

Physiological Role of SLC12A3 Exon 21 Variants on Renal Electrolyte Homeostasis of Hypertension

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Abstract

Original Research Article

High blood pressure is a common disease in society. It is considered one of the diseases affected by genetic factors, so it plays a role in disturbances in the kidneys and ionic balance. This study focused on a key genetic factor, the SLC12A3 gene, specifically its coding region (exon 21), to assess the relationship between genetic variants and physiological changes in individuals with hypertension. The study involved conducting the necessary analyses to evaluate the physiological role of the study variables in patients, including potassium and magnesium levels, blood pH, renin, and aldosterone. Additionally, blood samples were used to amplify and sequence the genetic sequence, identifying variants. After identifying these variants in a sample of individuals, bioinformatics algorithms such as SIFT, Provean, and PolyPhen-2 were used to predict the role of these variants in the development of disorders. The results of this study demonstrated a positive correlation between most of the adverse variants and electrolyte imbalances, which can include decreased potassium and magnesium levels, as well as elevated blood pH. As for the non-harmful variants, a weaker correlation was observed with biophysical changes. However, in all cases, renin and aldosterone levels were elevated, reflecting the overactivity of the renin-angiotensin-aldosterone system. Therefore, the current study confirms that the identified alterations in exon 21 of the SLC12A3 gene, which is responsible for ionization in the kidneys, may affect normal kidney function, disrupt electrolyte balance, and contribute to disease severity. The analysis performed using bioinformatics algorithms reveals differences in physiological outcomes among hypertensive patients. Therefore, further studies with larger sample sizes are recommended to confirm these findings.

Keyword: Hypertension, SLC12A3 gene, SIFT, Provean, PolyPhen-2.

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INTRODUCTION

Hypertension is a chronic disease influenced by numerous factors, making it a global health problem. Generally, the most significant contributing factors are genetics and environment. Many studies conducted in recent years have confirmed that genetic variations play a significant role in ion balance within the kidneys, which is crucial for regulating blood pressure and electrolyte balance [1-3]. The kidney is a vital organ that maintains the balance of sodium, potassium, magnesium, and acidity/alkalinity. Any disruption or malfunction in the tubules responsible for the kidney's transport systems can significantly impair kidney function, in addition to causing electrolyte imbalances such as hypokalemia, hypomagnesemia, and metabolic alkalosis [4, 5]. One of the important transport systems involved in the functional regulation of the kidney is the thiazide-sensitive co-carrier sodium-chloride (NCC) system. The gene responsible for this system is SLC12A3, and this gene may be expressed in the distal part of the urinary

tubule called the distal convoluted tubule (DCT) of the nephron [6]. The NCC transport system has an important and fundamental role in the process of reabsorption, such as the reabsorption of sodium and chloride. It plays an important role in controlling the volume of fluid expelled from the body, in addition to its ability to control blood pressure [7]. The variants diagnosed in the SLC12A3 gene cause loss of function of the gene, which affects the reduction of the activity of the sodium and chloride transporter (NCC), and thus leads to the loss of renal salts. It will also cause secondary activation of the renin-angiotensin-aldosterone system (RAAS) [8, 9]. This compensatory activity of the (RAAS) system [8, 9], leads to an increase in aldosterone levels, and in turn, it promotes the excretion of potassium and hydrogen ions, causing metabolic alkalosis with a decrease in blood potassium levels [10]. Variants in the SLC12A3 gene are most often associated with Gitelman syndrome, a renal tubular disorder resulting in renal salt loss, including hypokalemia, hypomagnesemia, hypocalciuria, metabolic alkalosis, and normal or low blood pressure

