia stimulates sodium re-absorption at the expense of increased potassium & hydrogen secretion that end up with hypokalemia and metabolic alkalosis (Gil-Pena et al., 2017).

Besides the Gitelman syndrome, many other clinical features like hyperthyroidism and primary hyperaldosteronism may also occur with hypokalemia and episodic muscular weakness (Zhang et al., 2016). Hyperthyroidism leads to muscular weakness which is associated with electrolyte disturbances and shows signs of throtoxicosis, whereas the muscular weakness of hyperthyroidism would also lead to hypertension, hypokalemia and increased aldosterone levels (Oguz et al., 2014). The current study was done to find the genetic causes of GS involved in a consanguineous Saudi family.

MATERIALS AND METHODS

We have recruited a consanguineous family with two affected individuals with Gentleman's syndrome and 100 unrelated control samples. The present study is approved from local ethical committee of Center of Excellence in Genomic Medicine Research. The study was conducted according to the guidelines of Helsinki Declaration. Blood samples were taken from affected and control participants and DNA was extracted for molecular analysis. Pedigree of family was made by interviewing the parents of the affected individuals. The detailed pedigree was shown in Figure 1.

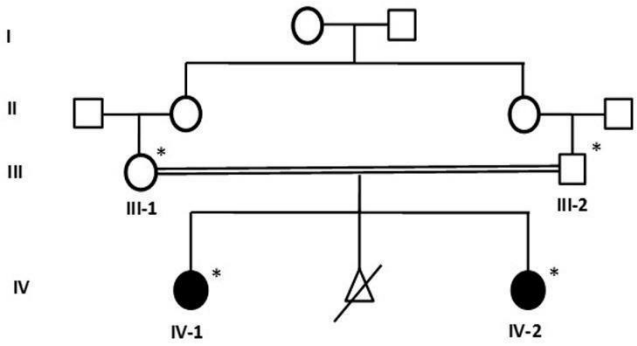

Figure 1. A pedigree of the family from Saudi Arabia showing consanguinity and the disease phenotype segregating in an autosomal recessive manner. The samples available for genetic testing were marked with asterisks.

Patient 1 (IV-1)

Patient 1 (IV-1) is a 12-year-old girl. She presented with developmental delay and dysmorphic features. She has difficulty and delayed in speech. She also seen delayed in walking (started walking after 6 years of age). Brain size is normal (50 cm head circumference). She also have tumor in the chest cage.

Patient 2 (IV-2)

Patient 2 (IV-2) is a 9-year-old girl. She had developmental delay and dysmorphic features. She also has difficulty in speech. She also has delayed in walking (started walking after 4 years of age). The head size is normal (50 cm head circumference).

Complete blood profile

Complete blood profile of both affected children (IV-1 and IV-2) was done from Saudi German Hospital Group in Jeddah. Clinical data and laboratory test results for the patients with GS are shown in Table 1.

Table 1: Important Biochemical characteristics in affected individuals of Gitelman's syndrome

Test	Patient 1 (IV-1)	Patient 2 (IV-2)	Reference Value
Calcium	10.3	10.3	8.6 – 10.3 mg/dl
24 h urine for CA	30	20	100 – 320 mg/24 h
Potassium	2.8	3.4	3.5 - 5.3 mmol/l
24 h urine for K	31	40	25 – 125 mmol/24 h
Magnesium	1.4	1.4	1.7 – 2.5 mg/dl
Sodium	142	141	135 – 145 mmol/l
Bicarbonate	23	18	22 – 30 mmol/l
Renin Level	184	368	3 – 24 pg/ml
Aldosterone	725	558	Early Morning, supine 20 – 180. Upright, 2 Hours 30 – 400 pg.mL

Whole exome sequencing

To identify the underlying pathogenic mutation behind this disease phenotype we did whole exome sequencing using Illumina HiSeq 2000/2500. Agilent Sure Select Target Enrichment Kit was used for samples preparation according to manufacture guide (Capture kit, SureSelect_v6). The libraries were sequenced with Illumina HiSeq 2000/2500 sequencer. These variants were filtered based on frequency, quality, protein effect, genomic position, pathogenicity and previous associations with the phenotype. We used different bioinformatics analyses to find out causative variant co-segregating with the *SLC12A3* phenotype in an autosomal recessive fashion. Raw data FASTQ files were aligned using the BWA Aligner (http://bio-bwa.sourceforge.net/) aligned to hg19 (NCBI build GRCh37) using SeqMan NGen 12 (DNASTAR). Copy number variation and insertion/deletion (InDel) detection were performed using SAMTOOLS (http://samtools.sourceforge.net/). The sequence reads were mapped against the hg19 (NCBI build GRCh37) human reference sequence (http://genome.ucsc.edu/) and compared against sequences in the dbSNP (http://www.ncbi.nlm. nih.gov/snp/) and the 1000 Genomes Project databases (http://www. 1000genomes.org/data). Candidate variants were expected to follow an autosomal recessive inheritance pattern (homozygous or compound heterozygous state) given the reported positive family history. Homozygous variants were of primary interest based on the positive consanguinity. A rare homozygous variant within the protein coding regions of all genes one of the symptoms provided one plausible candidate in the *SLC12A3* gene.

