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Association of RAGE gene polymorphisms (rs2070600 and rs2071288) with bone metabolism disturbances in patients with chronic kidney disease

Asma Ali Awad[1], Gholamreza Dehghan[1,2*], Hamid Tayebi Khosroshahi[3], Mahdi Haghi[1]

1. Laboratory of Biochemistry and Molecular Biology, Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.
2. Research Center of Biosciences and Biotechnology (RCBB), University of Tabriz, Tabriz, Iran.
3. Department of Nephrology, Tabriz University of Medical Sciences, Tabriz, Iran.

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***Corresponding**:
Gholamreza Dehghan
Address:
Laboratory of Biochemistry and Molecular Biology, Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
E-mail:
gdehghan@tabrizu.ac.ir

ABSTRACT

Chronic kidney disease (CKD) is frequently accompanied by disturbances in bone metabolism, leading to a heightened risk of fractures. The receptor for advanced glycation end-products (RAGE) plays a pivotal role in the pathogenesis of CKD. This case-control study included 50 CKD patients and 50 healthy controls to examine the association between RAGE gene polymorphisms (rs2070600 and rs2071288) and bone metabolism markers. Serum parathyroid hormone (PTH), alkaline phosphatase (ALP), and vitamin D levels were measured as indicators of bone metabolism. CKD patients exhibited significantly higher ALP (317.54 ± 21.95 U/L) and PTH (331.33 ± 17.11 pg/mL) levels, along with lower vitamin D (26.92 ± 6.64 ng/mL) compared to controls (all $P < 0.05$). The CC genotype of rs2070600 was more prevalent among patients (52%) and was associated with an increased risk of CKD (OR = 2.79, 95% CI: 1.21–6.39, $P = 0.014$). In contrast, no significant association was found between rs2071288 and CKD risk. Notably, individuals carrying CC or CT genotypes for rs2070600 and rs2071288 had higher levels of PTH and ALP, alongside significantly lower levels of vitamin D. The RAGE gene's rs2070600 polymorphism may be connected to abnormalities in bone metabolism and the advancement of CKD. These results indicate that rs2070600 genotyping may be useful in identifying high-risk patients; however, additional research is required to validate its clinical significance.

Keywords: Chronic kidney disease, Bone metabolism markers, Genetic polymorphism, Receptors for advanced glycation end products

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Introduction

Chronic kidney disease (CKD), a global health issue, is a multifactorial and irreversible pathophysiologic process characterized by the gradual loss of kidney function. It serves as a major risk factor for end-stage renal disease (ESRD), affecting 5–10% of the world's population (1, 2). CKD is directly attributed to both types 1 and 2 diabetes mellitus, with 21 million people diagnosed with type 2 diabetes in the USA, where approximately one in three adults with diabetes also has CKD (3). The majority of CKD patients are at an increased risk of bone and mineral metabolism disorders, characterized by abnormal bone metabolism, laboratory findings (e.g., calcium, phosphorus, parathyroid hormone (PTH), or vitamin D metabolism), and vascular or soft tissue calcification (4, 5). It has been reported that the primary causes of death among people with CKD are closely related to cardiovascular disease (CVD), progression to ESRD, and uremic toxins (6).

During CKD, metabolic byproducts known as uremic toxins accumulate in the body. Among these, advanced glycation end-products (AGEs) are significant uremic toxins that contribute to various CKD-associated complications (7). These substances result from non-enzymatic interaction between reducing sugars with an amino group of proteins, lipids, and nucleic acids. Oxidative stress, impaired renal excretion, and diet consumption stimulate the production of AGEs (8). AGEs bind to receptors for advanced glycation end products (RAGE), triggering diverse biological consequences, such as apoptosis, oxidative stress, inflammation, and altered gene expression (9). Excessive oxidative stress and chronic inflammation further enhance AGE synthesis, which exacerbates CKD morbidity and mortality.

AGEs bind to RAGE, activating immune cells and triggering the production of inflammatory mediators and chemokines, which initiate inflammatory responses. In addition, RAGE-AGEs interaction also induces oxidative stress, leading to cellular oxidative damage (10). Therefore, the AGE-RAGE axis has been implicated in various disorders, including Alzheimer's disease, diabetes mellitus, CKD, CVD, and schizophrenia (11). The RAGE gene, located on chromosome 6p21.3, exhibits several polymorphic sites, which have been investigated in relation to various diseases, including CKD. These polymorphisms have been studied in relation to various diseases, including CKD (12).

AGEs play a key role in bone metabolism disorders through their interaction with their receptor (RAGE). The accumulation of AGEs in the bone matrix is associated with structural changes in the collagen network and increased bone fragility. Activation of the AGE–RAGE axis increases oxidative stress and cellular inflammation, which ultimately inhibits osteoblast differentiation and enhances osteoclast activity. These processes lead to reduced bone remodeling and deterioration of bone tissue quality in diseases such as CKD (13). The expression or function of RAGE may be changed by variations in this gene, such as the rs2070600 and rs2071288 polymorphisms. These differences may have an impact on important oxidative stress and inflammatory pathways. These differences may cause alterations in bone metabolism in CKD patients, which may result in conditions like renal osteodystrophy.