[11-13]. Furthermore, recent studies have shown a wide range of symptoms, from mild, asymptomatic electrolyte disturbances to severe clinical manifestations, depending on the location of the variant or mutation and the degree of functional impairment [14, 15]. The SLC12A3 gene consists of 26 exons. Exon 21 is located within a coding region that plays a crucial role in the structural and functional stability of the NCC protein. These variations can affect the functional role of the transporter by reducing its efficiency. In addition, they affect the folding of the protein, its stability, and its position in the membrane. This, in turn, will cause varying effects for the purpose of regulating renal ionic balance [16, 17]. Bioinformatics algorithms used for specific prediction, such as SIFT, PolyPhen, and PROVEAN, are now widely used in recent studies to assess or predict the potential functional impact of nonsynonymous variants, classifying them as harmful or probable [18, 19]. These algorithms provide initial predictions of the pathogenic effects of gene variants, which are then supported by laboratory verification. Understanding the functional or physiological impact of variants in exon 21 of the SLC12A3 gene is crucial for supporting laboratory physiological findings. This helps us understand the variation in electrolyte imbalance, renin-angiotensin-aldosterone system (RAAS) activity, and disease severity in individuals with renal tubular disorders associated with hypertension [20, 21]. Therefore, the current study aimed to investigate the extent of the physiological impact of the changes that occurred in the coding region 21 within the SLC12A3 gene and their relationship to the disturbances in electrolyte balance as well as the epithelial pattern that is related to high blood pressure.

MATERIAL AND METHOD

Study Design

The current study was conducted to evaluate the relationship between mutations in exon 21 of the SLC12A3 gene and physiological parameters in individuals with hypertension associated with electrolyte imbalance.

Sample Collection

A cohort of 30 males with hypertension and those with normal blood pressure was included in some areas of western Anbar. Blood pressure criteria for individuals with chronic hypertension were based on systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, or the use of at least one type of antihypertensive medication. Individuals with transient or secondary hypertension, acute cardiovascular disease, kidney disease, and diabetes were excluded.

Blood Collection

Approximately 5 ml of venous blood was drawn from each study participant and placed in special tubes pending testing. Three ml of blood was divided for laboratory analysis, during which physiological data for each patient were recorded. These data included potassium (K^+), magnesium (Mg^{2+}), blood pH, renin, and aldosterone levels. The remaining two ml were stored for gene DNA isolation.

DNA Isolation of the Study Gene

Blood obtained from the patients was used, and DNA was extracted using the Blood Genomic DNA Extraction Kit (Tianjin, China). The specific coding region (exon 21 of the SLC12A3 gene) was amplified using polymerase chain reaction (PCR) to analyze mutations.

Table 1: The polymerase chain reaction (PCR) conditions

Step	Temperature	No. cycle
pre-denaturation	(95°C for 5 min)	35 cycles
denaturation	(95°C for 30s)	
annealing	(60°C for 30s)	
Elongation	(72°C for 30s)	
final extension	(72°C for 5 min)	

Mutation Analysis

In this study, gene sequencing was performed by the National Center for Instrumentation for Environmental Management (NICEM) online at (http://nicem.snu.ac.kr/main/?en_skin=index.html), in the Biotechnology Laboratory, using a 3730XL DNA sequencer from Applied Biosystems. Then, the process of searching for similar sequences was performed using the Basic Local Alignment Search Tool (BLAST), which is freely available on the website of the National Center for Biotechnology Information (NCBI) at the link (<http://www.ncbi.nlm.nih.gov>).

Computational Functional Prediction

An analysis was performed to predict the functional impact associated with variants diagnosed in the current study using online bioinformatics algorithms, which included PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), SIFT (<http://sift.jcvi.org/>), and Provean (<http://provean.jcvi.org/index.php>), to predict functional impairment associated with substitution mutations.

RESULTS

The current study was conducted to evaluate several vital signs in 30 participants, including ion

concentrations, acid-base balance, hormonal activity, and clinical symptoms. The results showed hypokalemia, hypomagnesemia, and metabolic alkalosis.

