How to Cite:
Qahtan, E. Y., & Al-Husseini, A. M. H. (2022). Study of single nucleotide polymorphism of aquaporin 2 in hypertension and CKD patients. *International Journal of Health Sciences*, *6*(S6), 10240–10248. https://doi.org/10.53730/ijhs.v6nS6.12584

Study of single nucleotide polymorphism of aquaporin 2 in hypertension and CKD patients

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***Abstract*---**The present study aims to examine the genetic variation of the Aquaporin-2 gene concerning hypertension and chronic kidney disease CKD. (120) blood samples from participants were obtained and divided into three groups: a first group representing hypertensive patients (hyper), a second group representing Hypertensive CKD patients (hyper with CKD), and a third group representing a control group. DNA was extracted from all blood samples and then converted to cDNA and the ARMS-PCR technique was used to investigate the single nucleotide polymorphism (SNP) from the *AQP-2*, genes. The result of this study investigated that SNP rs3759126 located in *AQP-2* is a significant association with hypertension and CKD.

***Keywords*---**aquaporin-2, CKD, hypertension, polymorphism.

Introduction

AQPs represent a family of ubiquitous membrane channels. Osmoregulation and the preservation of bodily water equilibrium depend on the regulation of AQPs. Eight AQPs are expressed in the kidney, and five of them AQP1, AQP2, AQP3, AQP4, and AQP7 have been implicated in the regulation of body water balance. Vasopressin, in particular, controls AQP2 (Kortenoeven and Fenton, 2014). Out of the seven aquaporins (AQPs) expressed in the kidney, AQP1-4 are important for renal water absorption and transcellular water transport. The collecting duct primary cells apically expressed AQP2 and basolaterally expressed AQP3 and AQP4 use this gradient as the force behind transepithelial water transport. Antidiuretic vasopressin (AVP) also boosts AQP2-4 expression to enhance water absorption during chronic dehydration (Jung and Kwon,2016). The translocation of AQP2 from intracellular reserves to the apical plasma membrane is also

induced by AVP. This effectively and quickly enhances the collecting duct primary cell's water permeability and enables fine-tuning of body water balance in response to even minute variations in plasma osmolality. At the molecular level, understanding AQP2 trafficking is crucial because it reveals mechanisms that might be pharmacologically targeted to either boost or inhibit water absorption through AQP2 activity (Cheung *et al.,* 2020). The renal urine concentration and body water balance are significantly influenced by the normal expression of AQP2 in the apical plasma membrane.

Nephron-genic diabetes insipidus (NDI), which is caused by AQP2 mutations, is characterized by the production of a significant amount of hypotonic urine. Recessive mutations that affect the water-conducting pore, the loop sections, and the transmembrane helices might change the structure of the AQP2 protein (Frick *et al.,* 2014), causing ER retention, AQP2 misfolding or disrupted tetramer formation, higher rates of degradation (Milano et al., 2017). There are now 11 mutations in the AQP2 carboxyl terminus known to cause autosomal dominant NDI, including deletions, insertions, or substitutions of amino acids (Dollerup *et al.,* 2015). all of which have varying effects on AQP2 trafficking (Moeller *et al.,* 2013). It has been acknowledged that urinary excretion of AQP2 is a helpful marker for the detection of renal disorders (Krais *et al.,* 2018).

Material and Methods

Collection of samples

(1ml) of blood was collected from 120 individuals, divided into three groups, each group included forty individuals. The first group included Hypertensive patients and the second group included Hypertensive with chronic kidney diseases patients (while the last group Included healthy individuals as a control group, then immediately blood Samples were drawn and placed into Dipotassium-EDTAVacutainer® Tubes for use in ARMS-PCR Technique.

DNA extraction and ARMS –PCR (primer amplification refractory mutation system–polymerase chain)

ARMS-PCR primers for Aquaporin-2(rs3759126) gene polymorphism were designed in this study using the NCBI-SNP database and Primer1 ARMS-PCR primers were designed online. These primers were provided by (Scientific Researcher. Co. Ltd. Iraq) as following tables:

Table 1
The ARMS-PCR primers for Aquaporin-2 (AQP-2) A/G Promoter rs3759126 Polymorphism with their sequence and amplicon size

Primer	Sequence (5'-3')	Product size
Wildtype Forward Primer	GCTTCCTCAGCCGCCCCA	276bp
Mutant Forward Primer	GCTTCCTCAGCCGCCCCG	
Common Reverse Primer	ATGAGTGCTGCTCCTTCTGG	

Statistical analysis

The data of genotype and allele frequencies of rs3759126 SNP barcode with SNP (AQP-2 rs3759126) gene was estimating of Odds ratios for genetic variants that are associated with diseases and provided the 95% confidence interval (CI), corresponding to the effect of each specific SNP barcode on the occurrence of hypertension and CKD.SPSS for windows version (2010) was used for statistical analysis of the molecular study.