Exon 3 of the *RAGE gene* has the non-synonymous polymorphism rs2070600 (Gly82Ser). The amino acid Gly is produced by the more common allele G, whereas Ser is produced by the less common allele A. The sRAGE and esRAGE levels have been linked to the rs2071288 single-nucleotide polymorphism (SNP), which is found on chromosome 6 and at a splice site in intron 9. The common alleles for rs2071288 are T and C, resulting in the genotypes TT, CT, and CC (14, 15). Given that RAGE is involved in inflammatory processes and oxidative stress, which are highly increased in CKD, polymorphisms such as rs2070600 and rs2071288 could alter the severity of bone complications by affecting the ability of RAGE to regulate these pathways. Therefore, this study aimed to investigate the association between two specific polymorphisms, rs2070600 and rs2071288, and their potential impact on bone metabolism markers in patients with advanced CKD. Understanding the relationship between these genetic variations and bone health in CKD patients could provide insights into the mechanisms underlying CKD-mineral and bone disorder (CKD-MBD) and contribute to better management and therapeutic strategies for CKD-related bone disorders. Using a case-control design, we analyzed key biochemical markers, including PTH, ALP, calcium, phosphorus, and vitamin D, alongside genotyping for the selected polymorphisms. Our findings indicate that the rs2070600 CC genotype is

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more frequent in CKD patients and is associated with elevated PTH and ALP levels and decreased vitamin D, suggesting that this polymorphism may influence CKD progression and bone metabolism alterations. This approach provides context for understanding the genetic factors contributing to CKD-related bone disorders and may inform future strategies for patient risk assessment and management.

Methods

Study design and participants

This case-control study received ethical approval from the Ethics Committee of the University of Tabriz and informed written consent from each participant or their legal guardian prior to the initiation of blood sample collection. Blood sampling was conducted from a total of 100 study individuals, 50 CKD patients at the Dialysis Center of Tabriz University of Medical Science (aged between 29 and 87 and 50 control participants (aged between 25 and 81). All CKD patients included in this study were at the ESRD stage. Based on minor allele frequencies (MAF) obtained from the NCBI dbSNP database (https://www.ncbi.nlm.nih.gov/snp) and an assumed moderate effect size (Cohen's d ≈ 0.4), power calculations were performed. These calculations indicated that a minimum of 45 individuals per group were needed to attain 80% statistical power at the 5% significance level. In the present study, to improve statistical reliability and take potential participant attrition into consideration, the final sample size of each group was increased to 50 people. We certify that all procedures followed the Declaration of Helsinki and all other applicable rules and laws.

Individuals with a history of prolonged medication use for comorbidities, any other medical conditions, active infections, a history of a previous cardiovascular event, any organ transplantation, and pregnant women were excluded. The subjects diagnosed with advanced CKD were selected for this study (inclusion criteria). The control group consisted of fifty healthy individuals who were age- and sex-matched to the patients, with no history of diabetes mellitus, CKD, or any metabolic bone diseases. They were voluntarily selected from people who regularly visited the Hakim Laboratory in Tabriz for check-ups. Using questionnaires, other characteristics such as eating habits, medication schedules, and patterns of physical activity were recorded and incorporated into the statistical framework. Other factors, such as dietary habits, medication schedules, and patterns of physical activity, were recorded by questionnaires and incorporated into the statistical model. We used a matching technique between the control participants and CKD based on 5 key features: sex distribution, body composition indices, tobacco use patterns, metabolic health indicators, and chronological age to increase the validity of our comparison research. Structured surveys that recorded information on exercise habits, dietary habits, and medication use were used to document other lifestyle and health factors. These additional variables were included in the analysis to increase the reliability of our results and reduce the possibility of confounding effects.

Blood sampling and biochemical analysis

Body mass index was calculated using established methods based on height and weight values. Structured patient interviews and evaluations of medical records with an emphasis on tobacco use, genetic susceptibility, and current medications were used to gather additional health information. For blood collection, approximately 5 mL of blood was collected from patients and control subjects. To extract the genomic DNA, 2.5 mL of the drawn blood was transferred into EDTA tubes. Also, approximately 2.5 mL of blood was placed in a vacutainer containing a gel clot activator for serum seperation and biochemical analysis. To separate the serum and plasma components, centrifugation of the prepared blood samples was performed in the laboratory (4,000 rpm for 10 minutes at 4°C). For further testing, these separated samples were subsequently portioned and stored at -80°C. A number of parameters, including fasting blood sugar (FBS), urea, uric acid, creatinine, cholesterol, triglycerides (TG), alkaline phosphatase (ALP), parathyroid hormone (PTH), Ca, Na, vitamin D, K, P, and Fe, were measured during further blood chemistry tests using advanced analytical equipment (Roche Cobas C311 system).

DNA extraction and genotyping

The extraction of genomic DNA from the collected blood samples was performed via the SimEX™ Blood DNA Extraction Kit (Simbiolab®, Iran) according to the manufacturer's instructions. The quantitative and qualitative analysis of the purity of the

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extracted DNA was carried out using the NanoDrop method by recording the A_{260}/A_{280} ratio and gel electrophoresis. Protein contamination of the extracted DNA was indicated by a low A_{260}/A_{280} ratio. Generally, a ratio of about 2.0 indicates high-purity DNA, suggesting minimal contamination from proteins or other impurities (16). Tetra-primer amplification refractory mutation system-PCR (T-ARMS-PCR) was utilized to investigate polymorphisms in the RAGE gene (rs2070600 and rs2071288). This method employed four specific primers to enable simultaneous detection of wild-type and mutant alleles in a single reaction tube. Two outer primers, forward outer (FO) and reverse outer (RO) were designed as allele-independent to amplify the polymorphic site, while two inner primers, forward inner (FI) and reverse inner (RI) annealed to one of the outer primers to generate allele-specific fragments, distinguishing homozygotes and heterozygotes. The exact sequences of the primers are provided in Table 1. The PCR reaction mixture was prepared in a total volume of 12 µL, containing 1.0 µL of template DNA, 0.7 µL of each primer (FO, RO, FI, and RI), 6.0 µL of PCR Master Mix, and 4.3 µL of H_2O. The PCR cycling conditions were as follows: an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 64 °C (rs2070600) and 60 °C (rs2071288) for 30 seconds, and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 10 min in a thermo-cycler (Sensoquest, GmbH, Germany). The PCR products were visualized by electrophoresis on a 2.0% agarose gel under UV light. Genotypes were determined based on the presence of allele-specific bands.

