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The Association of Calcium- Sensing Receptor (*CaSR*) Gene Polymorphism and the Risk of Kidney Stone Disease

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Abstract

Urolithiasis or kidney stone is a multiple factorial disorder caused by a complex interaction of genetic predisposition, lifestyle, and dietary habits that lead to the formation of kidney stones. Calcium stones, including calcium oxalate and phosphate, are the most common kidney stones. The calcium-sensing receptor (*CaSR*) is a protein containing 1078 amino- acids that potentially affects calcium homeostasis and stones formation. Since most stones are calcium-dependent, this represents a starting point for research into calcium status and the genetic polymorphisms affecting it. We investigated the association of the Single nucleotide polymorphisms (SNPs) of the calcium-sensing receptor gene (*CaSR*), such as rs1801725 A986S and rs1042636 R990G SNPs in the Iraqi kidney stones population. This study included (96) samples, 64 patients with kidney stones, and the control group consisted of 32 healthy individuals. A polymerase chain reaction technique and DNA sequencing were used for genotyping *CaSR* SNPs. It was found that *CaSR* rs1801725 GG genotypes (OR 1.0000, 95% CI 0.4273-2.340) have no effect on the disease compared with GT genotypes (OR 1.053, 95% CI 0.4533-2.5035) which is related with disease. *CaSR* rs 1042636 AA genotypes (OR 1.9676, 95% CI 0.5936-6.5217) are related to disease. Compared with AG genotypes (OR 0.7855, 95% CI 0.2346-2.6303), which reduces the chance of disease.

Keywords: Calcium kidney stones, calcium-sensing receptor (*CaSR*), Single nucleotide polymorphisms, Stone formation,

العلاقة بين تعدد اشكال جين مستقبل استشعار الكالسيوم وخطر الاصابة بمرض حصي الكلى

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الخلاصة

حصوات الكلى هو اضطراب متعدد الاسباب، حيث تلعب الاستعدادات الوراثية ونمط الحياة والعادات الغذائية دورا في تكوين حصي الكلى. تعد حصوات الكالسيوم التي تشمل اكرالات الكالسيوم وفوسفات الكالسيوم أكثر انواع حصي الكلى شيوعا. تلعب تعددات الاشكال الجينية دورا مهما في تطوير حصي الكلى، ونظرا لان معظم حالات الحصى تعتمد على الكالسيوم، اعتبرت نقطة انطلاق للبحث في مستوى الكالسيوم والتغيرات الوراثية المؤثرة عليه. لقد بحثنا في دراستنا تعدد اشكال النيوكليوتيدات المفردة SNPs لمستقبل استشعار

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الكالسيوم (CaSR) مثل SNPs rs1801725 A986S and rs1042636 R990G للمرضى حصى الكلى في المجتمع العراقي في هذه الدراسة تضمنت المجموعة التجريبية (96 عينة: 64 مريض مصاب بحصى الكلى و 32 فردا سليما). تم استخدام تقنية تفاعل البوليميراز المتسلسل لتحديد النمط الجيني لتعدد اشكال النيوكليوتيدات المفردة ، ثم تتبع بتحديد تسلسل الحامض النووي DNA لمعرفة وتحديد الانماط الوراثية. وجد ان النمط الوراثي GG لتعدد اشكال النيوكليوتيدة المفردة للموقع *CaSR* rs1801725 A986S وكانت نسبة الارجحية (OR 1.0000, 95% CI 0.4273–2.3405) وهذا يعني انه لايمك تايثير على المرض مقارنة ب النمط الوراثي GT الذي يحمل الاليل T كانت نسبة الارجحية (OR 1.053, 95% CI 0.4533–2.5035) والذي يرتبط بزيادة خطر الاصابة بالمرض، بينما كان النمط الوراثي TT بقيمة ارجحية (OR 2.5035) والتي ترتبط بزيادة خطر الاصابة بالمرض مقارنة مع النمط الوراثي AG (OR 0.7855, 95% CI 0.2346–2.6303) والذي يقلل من نسبة الاصابة بالمرض: في حين كان النمط الوراثي GG مفقود لهذا يمكن اعتباره عامل حماية ضد المرض.

