Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2017, 6(6): 144-149

Whole-Exome Sequencing Reveals an M268T Mutation in the Angiotensinogen Gene of Four Unrelated Renal Failure Patients from the Hail Region of Saudi Arabia

Nuglozeh Edem[1]*, Fazaludeen F Mohammad[1,2], Mbikay Majambu[3,4], Hussain A Gadelkarim[1], Al-Hazimi Awdah[1] and Ashankyty Ibraheem[1,2]

[1] *Molecular and Diagnostics Personalized Therapeutic Unit, University of Hail School of Medicine, Hail, Kingdom of Saudi Arabia*

[2] *University of Hail, College of Applied Medical Sciences, Hail, Kingdom of Saudi Arabia*

[3] *Chronic Disease Program, Ottawa Hospital Research Institute, University of Ottawa, Ottawa, Ontario, Canada*

[4] *Functional Endoproteolysis Laboratory, Clinical Research Institute of Montreal, Montreal, Quebec, Canada*

**Corresponding e-mail: a.nuglozeh@uoh.edu.sa*

ABSTRACT

Aims: *Chronic kidney disease and renal failure are major health concerns in Saudi Arabia, especially in the Hail region. These diseases are commonly coupled with hypertension and hypercholesterolemia. Genetic factors strongly contribute to them. This study was undertaken in an effort to identify genetic variations that might contribute to renal failure in the Hail population.* ***Methods:*** *We performed Whole Exome Sequencing (WES) on genomic DNA of four unrelated Hail patients afflicted by renal failure. Exonic variations located in genes known to be implicated the disease were selected and validated by Sanger sequencing.* ***Results:*** *In all four patients, we identified a c.C803T transition in exon 2 of the angiotensinogen gene (AGT), causing an M268T amino acid substitution in the protein. All four patients were homozygous for T268 allele.* ***Conclusion:*** *AGT is implicated in blood pressure regulation; it contributes to normal kidney function. The M268T AGT mutation has been known to be associated with hypertension and kidney disease. Whether or not it is a determinant of the kidney failure of our patients could be established if family studies show segregation of the mutant allele with the disease. This study illustrates how WES can be used to identify candidate genetic variations for inherited kidney diseases.*

Keywords: Whole-exome sequencing, Renal failure, Angiotensinogen, Saudi Arabia

INTRODUCTION

Renal diseases are a congruence of many vascular and metabolic diseases, particularly hypertension, diabetes and obesity. They cover a large spectrum of diseases ranging from relatively common to rare, and from benign disorders to high-morbidity/high-mortality disorders [1]. There are no definite epidemiological data on the prevalence of chronic kidney disease (CKD) and its risk factors in Saudi Arabia. In a pilot community-based screening of young Saudi conducted in Riyadh in 2010, the prevalence was estimated at 5.7% [2]. In the Hail region of the kingdom, a 75% overall prevalence of risk factors for CDK was observed in a cross-sectional survey of 5000 Saudis. The factors included cardiovascular disease, use of non-steroidal anti-inflammatory drugs and cigarette smoking [3].

End-stage renal disease (ESRD) is the ultimate complication of CKD; it is managed by dialysis or renal transplantation. With 28 million inhabitants (including 5.5 million expatriates), Saudi Arabia has an incidence of dialysis of 140 new cases per million population (PMP) [4], comparable to that in India (163 PMP) [5], but lower than that in the United States (360 PMP) [6], or Europe (585 PMP) [7].

Only 10% of CDK cases are known to due to Genetic Renal Disease (GRD); a large proportion is yet to be identified as such; for nearly half of those identified as GRD, the gene implicated remains unknown [8,9]. A typical example of GRD is Autosomal Dominant Polycystic Kidney Disease (ADPKD), a common, potentially lethal disease [10].