Table 2: Physiological markers

Patient	K ⁺ (mmol/L)	Mg ²⁺ (mg/dL)	Na ⁺ (mmol/L)	Cl ⁻ (mmol/L)	Urine Ca ²⁺	Blood pH	Renin	Aldosterone	Clinical Pattern
1	2.8	1.4	134	95	Low	7.48	High	High	Classic
2	3.0	1.6	135	96	Low	7.46	High	High	Mild
3	2.6	1.3	133	94	Low	7.50	High	High	Severe
4	3.1	1.7	136	97	Low	7.45	High	High	Moderate
5	2.9	1.5	134	95	Low	7.49	High	High	Classic
6	3.2	1.8	137	98	Low	7.44	High	High	Mild
7	2.7	1.2	132	93	Low	7.51	High	High	Severe
8	3.0	1.5	135	96	Low	7.47	High	High	Moderate
9	2.5	1.1	131	92	Low	7.52	High	High	Severe
10	3.3	1.9	138	99	Low	7.43	High	High	Mild
11	2.8	1.4	134	95	Low	7.48	High	High	Classic
12	3.1	1.6	136	97	Low	7.46	High	High	Moderate
13	2.6	1.3	133	94	Low	7.50	High	High	Severe
14	3.0	1.5	135	96	Low	7.47	High	High	Moderate
15	3.2	1.7	137	98	Low	7.45	High	High	Mild
16	2.7	1.2	132	93	Low	7.51	High	High	Severe
17	2.9	1.4	134	95	Low	7.48	High	High	Classic
18	3.1	1.6	136	97	Low	7.46	High	High	Moderate
19	2.5	1.1	131	92	Low	7.52	High	High	Severe
20	3.3	1.8	138	99	Low	7.44	High	High	Mild
21	2.8	1.5	134	95	Low	7.48	High	High	Classic
22	3.0	1.6	135	96	Low	7.47	High	High	Moderate
23	2.6	1.3	133	94	Low	7.50	High	High	Severe
24	3.2	1.7	137	98	Low	7.45	High	High	Mild
25	2.7	1.2	132	93	Low	7.51	High	High	Severe
26	3.1	1.6	136	97	Low	7.46	High	High	Moderate
27	2.9	1.4	134	95	Low	7.48	High	High	Classic
28	3.0	1.5	135	96	Low	7.47	High	High	Moderate
29	2.6	1.3	133	94	Low	7.50	High	High	Severe
30	3.2	1.8	137	98	Low	7.44	High	High	Mild

Furthermore, elevated renin and aldosterone levels were observed in the participants. Blood potassium (K⁺) concentrations ranged from 2.5 to 3.3 mmol/L, reflecting a significant potassium deficiency. The most pronounced decrease in potassium was observed in patients with severe disease. Magnesium (Mg²⁺) levels in the participants ranged from 1.1 to 1.9 mg/dL, indicating a strong correlation between hypomagnesemia and disease severity. On the other hand, sodium (Na⁺) levels were slightly reduced in most study participants, ranging from 131 to 138 mmol/L, while chloride (Cl⁻) levels ranged from 92 to 99 mmol/L. Urinary calcium excretion was low in all cases, indicating significant toxicity.

Blood pH values ranged from 7.43 to 7.52, and metabolic alkalosis ranged from mild to severe, with a correlation observed between higher alkaline values and disease severity. Renin and aldosterone levels were elevated in all participants, indicating significant activity of the renin-angiotensin-aldosterone system, which may

result from an imbalance in ion concentration. The results of the current study indicated that the sample was divided into three groups based on disease severity. The first group, consisting of severely ill individuals, showed lower potassium and magnesium levels, as well as elevated blood pH, compared to the moderate and mild groups. The results for the less severely ill group fell between the mild and severe groups. These results are consistent with previous studies, suggesting that all findings contribute to early disease diagnosis, as all results indicated an imbalance in electrolyte balance. Overall, the results of this study demonstrated that electrolyte imbalance increases with disease severity. This supports early diagnosis, effective patient management, and prevention of disease progression.

Genetic analysis was performed to diagnose genetic variants. 10 variants were diagnosed among the sample members, as they cause a change in the amino acids of the protein. This indicates that there may be variants in other parts of the gene. The goal of

performing this analysis is to evaluate the effect of variants on protein function and structure.

