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Influence of vitamin D and calcium-sensing receptor gene variants on calcium metabolism in end-stage renal disease: insights from machine learning analysis

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Abstract. – OBJECTIVE: End-stage renal disease (ESRD) commonly manifests with disrupted calcium balance, leading to renal osteodystrophy. We posited that variations in the genetic makeup of vitamin D and calcium-sensing receptors, specifically single nucleotide polymorphisms (SNPs), could affect calcium homeostasis. This study aimed to identify the genetic predictors related to vitamin D and calcium-sensing receptors on calcium metabolism using machine learning algorithm analysis in ESRD.

PATIENTS AND METHODS: We conducted a cross-sectional analysis on adults with ESRD. We gathered comprehensive demographic data and medical history. Blood samples were collected to measure SNPs, and a panel of calcium metabolism biomarkers associated with the calcium-sensing receptor and vitamin D receptor. The biomarkers included calcium, phosphate, vitamin D, parathyroid hormone (PTH), sclerostin, procollagen type 1 alpha 1, osteocalcin, and bone-specific alkaline phosphatase. We utilized machine learning algorithms to pinpoint genetic markers predictive of vitamin D deficiency.

RESULTS: We found a notable decrease in serum procollagen type 1 alpha 1 levels among individuals with the CC of rs10190 (related to the calcium-sensing receptor) compared to those with the TT genotype and in those with the TT of rs739837 (pertaining to the vitamin D receptor) compared to the GG genotype. Similarly, the TT genotype of rs10190 was associated with significantly lower serum phosphate levels compared to CC and CT genotypes. Additionally, a lower serum PTH level was noted in individuals with the CT of rs1802757 (calcium-sensing receptor) compared to those with the CC genotype. Our machine learning analysis identified rs2221266 and rs1042636 as the most significant SNPs linked to vitamin D deficiency, demonstrating considerable predictive accuracy.

CONCLUSIONS: Our findings indicate that specific single nucleotide polymorphisms in the vitamin D and calcium-sensing receptors significantly influence calcium metabolism biomarkers in ESRD patients. Assessing the clinical implications of these genetic variations is crucial for advancing personalized medicine in renal care.

Key Words:
SNPs, Genetic polymorphisms, Calcium, CKD, CRF, ESRD, Chronic renal failure.

Introduction

Chronic kidney disease (CKD) is increasingly recognized as a significant global health challenge, with its prevalence estimated at 13.4% worldwide[1]. The Global Burden of Disease Study 2017[2] highlighted a mortality rate [95% confidence interval] of 133 [118, 448] per 1,000 individuals in Bahrain, underscoring the severity of the condition. The kidneys play an indispensable role in maintaining the balance of calcium and phosphate metabolism, crucially by aiding the reabsorption of calcium and the excretion of phosphate[3]. Parathyroid hormone (PTH) not only facilitates the reabsorption of calcium and the excretion of phosphate in the urine but also catalyzes the transformation of 25-hydroxy vitamin D into its final, active form, 1,25-dihydroxy vitamin D, within the kidneys[4]. In the context of CKD, particularly at the end-stage renal disease

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(ESRD) phase, there is a profound disruption in the reabsorption of calcium and the excretion of phosphate. This disturbance leads to an increased release of PTH, culminating in secondary hyperparathyroidism[4]. Furthermore, PTH exerts its effects on the bones, extracting calcium and leading to osteodystrophy, which is marked by bone pain and an elevated risk of fractures[5]. The secondary hyperparathyroidism observed in CKD patients leads to osteodystrophy, deranged cardiac function, and altered peripheral nerve conduction, along with a decrease in lean body mass, particularly in post-menopausal women[6]. Hypercalcemia, arising from hyperparathyroidism, has been linked to pathological calcifications, such as nephrocalcinosis. Observations indicate that nearly 40% of patients with stage 3 CKD and a staggering 82% of those in stage 4 experience secondary hyperparathyroidism[7]. This condition is significantly associated with an increased mortality risk in CKD patients, with odds ratios ranging from 1.5 to 3.8[8].