In recent years, enormous progress has been achieved in the field of molecular genetics and genomics. Next-Generation Sequencing (NGS) collectively describes genetic technologies that enable rapid and cost-effective sequencing of large amounts of DNA. NGS facilitates rapid identification of disease-causing genetic variants in patients with a suspected Mendelian disorder. Because of its reduced cost and time, Whole Exome Sequencing (WES) has become the preferred initial method for disease gene identification in small pedigrees, replacing previous methods, such as linkage analysis and association studies. In nephrology, NGS has identified disease-causing mutations responsible for atypical haemolytic uremic syndrome [11,12], steroid-resistant nephrotic syndrome [13-15] and nephronophthisis [16-25].

Here, we used WES by the Ion Torrent Technology with its automated Bioinformatics platform to identify causative genes of GRD in the Saudi population. We sequenced the exome of four unrelated ESRD patients, analyzed the data for variations or mutations of genes known to be associated with CDK risk factors, and validated the identified mutations by Sanger sequencing.

MATERIALS AND METHODS

Genomic library construction and ion proton sequencing

Genomic DNA (gDNA, 50-100 ng) was used to prepare the genomic library which was amplified by PCR using the Ion ampliseq exome kit (Ion torrent). The PCR products were pooled, blunted, ligated to adaptor and purified using Agencourt® AMPure® XP Reagent from (Ion torrent). Validation of the library was done on an Agilent Bio-Analyzer to assess fragment distribution. The analyzed product was quantitated using a Qubit fluorometer (Invitrogen).

Each molecule of the validated library was amplified on an Ion OneTouch™ by emulsion PCR methodology, to recover template-positive Ion Sphere Particles (ISPs) harboring the desired fragment. The enriched PCR product was cleaned, loaded on Ion PITM Chip V2 and sequenced on an Ion proton platform.

Bioinformatics analysis

Bioinformatics tools used to analyze the sequence for variants of interest are summarized in Figure 1. Briefly, they included, successively, signal processing, base calling, base recalibration and reference alignment; mapping of raw reads to the human genome reference assembly (GRCh37/hg19); variant calling and filtering for minimum allele frequency, quality and coverage as well as maximum length of homopolymer and strand bias; quality scoring (e.g. FreeBayes); and, finally, annotation of variants for known or putative functional importance and disease association.

Variant sequence validation

The selected variants were validated by Sanger sequencing.

RESULTS

The four ESRD patients were identified by numbers 35, 4303, 5344 and 5346. Their anthropomorphic, clinical, and biochemical parameters are described in Table 1. The normal glomerular filtration rate (GFR) as assessed via cystatin (GRFcy) and creatinine clearance (GFRcr) is above 90 mL/min/1.73 m^2; in the four patients, it ranged from 17 to 25 mL/min/1.73 m^2, representing a 72% to 79% decrease in renal function. The patients showed an accumulation of creatinine in the bloodstream (188-261 µmol/L compared to normal value of 60-110 µmol/L) and, most dramatically, of uric acid (104 - 443 µmol/L compared to the normal range of 2-8 µmol/L). The alteration of the above parameters represents a big deterioration in the renal function characteristic of ESRD.

WES was applied on genomic DNA from the four patients. The number of variants called from the sequencing data are described in Table 2. After stringent filtering and annotations using the different bioinformatics algorithms (Figure 1), we identified the M268T mutation caused by a c.C803T transition in exon 2 of the angiotensinogen gene (AGT) (Figure 2) as potentially causative of GRD in the four patients (Table 2). They were all homozygous for T268 allele.