Table 1. Designed T-ARMS-PCR primers for the rs2070600 and rs2071288 genes

SNP ID	Primer set and sequence (5′→3′)	Tm (°C)	Annealing T (°C)
rs2070600	FO: ACACCAGCCGTGAGTTCAGAGG	66	
	RO: CTCTGCCTCACAGTCCTTTCCCA	66	64
	FI: GCCGGAAGGAAGAGGGAGCC	67	
	RI: TGGCTCGTGTCCTTCCCAACA	63	
rs2071288	FO: CCACTCACCTCTCCTCTCCTC	65	
	RO: CTCCCACTAAATAACCCTCTCTCAAC	66	60
	FI: TGATCCTCCCACAGAGCCTATAC	65	
	RI: GCTCTCAATTTTCCCTGTCTCCA	63	

Assessment of bone metabolism markers

In this study, several key markers of bone metabolism, including serum levels of Ca, P, PTH, and ALP, were evaluated to explore their association with RAGE gene polymorphisms. Blood serum was separated by centrifugation at 3500 rpm for 10 minutes and subsequently stored at –80 °C for further biochemical analysis. Serum Ca and P levels were determined using standard colorimetric methods, while ALP activity was measured via an enzymatic approach.

Statistical analysis

Statistical analyses were performed using the SPSS Statistics package 25 (IBM). The findings were displayed using the standard format, which includes median values with interquartile ranges for non-normal distributions and arithmetic means with standard deviations for data that are regularly distributed. A *p*-value of < 0.05 was considered to be statistically significant. The normality of the distribution of the continuous data was assessed using the Kolmogorov-Smirnov test. The independent samples t-test and Mann-Whitney U test (the non-parametric version of the t-test) were used to examine the significant difference between the mean variables of the two groups of normal and nonnormal variables, respectively, and the chi-square (χ^2) test for categorical variables. Bonferroni correction was applied to adjust for multiple comparisons when analyzing associations between genotypes and multiple biochemical parameters. Pearson's χ^2-square test was applied to compare the genotypes and allele distributions between CKD subjects and healthy individuals. The obtained odds ratios (ORs) with 95% confidence intervals (95%

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CIs) were used to evaluate the correlation between the RAGE gene polymorphisms and CKD. Furthermore, Hardy-Weinberg equilibrium (HWE) was checked in the reference group based on Pearson's χ^2-square test with a threshold of $P < 0.05$.

Results

Characteristics of patients and controls

A total of 100 individuals participated in this study, including 50 patients with CKD and 50 healthy controls who were matched for age and sex. In general, patients with CKD showed noticeable alterations in both biochemical and bone metabolism indicators compared with healthy participants. Higher levels of serum PTH, ALP, and markers of renal impairment were observed in the patient group, whereas vitamin D and calcium levels tended to be lower. These overall patterns formed the basis for the more detailed analyses described in the following sections.According to the demographic analysis, the group characteristics were balanced. The age of the subjects with CKD and those of controls ranged from 29 to 87 years (54.87±14.92) and from 25 to 81 years (52.38±13.01), respectively.

According to Table 2, the CKD patient group consisted of 23(56%) males and 22 (44%) females, and the healthy control group consisted of 24 (58%) males and 21 (42%) females. Analysis of biochemical and bone metabolism markers showed many unique patterns among the groups. In CKD patients, vitamin D levels significantly decreased compared to the control group ($P < 0.001$).

Table 2. The clinical and laboratory findings of the population studied (CKD patients and control subjects)

Parameters		CKD patients Mean±SD (n=50)	Control Mean±SD (n=50)	P-value
Age (years)		54.87±14.92	52.38±13.01	0.371
Gender	Female; n (%)	22 (44)	21 (42)	0.841
	Male; n (%)	23 (56)	24 (58)	
FBS (mg/dL)		124.86±9.56	84.88±9.03	0.0001***
Urea (mg/dL)		112.06±9.35	28.54±7.95	0.0001***
Uric acid (mg/dL)		5.41±1.38	5.12±1.27	0.293
Creatinine (mg/dL)		7.31±1.63	1.12±0.43	0.0001***
Cholesterol (mg/dL)		147.27±21.58	127.5±20.72	0.0001***
TG (mg/dL)		125.03±17.49	103.36±18.93	0.0001***
Na (meq/L)		137.85±3.06	137.19±2.70	0.420
Fe (µg/dL)		70.34±14.65	75.64±12.59	0.071

Furthermore, no significant difference was detected in serum Ca and P levels between CKD patients and controls. PTH and ALP, one of the markers of bone activity, especially in the processes of bone formation and resorption, significantly elevated in CKD patients compared to the control group ($P < 0.001$) (Figure. 1), indicating the increased activity of bone regeneration and bone-related disorders in CKD patients. The CKD patients exhibited increased levels of FBS, urea, creatinine, cholesterol, and TG (all $p < 0.05$). After adjustment for multiple testing, the association remained significant. Other markers, including uric acid, Na, and Fe, remained consistent between the groups. The relationship between biochemical factors and different bone metabolism markers in CKD patients was studied. The results showed that P levels positively correlated with urea (r=0.305; P=0.044) and creatinine (r=0.360; P=0.018) and negatively correlated with ALP (r=-0.402; P=0.009). In addition, significantly negative correlations were detected between the serum levels of Na and FBS (r=-0.373; P = 0.011), TG and cholesterol (r=-0.422; P = 0.007), TG and ALP (r=-0.388; P = 0.016), and PTH and Ca (r=-0.376; P = 0.015) in the CKD group. Also, a positive relationship was observed between Fe and cholesterol (r=0.310; P = 0.046) (Figure 2).

