



# Role of Calcium Sensing Receptor Gene Polymorphism r1801725 in the Evaluation of kidney Disease

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**Abstract:** "kidney disease" refers to a diverse range of conditions that affect the structure and operation of the kidneys. It is now understood that even minor deviations in kidney structure and function are linked to a higher risk of mortality as well as consequences in other organ systems. In the kidney it has an inhibitory effect on the reabsorption of calcium, potassium, sodium, and water depending on which segment of the tubule is being activated. In this study we aimed to investigate the genotypes prevalent for the (CASR) rs1801725 variant is responsible for a non-conservative amino-acid change (A986S) in the calcium-sensing receptor cytoplasmic tail. This study involved 50 blood samples were collected as patient with kidney disease and (50) apparently healthy controls. Molecular study was performed in order to investigate the single nucleotide polymorphisms (SNPs) for CASER gene in chromosome 3 (rs1801725) for studied groups which have determined using high resolution method (HRM) genotyping assay by real time polymerase chain reaction (RT-PCR) using the Qiagen rotor gene Q real-time PCR system. . Data obtained was subjected to statistical analysis using the SPSS 25 software. The rs1801725 G/T genotype was associated with increased risk for kidney disease in Iraqi patients with highly significant difference. The average age of a patient with kidney disease is between 21 and 69 years old The Genotypes and allele frequencies of (rs1801725, G>T, A986S) in patient with kidney disease groups 48% (n=24) wild (TT), and 14% (n=7) heterozygous (TG) and 38% (n=19) homozygous (GG). Allele frequencies for T and G were 55% and 45% respectively It was concluded that the variants of CASR SNP, namely, rs1801725 might be involved in susceptibility to kidney disease.

**Keywords:** The calcium-sensing receptor gene (CASR), kidney disease (KD)

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## Introduction

The kidneys are the main organ of the human urinary system, responsible for filtering the blood, and concentrating metabolic waste into urine by means of the renal glomerulus (1) Kidney disease is a complex condition that can result from various factors, including genetic predisposition, lifestyle factors, and environmental exposure Autosomal dominant polycystic kidney disease

(ADPKD) is the most common hereditary kidney disease and leads to end-stage renal disease. Kidney failure has a substantial impact on life expectancy and health related quality of life (HRQoL). Although dialysis prolongs life for patients with End-Stage Kidney Disease (ESRD), morbidity and mortality remain high in this population. (2,3). The worldwide burden of kidney disease is rising, but public awareness remains limited,

underscoring the need for more effective communication by stakeholders in the kidney health community (4). The calcium-sensing receptor (Caser) is a calcium ( $\text{Ca}^{2+}$ ) sensitive G protein-coupled receptor implicated in various biological processes (5). It was molecularly identified in 1993 by Brown *et al.* in the laboratory of Dr. Steven Hebert with an expression cloning strategy (6). Calcium-sensing receptors (Caser) is found in the membrane of parathyroid cells, these sensors are responsible for increasing and lowering of PTH secretion based on the calcium concentrations (7,8). The two primary hormones involved in calcium balance are parathyroid hormone (PTH) and 1,25 vitamin D (1,25D)(9). They play a key role in calcium homeostasis, by sensing free calcium levels in blood and regulating parathyroid hormone secretion in response (10). Vitamin D is responsible for enhancing intestine absorption of calcium, magnesium, and phosphate (11). The Caser is also located in other organs imperative to  $\text{Ca}^{2+}$  homeostasis including the kidney and intestine, where it modulates  $\text{Ca}^{2+}$  reabsorption and absorption, respectively. (12). The human calcium-sensing receptor gene (*CASR*) has 8 exons, and localizes to chromosome 3q. Exons 1A and 1B encode alternative 5'-untranslated regions (UTRs) that splice to exon 2 encoding the AUG initiation codon. Exons 2–7 encode the CaSR protein of 1078 amino acids. Promoter P1 has TATA and CCAAT boxes upstream of exon 1A, and promoter P2 has Sp1/3 motifs at the start site of exon 1B (13).

#### Materials and methods

The study includes 100 Iraqi participation divided into two

groups: 50 patients and 50 control. EDTA (Ethylene Diamine Tetracetic Acid) blood samples from patients and controls were kept at  $-20^{\circ}\text{C}$ . ReliaPrep™ Blood gDNA Miniprep System was utilized to extract genomic DNA. Both, in the case of DNA purity and Genotyping of polymorphism (rs1801725) of the CASER gene was done, by using High Resolution Melting (HRM). Used master mixes were containing EVA-Green, HRM Master Mix Synthetic SNP sequences was tested using duplicates. The DNA was extracted, using DNA extraction kit EasyPure® Genomic (TransGen, biotech. EE101-01). The concentration and DNA purity were determined by using a Nanodrop spectrophotometer one c (Thermo Fisher Scientific). The concentration was in the range of (66–182 ng/ $\mu\text{l}$ ). In the case of DNA purity, the Nanodrop spectrophotometer 2000c was also used in which the absorbance of sample was read at two wavelengths (260 and 280nm). The A260/A280 ratio was within the range 1.7–1.9. (Figure 1).