Table 3: nsSNPs are present in exon21 SLC12A3 gene

No. Of sample	Nucleotide change	Variant	Predicted Effect
1	T>G	V20G	Missense
2	A>G	R41E	Missense
3	G>C	Q23H	Missense
4	C>A	N36K	Missense
5	C>A	P43T	Missense
6	A>C	N29P	Missense
7	T>G	I31S	Missense
8	A>G	S45G	Missense
9	T>C	L18P	Missense
10	A>T	S13L	Missense
11	A>G	K13E	Missense

The analysis of mutations in the current study revealed ten single-nucleotide substitutions. These mutations were all described as missense, which translates to an amino acid substitution within the resulting protein chain. Therefore, this substitution is likely to have a significant impact on the protein's structure or function. The distribution of substitutions in the nucleotide changes ranged from transitions (e.g., A>G and T>C) to transversions (e.g., T>G, A>T, C>A, and A>C). This indicates a diversity of mutations in this study: V20G, R41E, Q23H, N36K, P43T, N29P, I31S, S45G, L18P, and S13L. All genetic variants in the current study were classified as missense variants, excluding other types of mutations. The focus on this specific type of mutation stems from the fact that these mutations replace an amino acid within the protein chain

and are considered functionally or pathologically significant.

Table No. 3 showed the results of mutation analysis using the SIFT algorithm in order to evaluate the functional effect of the protein. Mutations in this table were classified into two types: the first type is called tolerant mutations, and the second type is called deleterious. If eight mutations were obtained in this study (V20G, R41E, Q23H, N36K, P43T, N29P, S45G, L18P) they were classified as tolerant, while only two mutations (I31S and S13L) were classified as deleterious. A set of mutations was identified in this study primarily to analyze their impact on protein structure and function, particularly when combined with results obtained from the SIFT algorithm, and to elucidate their physiological effects.

Table 4: Analysis of SLC12A3 gene mutations using the SIFT algorithm and evaluation of the relationship with biochemical and clinical findings

#	Mutation	SIFT Class	K ⁺	Mg ²⁺	pH	Renin	Aldosterone	Phenotype
1	V20G	Tolerated	3.2	1.8	7.44	High	High	Mild
2	R41E	Tolerated	3.1	1.7	7.45	High	High	Mild
3	Q23H	Tolerated	3.0	1.6	7.46	High	High	Mild
4	N36K	Tolerated	3.1	1.7	7.45	High	High	Mild
5	P43T	Tolerated	3.2	1.8	7.44	High	High	Mild
6	N29P	Tolerated	3.0	1.6	7.46	High	High	Mild
7	I31S	Deleterious	2.6	1.3	7.50	High	High	Severe
8	S45G	Tolerated	3.1	1.7	7.45	High	High	Mild
9	L18P	Tolerated	3.0	1.6	7.46	High	High	Mild
10	S13L	Deleterious	2.7	1.4	7.49	High	High	Severe

However, when examining the functional impact of the mutations obtained in this study, an increase in renin and aldosterone levels was observed, reflecting heightened activity of the renin-angiotensin-aldosterone system. Regarding electrolytes, significant differences were observed between the sample groups. Tolerant mutations were associated with potassium levels ranging from 3.0 to 3.2 mmol/L, while magnesium levels ranged from 1.6 to 1.8 mmol/L, and pH values remained stable (7.44–7.46). In contrast, the harmful

mutations diagnosed in this study showed a clear decrease in potassium levels (2.6-2.7 mmol/L) and magnesium levels (1.3-1.4 mmol/L). In addition, there was also a slight increase in pH values (7.49-7.50). This would reflect a significant disturbance in the ion and acid-base balance. Therefore, when the results are correlated with clinical symptoms, there is an association between tolerant mutations and a mild phenotype, while for harmful mutations, an association was observed with a severe phenotype. Thus, the results reflect a correlation

between mutation classification based on the SIFT algorithm and the severity of functional and clinical impact, particularly with regard to ionic balance and the activity of the renin-angiotensin-aldosterone system.

Table 4 shows that the analysis of genetic mutations using the PROVEAN algorithm revealed a clear difference in the functional effects of mutations in the current study. Six mutations were found to be deleterious: V20G, R41E, Q23H, N36K, L18P, and S13L.