Table 1 Anthropomorphic and biochemical parameters of the patients [a]

Parameters [b]	Units	Normal Range	Patient ID			
			35	4303	5344	5346
Gender	F/M	-	F	F	M	F
Age	Years	-	72	56	61	63
Glucose	mmol/L	3.3-5.6	10.8	13	5	9

GFRcy	**mL/ min/1.73m²**	**≥ 90**	**17**	**24**	**22**	**17**
GFRcr	**mL/ min/1.73m²**	**≥ 90**	**18**	**25**	**22**	**17**
Creatinine	**µmol/L**	**60-110**	**213**	**261.7**	**251.8**	**188**
Uric acid	**mmol/L**	**2.0-8.5**	**443.56**	**131**	**124.3**	**103.9**
Blood pressure	**mmHg**	**N/A**	**THT***	**THT***	**THT***	**THT***
Urea	**mmol/L**	**2-7**	**9.3**	**7.18**	**9.42**	**5.89**
K^+	mmol/L	3.5-5.1	4.8	3.76	4.1	4.7
Na^+	mmol/L	135-147	134	136	135.1	132.6
Cl^-	mmol/L	95-110	104	100	99	99
Ca^{2+}	mmol/L	2.1-2.8	2.14	2.45	2.54	2.31

[a] The bold highlighted are parameters most symptomatic of kidney failure. [b] Abbreviations: GFR, glomerular filtration rate as assessed via cystatin (GFRcy) and creatinine clearance (GFRcr); F/M: female/male; THT*: Treated Hypertension

Table 2 Variant calling, filtering and validation leading to the identification of the AGT missense mutation

Samples ID	Torrent variant calling	Free Bayes	Disease Association Filter	Sanger Validation	Validated SNPs	Gene	Mutation	Amino Acid Change
35	51019	17949	35	7	1	AGT	c.803T>C	Met268Thr
4302	42856	24129	7	7	1	AGT	c.803T>C	Met268Thr
5344	292293	23896	7	7	1	AGT	c.803T>C	Met268Thr
5346	16971	19258	7	7	1	AGT	c.803T>C	Met268Thr

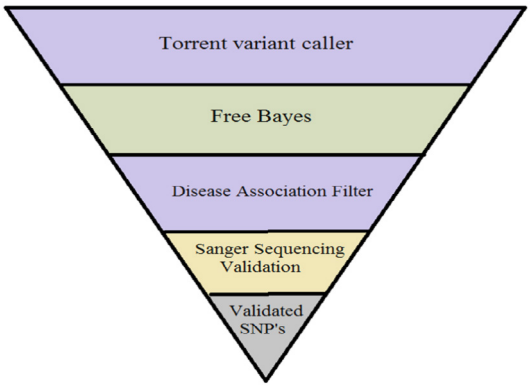

Figure 1 Schematics of the steps illustrating variant reduction protocol for the samples

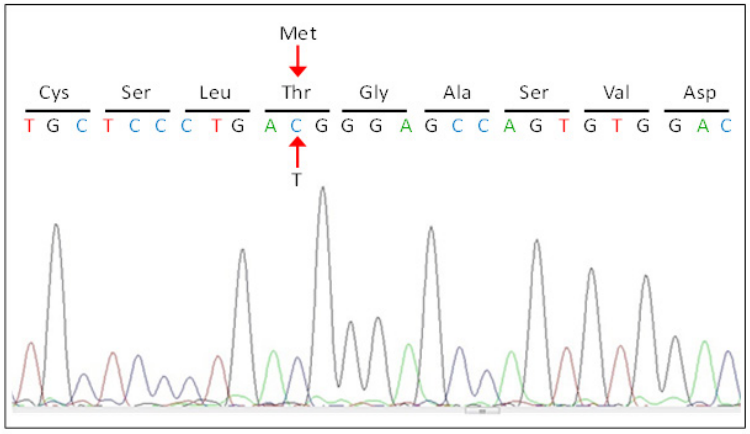

Figure 2. Sanger sequencing result characterized by conversion of Methionine to Threonine

Figure 2 shows Sanger sequencing the AGT gene. Above the nucleotide sequence profile are shown the codon and the amino acid sequences. The T/C nucleotide transition and Met/Thr amino acid substitution are indicated by upwards and downwards arrows, respectively.