Results

Analysis of genotype and Allele

The results of genotype and allele frequencies of SNP3759126 that are located on the *AQ-2* gene are shown in table (2) and figure 1 and 2. The homozygous genotype AA was more frequent in hyper patients group 16(40 %) in comparison with a control group 10(25%) and hyper with CKD group 12 (30%). our study confirmed there were no significant differences in the hyper Group versus hyper with CKD group (p = 0.34), [OR (CI) = 1.5556 (0.6 162 to 3.9269)] . in the hyper versus control group, the results showed significant differences between them(p = 0.04) and the Odd value was [OR(CI) = 2(0.7695 to 5.1983)] this indicated that genotype AA as a risk factor for disease take place and associated with hypertension. in hyper with CKD versus control recorded non significant differences between them (p = 0. 61), the Odd value was [OR(CI) = 1.2857(0.4803 to 3.4417)] .

On the other hand, the heterozygous genotype AG was more frequent in the control group 24(60%) compared with hyper group 18(45%) and hyper with CKD 22(55%), respectively, table (2) and (figure 1). [(p =0.37) and OR(CI) = 0.6694 (0.2774 to 1.6154)] in hyper and hyper with CKD means that was non-significant differences, that was represented resistant factor for disease. in hyper versus control [(p = 0.18) and OR(CI) = 0.5455 (0.2245 to1.3253)]. That means were no significant differences, and the heterozygous genotype AG represented as a resistant factor for disease.[(p = 0.18) and OR(CI) = 0.5455 (0.2245 to 1.3253)] in hyper with CKD versus control means that was found no significant differences, that was represented resistant factor for disease. GG genotype was 6(15%) in three groups hyper, hyper with CKD, control, [(p = 1) and OR(CI) = 1(2931 to 3.4123)] means that was found no significant differences among them.

Allele A was more frequent in hyper group 50(62.5 %) compared with hyper and CKDgroup 46(57.5%) and control group 44(55%). (table 2)and figure (2) [(p= 0.51) and OR (CI) = 1.2319(0.6538 to 2.3212)] in hyper group versus hyper with CKD group , in hyper group versus control group, and in hyper with CKD group versus control group , P value and Odds ratio were [(p= 0.51) and OR (CI) = 1.2319(0.6538 to 2.3212)], [(p= 0.33) and OR (CI) = 1.3636(0.7251 to 2.5645)], and [(p= 0.75) and OR (CI) = 1.1070(0.5926 to 2.0679)], respectively. all these indicate that non-significant differences among groups that was found no significant differences, that was found no significant differences.

[(p= 0.33) and OR (CI) = 1.3636(0.7251 to 2.5645)] in hyper group versus control group means that was found no significant differences, ,OR (CI) = 1.3636(0.7251 to 2.5645) means no significant differences.[(p= 0.75) and OR (CI) = 1.1070(0.5926 to 2.0679)] in hyper with CKD group versus control group means that was found no significant differences, means no significant differences. Allele G was more frequent in the control group 36(45%) compared with hyper and CKD group 34(42.5%) and hyper group 30(60 %). (table 2)and figure (2). [(p= 0.51)and OR (CI) = 0.8118 (0.4308 to 1.5296)] in the hyper group versus hyper with CKD group that was found no significant differences, that was represented resistance factor for disease. [(p= 0.33) and OR (CI) =0.7333(0.3899 to 1.3791)] in the hyper group versus control group means that were found no significant differences, and that was represented resistance factor for disease.

Table 2
Genotype and Allele Frequency for Aquaporin 2

Genotype and Allele	Groups			G1 vs G2		G1 vs G3		G2 vs G3	
	G1 Hyper. N=40	G2 Hyper with CKD N=40	G3 Control N=40	P value	OR(CI)	P value	OR(CI)	P value	OR
Genotypes									
AA	16(40)	12(30)	10(25)	0.34	1.5556(0.6162 to 3.9269)	0.04	2(0.7695 to 5.1983)	0.61	1.2857 3 to 3
AG	18(45)	22(55)	24(60)	0.37	0.6694(0.2774 to 1.6154)	0.18	0.5455(0.224 5 to 1.3253)	0.18	0.5455 to 1
GG	6(15)	6(15)	6(15)	1	1(2931 to 3.4123)	1	1(2931 to 3.4123)	1	1(29 3.4
Alleles									
A	50(62.5)	46(57.5)	44(55)	0.51	1.2319(0.6538 to 2.3212)	0.33	1.3636(0.725 1 to 2.5645)	0.75	1.107(6 to 2
G	30(60)	34(42.5)	36(45)	0.51	0.8118 (0.4308 to 1.5296)	0.33	0.7333(0.389 9 to 1.3791)	0.75	0.9034 6 to 1

OR: Odd ratio, CI: Confidence interval.