1. Introduction

Urolithiasis, or kidney stone disease, is a multifactorial condition resulting from a complex interaction of the metabolism , genetic and environmental factors [1,2]. With significant morbidity and significant expenditures to the world economy and health system , it is considered one of the most prevalent health problems[3]. An increase in global concern for kidney stones is associated with the rise in morbidity and prevalence rate around the world, its estimated at 1-15% of individuals[4]. According to statistics issued by the Iraqi Ministry of Health, the infection statistics in the last five years have been continuously increasing, reaching (85835) in 2018, (93635) in 2019, (63602) in 2020, (81160) in 2021, (110818) in 2022 and (123880) in 2023[5].

There are five different types of stones. Calcium stones, which mainly contain calcium, were subdivided into calcium oxalate (CaOX) and calcium phosphate (CaP), which account for 80 % of all urinary calculi in most of the world's countries, while other types of kidney stones constitute a smaller percentage, such as struvite (magnesium ammonium phosphate)stones(~10–15%), Uric acid stones or urate accounts for (~ 3–10%), cystine stones accounts less than 2% of all stone types and drug-induced stones accounts for about 1% of all kidney stones types[6,7].

Stone formation is a slow and complex process resulting from an imbalance of inhibitors and promoters of crystallization ; it includes several physicochemical events, including supersaturation, nucleation, growth, aggregation, and retention of urinary stone constituents within tubular cells [8, 9]. The initiation of crystals is considerably controlled by various regulators, such as the calcium-sensing receptor (CaSR) and vasopressin V2 receptor, inhibitors specifically citrate, and uromodulin [10], while some portions promote the crystallization process [11].

Kidney stone formation is significantly influenced by genetic factors that regulate calcium metabolism directly or indirectly. The calcium-sensing receptor (*CaSR*) gene is considered as a major player in maintaining Ca^{+2} homeostasis as its protein receptor regulates the calcium levels in the serum and markedly contributes to kidney stone formation [12,13].

The CaSR protein is a member of the largest cell plasma membrane receptor family, containing 1078 amino- acids, and is considered a member of the G-protein coupled receptor

(GPCR) that potentially affects calcium homeostasis [14]. It senses to minor changes in the concentration of free calcium levels in the blood, and activates signalling events within the cell to restore the level of calcium ions to normal and maintain serum calcium concentrations in a normal physiological range [15]. The structure of the receptor of CaSR is comprised of a large amino-terminal extracellular domain (ECD) that contains a ligand-binding venus flytrap (VFT) domain which contains two lobes (lobe one and lobe 2) and a cysteine-rich domain (CRD) that links a seven-trans membrane domain (7 TMD) to carry signals from VFT domain to downstream G proteins, and long C-terminal intra- cellular tail cytoplasmic tail [16-18].

When the receptor is inactive it is open conformation, and activated by homodimer formation by binding the fourth binding site of two polypeptide chains ; the structure of the receptor changes to a close conformation. In the presence of high calcium concentration, ionized calcium binds between the two lobes of the VFTD, and the change in the structure conformation leads to activation of CaSR, leading to changes in its signal transduction and increased intracellular concentration of calcium [19]. The *CaSR* gene is expressed in a specific manner specifically in the parathyroid gland and kidney, where its role is well tissue characterised. Variations in gene expression of the *CaSR* gene by gain or loss of functions mutations may lead to Ca^{2+} homeostatic disorders [20, 21].

The *CaSR* gene is located on chromosome 3q13.3-21, composed of eight exons, seven introns, and passes over approximately ~100-kb. The first exon (1A and 1B) encodes for alternative 5'-untranslated regions (UTRs), and exons 2–7 encode the CaSR protein [22,23]. This gene exhibits at least 200 non-synonymous single nucleotide polymorphisms that influence the formation and development of kidney stones, as they affect the Ca^{2+} homeostatic. Several of these genetic variations were associated with an increased risk of stones formation. It was demonstrated that, the most frequent SNPs were located in exon 7 of the *CaSR* gene, including guanine–thymine substitution at codon 986 (A986S; rs1801725), and the substitution of an adenine–guanine at codon 990 (R990G; rs1042636) [17, 24].

Due to the limited research and studies on the role of genetic changes in the CaSR protein in the development of kidney stones in the Iraqi population. Our current study aims to shed light on the association between the most frequent genetic variations of the *CaSR* gene (*CaSR* rs1801725 A986S and rs1042636 R990G) SNPs and the risk of kidney stones in a cohort of Iraqi patients.