DISCUSSION

In this WES study, we have identified an AGT gene missense mutation as a potential cause of ESRD in four unrelated residents of the Hail region of Saudi Arabia. The AGT gene is localized on the chromosome 1 at the position 1q42.2. It codes for the angiotensinogen protein, a crucial player of the renin-angiotensin-aldosterone system (RAAS), which regulates blood pressure and the balance of fluids and salts in the body. In a cascade of proteolytic events, angiotensinogen is converted by renin to angiotensin I, which is in turn converted to angiotensin II (AngII) via angiotensin converting enzyme (ACE). Angiotensin II (Ang II) causes vasoconstriction provoking high blood pressure. This molecule also stimulates production of the hormone aldosterone, which triggers the absorption of salt and water by the kidneys [25]. In addition, Ang II may play a more direct role in kidney development by affecting growth factors involved in the development of kidney structures in mice [26]. At least six mutations in the AGT gene have been found to cause a severe kidney disorder called renal tubular dysgenesis, a condition characterized by abnormal kidney development before birth, anuria, and severe hypotension [27].

We have characterized single nucleotide polymorphism (SNP, rs699) located in exon 2 of the AGT gene and corresponding to c.T803C transition and leading to p.M268T amino acid substitution. The minor allele frequency of T268 is 88% in African Americans, 83% in Asians, 64% in all Americans and 41% in Europeans [28]. This genetic variant gene leads to increased production of angiotensinogen [30]. People carrying this variant are salt-sensitive and this increased salt sensitivity provokes sodium retention with increases blood volume, leading to high blood pressure [30]. The variant is also associated with a higher risk for non-dipper, a condition characterized by high blood pressure during sleep time putting a greater, potentially pathogenic burden on their cardiovascular system and kidneys function [31].

It is known that Ang II, like insulin, has trophic effect on tissues. It is probable that the M268T ATG mutation which leads to increased production of Ang II might induce abnormal growth of collecting tubule cells constituting the nephrons. Nephron hypertrophy might provoke more sodium reabsorption increasing blood pressure. Indeed, in experimental animals, Ang II infusion leads to activation of the kidney RAAS, including an increased intrarenal Ang II production; this results in elevated blood pressure and injury to the kidneys and other tissues. Blockade of prominent type 1 Ang II receptors prevents this phenomenon [32].

The M268T variation is also known in the scientific literature as M235T. The latter nomenclature ignored the 33 amino acids of the signal peptide of the AGT preproprotein. In recent years, the T268 allele has been associated with negative prognostic of renal function [33], atherosclerosis [34], diabetic nephropathy [35], essential hypertension [35], renal cysts [36], and ESRD [37].

CONCLUSION

In conclusion, we have characterized AGT mutation (rs699, c.803T>C, p.M268T) in four ESRD patients. Based on previous studies on this mutation, it is likely that it may have contributed to their condition. A family study showing the co-segregation of the minor allele with the disease should corroborate this presumption. The fact that four unrelated ESRD patients of the Hail region of Saudi Arabia carried this mutation suggests that the latter might be relatively frequent in this region. Thus, regional genotyping for this SNP might be warranted. It would allow the genetic counselling of carriers for lifestyle modification aimed at reducing the risk factors of a renal pathology and prevent its onset. The study also illustrates how WES could be applied to identify candidate genes for a given pathology in a particular population.

DECLARATIONS

ACKNOWLEDGEMENTS

The authors thank The Renal chair of KSA for supporting this work

Author Disclosure Statement

No competing financial interest exists

REFERENCES

[1] Nanayakkara, Shanika, et al. “Whole-exome sequencing reveals genetic variants associated with chronic kidney disease characterized by tubulointerstitial damages in North Central Region, Sri Lanka.” *Environmental Health and Preventive Medicine* 20.5 (2015): 354-359.

[2] Alsuwaida, Abdulkareem O., et al. “Epidemiology of chronic kidney disease in the Kingdom of Saudi Arabia

(SEEK-Saudi investigators)-a pilot study." *Saudi Journal of Kidney Diseases and Transplantation* 21.6 (2010): 1066.