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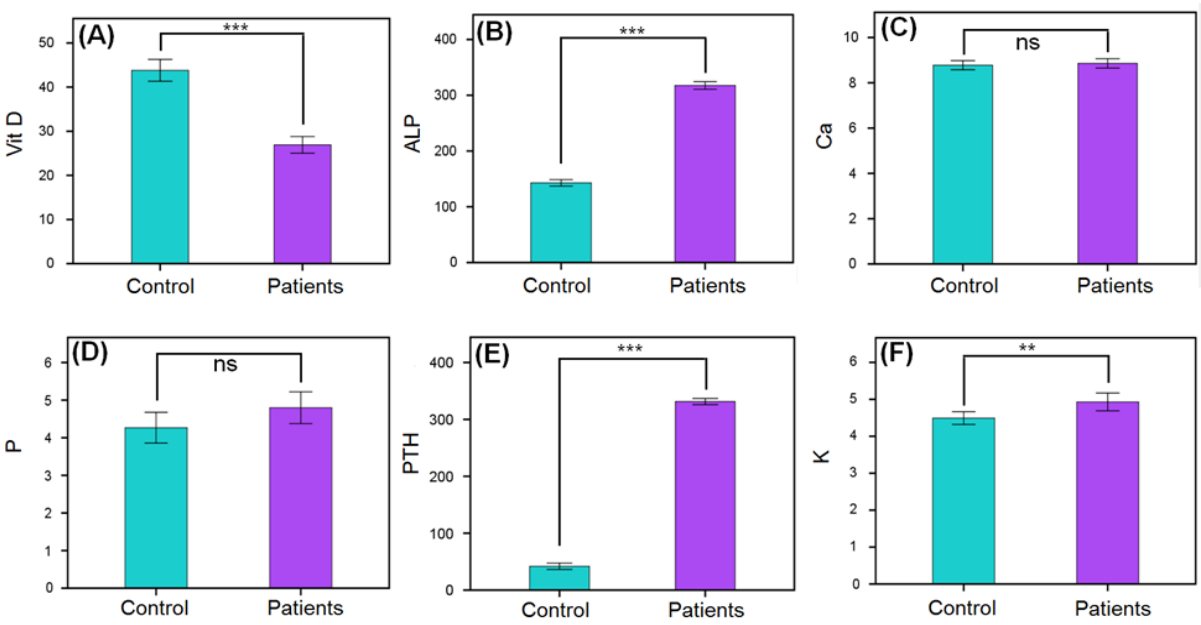


Figure. 1 Changes in the serum level of main bone metabolism biomarkers, including (A) vitamin D, (B) ALP, (C) Ca, (D) P, (E) PTH, and (F) K in the CKD patient group compared to control subjects.