Sanger sequencing

SLC12A3 gene was amplified by polymerase chain reaction (PCR). Purified PCR products were Sanger sequenced by the Big Dye terminator method to detect any mutation. To confirm the mutation in patients and family, we did Sanger sequencing using (ABI 3700). Further to confirm the mutation as pathogenic, we also sequenced this DNA variant in unrelated 100 control people.

In silico functional prediction of mutations

Functional prediction of the current mutation was performed using the online *in silico* prediction software packages like PhyloP (https://www.ncbi. nlm.nih.gov/pmc/articles/ PMC4702902/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) PROVEAN/SIFT (http://provean.jcvi.org/) and PhastCons (http://compgen.cshl.edu/phast/).

RESULTS

Blood profile analysis

The detailed laboratory test results for the patients IV-1 and IV-2 with GS was done and important biochemical parameters measured in this study are summarized in Table 1. The both patients IV-1 and IV-2 were suffering from hypokalemia, low urinary calcium (24 h) and high aldosterone levels. These all parameters confirmed the presence of GS biochemically.

Whole exome sequencing

Whole exome sequencing was done using Illumina HiSeq 2000/2500. The variant call format (VCF) file was as a result contains 76330 variants. These variants are filtered based on genomic position, quality, protein effect, frequency, pathogenicity and previous associations with the phenotype. On average, 91 % of bases had a pared score>20; the total number of bases in the reads was 11.2 Gb; and mean depth of the target region was 71. The resulting VCF file contains 76330 variants those met quality control criteria (minimum Q call >20, minimum depth >10) of these, 10,420 were silent, missense, nonsense, or splice variants. After filtering those variants, we identify a novel homozygous stop gained mutation in exon 23 of the *SLC12A3* where c.2686C>T as a result in p. Arg896* as shown in Figure 1.

In silico functional prediction of mutations

Functional prediction of mutations was performed using the online *in silico* prediction software package PhyloP, PolyPhen-2, PROVEAN, and PhastCons as shown in Table 2. All the software's predicted this mutation to be disease causing.

Table 2: Results of *insilco* tools used for prediction of pathogenicity of missense variant

S. No	Online tools	Pathogenicity Score
1	Mutation Tester (http://www. mutationtaster.org/)	1.0
2	1000 genomes (http://www. internationalgenome.org/)	0.0
3	PhyloP (https://www.ncbi. nlm.nih.gov/pmc/articles/ PMC4702902/)	-0.04
4	GERP++ (http://mendel. stanford.edu/SidowLab/ downloads/gerp/)	3.92
5	ExAC (http://exac.broadinstitute.org/)	0.0
6	PhastCons (http://compgen.cshl.edu/phast/)	1.0

Sanger sequencing

Our Sanger sequencing results showed that *SLC12A3* mutation in the exon 23 of *SLC12A3* gene was further confirmed the results of exome sequencing. The mutation was also ruled out in 100 unrelated healthy controls with same ethnic background as shown in Figure 2. The affected members were homozygous while the parents of the affected members were heterozygous, which also confirms the autosomal recessive mode of inheritance.