[3] Ginawi, Ibrahim Abdelmajeed, Hussain Gadelkarim Ahmed, and Awdah M. Al-hazimi. "Assessment of risk factors for chronic kidney disease in Saudi Arabia." *Hypertension* 1.1.4 (2014).

[4] Al-Sayyari, Abdulla A., and Faissal A. Shaheen. "End stage chronic kidney disease in Saudi Arabia. A rapidly changing scene." *Saudi Medical Journal* 32.4 (2011): 339-346.

[5] Modi, G. K., and V. Jha. "The incidence of end-stage renal disease in India: A population-based study." *Kidney international* 70.12 (2006): 2131-2133.

[6] Collins, Gary S., et al. "A systematic review finds prediction models for chronic kidney disease were poorly reported and often developed using inappropriate methods." *Journal of Clinical Epidemiology* 66.3 (2013): 268-277.

[7] Grassmann, Aileen, et al. "ESRD patients in 2004: global overview of patient numbers, treatment modalities and associated trends." *Nephrology Dialysis Transplantation* 20.12 (2005): 2587-2593.

[8] Mallett, Andrew, et al. "The prevalence and epidemiology of genetic renal disease amongst adults with chronic kidney disease in Australia." *Orphanet Journal of Rare Diseases* 9.1 (2014): 98.

[9] Levy, Micheline, and Josué Feingold. "Estimating prevalence in single-gene kidney diseases progressing to renal failure." *Kidney International* 58.3 (2000): 925-943.

[10] Lemaire, Mathieu, et al. "Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome." *Nature genetics* 45.5 (2013): 531-536.

[11] Bu, Fengxiao, et al. "Comprehensive genetic analysis of complement and coagulation genes in atypical hemolytic uremic syndrome." *Journal of the American Society of Nephrology* 25.1 (2014): 55-64.

[12] Hinkes, Bernward G., et al. "Nephrotic syndrome in the first year of life: two thirds of cases are caused by mutations in 4 genes (NPHS1, NPHS2, WT1, and LAMB2)." *Pediatrics* 119.4 (2007): e907-e919.

[13] Ashraf, Shazia, et al. "ADCK4 mutations promote steroid-resistant nephrotic syndrome through CoQ 10 biosynthesis disruption." *The Journal of Clinical Investigation* 123.12 (2013): 5179-5189.

[14] McCarthy, Hugh J., et al. "Simultaneous sequencing of 24 genes associated with steroid-resistant nephrotic syndrome." *Clinical Journal of the American Society of Nephrology* 8.4 (2013): 637-648.

[15] Otto, Edgar A., et al. "Hypomorphic mutations in meckelin (MKS3/TMEM67) cause nephronophthisis with liver fibrosis (NPHP11)." *Journal of Medical Genetics* 46.10 (2009): 663-670.

[16] Otto, Edgar A., et al. "Candidate exome capture identifies mutation of SDCCAG8 as the cause of a retinal-renal ciliopathy." *Nature Genetics* 42.10 (2010): 840-850.

[17] Chaki, Moumita, et al. "Genotype–phenotype correlation in 440 patients with NPHP-related ciliopathies." *Kidney International* 80.11 (2011): 1239-1245.

[18] Otto, Edgar A., et al. "Mutation analysis of 18 nephronophthisis associated ciliopathy disease genes using a DNA pooling and next generation sequencing strategy." *Journal of Medical Genetics* 48.2 (2011): 105-116.

[19] Chaki, Moumita, et al. "Exome capture reveals ZNF423 and CEP164 mutations, linking renal ciliopathies to DNA damage response signaling." *Cell* 150.3 (2012): 533-548.

[20] Halbritter, Jan, et al. "High-throughput mutation analysis in patients with a nephronophthisis-associated ciliopathy applying multiplexed barcoded array-based PCR amplification and next-generation sequencing." *Journal of Medical Genetics* 49.12 (2012): 756-767.