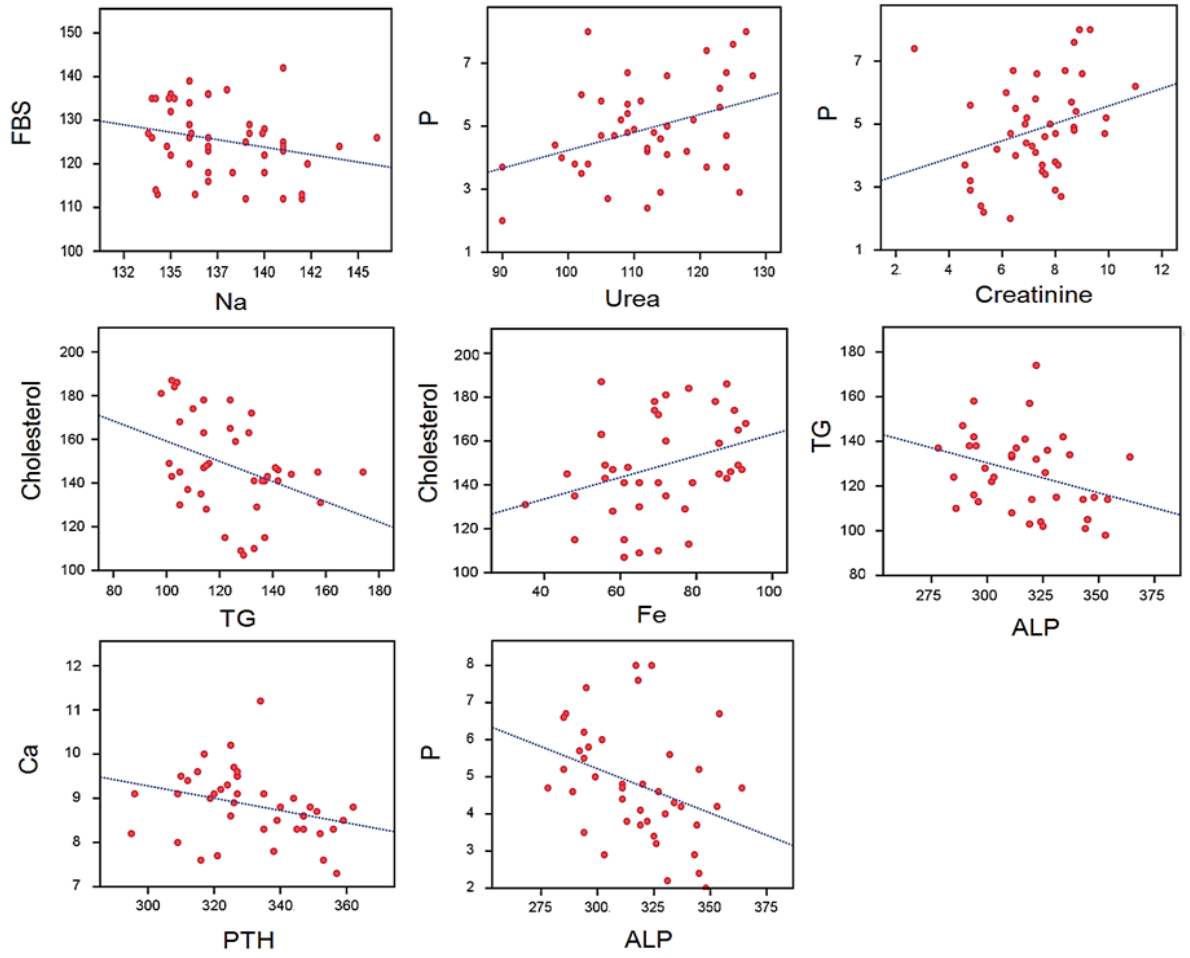


Figure 2. Relationship between biochemical factors and different bone metabolism markers in CKD patients.

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Genotyping of rs2070600 and rs2071288 gene polymorphisms

Table 3 presents the distribution and frequency of rs2070600 and rs2071288 polymorphisms in CKD patients and control groups. Analysis of Hardy-Weinberg Equilibrium (HWE) confirmed that genotype distributions for both polymorphisms were in equilibrium in both groups (P > 0.05). For the rs2070600 polymorphism, the CC genotype was more frequent in CKD patients (52%) compared to controls (28%), with an odds ratio (OR) of 2.79 (P = 0.014), indicating an increased CKD risk. Conversely, the CT and TT genotypes were more frequent in controls than in CKD patients, suggesting a potential protective effect, though these differences were not significant (P = 0.108 and P = 0.249, respectively). For the rs2071288 polymorphism, the CC and CT genotypes were more prevalent in CKD patients than in controls, but these differences lacked statistical significance (P = 0.548 and P = 0.840, respectively). Notably, the TT genotype was absent (0%) in CKD patients but present in 8% of controls, a statistically significant difference (P = 0.041), indicating that the TT genotype may serve as a protective factor against CKD. For the rs2070600 polymorphism, the CC genotype was more prevalence in CKD patients (52%) compared to the control group (28%), with an odds ratio (OR) of 2.79, indicating a 2.79-fold increased risk of CKD associated with this genotype (P = 0.014). Conversely, the CT and TT genotypes were observed at higher frequencies in the control group than in CKD patients, suggesting a potential protective effect; however, these differences were not significant (P = 0.108 and P = 0.249, respectively). Furthermore, the results confirmed that the probability of the C allele in CKD patients is higher than in the control group, while the OR rates for T/C were equal to 0.50 and 0.69 for the rs2070600 and rs2071288 genes, respectively (OR<1).

Therefore, compared to C, the T allele reduces the risk of CKD by 0.50 and 0.69 times. This means that the T allele is potentially a protective allele. The observed difference was statistically significant in the rs2070600 gene polymorphism (P =0.019), but no significant difference was detected for the rs2071288 gene (P =0.256). The association between the CC genotype of rs2070600 and CKD remained statistically significant after Bonferroni correction. No significant differences were detected for rs2071288 after correction for multiple testing. Between-group comparisons were conducted to evaluate differences in genotype and allele frequencies between CKD patients and healthy controls.

Table 3. Genotype and allele frequencies of rs2070600 and rs2071288 polymorphisms in CKD patients and control group

SNP	Frequencies n (%)		OR (95% CI)	*P*-value	*P*-value for HWE
	CKD (50)	Control (50)			
rs2070600					
CC	26 (52)	14 (28)	2.79 (1.21-6.39)	0.014*	0.976
CT	19 (38)	27 (54)	0.52 (0.23-1.16)	0.108	
TT	5 (10)	9 (18)	0.50 (0.16-1.63)	0.249	
Alleles					
T	29 (29)	45 (45)	-	-	
C	71 (71)	55 (55)	0.50 (0.28-0.90)	0.019*	
CC	26 (52)	14 (28)	-	-	
CT+TT	24 (48)	36 (72)	2.79 (1.21-6.39)	0.014*	
CT	19 (38)	27 (54)	-	-	
CC+TT	31 (62)	23 (46)	0.52 (0.23-1.16)	0.108	
TT	5 (10)	9 (18)	-	-	
CC+CT	45 (90)	41 (82)	0.50 (0.16-1.63)	0.249	
rs2071288					
CC	28 (56)	20 (50)	1.27 (0.58-2.79)	0.548	0.548
CT	22 (44)	21 (42)	1.08 (0.49-2.39)	0.840	
TT	0 (0)	4 (80)	-	0.041*	

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SNP	Frequencies n (%)		OR (95% CI)	*P*-value	*P*-value for HWE
	CKD (50)	Control (50)			
Alleles					
T	22 (22)	29 (29)	-	-	
C	78 (78)	71 (71)	0.69 (0.36-1.31)	0.256	
CC	28 (56)	25 (50)	-	-	
CT+TT	22 (44)	25 (50)	1.27 (0.58-2.79)	0.548	
CT	22 (44)	21 (42)	-	-	
CC+TT	28 (56)	29 (58)	1.08 (0.49-2.39)	0.840	
TT	0 (0)	4 (8)	-	-	
CC+CT	50 (100)	46 (92)	-	0.041*	

Impact of rs2070600 and rs2071288 polymorphisms on clinical parameters and bone metabolism biomarkers

In the present study, we studied the effects of rs2070600 and rs2071288 genetic variants on different biochemical and bone metabolism biomarkers, and the results are reported in Tables 4 and 5.