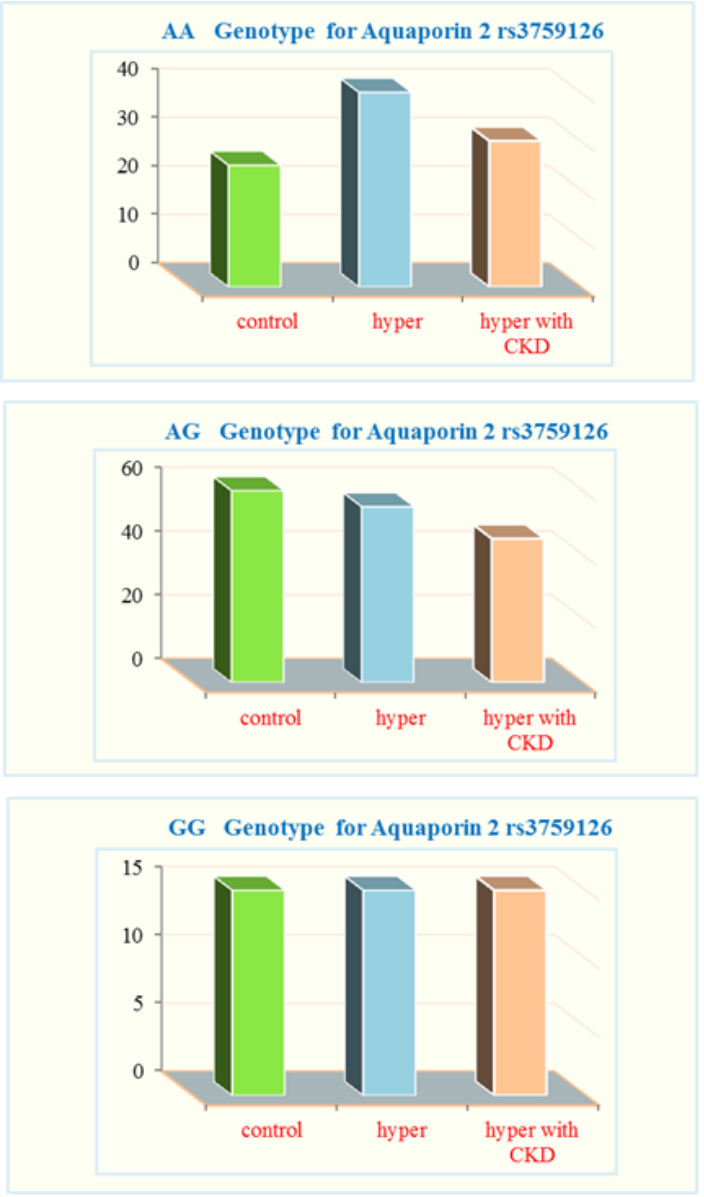
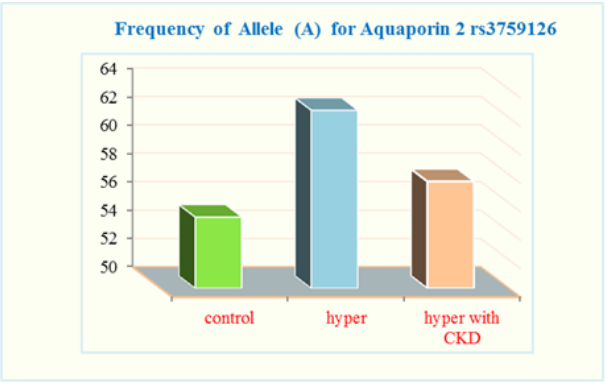
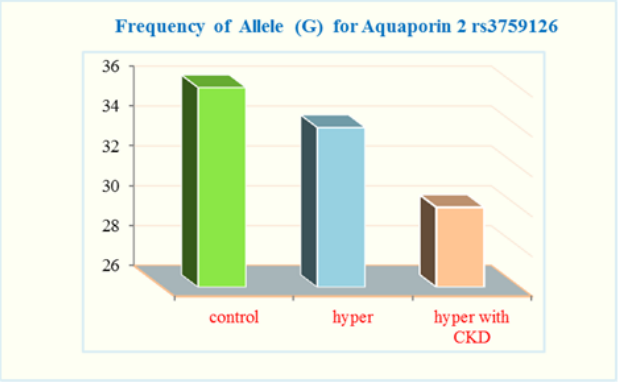