[21] Halbritter, Jan, et al. "Defects in the IFT-B component IFT172 cause Jeune and Mainzer-Saldino syndromes in humans." *The American Journal of Human Genetics* 93.5 (2013): 915-925.

[22] Hoff, Sylvia, et al. "ANKS6 is a central component of a nephronophthisis module linking NEK8 to INVS and NPHP3." *Nature Genetics* 45.8 (2013): 951-956.

[23] Taskiran, Ekim Z., et al. "Mutations in ANKS6 cause a nephronophthisis-like phenotype with ESRD." *Journal of the American Society of Nephrology* (2014): ASN-2013060646.

[24] Kotchen, Theodore A., and Gordon P. Guthrie Jr. "Renin-angiotensin-aldosterone and hypertension." *Endocrine Reviews* 1.1 (1980): 78-99.

[25] Oliverio, Michael I., et al. "Reduced growth, abnormal kidney structure, and type 2 (AT2) angiotensin receptor-mediated blood pressure regulation in mice lacking both AT1A and AT1B receptors for angiotensin II." *Proceedings of the National Academy of Sciences* 95.26 (1998): 15496-15501.

[26] Gribouval, Olivier, et al. "Mutations in genes in the renin-angiotensin system are associated with autosomal recessive renal tubular dysgenesis." *Nature Genetics* 37.9 (2005): 964-968.

[27] Hunt, Steven C., et al. "Angiotensinogen genotype, sodium reduction, weight loss, and prevention of hypertension." *Hypertension* 32.3 (1998): 393-401.

[28] Bloem, Laura J., et al. "The serum angiotensinogen concentration and variants of the angiotensinogen gene in white and black children." *Journal of Clinical Investigation* 95.3 (1995): 948.

[29] Blaustein, Mordecai P., et al. "How does salt retention raise blood pressure?." *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 290.3 (2006): R514-R523.

[30] Tohru, Fujiwara, et al. "T+ 31C polymorphism of angiotensinogen gene and nocturnal blood pressure decline: the Ohasama study." *American Journal of Hypertension* 15.7 (2002): 628-632.

[31] Doria, Alessandro, et al. "Angiotensinogen polymorphism M235T, hypertension, and nephropathy in insulin-dependent diabetes." *Hypertension* 27.5 (1996): 1134-1139.

[32] Campbell, Catherine Y., et al. "Associations between genetic variants in the ACE, AGT, AGTR1 and AGTR2 genes and renal function in the Multi-ethnic Study of Atherosclerosis." *American Journal of Nephrology* 32.2 (2010): 156-162.

[33] Al-Najai, Mohammed, et al. "Association of the angiotensinogen gene polymorphism with atherosclerosis and its risk traits in the Saudi population." *BMC Cardiovascular Disorders* 13.1 (2013): 17.

[34] Shaikh, Rozeena, et al. "Genetic variants of ACE (Insertion/Deletion) and AGT (M268T) genes in patients with diabetes and nephropathy." *Journal of Renin-Angiotensin-Aldosterone System* (2014): 1470320313512390.

[35] Shamaa, Marium M., et al. "Association between the Angiotensinogen (AGT) gene (M235T) polymorphism and Essential Hypertension in Egyptian patients." *The Egyptian Heart Journal* 67.1 (2015): 1-5.

[36] Tabei, S. M. B., et al. "Simple renal cysts and hypertension are associated with angiotensinogen (AGT) gene variant in Shiraz population (Iran)." *Journal of the Renin-Angiotensin-Aldosterone System* 16.2 (2015): 409-414.

[37] Sarkar, Saumya, et al. "M235T Polymorphism in the AGT Gene and A/Gi8-83 Substitution in the REN Gene Correlate with End-Stage Renal Disease." *Nephron* 129.2 (2015): 104-108.