According to the results reported in these tables, individuals with the rs2070600 and rs2071288 CC genotypes showed a significant enhancement in serum levels of urea, creatinine, PTH, and ALP (P <0.01) and a significant reduction in vitamin D levels (P<0.01). In addition, the levels of Na, Ca, K, and P also significantly increased in individuals carrying the rs2070600 CC genotype (P-value <0.05). Furthermore, a significant increase was detected in the concentrations of cholesterol (P<0.05), K (P<0.01), and P (P<0.05) in subjects with the 2071288 CC genotype. On the other hand, subjects carrying the rs2070600 and rs2071288 CT genotypes had increased serum levels of urea, creatinine, PTH, and ALP with a P-value <0.05, while the content of vitamin D decreased. The concentrations of urea, creatinine, and PTH significantly increased (P<0.01).

However, a significant decrease was shown in the serum levels of cholesterol, Ca, and vitamin D (P<0.05). Correlation analyses were corrected for multiple comparisons using the Bonferroni method; significant correlations are reported. Within-group analyses were performed to investigate the effect of different genotypes on clinical and biochemical parameters within each group.

Table 4. Comparison of clinical characteristics and bone metabolism parameters according to rs2070600 genotypes in patients with chronic kidney disease (CKD) and healthy controls

Parameters	CC (mean±SD)			CT (mean±SD)			TT (mean±SD)		
	Control	CKD	*P-value*	Control	CKD	*P value*	Control	CKD	*P value*
FBS	83.37±5.80	120.77±10.67	0.0001***	86.76±8.08	122.00±7.45	0.0001***	82.80±8.81	130.50±5.91	0.005**
Urea	29.02±7.50	114.22±8.27	0.0001***	25.23±6.40	107.83±12.40	0.0001***	28.80±8.64	111.50±15.11	0.001***
Uric acid	4.80±0.91	4.94±1.45	0.278	5.52±1.25	5.51±1.95	0.833	4.44±1.40	4.72±1.15	0.841
Creatinine	1.06±0.07	6.61±2.01	0.0001***	1.11±0.44	7.96±1.06	0.0001***	1.18±0.65	7.65±2.53	0.003**
Cholesterol	125.25±23.38	150.88±22.85	0.002*	126.47±20.40	139.66±28.46	0.040*	127.80±13.73	138.25±10.78	0.463
TG	102.87±19.44	122.11±13.37	0.009*	105.52±18.97	133.33±17.44	0.0001***	126.22±50.16	115.75±18.46	0.205
PTH	49.62±12.21	332.77±16.59	0.0001***	41.00±22.88	335.01±17.88	0.0001***	44.60±11.88	333.00±14.94	0.003**
Na	135.41±1.89	138.15±2.58	0.010**	138.18±2.74	138.95±4.03	0.485	137.28±2.85	135.50±1.73	0.081

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Parameters	CC (mean±SD)			CT (mean±SD)			TT (mean±SD)		
	Control	CKD	*P-value*	Control	CKD	*P value*	Control	CKD	*P value*
Ca	8.50±0.40	8.96±0.94	0.021*	8.67±0.68	8.83±0.76	0.829	9.34±0.24	8.37±0.59	0.019*
K	4.27±0.69	4.87±1.09	0.015*	4.54±0.52	4.89±0.77	0.143	4.60±0.12	4.69±1.05	0.641
P	4.01±1.63	4.97±1.60	0.029*	4.38±1.74	5.68±1.84	0.539	3.68±1.20	3.57±1.89	0.688
Fe	77.75±10.74	69.11±12.12	0.082	76.52±14.04	77.66±12.33	0.279	71.00±11.24	75.75±16.72	0.569
ALP	148.01±19.55	309.88±18.89	0.0001***	144.76±18.37	316.83±28.65	0.0001***	144.40±13.10	332.75±25.88	0.004**
Vitamin D	44.31±6.73	29.33±7.59	0.0001***	40.67±7.91	28.50±7.55	0.0001***	46.30±7.89	25.00±5.77	0.004**

Table 5. Comparison of clinical characteristics and bone metabolism parameters according to rs2071288 genotypes in patients with chronic kidney disease (CKD) and healthy controls