Primer sequences were designed according to their reference sequence (rs) in the National Center for Biotechnology Information database (NCBI). The forward-primer GCACGGTCACCTTCTCACTG and the Reverse-primer

AGGGAGTTCTGGTGCGTAGA (Table 1). The thermal cycling program was as follows: enzyme activation in  $94^{\circ}\text{C}$  for 30 sec, (first one was denaturation  $94^{\circ}\text{C}$  for 10 sec and second step of annealing  $60^{\circ}\text{C}$  for 15 sec (40 cycle) and extension  $72^{\circ}\text{C}$  for 20 sec. SNP Genotyping Assay HRM analysis with ramping by 2sec from  $55$  to  $95^{\circ}\text{C}$  (Figure 1).

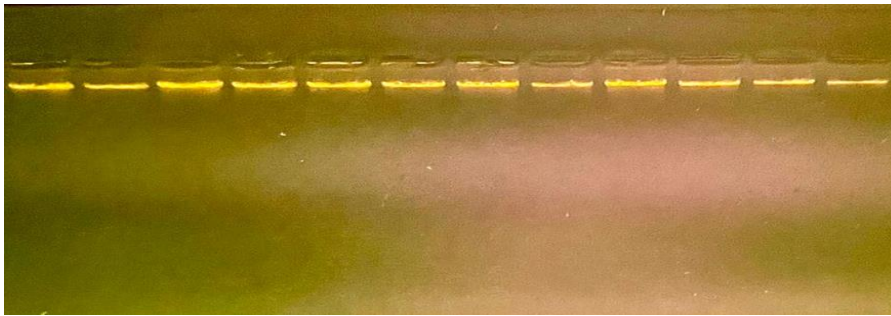


Figure (1): Genomic DNA gel electrophoresis for 10 Samples on Agarose gel with a concentration of 1% for 70 min and 70 volts.

Table (1): Specific primers for CASER single nucleotide polymorphisms.

	Primer Sequence(5'-3')	Primer size	Product size bp
F-rs1801725	GCACGGTCACCTTCTCACTG	20	82
R-rs1801725	AGGGAGTTCTGGTGCCTAGA	20	82

### Statistical analysis

The Statistical Analysis System-SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference -LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Estimate of Odd ratio and CI in this study (SAS, 2018).

### Results and discussion

The CASR gene, containing seven exons, was located on chromosome 3q13. A single nucleotide polymorphism (SNP) of CASR gene in this study found that the Genotypes and allele frequencies of rs1801725 in (HWE) in patient with kidney disease groups 48% (n=24) normal (TT), and 14% (n=7) heterozygous (TG) and 38% (n=19) homozygous (GG). the Genotypes and allele frequencies of rs1801725 in Hardy Weinberg equilibrium (HWE) in healthy control was 64% (n=32) normal (TT), and 32%(n=16) heterozygous (TG) and 4% (n=2) homozygous (GG). Allele frequencies for T and G were 55% and 45% respectively. In patient The genotype distribution is within Hardy

Weinberg equilibrium (Goodness of fit  $X^2 = 1.78$ ,  $df = 2$ ,  $P = 0.07$ ). Genotype and allele frequencies of the patient and control are presented in (Table 2).

Genotype and allele frequencies of the patient and control are presented in patients and control subjects show no Significant differences in the frequency of CASER rs1801725 allele (TT) and genotypes were observed between patients and controls  $P$ -value (0.3285) but it showed highly significant different in patients and control subjects in homozygous (GG)  $P$ -value were observed (0.0002) and heterozygous (TG) showed highly significant different in patients and control  $P$ -value(0.06) G allele frequency values were (0.20 and 0.45) for apparently healthy subjects and patients, respectively, the values of allele frequency of the T allele were (0.80 and 0.55) of apparently healthy with patients, respectively. The TT percentage in healthy controls and patients (64%, 48%), the TG percentage (32%, 14%) while GG percentage (4%, 38%) respectively. As showed in (Table 2) and (Figure 2) there was a positive correlation(etiological factor) between GG genotype with kidney disorder, this mean that men who caring GG genotype

have higher risk of kidney problem than other genotypes, Although a decreased frequencies of G allele (45 vs. 20%) and an increased frequencies of T allele (55% vs. 80 %) were observed in patients compared to controls and the difference was highly significant (Figure 3).

The present study supports a similar study (14) who found that the variant allele of *CASR* rs1801725 solely and together with the variant allele of rs7652589 increases risk of more advanced sHPT. Homozygosity of the rs1801725 variant allele contributes to infection-related mortality in HD patients.