Single nucleotide polymorphisms (SNPs) in calcium-sensing receptors, specifically rs10190, rs1802757, rs2221266, and rs1042636, have been identified as significant determinants of calcium metabolism markers. A study[9] within the Thai population demonstrated that individuals carrying the rs1042636 SNP exhibited lower parathyroid hormone (PTH) levels compared to those without this SNP. Research conducted in the United States[10] showed that individuals with a homozygous SNP in the calcium-sensing receptor exhibited a markedly higher sensitivity, leading to a profound decrease in serum PTH levels in comparison to non-carriers. In the Korean demographic, a significant correlation was found with rs1042636 (odds ratio: 0.066, p=0.027) and rs1802757 (odds ratio: 10.532, p=0.042), indicating a notable association of these polymorphisms with PTH levels[11]. Importantly, a genome-wide association study spanning European and Asian populations pinpointed rs1801725, a missense mutation, as having the most pronounced association with altered serum calcium levels. This effect is attributed to its impact on modulating the response of PTH release via calcium-sensing receptors[12]. Likewise, SNPs in the vitamin D receptors, including rs7975232, rs2228570, and rs739837, have been shown to influence serum PTH and vitamin D levels, as well as the risk of developing osteodystrophy[13,14]. Moreover, several novel biomarkers, including sclerostin, have been found to have a direct connection to renal hypofunction in CKD patients, especially in the later stages and end-stage renal dysfunction. Notably, changes in sclerostin levels occurred earlier than those of several other biomarkers and were also linked to cardiac calcification[15].

A recent investigation[16] into the Arab population, especially those from the Arabian Peninsula, unveiled a considerable genetic diversity, underscoring the region's high genetic heterogeneity. In our earlier research[17,18], focusing on the genetic polymorphisms of critical metabolizing enzymes in relation to warfarin and acetaminophen, we discovered a notable variance in the occurrence of pharmacologically relevant SNPs within our demographic. Given the promising capabilities of machine learning algorithms (MLAs) in pinpointing predictor variables, their role becomes increasingly pivotal in the realm of personalized medicine. Motivated by this potential, we embarked on the current study to explore the connection between SNPs in vitamin D and calcium-sensing receptors and the markers of calcium metabolism in patients with ESRD.

Patients and Methods

Study Ethics and Design

An observational, cross-sectional study was carried out in the Department of Nephrology, Salmaniya Medical Complex, Bahrain, between December 2021 and August 2023. Approvals were obtained from the Research Ethics Committee, College of Medicine & Medical Sciences, Arabian Gulf University (E09-PI-11-21), and Salmaniya Medical Complex (number: 150061221). Written consent was obtained from the study participants.

Study Procedure

We enrolled adults over the age of 21, of both sexes, who had been diagnosed with ESRD for at least 12 months. Participants undergoing treatment with rifampicin, phenobarbital, phenytoin, clarithromycin, diltiazem, or verapamil were excluded from the study. We meticulously collected their demographic information, including age, weight, height, and sex, along with their primary and secondary diagnoses. A single blood sample was obtained from each participant to analyze SNPs in the CYP and target enzymes, as well as biomarkers pertinent to calcium metabolism. For this study, serum vitamin D levels below 20 ng/

ml were classified as vitamin D deficiency; levels ranging from 21-29 ng/ml were deemed insufficient, and levels above 30 ng/ml were considered normal[19].

Estimation of Biomarkers of Calcium Metabolism

The assessment of calcium metabolism biomarkers was conducted using the enzyme-linked immunosorbent assay (ELISA) technique in strict adherence to the manufacturer's guidelines. The following ELISA kits were utilized for biomarker estimation: ab210966 from Abcam© (Cambridge, UK) for procollagen 1 alpha 1; ab221836 from Abcam© for sclerostin; ab230931 from Abcam© for serum parathyroid hormone (PTH); ab213966 from Abcam© for vitamin D3; MBS9716394 from MyBiosource© (San Diego, CA, USA) for osteocalcin; and MBS285496 from MyBiosource© for bone-specific alkaline phosphatase. Additionally, serum calcium and phosphate levels were quantified through calorimetric assays using kit MBS2540479 from MyBiosource© for calcium and ab65622 from Abcam© for phosphate.