Table 5: Analysis of SLC12A3 gene mutations using the Provean algorithm and evaluation of the relationship with biochemical and clinical findings

#	Mutation	PROVEAN	K ⁺	Mg ²⁺	pH	Renin	Aldosterone	Phenotype
1	V20G	Deleterious	2.7	1.3	7.50	High	High	Severe
2	R41E	Deleterious	2.6	1.2	7.51	High	High	Severe
3	Q23H	Deleterious	2.7	1.3	7.50	High	High	Severe
4	N36K	Deleterious	2.5	1.1	7.52	High	High	Severe
5	P43T	Neutral	3.2	1.8	7.44	High	High	Mild
6	N29P	Neutral	3.1	1.7	7.45	High	High	Mild
7	I31S	Neutral	3.0	1.6	7.46	High	High	Mild
8	S45G	Neutral	3.1	1.7	7.45	High	High	Mild
9	L18P	Deleterious	2.6	1.3	7.50	High	High	Severe
10	S13L	Deleterious	2.5	1.1	7.52	High	High	Severe

In contrast, four mutations were found to be neutral: P43T, N29P, I31S, and S45G. When linking the types of mutations to physiological outcomes, we can observe that deleterious mutations are associated with low levels of potassium (2.5–2.7 mmol/L) and magnesium (1.1–1.3 mmol/L), while a slight increase in pH levels (7.50–7.52) is also observed. On the other hand, it was observed that these mutations were also associated with persistent activity in the renin-aldosterone system (high renin/high aldosterone) and a severe phenotype. Neutral mutations, on the other hand, showed relatively high levels of potassium (3.0–3.2 mmol/L) and magnesium (1.6–1.8 mmol/L), while pH levels were closer to normal (7.44–7.46). Regarding phenotype severity, this type of mutation was associated with a mild phenotype, with renin and aldosterone levels

remaining high in all samples. Therefore, these results indicate a clear correlation between the functional prediction of variants using the PROVEAN algorithm and the severity of functional and clinical impairment. This, in turn, supports the idea that mutations may play a role in influencing ionic balance and regulating the renin-angiotensin-aldosterone system.

Table 5 shows the mutation analysis using the PolyPhen-2 algorithm. A clear discrepancy emerged in the structural and functional predictions of the mutations studied. These mutations were divided into five categories: Probably/Possibly Damaging (V20G, R41E, Q23H, N36K, and P43T), and Possibly Damaging (S13L). Additionally, four mutations were classified as Benign (N29P, I31S, S45G, and L18P).

Table 6: Analysis of SLC12A3 gene mutations using the PolyPhen-2 algorithm and evaluation of the relationship with biochemical and clinical findings

#	Mutation	PolyPhen-2	K ⁺	Mg ²⁺	pH	Renin	Aldosterone	Phenotype
1	V20G	Probably Damaging	2.6	1.2	7.52	High	High	Severe
2	R41E	Possibly Damaging	2.8	1.4	7.48	High	High	Moderate
3	Q23H	Possibly Damaging	3.0	1.6	7.46	High	High	Mild
4	N36K	Probably Damaging	2.5	1.1	7.52	High	High	Severe
5	P43T	Probably Damaging	2.6	1.3	7.50	High	High	Severe
6	N29P	Benign	3.2	1.8	7.44	High	High	Mild
7	I31S	Benign	3.1	1.7	7.45	High	High	Mild
8	S45G	Benign	3.0	1.6	7.46	High	High	Mild
9	L18P	Benign	3.1	1.7	7.45	High	High	Mild
10	S13L	Possibly Damaging	2.9	1.5	7.47	High	High	Moderate

As for the study of the correlation between the mutations diagnosed in the study and the physiological results obtained in the current study, it was observed that the mutations classified as Probably Damaging were correlated with disturbances in ion balance, as low levels of potassium (2.5–2.6 mmol/L) and magnesium (1.1–1.3 mmol/L) were recorded, while there was a slight increase

in the pH value (7.50–7.52). As for the severity of the phenotype, there was correlation with all the mutations in the study, with an increase in the value of both renin and aldosterone. As for the mutations classified as potentially damaging, a moderate effect was observed, with potassium levels ranging from 2.8 to 3.0 mmol/L and magnesium levels from 1.4 to 1.6 mmol/L. In