2. Material and methods

2.1. Subjects and samples

This study was conducted from November 2023 to July 2024 at Yarmouk Hospital in Baghdad, Iraq; the study was designed to find an association between *CaSR* gene variations in patients who are suffering from recurrent kidney stones. The samples were obtained from 96 individuals (60 male and 36 female) with vitamin D deficiency, and were divided into two groups: the patients were 64 (42 male and 22 female), and the healthy groups were 32 (18 male and 14 female). Official approvals were obtained to conduct this research according to the ethics of scientific research for human studies. Both patients and control participants were diagnosed by kidney ultrasound, and X- ray to confirm the presence or absence of kidney stones. A general urine examination and biochemical analysis were performed for renal functional investigation. The kidney stones were collected from the patients and subjected to a chemical quantitative method to determine the main mineral components of urinary stones. According to the kit (Bioloabo/France).

Subjects were excluded from this study, patients diagnosed with autoimmune disorders, diabetes, cancer, high blood pressure, auto immune disease and if they take vitamin D and calcium supplementations.

Five ml of venous blood was collected from each patient and control; each blood sample was divided into two sections: Two ml of whole blood was kept in EDTA tubes for DNA extraction and used for the genetic study and the other (3 ml) was poured in GEL tubes, then centrifuged at 5000 rpm for 5 minutes. Biochemical analysis for kidney function, including investigation of serum uric acid, urea, and creatinine, was performed in hospital laboratories for both patient and control participants, and the sera were obtained for investigation of serum vitamin D3. On the other hand, urine samples (10-30 ml) were collected from patients and control groups for the microscopic examination to confirm the presence or absence of the crystals in the urine. Also complete blood count (CBC) tests and biochemical tests (urea, uric acid, Ca^{++} , and vitamin D level) for each patient and control group. Family history of kidney stones disease (KSD) was recorded from all patients, and make sure no family history of KSD was found for any people in the control group. Also, all the details related to the disease were collected by conducting a questionnaire that included details about the nature of the food, the amount of fluids taken, and weight, and ensured that there were no chronic, genetic, or immune diseases.

2.2. Genetic study

The genetic study included the extraction of genomic DNA from peripheral blood samples according to the kit (ReliaPrep (TM)) Blood DNA Miniprep system/Promega/USA), then DNA concentration and purity were measured by Nano-Drop spectrophotometers (Quawell Technology Inc./ USA),. The targeted DNA sites were amplified using design-specific primers provided by (MacroGen/ South Korea), which was designed by (Geneious primer) software (MacroGen – South Korea), (<http://WWW.macrogen.com>). The primer for *CaSR* gene rs1801725 and rs1042636 were: the forward primer (5'- AAGAGCAACAGCGAAGAC-3') and reverse primer (5'- TATCCCTTCTTCCTCAGAGG-3'). The final volume of the reactions was 25 μ l, composed of 1.5 μ l of each forward and reverse primer, 12.5 μ l of OneTaq (NEB®) master Mix, three μ l of genomic DNA (concentration of 7.8 ng/ μ L, A260/A280 = 1.8), and 6.5 μ l of free-nuclease water added to the reaction volume of PCR. DNA had been amplified under the optimal PCR conditions for this gene. Amplification was conducted in a Gradient thermo cycler (Eppen-drop, Germany). PCR thermal program was (35) cycles with the following settings: Initial denaturation stage at 94°C for 3 minutes., denaturation stage at 94°C for 30 second, annealing stage at 51°C for 45 seconds and extension stage at 68°C at 30 seconds (according to the manufacturer's instruction for the DNA polymerase enzyme), at last, final extension stage at 68°C for 5 minutes, the amplified PCR products was (620bp). After completing the reaction, PCR products were electrophoresed in 2% agarose at 80 V for 80 minutes using apparatus gel electrophoresis (Cleaver Scientific – UK) and visualized with μ l Red safe dye (Intron Red Safe Nucleic Acid Staining Solution/South Korea). A gel documentation system with a high-resolution camera (Cleaver Scientific –UK) was used to capture the images.