Estimation of Single Nucleotide Polymorphisms

Total genomic DNA was extracted from peripheral blood leukocytes using the QIAamp® (Hilden, Germany) DNA Blood Mini Kit (Qiagen). The concentration of the extracted DNA was quantified with a Nanodrop spectrophotometer. The genotyping of SNPs was performed through the allelic discrimination technique employing VIC- and FAM-labeled probes on the StepOne Plus® real-time PCR system (Applied Biosystems; Foster City, CA, USA). This process utilized commercially available TaqMan® (Vilnius, Lithuania) assays designed specifically for the following SNPs: rs10190, rs1802757, rs2221266, rs1042636, rs7975232, rs2228570, and rs739837.

Statistical Analysis

Descriptive statistics were employed to summarize demographic data. The association between categorical variables was examined using the Chi-square test. The distribution of numerical variables was assessed, and based on their distribution, non-parametric tests were employed for analysis. Specifically, the Kruskal-Wallis H test was utilized for statistical comparisons across all genotypes except for rs1802757. The Mann-Whitney U test was applied for rs1802757, which had only two categories. To adjust for the risk of type I error due to multiple comparisons, Bonferroni's correction was implemented. A p-value threshold ≤ 0.05 was set to determine statistical significance.

For the analysis involving machine learning algorithms, the dataset was divided into training and testing cohorts with a ratio of 75:25, respectively. The C5.0 algorithm, a decision tree-based method, was utilized to identify predictors of vitamin D deficiency, incorporating genotypes and calcium-related biomarkers as covariates. This algorithm was particularly used for evaluating variables' significance and developing a model tree. Important predictors were visualized in a horizontal bar chart, and a gains plot was executed to ascertain the fraction of information gained through the machine learning model.

Statistical analyses were conducted using SPSS version 28 (IBM Corp., Armonk, NY, USA), while SPSS Modeler version 18 was employed for the machine learning analysis.

Results

Demographic Characteristics

One hundred fifty participants were recruited, and Table I summarizes their demographic characteristics. Most of the participants were in the older adult category, with a mean age of 61 years, and were predominantly males, with multiple comorbidities such as systemic hypertension, diabetes, and dyslipidemia.

Biomarkers of Calcium Metabolism in the Study Participants

Table II presents a comprehensive summary of biomarkers associated with calcium metabolism. Among the participants, 77 individuals (60.6%) were found to have normal levels of vitamin D,

Table I. Demographic characteristics of the study participants (N = 150).

Variables	Values
Age (Years)	61 (21-84)
Male: Female	99: 51
Body weight (kg)	69 (35-121)
Concomitant diagnoses (n)	
Diabetes mellitus	107
Systemic hypertension	138
Dyslipidemia	126
Hyperuricemia	19

Values are mentioned in median (range).

Table II. Summary of blood biomarkers related to calcium metabolism.

Variables	Values
Parathyroid hormone (pg/ml)	30.1 (1.9-257)
Calcium (mmol/L)	2.1 (0.4-3.9)
Phosphate (mmol/L)	1.7 (0.4-3.5)
Vitamin D (ng/ml)	33 (2-141)
Bone specific alkaline phosphatase (ng/ml)	67.3 (3-385.3)
Osteocalcin (ng/ml)	1.4 (0.3-14.1)
Sclerostin (pg/ml)	638.5 (75-3,189)
Procollagen 1 alpha 1 (ng/ml)	314.8 (63.2-625.6)

Values are stated in median (range).

while 42 (33.1%) exhibited vitamin D insufficiency, and 8 (6.7%) were classified as vitamin D deficient. Upon analysis, no statistically significant differences were detected among the biomarkers when comparing the various vitamin D status categories, as illustrated in Figure 1.