Parameters	CC (mean±SD)			CT (mean±SD)		
	Control	CKD	*P- value*	Control	CKD	*P-value*
FBS	85.50±6.03	127.30±8.66	0.0001***	84.00±6.44	120.40±9.48	0.0001***
Urea	25.83±8.32	111.30±14.40	0.0001***	27.85±6.53	113.20±6.72	0.0001***
Uric acid	5.49±1.50	5.40±1.59	0.299	4.98±0.99	4.61±1.41	0.864
Creatinine	1.07±0.44	8.18±1.62	0.0001***	1.16±0.45	6.44±1.72	<0.001***
Cholesterol	127.33±19.96	150.90±22.33	0.019*	125.28±17.29	141.80±24.48	0.774
TG	104.33±20.48	123.90±18.53	0.002**	102.57±17.08	123.70±14.17	0.0001***
PTH	46.33±21.51	332.50±15.81	0.0001***	40.92±15.15	332.90±16.66	0.0001***
Na	137.72±2.99	136.84±3.46	0.985	136.98±2.29	138.57±2.55	0.188
Ca	8.73±0.78	8.79±0.77	0.795	8.80±0.50	8.93±0.94	0.741
K	4.55±0.63	5.24±0.71	0.001**	4.47±0.49	4.58±1.03	0.461
P	4.45±2.11	5.57±1.98	0.068	4.05±1.35	4.42±1.49	0.710
Fe	69.50±11.27	67.70±16.16	0.387	77.92±10.88	70.90±8.72	0.300
ALP	141.08±17.00	319.90±23.04	0.0001***	149.50±15.62	317.60±27.73	0.0001***
Vitamin D	43.90±9.30	27.40±7.10	0.0001***	40.77±5.85	28.60±7.05	0.0001***

Discussion

It has been reported that the AGE-RAGE axis plays a main role in the development of type II diabetes mellitus and its complications by mediating oxidative stress, cell dysfunction, etc. (17). Therefore, RAGE plays a role in the development of a number of renal disorders, such as hypertensive kidney injury, diabetic kidney injury, and obesity-related nephropathy (18). Cipollone et al. (19) found that the RAGE gene presented an up-regulation in diabetic atherosclerosis, suggesting the potential of RAGE genes in the development of various vascular diseases. CKD is a commonly occurring and debilitating complication of diabetes mellitus, which is caused by the damage of kidneys and nephrons blood vessels due to the high blood sugar level (20). Considering the potential pathological role of RAGE in diabetes-associated kidney diseases, the present study investigated the association between two gene polymorphisms

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(rs2070600 and rs2071288) with various biomarkers of bone metabolism and clinical characteristics of patients with CKD and controls.

The results provide important insights into the effects of the mentioned polymorphisms on factors related to CKD and bone metabolism. Statistically significant differences were observed between CKD patients and controls in biochemical factors such as FBS, creatinine, urea, cholesterol, PTH, Fe, and ALP. These findings indicate the systemic effects of CKD on total body metabolism and bone-related indices. The demographic characteristics, clinical, and laboratory findings of CKD patients and the reference group were thoroughly examined, and as shown in Table 2, the serum levels of FBS, creatinine, urea, PTH, and ALP significantly increased in the CKD patients ($P<0.05$). While a statistically significant decrease was observed in the serum concentrations of cholestrol and Fe ($P<0.05$). Increased levels of PTH and ALP in CKD patients reveal higher activity of bone remodeling and destruction processes, which is attributed to changes in bone metabolism in these patients. Few investigations have confirmed that different phases of CKD were associated with significant alterations in the levels of biological markers such as growth hormone, fibroblast growth factor-23 (FGF-23), calcitriol, calcidiol, and PTH (21, 22). Vitamin D deficiency also plays a significant role in the development and progression of CKD (23). In accordance with our results, Block et al. (24) and Naves Diaz et al. (25) observed hyperphosphatemia in CKD cases. One of the primary requirements for the mineralization and the development of bone is P. In CKD, hyperphosphatemia is a major contributor to cardiovascular events and vascular calcification. In CKD, hyperphosphatemia results from the kidneys' inability to excrete P.

It migrates from the bones to the blood in CKD due to inadequate and faulty bone remodeling, resulting in hyperphosphatemia. Increased phosphate release from bone into the blood is the outcome of elevated PTH levels (26). PTH acts as one of the key indicators in regulating Ca and P metabolism and maintaining bone balance. Also, it acts to defend plasma Ca concentrations and participates with Ca, P, and vitamin D to maintain skeletal integrity (27). The increase of PTH in CKD patients can be due to the development of secondary hyperparathyroidism, which is a common complication in patients with kidney diseases (28). This situation, as a result of the reduction of phosphate excretion and impairment of vitamin D absorption, leads to a decrease in Ca levels, which ultimately stimulates the production of PTH. ALP levels have been linked to mortality risk in both the general population and CKD patients, according to numerous recent large population cohort studies (29).

The higher activity of ALP may be due to kidney failure and subsequent disturbances in the balance of Ca and P, which leads to the destruction of bone tissue. It has been reported that CKD patients are more prone to oxidative stress and chronic inflammation, which increase the generation of AGEs. The AGE accumulation can be due to an increase in its production and decreased kidney clearance (30). AGEs-RAGE interaction initiates inflammatory responses and leads to oxidative stress. These conditions are important in many diseases, including CKD, diabetes, and atherosclerosis (10). The increased oxidative stress can lead to increased systemic inflammation, dysregulation of calcium and phosphorus, and further damage to bone tissues. In addition, oxidative stress associated with RAGE may exacerbate PTH production through indirect mechanisms such as decreased serum Ca (31).

Positive correlation between P and urea and creatinine showed the effect of kidney function on P accumulation in the body. An increase in urea and creatinine as indicators of decreased kidney function can cause an increase in P levels (32, 33). This condition may lead to hyperphosphatemia and impaired bone metabolism, which are common in CKD. The results showed that the CC genotype at rs2070600 was more common in CKD (52% vs. 28% in the control group). This finding was associated with an increased odds ratio (OR = 2.79), indicating an approximately threefold increase in the risk of developing CKD in individuals with this genotype. On the other hand, the TT genotype at rs2071288 was significantly observed only in the control group, possibly indicating a protective role of this genotype against CKD. Furthermore, the findings showed that the T allele in both polymorphisms may play a protective role, as it reduces the risk of CKD compared to the C allele (OR = 0.50 and OR = 0.69 for rs2070600 and rs2071288, respectively).