2.3. Sequencing

For each sample, the PCR products were subjected to Sanger sequencing (MacroGen company/ South Korea) for both the forewords and reverse primers to identify the single nucleotide polymorphism. Analysis of the sequences in FASTA files has been done by Geneious Prime software (MacroGen – South Korea) <http://WWW.macrogen.com> and aligned to Ref Seq of (*CaSR*) gene.

2.4. Statistical

Statistical analyses have been done using SPSS statistical software (version 26; SPSS IBM). The effect of various factors on study parameters was assessed using the *t*-test and least significant difference (LSD) test. The genetic statistical analyses have been done by the medical online, odd ratio calculator software (https://www.medcalc.org/calc/odds_ratio.php) to compare the association between the odd ratios for each genotype with kidney stone disease. Statistical significance was determined at the p-value of 0.05 and chi-square at $\chi^2 < 3.84$.

3. Results and Discussion

3.1 Clinical and demographic characteristics of patients

This study included 64 patients (42 male and 22 female) diagnosed with kidney stones, aged between (20 -78 years), with an average age of (39 years).

The results of the questionnaire regarding dietary habits showed that (37.5%) of patients follow a diet based on meat and dairy products, while (34.4.%) follow a mixed diet, and (28.1%) follow a vegetarian diet. Regarding fluid intake, (35.9%) of participants reported that their fluid intake was good, while (64.1%) described their fluid intake as low. Finally, (48.4%) of patients reported having a family history of the disease, while (51.6%) reported that the disease was not found in their families, as shown in (Table 1).

Table 1: Distribution of dietary habits, fluid intake, and family history among patients with kidney stones.

Variable	Group	Frequency	Percentage (%)
Dietary habit	Meat and dairy products	24	37.5
	Vegetables	18	28.1
	Meat and Vegetables	22	34.4
Fluid intake	Low	41	64.1
	Normal	23	35.9
Family history	Have not a family history	33	51.6
	Have a family history	31	48.4

3.2 Biochemical tests

The results of kidney stone analyses showed that 82% of the stones were (CaOX) stones, while uric acid stones constituted 18%. The results of the biochemical tests found no differences in patients with kidney stones compared to controls in terms of most variables (calcium, vitamin D, uric acid, and creatinine). The lack of significant differences between the patient group and the control group in most biochemical tests may be due to several reasons, including the small sample size, which may not be sufficient to detect significant differences, or the patient may have followed a diet after diagnosis of the disease. However, they showed differences in urea between the patient and control group. The results of calcium were (9.638 ± 0.831) in the patients compared to (9.519 ± 0.3167) in control with a p-value of (0.317); the standard normal ranges are (8.5-10 mg\dl). The results of vitamin D were (15.713 ± 5.804) in the patients compared (to 16.1662 ± 6.49197) in control with a p-value (of 0.740); the normal ranges are (30-70 ng\ml). The results of Uric Acid were (4.9203 ± 1.52173) in the patients compared to (6.262 ± 0.995) in the control with a *P* value (of 0.077); the standard normal ranges are (2.4-6 mg\dl). The results of creatinine were (0.948 ± 0.420) in the patients compared to (0.890 ± 0.248) in the control with p-value (0.471); the standard normal ranges are (0.5-1.2 mg\dl). The results of urea were shown high significance (30.514 ± 9.456) in the

patients compared to (26.753 ± 5.527) in control with a p-value (0.016); the standard normal ranges are (7-37 mg\dl), as shown in (Table 2).

Table 2: Mean $\pm$ S.D. of Serum calcium, vitamin D3, Urea, Uric Acid and Creatinine of the study groups

Variables	Patients (N=64) (mean $\pm$ SD)	Control (N=32) (mean $\pm$ SD)	Normal ranges	p-value	Significance
Calcium (mg/dL)	9.638 $\pm$ 0.831	9.519 $\pm$ 0.316	8.5-10 mg\dl	0.317	N.S
Vitamin D (ng/ml)	15.713 $\pm$ 5.804	16.166 $\pm$ 6.491	30-70 ng\ml	0.740	N.S
Urea (mg/dl)	30.514 $\pm$ 9.456	26.753 $\pm$ 5.527	7-37 mg\dl	0.016	S
Uric Acid (mg/dl)	4.920 $\pm$ 1.521	6.262 $\pm$ 0.995	2.4-6 mg\dl	0.077	N.S
Creatinine (mg/dl)	0.948 $\pm$ 0.420	0.890 $\pm$ 0.248	0.5-1.2 mg\dl	0.471	N.S

S: Significant **NS: Non- significant**

3.3. Genetic study results

The extracted genomic DNA samples of patients and controls were separated in agarose gel (1 % agarose) using an electrophoresis device (1 % agarose, 75 volt, for 1 hour), as shown in (Figure 1).