Prevalence of SNPs in the Study Population

Table III provides a summary of distribution of genotypes amongst the study population. CC genotypes were observed to be the predominant type in the following SNPs: rs10190, rs1802757, rs2228570 and rs2221266. In the rs1042636, AA genotype was the predominant as like the G alleles in rs739837 and A alleles in rs7975232.

Table III. Prevalence of SNPs in the study population.

SNP	Genotype	Numbers (%)
rs10190	CC	84 (56.4)
	CT	54 (36.2)
	TT	11 (7.4)
rs1802757	CC	124 (82.7)
	CT	26 (17.3)
rs739837	GG	22 (14.9)
	GT	73 (48.9)
	TT	54 (36.2)
rs7975232	AA	62 (41.3)
	AC	64 (42.7)
	CC	24 (16)
rs1042636	AA	129 (86)
	AC	18 (12)
	CC	3 (2)
rs2228570	CC	87 (58)
	CT	55 (36.7)
	TT	8 (5.3)
rs2221266	CC	82 (54.7)
	CT	55 (36.7)
	TT	13 (8.7)

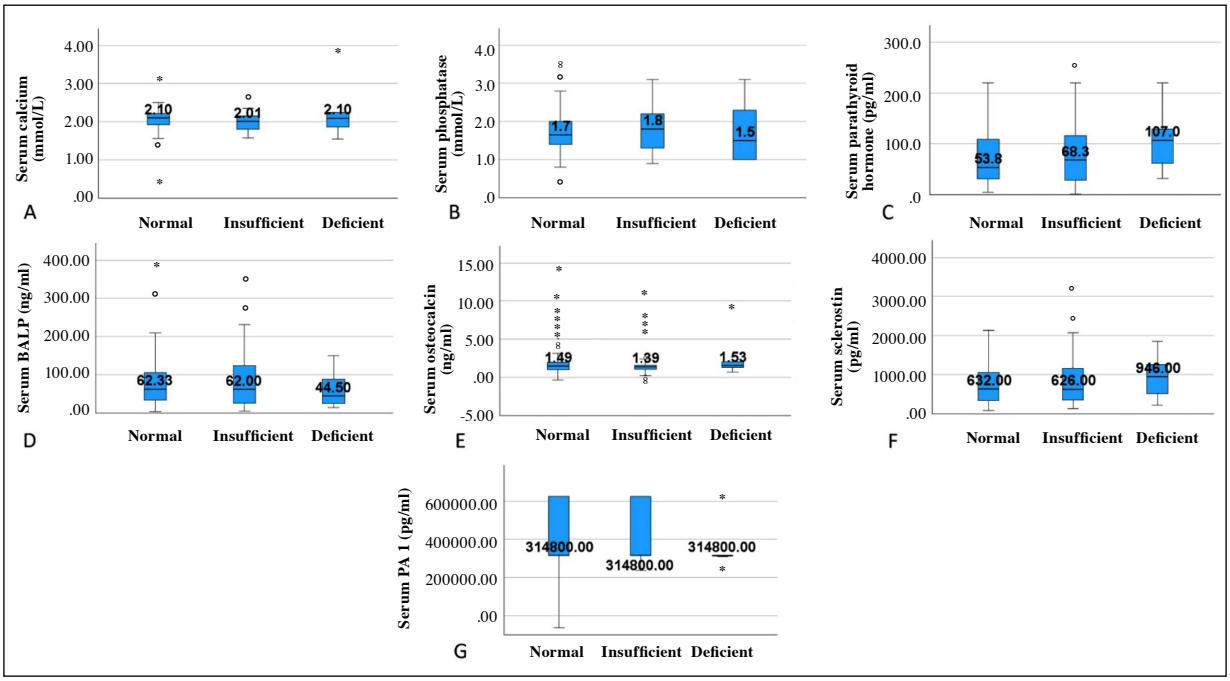

Figure 1. Biomarkers of calcium metabolism in various categories of vitamin D. The boxplots in (**A-G**) figures depict the distributions of various biomarkers related to calcium metabolism with respect to different categories of vitamin D. No significant differences were observed between any of the biomarkers, and the asterisks and circles represent the outliers for each of the respective groups. BALP: bone-specific alkaline phosphatase; PA: procollagen alpha.