In Iraq study by Isama (15) found that variants of *CASR* SNP, namely, rs1801725 might be involved in susceptibility to kidney stone disease. While in Egypt Another study by (16) found that a significant association with the risk of kidney stone disease (KSD) in the Egyptian population. Furthermore, the rs 1801725 might have an impact on the genetic susceptibility to develop KSD. Homozygosity of the rs1801725 variant allele contributes to infection-related mortality in HD patients. in West Indian Population (17) found that *CaSR* gene variants, rs1801725 (GG) and rs1042636 (AA), both have shown significant association with renal stone disease. Another study showed that *CASR* polymorphisms (rs1801725,

rs7652589) are associated with dyslipidemia in HD patients.(18) another study in Iraq which found that the *CaSR* polymorphism A986S was one of the genetic susceptibility factors for the premenopausal and postmenopausal in Iraqi women with osteoporosis and had little effects on mineral levels (19). The recognition of SNPs was achieved by using HRM (high resolution melting) real-time PCR (20).

The SNP frequency is significant in the samples under study, and the result varies according to the samples size. However, there are some contradictory conclusions. Limited sample sizes, low statistical power, ethnic variations, extensive geographic variation, interactions with other genetic or environmental factors, and clinical variability may all contribute to this difference.

### Conclusion

In conclusion, it appears that there was a positive correlation (etiologically factor) between GG genotype with kidney disorder, this mean that men who carrying GG genotype have higher risk of kidney problem than other genotypes, Although a decreased frequencies of G allele (45 vs. 20%) and an increased frequencies of T allele (55% vs. 80 %).

**Table (2): Genotype and allele frequencies of CASER gene of Control group and kidney disorder group in blood samples.**

Genotype	Control No.50 (%)	Patients No50. (%)	$\chi^2$	P-value	O.R. (C.I.)
TT	32 (64.00%)	24 (48.00%)	1.142 NS	0.285	Ref. =1
TG	16 (32.00%)	7 (14.00%)	3.521 NS	0.061	0.583 (0.29-1.28)
GG	2 (4.00%)	19 (38.00%)	13.761 **	0.0002	12.66 (5.92-17.51)
Total	50	50	--	--	--
T	80 (0.80)	55 (0.55)	P-value = 0.031 *		
	20 (0.20)	45 (0.45)	P-value = 0.0019 **		
** (P≤0.01), NS: Non-Significant.					

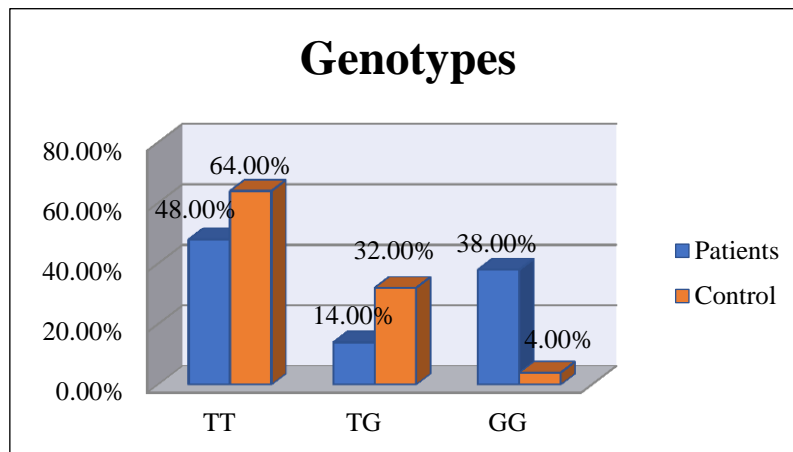
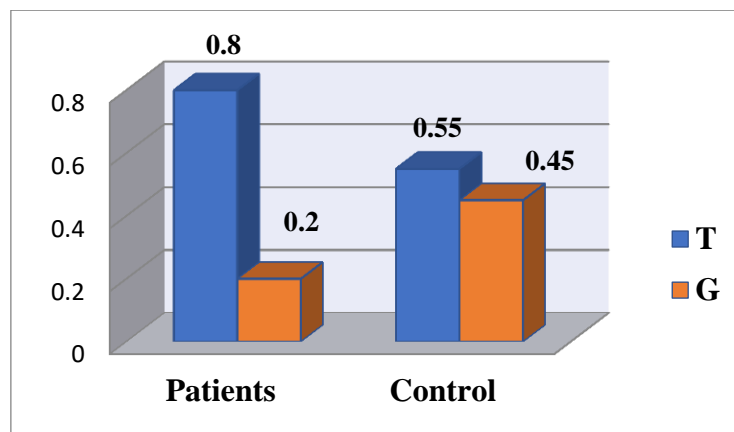


Figure (2): Comparison of genotypes between patient and control group



Figure(3): Allele Frequency in patient and control groups

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