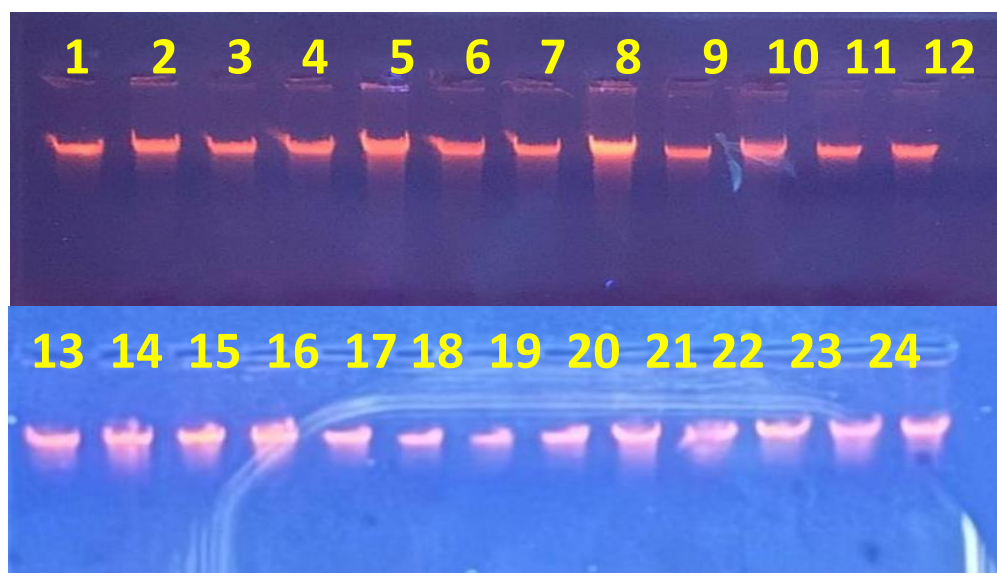


Figure 1: Gel electrophoresis of extracted genomic DNA from patient and control groups in 1% agarose, at 75 V, for 1 hour, stained by Red safe dye.

PCR products were separated in 2% agarose gel in TBE with an electrophoresis device at (80 Volt for 80 minutes), as shown in Figure 2.



Figure 2: PCR product gel electrophoreses at 2% agarose in TBP for 80 `minutes at 80 volt for the patient group, band size 620 bp. Line 1 is the DNA ladder(100bp).

3.2.1 Analysis of polymorphism in *CaSR* gene rs1801725 (G>T)

Analyzing the sequence results for the *CaSR* amplified fragment gene shows that the rs1801725 (G>T) single nucleotide polymorphism displays the genotype and allele frequency of both control and patient groups. The observed value is the same as the expected value in both control and patient groups, and it was found that the Chi-Square χ^2 values were statistically low when compared to the tabular Chi-square values of 3.84., therefore the alleles are consistent with Hardy Weinberg law in this population, as shown in (Table 3).

Table 3: Expected frequencies of *CaSR* rs1801725 genotypes using Hardy-Weinberg equilibrium for the expected frequencies of genotypes to determine whether the distribution of genotypes of the study groups remains conserved.

Groups	genotype	GG	GT	TT	Chi-square χ^2
Controls	Observed genotype	15	14	3	0.0106 C
	Expected genotype	15.12	13.75	3.12	
Patients	Observed genotype	30	29	5	0.3085 C
	Expected genotype	30.94	27.11	5.94	

C: Distribution consistent with Hardy Weinberg law at the level significance $\chi^2 < 3.84$.