Figure 1. Ratio of Genotype for Aquaporin 2rs3759126 of hypertension and patients with CKD hypertension in comparison with control





Figure(2) Ratio of Allele Frequency for Aquaporinrs3759126 of hypertension and patients with CKD hypertension in comparison with control .

Control **hypertension** **hypertension with CKD**

Detection of Genes Polymorphisms

Detection of *(* rs3759126*)* Polymorphism, the distribution of ARMS-PCR for AQP-2gene polymorphisms (rs3759126) was detected by the ARMS-PCR technique. At this locus, there are three genotypes; AG, GG and AA. The wild-type homozygote genotype AA showed only A allele amplification at 276 bp product size. the (GG) mutant type homozygote was shown in the G allele only, whereas the (G/A) heterozygote was shown in both G and A allele. The presence of the A or G allele was observed at bp 276 bp product size.

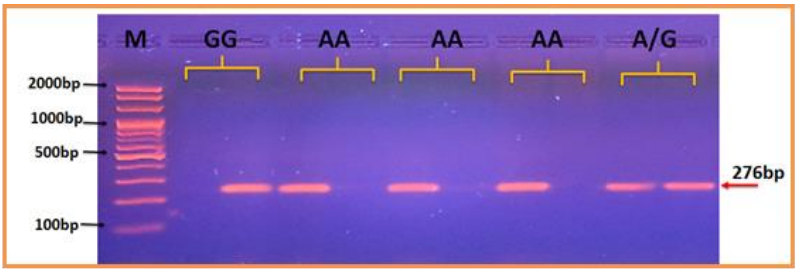


Figure (3): Agarose gel electrophoresis image that showed the ARMS-PCR product analysis of Aquaporin-2 (AQP-2) A/G Promoter rs3759126 gene polymorphism.

Discussion

In our study, we examined variation in one SNP rs3759126 located on *AQP-2gene* that affects water channel transport and function of these proteins upon expression in cell systems *of the kidney which is* similar to the previous study (Deen and Knoers, 1998), thus causing dysregulation of AQP-2 release is associated with hyponatremia, hypokalemia, or water retention in human disease(Schrier and Cadnapaphornchai,2003). All dominant mutations identified so far are located within the C-terminus of AQP2, which is a region important for the regulation of intracellular trafficking (Bichet, 2006). The findings of this study establish that genotypes AA was more frequent with Hypertension and Hypertension with CKD patients in comparison with control ,that suggest genotypes AA of rs3759126 *AQP-2* in relation water channels function of kidney. Wich is similar with Bichet ,(1996) who proved mutations in AQP-2 gene leading to dysfunction of AQP2 Trafficking Causes Nephrogenic Diabetes Insipidus (NDI) Congenital NDI is a genetic disease characterized by the inability of the kidney to respond to AVP, which results in a massive loss of hypotonic urine and causes polydipsia.

Considarad is an autosomal disease caused by mutations in the gene encoding AQP2 Autosomal dominant NDI, discovered in a few families, is caused by mutations that affect the intracellular trafficking of the protein. In autosomal dominant NDI, AQP2 is either retained in the Golgi, sorted to late endosomes, lysosomes, or to the basolateral plasma membrane. The mutants form heterotetramers with wild-type AQP2 and thereby prevent the wild-type protein from reaching the plasma membrane (Bichet 2006; Sohara et al. 2006). Also the most commonly used treatments paradoxically include diuretics such as hydrochlorothiazide and amiloride effects aquaporin channals. The thiazide diuretics inhibit the NaCl cotransporter in the distal convoluted tubule, decreasing sodium reabsorption. The consequent increase of sodium excretion leads to extracellular volume contraction, decreasing the glomerular filtration rate and increasing the sodium and water reabsorption in the proximal tubule (Earley and Orloff ,1962; Kennedy and Crawford,1961).

Conclusion

We conclude from the results of our study, and previous studies confirming the presence of single nucleotide polymorphism in AQP-2 lead to defects in the water channel and distribution and function through kidney tubular cells.

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