These findings are consistent with some previous reports that have reported the role of similar genotypes in kidney function and related diseases. In accordance with our findings, Jin et al. (34) analyzed the RAGE

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SNP (rs2070600) association with diabetic retinopathy in type 2 diabetic patients, and the results showed that the TT genotype or T allele reduces the risk for diabetic retinopathy. In addition, Balasubbu et al. (35) reported the association of the rs2070600 SNP in the RAGE gene with diabetic retinopathy. In the case of the rs2071288 polymorphism, the absence of the TT genotype in CKD patients may indicate the protective role of this genotype.

The present work evaluated the possible impact of two studied polymorphisms on biomarkers of bone metabolism, including levels of PTH, ALP, Ca, vitamin D, and other related variables. The results revealed that these polymorphisms not only affect the risk of CKD but also play an important role in the severity of bone-related metabolic disorders. Patients with the rs2070600 polymorphism had higher levels of urea, creatinine, PTH, and ALP, and lower level of vitamin D. These changes indicate more severe impairments in renal function and bone remodeling in this group. PTH was significantly higher in patients with rs2070600 and rs2071288 CC genotypes. This increase is due to hypocalcemia and impaired Ca reabsorption by the kidneys. Increased PTH can lead to increased bone formation and bone turnover (36, 37).

High ALP and PTH levels indicate increased bone resorption activity. This increase may be due to the body's response to bone destruction caused by secondary hyperparathyroidism. Reduced vitamin D concentrations in patients with rs2070600 and rs2071288 polymorphisms reflect a reduced ability of the kidneys to activate vitamin D. This leads to reduced Ca absorption from the intestines and hypocalcemia, which in turn stimulates further PTH secretion (31, 38). These changes are likely due to changes in the expression of the RAGE gene. Activation of RAGEs can increase inflammatory pathways and tissue damage, thereby affecting bone metabolism (10).

However, our results indicated that in individuals with the TT genotype, fewer changes in bone biomarkers were observed. This finding supports the protective role of the T allele. This allele may reduce the deleterious effects of RAGE on inflammatory and metabolic pathways. As a result, individuals with the CC and TT genotypes showed the greatest and least changes in bone biomarkers for both polymorphisms, respectively. This suggests that the effects of the genotypes may be additive. The heterozygous CT genotype also demonstrated alterations, although the extent of these changes was less pronounced compared to the CC genotype. This may be related to the role of the T allele in reducing the deleterious effects of the C allele. The positive correlation between PTH and ALP indicates that increased PTH activity is directly related to increased bone remodeling activity. In fact, PTH affects ALP levels as an indicator of bone formation activity. The observed associations between RAGE polymorphisms, particularly rs2070600 CC, and alterations in bone metabolism markers (increased PTH and ALP, decreased vitamin D) may be explained by functional mechanisms involving the RAGE pathway. When AGEs bind to RAGE, they trigger downstream signaling, such as NF-κB, which can alter osteoclast and osteoblast activity and cause dysregulated bone remodeling (39). High-risk genotype carriers may be more sensitive to AGEs, which can intensify inflammatory signaling and lead to disruptions in calcium and phosphate balance. Such interactions may worsen bone metabolism abnormalities in people with CKD, when AGE buildup accumulation high. However, more experimental confirmation is needed for these suggested pathways.

Like any scientific investigation, this study has some limitations, which must be considered when interpreting the findings. First, we can only infer connections between RAGE gene polymorphisms and CKD or related biochemical and bone metabolism markers; we are unable to demonstrate causal relationships due to the cross-sectional approach. Second, the results might have been impacted by additional unmeasured variables even though we adjusted for several possible confounders such age, sex, and lifestyle characteristics. Lastly, the sample size in our study was small. Our results may be limited by the short sample size, which could also impact statistical power. To further test and confirm these findings, it is advised that bigger and more varied sample sizes be used in future research. Also, longitudinal studies can perform to investigate causal relationships between RAGE polymorphisms and CKD progression, as well as bone metabolism alterations.

The results of this study show that the rs2070600 polymorphism in the RAGE gene is strongly associated with the risk factors of CKD as well as changes in bone metabolism in these patients. While the rs2071288 polymorphism has not significant correlation with CKD complications. The results for rs2070600 showed

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that the CC genotype was associated with increased risk of CKD. Specifically, subjects with the CC genotype had a higher risk of developing CKD compared to individuals with other genotypes (CT or TT), which was also consistent with increased levels of urea, creatinine, and ALP in patients. The study also showed that changes in bone metabolism indicators, such as elevated ALP activity, was greater in patients with CKD. Since bone disorders are common in CKD patients and can lead to bone fragility and increased fracture risk, the investigation of genetic polymorphisms such as rs2070600 can provide valuable information on the prediction and management of these complications in end stage renal failure disorders. The results of this study indicate that the rs2070600 polymorphism is not only associated with the incidence of CKD but may also be correlated with bone metabolism disorders. It is suggested that future studies investigate the more detailed function of these polymorphisms in RAGE-related signaling pathways and their effect on bone metabolism. Also, increasing the number of samples and examining patients in different stages of CKD can help to better understand the role of these polymorphisms. These results can help to develop new diagnostic and therapeutic strategies for patients with CKD, as well as to reduce the risks associated with bone disorders in these people.

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