By analysis of genotype distribution between the control and patient groups, the study found three genotypes (GG, GT, and TT.) at the position rs1801725 in both groups. It was found that the GG genotype in the studied population was almost equal, appearing as (47%) in both control and patient groups; the p-value is (1.000) significantly greater than 0.05. Therefore, showed no significant differences between control and cases in the GG genotype; the odds ratio (OR) for the GG genotype was (1.000) with a confidence interval (CI) (0.427 - 2.340), which indicates that there was no association between the GG genotype and stones formation, the wide CI range indicates a degree of uncertainty, suggesting that a larger sample size might be needed to confirm this finding with higher confidence. GT genotype was (45%) in patient and (44%) in control with a p-value of (0.884), showing no significant differences between control and cases in the GT genotype; the OR for the GT genotype was (1.065) > 1, with a CI of (0.453 - 2.503). Therefore, this genotype may contribute to stone formation. The TT genotype frequency was 8% in patients and 9% in controls with a p-value (0.794), showed no significant differences between control and cases in TT genotype, the OR for the GT

3.2.2. Analysis of polymorphism in *CaSR* gene rs1042636 (A>G).

The sequence analysis results of rs1042636 (A>G) single nucleotide polymorphism of the *CaSR* gene display the genotype and allele frequency of both control and patient groups. The observed value was the same as the expected value in both control and patient groups, and the Chi-Square χ^2 values were statistically low compared to the tabular chi-square values of 3.84. Therefore, the alleles are consistent with Hardy Weinberg law in this population, as shown in Table 6.

Table 6: Expected frequencies of *CaSR* rs1042636 genotypes using Hardy-Weinberg equilibrium for the expected frequencies of genotypes to determine whether the distribution of genotypes of the study groups remains conserved.

Groups	Genotypes	AA	AG	GG	Chi square χ^2
Controls	Observed genotype	27	5	0	0.229 C
	Expected genotype	27.19	4.60	0.19	
Patients	Observed genotype	55	8	0	0..229 C
	Expected Genotype	55.25	7.49	0.25	

C: Distribution consistent with Hardy Weinberg law at the level significance $\chi^2 < 3.84$.

When analyzing the genotypes of the control and patient groups, we found that AA and AG genotypes in both the patient and control groups, but the absence of the GG genotype in both the patient and control group; this indicates that the disease does not appear in people who carry the GG genotype. The AA genotype in the studied population was (86%) in patient groups while (84%) in controls. This indicates that genotype may contribute to the disease. The p-value was (0.268) significantly greater than 0.05. Therefore, there were no significant differences between the control and cases in the AA genotype; the OR for the AA genotype was (1.967) with a CI of (0.593 - 6.521), which indicates that the AA genotype was associated with an increased risk of disease. AG genotype was (13%) in patients and (16%) in control with a P-value of (0.695). It showed no significant differences between control and cases in the AG genotype. The OR for the AG genotype was (0.785) with a CI of (0.234 - 2.630). This means that the AG genotype is associated with a lower risk of stone formation, while the GG genotype was not found in the patients or the control group, as shown in Table 7.

Table 7: Genotype frequency of *CaSR* rs1042636 (A>G) SNP in control and patients groups

Genotype	Cases N=64(%)	Control N=32(%)	OR	95%CI	P-value
AA	55 (86%)	27 (84%)	1.967	0.593- 6.521	0.268
AG	8 (13%)	5 (16%)	0.785	0.234- 2.630	0.695
GG	0 (%)	0(%)	0.511	0.009- 26.388	0.739

OR odd ratio, CI confidence interval $P \leq 0.05$

The allele frequency of the A allele in the patient (94%) was close to the allele frequency of the A allele in control (92%), while the G allele frequency was (6%) in the patient group compared to the control group (8%), this indicates that the A allele frequency constitutes the vast majority compared to the G allele frequency in both groups. The p-value is (0.706) significantly greater than 0.05. Therefore, showed no significant differences between control and cases in both A, and G allele. The OR of the A allele was (1.25) with a CI of (0.391- 3.988), while the odds ratio (OR) of the T allele was (0.80) with a CI of (0.250 - 2.552). Therefore, the A allele may be a contributing factor to kidney stone disease, as shown in

(Table 8). The sequencing results showed several SNPs in the exon7 of the *CaSR* gene at codon 990 (R990G; rs1042636), as shown in Figure 4.

Table 8: Allele Frequency of *CaSR* rs1042636 (A>G) SNP in control and patients groups.