Association Between SNPs and Biomarkers of Calcium Metabolism

Individuals carrying the CC genotype of rs10190 exhibited significantly lower levels of serum procollagen 1 alpha 1 when compared with those possessing the TT genotype, and those with the TT genotype of rs739837 also had significantly lower levels compared to individuals with the GG genotype, as depicted in Figure 2. Likewise, the TT genotype of rs10190 was associated with significantly reduced serum phosphate levels compared to both CC and CT genotypes. Furthermore, a lower serum parathyroid hormone (PTH) level was noted in individuals with the CT genotype of rs1802757 compared to those with the CC genotype. However, no significant differences were found among any of the calcium metabolism biomarkers when comparing the SNPs related to rs7975232, rs1042636, rs2228570, and rs12221266, as outlined in Table IV.

Machine Learning Analysis for Identifying the Predictor of Vitamin D Deficiency

The C5.0 algorithm identified the rs2221266 polymorphism, closely followed by rs1042636, as the most crucial predictor among others, as illustrated in Figure 3. The C5.0 decision tree model, depicted in the Electronic **Supplementary Figure 1**, showcases the initial nodal split occurring with the rs1042636 polymorphism. In this split, individuals possessing homozygous mutant alleles (CC) were more likely to be identified with vitamin D deficiency. The subsequent significant nodal split involves the rs2221266 polymorphism, where patients carrying CT or TT alleles were found to be predisposed to vitamin D deficiency. Figure 4 presents the gains plot, indicating that the information gained from the test cohort is comparable to that from the training cohort. The model exhibited a predictive accuracy of 90.9% for the normal vitamin D category and 81.6% for the category combining vitamin D insufficiency and deficiency.

Discussion

Key Findings of the Present Study

In our study, we investigated the correlation between specific single nucleotide polymorphisms (SNPs) associated with vitamin D and calcium-sensing receptors and markers of calcium metabolism in individuals diagnosed with end-stage renal disease (ESRD). We found that approximately 40% of the participants were ei-