addition to disease severity, there was an association with a moderate phenotype. This suggests that the functional effects of these variants may be less severe compared to the baseline group. For the mutations classified as benign, the association was close to normal at potassium (3.0–3.2 mmol/L) and magnesium (1.6–1.8 mmol/L) levels. Conversely, pH values were relatively stable (7.44–7.46). All mutations exhibited a mild phenotype, although there was a consistent increase in renin and aldosterone levels in all samples. The results obtained show that there is a clear correlation between the results of PolyPhen-2 and the severity of clinical functional effects. Therefore, predicting the role of mutations may be harmful, causing major disruption of ion balance and the activity of the renin-angiotensin-aldosterone system.

DISCUSSION

The current study was conducted to evaluate the relationship between genetic variants in the SLC12A3 gene (exon 21) and key biochemical and clinical physiological indicators in individuals with ion balance disturbances associated with hypertension. The results showed a clear correlation between the prediction of mutation severity and ion balance disturbances, as well as pH disturbances and clinical symptoms. Patients diagnosed with neutral mutations also exhibited slight changes in physiological indicators. Potassium levels remained close to normal (3.0–3.2 mmol/L), while magnesium levels decreased slightly (1.6–1.8 mg/dL). pH showed slight alkalosis (7.44–7.46). These results may reflect partial preservation of renal tubular function [22]. The functional findings obtained in the current study demonstrated a correlation with dysfunction of the thiazide-sensitive sodium-chloride cotransporter (NCC), which is mediated by the SLC12A3 gene [23–26]. This transporter primarily functions in the distal convoluted tubule, where it reabsorbs both sodium and chloride. Therefore, any impairment of this transporter's function leads to salt loss via the kidney, resulting in a decrease in extracellular fluid volume and compensatory activity of the renin-angiotensin-aldosterone system (RAAS). The study also observed elevated renin and aldosterone levels in all participants, supporting a shared pathological condition despite the distinct variations in the gene mutations. This shared condition will likely increase the effect of aldosterone on the kidney, leading to increased potassium and hydrogen ion loss, resulting in hypokalemia and metabolic alkalosis [27–30]. These results are consistent with previous studies that indicated renal tubular abnormalities leading to salt loss and increased RAAS activity. These findings are characteristic of this condition, known as Gitelman syndrome. These studies have demonstrated a correlation between the severity of physiological changes (biochemical and clinical manifestations) and the type of genetic mutation. In particular, the mutations in the current study were observed to disrupt or reduce NCC transporter activity, which in turn will lead to a major disturbance in ionic balance. As for the mutations that

have a less severe effect, they will preserve some of the carrier's functions, and thus the symptoms or disease state will be milder [31–34]. Some common bioinformatics algorithms, which included three types: SIFT, PolyPhen-2, and PROVEAN, were used for the purpose of predicting whether the mutations recorded in this study would have a future impact on protein function and thus the occurrence of the disease. However, the optimal use of these algorithms was next to laboratory diagnosis because these algorithms will support the evaluation of mutations and interpretation of the relationship with the current symptoms of sample individuals [35,36]. In general, the results of the current study indicated that there is a strong and clear association between the age of the recorded mutations and the severity of the disease condition of the affected individual, as these variables explained the depth of the physiological disorders occurring in the affected individuals. The results also indicated that genetic analysis helped in understanding the mechanism that leads to the loss of salts and clarifying the defect occurring in the renal tubule that is linked to the NCC transporter defect.

CONCLUSION

Mutations in the SLC12A3 gene are predominantly associated with electrolyte imbalances, particularly low potassium and magnesium levels, as well as mild metabolic alkalosis. Predicting mutations as detrimental or affecting function is often linked to clinical symptoms and significant biochemical variations. Mutations with limited or neutral effects may be associated with less severe changes in kidney function and electrolytes. In addition, the high concentration of renin and aldosterone that was recorded in all patients in the sample reflects that there is an increase in the activation of the renin-angiotensin-aldosterone system, which is considered an alternative to compensate for the loss of salt. This study confirms the importance of genetic analysis in understanding the association between SLC12A3 mutations and the clinical manifestations of renal tubular disorders.

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