Allele Frequency	Cases N=64(%)	Control N=32(%)	OR	95%CI	p-value
Risk allele A	118(94%)	59(92%)	1.25	0.391 - 3.988	0.706
Protective allele G	8 (6%)	5 (8%)	0.80	0.250 - 2.552	

OR odd ratio, CI confidence interval $P \leq 0.05$

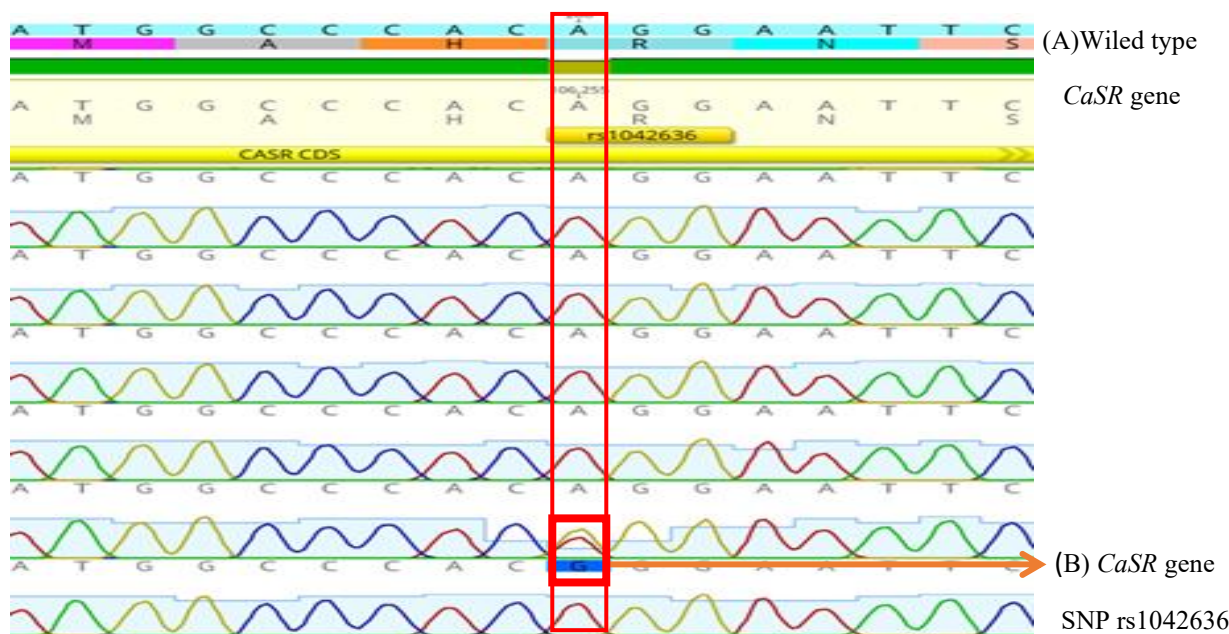


Figure 4: Chromatograms of *CaSR* gene, (A): Wild type *CaSR* gene, (B): *CaSR* gene SNP at (rs1042636) from(A>G)

Most cases of stones depend on calcium concentration in serum such as calcium oxalate stones and calcium phosphate stones; it represents a starting point for research into calcium status and the genetic polymorphisms of the *CaSR* gene affecting stone formation [25]. This study is one of the first to address the relationship between genetic variations in the calcium-sensing receptor and the formation and development of kidney stones in the Iraqi population from a genetic and functional perspective, in an attempt to bridge a knowledge gap and gain a deeper understanding of the mechanisms of mineral regulation in the body. *CaSR* protein can play a substantial role in the regulation of serum calcium level; when the level of calcium in the blood rises, the *CaSR* protein is activated, which reduces calcium reabsorption in the renal tubules; this, in turn decreases calcium levels in the blood and increases calcium excretion in the urine, which increases the possibility of calcium salts being deposited inside the kidneys and the formation of stones. When the level of calcium in the blood decreases, the *CaSR* protein is inhibited, which leads to increased calcium reabsorption in the renal tubules; this, in turn, increases calcium levels in the blood and decreases calcium excretion in the urine, which reduces the possibility of stone formation [26]. The genetic variants in the gene coding region may be functional, altering the resulting amino acid and affecting the expression level of the resulting protein, making it more associated with the disease[27,28]. The genetic variants of the *CaSR* gene are associated with pathophysiological changes associated with altered serum calcium levels through their control of renal calcium reabsorption, either by increasing or inhibiting the calcium reabsorption of calcium in the kidney, loss-of-function variants of the