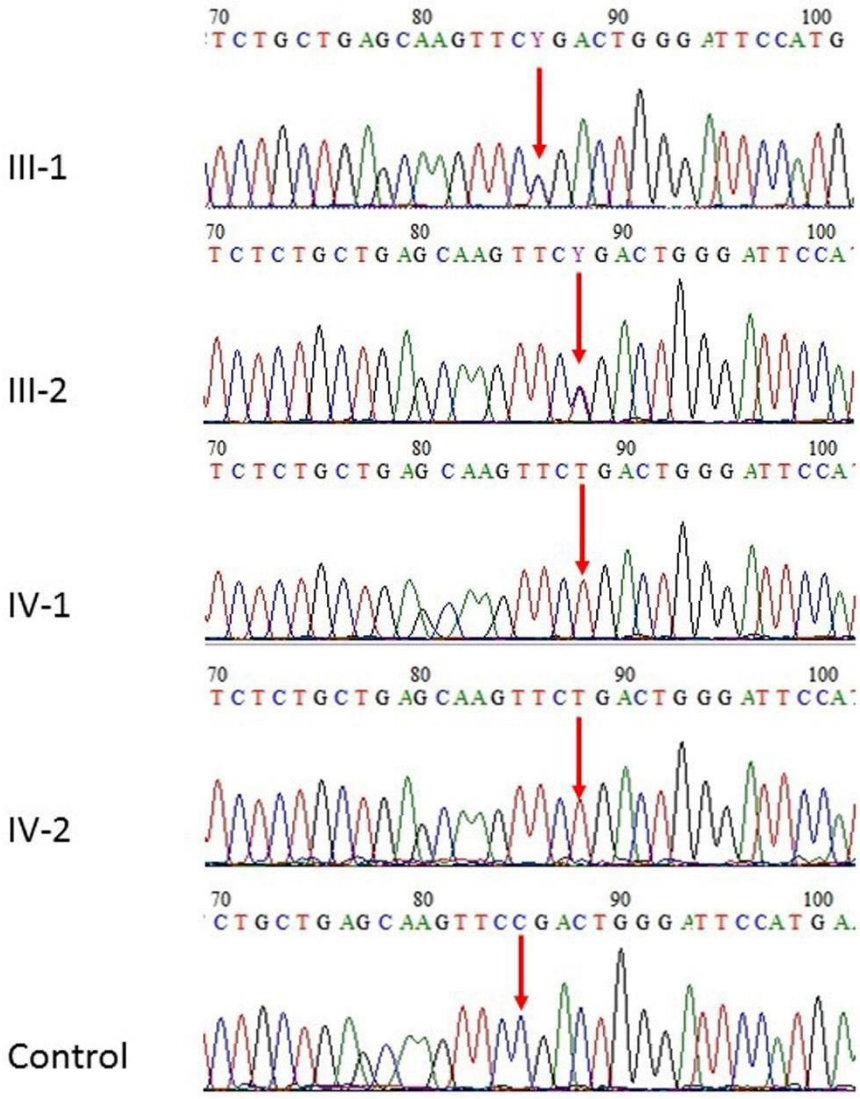

Figure 2: Sanger sequence analysis: (III-1 and III-2) are normal parents showing heterozygosity while (IV-1 and IV-2) are affected children showing only homozygous T in exon 23 of the *SLC12A*367 gene.

DISCUSSION AND CONCLUSION

This gene encodes a renal thiazide-sensitive sodium-chloride cotransporter that is important for electrolyte homeostasis. This cotransporter mediates sodium and chloride reabsorption in the distal convoluted tubule. Mutations in this gene cause Gitelman syndrome, a disease similar to Bartter's syndrome, that is characterized by hypokalemia alkalosis combined with hypomagnesemia, low urinary calcium, and increased renin activity associated with normal blood pressure (Geven et al. 1994). This cotransporter is the target for thiazide diuretics that are used for treating high blood pressure. Multiple transcript variants encoding different isoforms have been found for this gene.

A nonsense variant was detected in homozygous state in the *SLC12A3* gene. Based on the homozygous state of the variant, this implicates that this patient suffers from Gitelman syndrome, also known as familial hypokalemia hypomagnesemia, an autosomal recessive condition. *SLC12A3* encodes a sodium-chloride cotransporter that is expressed in the kidney. Recessive mutations in *SLC12A3* are the cause of GS, a kidney disorder with onset usually after 6 years of age, that causes an imbalance of charged atoms, including potassium, magnesium, and calcium, in the body. This can cause muscle weakness, tingling sensations in the skin, fatigue, low blood pressure and joint conditions. The disease is generally mild, and some patients even remain without symptoms. Sustaining a potassium and sodium rich diet supplemented with magnesium, in combination with regular monitoring generally provides an excellent prognosis (Glaudemans et al. 2012). The detected variant in this study causes the introduction of a premature stop at codon 896 (in exon 23 out of 26 in totals).

This mutation has not been described in homozygous state previously. It was however identified in heterozygous state in a patient with Gitelman syndrome, in whom no second SLC13A2 mutation could be identified (Ji et al. 2008). In addition, it was detected in another patient, in compound heterozygous state with a pathogenic missense mutation (Vargas-Poussou et al. 2011). There are seemingly no functional studies on this variant yet, although it is expected to lead to a loss of function based on the nonsense effect. To date more than 400 deleterious mutations have been identified in *SLC12A3* gene so far (Nozu et al., 2014). The majority of pathogenic mutations in SLC13A2 are missense mutations, but nonsense substitutions, but frameshift, splice-site defects and gene rearrangements have also been described. Nonsense mutations, located both upstream and downstream of the here detected variant, identified in patients with Gitelman syndrome include E112X, K497X, Y513X, R977X, R1018X (Riveira-Munoz et al. 2007; Vargas-Poussou et al. 2011).

Regular clinical evaluation with respect to this genetic diagnosis of GS would be recommended. Patients are recommended to follow a high-sodium and potassium diet. The recommended therapy for patients with symptoms of GS includes oral supplementation of potassium and magnesium.

Currently the physicians treat this disease with prescribing combination of medications including potassium supplement, magnesium supplement, aldosterone antagonists and prostaglandin synthetase. As this mutation is inherited as autosomal recessive form, therefore, all the family members were advised to undergo genetic testing to know the carrier state. So they can take precautions when marrying within family.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

AUTHOR’S CONTRIBUTIONS

M.I.N conceived and designed the project. M.I.N, O.Y.M and A.A.A performed experiments and confirmed these results. M.I.N, M.R, P.N.P, G.K and A.G.C. analyzed and interpreted the data. M.I.N, M.R. and M.A.J provided and interpreted phenotypic details for the patients. M.H.A. and A.G.C. advised on the study design and writing of the manuscript. M.R and M.I.N wrote the manuscript.

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