CaSR protein lead to increased tubular reabsorption of calcium in the renal tubules, which raises the level of calcium in the blood and reduces the excretion of calcium in the urine, thus being less effective in the formation of kidney stones. While gain-of-function variants in the CaSR protein lead to decreased reabsorption of calcium by the urinary tubules and increased renal excretion of calcium in the urine, which increases the likelihood of calcium salt deposition in the kidneys and the formation of stones[17,29]. Therefore, the *CaSR* gene and its polymorphisms are candidates for explaining calcium nephrolithiasis and stone formation. The results of a questionnaire for patients with kidney stones revealed an overlap between behavioral and genetic factors in explaining the occurrence of the disease. It was found that patients who follow a diet based on eating meat and dairy products are the most common, which contributes to increased calcium excretion in the urine and thus increases the risk of stone formation. Fluid intake rates also showed that the majority of patients consume low amounts of fluid, which increases the concentration of salts and their deposition in the kidneys, leading to stone formation. These results are consistent with the results of stone analysis, which indicated that 82% of stones are (CaOX).

Regarding the results of family history of the disease, the questionnaire showed that approximately half of the patients had a family history, indicating a genetic role that may contribute to the predisposition to stone formation [30]. In this study, no deviation from Hardy-Weinberg Equilibrium (HWE) was found in the genotype distribution of the SNPs under study; the study did not find an increase in serum calcium in cases of idiopathic stones. Through the results of the OR, it becomes clear to us that there is no association between rs1801725 SNP and stones formation, and they were not associated with serum calcium; this indicates that these polymorphisms did not alter the function of the CaSR protein. At the same time, the *CaSR* polymorphism rs1042636 SNP is associated with an increased risk of disease but not with serum calcium, this is consistent with the results of both studies [31]. The results of the biochemical tests showed that the low level of vitamin D in both the patients and the control group did not affect the levels of calcium in the blood because the body follows mechanisms to compensate for this loss by reabsorbing it from the bones into the blood [32]. Although there were no statistical differences in biochemistry tests between the patients and the control group, the results remain of clinical importance because the disease is present and still poses a risk to the kidneys. When linking the results of biochemical tests with the results of the study of genetic variations in the *CaSR* gene at positions rs1801725 and rs1042636, These results indicate that these genetic polymorphisms are ineffective and have no role in regulating the function of calcium-sensing receptors in the body, in spite of the notion that they contribute to the formation and development of stones. The lack of association between serum calcium concentrations is consistent with the lack of association between genotype and disease because the increase in calcium levels activates the role of the CaSR protein. The results of genotype and allele frequency were often close between patients and the control group, and they disagreed with most researchers in many countries around the world, such as Russia, India, and Iran. Where rs1801725, and rs1042636 SNPs of the *CaSR* gene were significantly associated with kidney stone disease with $p\text{-value} \leq 0.05$, and serum calcium levels were significantly higher in patients [33,34]. The lack of statistical significance for most of the results of this research directs researchers to increase the sample size in future studies. Researchers also suggest including the research on *CaSR* gene expression to validate SNP effects and their role in the formation of kidney stones.

5. Conclusion

This study demonstrated a weak association between polymorphism rs1801725 and rs1042636 of the *CaSR* gene and the risk of calcium urolithiasis development in the Iraqi population. Another genetic and environmental factor may play a role in regulating calcium

levels and the formation of kidney stones. Therefore, it was suggested that more studies be conducted to determine the relationship between *CaSR* gene polymorphism and kidney stone disease or to identify genes and other factors that may contribute to the formation of kidney stones. However, there were no statistical differences in biochemistry tests between the patients and the control group.

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Author's declaration

The Institutional Scientific Committee at the University of Baghdad approved this study according to the Declaration of Helsinki for human studies, which is consistent with the instructions of the Iraqi Ministry of Health and Environment; under the official letter (No 73283 on 30/11/2023).

Conflict of Interest

The authors declare that they have no conflicts of interest

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