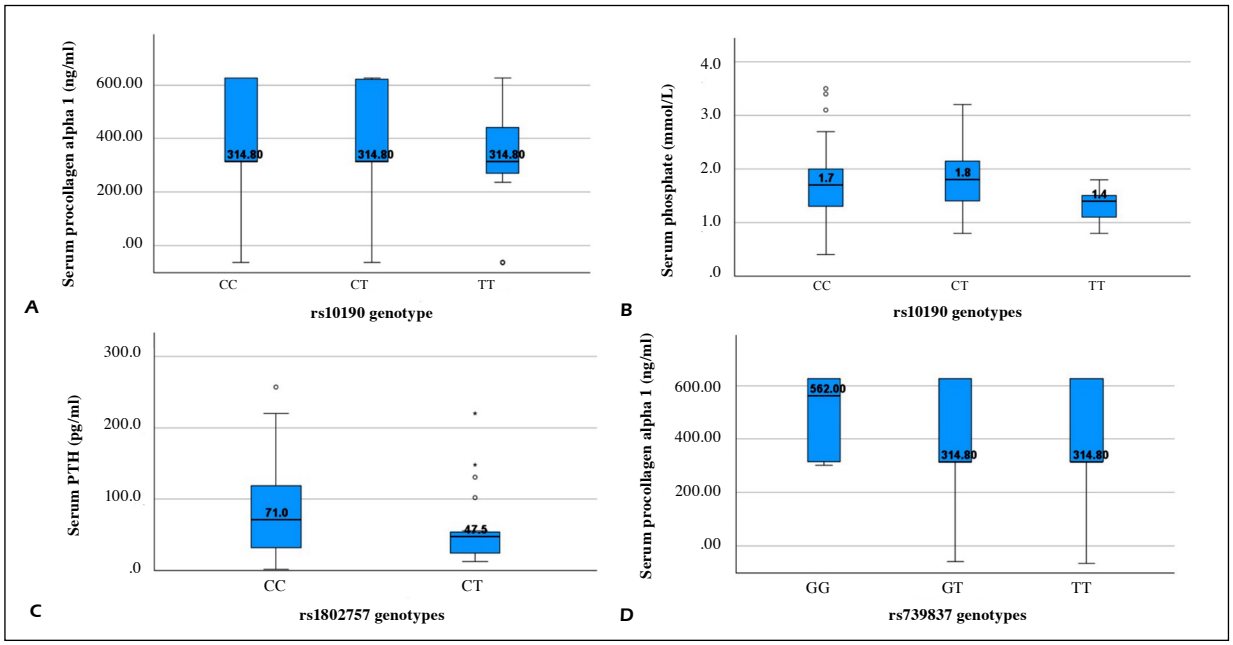

Figure 2. Significant associations between the biomarkers of calcium metabolism and genotypes. **A-D**, Represent the distribution biomarkers related to calcium metabolism in various genotypes. Statistically significant associations were observed between the biomarkers of calcium metabolism and the stated genotypes; the asterisks and circles represent the outliers for each of the respective groups.

Table IV. Associations between SNPs and biomarkers of calcium metabolism.

SNPs	Genotypes	Procollagen alpha 1 (ng/ml)	BALP (ng/ml)	Osteocalcin (ng/ml)	Sclerostin (pg/ml)	PTH (pg/ml)	Calcium (mmol)	Phosphate (mmol)	Vitamin D (ng/ml)
rs7975232	AA	314.8 (63.2-625.6)	81.5 (3-385.3)	1.47 (0.03-14.1)	567.5 (127-3189)	53.5 (8.7-220)	2.03 (1.5-3.9)	1.6 (0.4-3.5)	33 (2-141)
	AC	314.8 (58.6-625.6)	53.5 (5-311)	1.4075 (0.3-7)	688 (75-2267)	55.8 (1.9-257)	2.1 (1.4-2.68)	1.7 (0.8-3.4)	33 (2.9-107)
	CC	412.9 (301.8-625.6)	61.2 (3-350)	1.54 (0.2-11)	638.5 (125-2237)	91.5 (24.3-148)	2.09 (0.4-2.37)	1.75 (1.1-2.4)	36.5 (17-81)
rs1042636	AA	314.8 (63.2-625.6)	72.5 (3-385.3)	1.435 (0.3-14.1)	645 (75-3172)	56.1 (1.9-257)	2.08 (0.4-3.9)	1.7 (0.4-3.5)	33 (2-141)
	AC	521 (238.4-625.6)	63.5 (10-205.7)	1.4 (0.2-2.96)	551.5 (147-3189)	65.6 (17.7-220)	2 (1.5-2.43)	1.85 (0.8-3.2)	37 (14-84)
	CC	314.8 (256-314.8)	47.5 (27-79.5)	1.455 (0.9-1.85)	1103 (360-1223)	125.45 (30.9-220)	2.2 (2.1-2.24)	0.9 (0.7-3.1)	19.000
rs2228570	CC	314.8 (63.2-625.6)	77 (3-385.3)	1.4 (0.3-10.5)	559 (86-3189)	62.8 (5.5-220)	2.06 (0.4-3.1)	1.7 (0.4-3.5)	34.5 (2.9-141)
	CT	314.8 (62-625.6)	57 (3-350)	1.49 (0.2-10.98)	820 (88-3172)	60.2 (1.9-257)	2.075 (1.6-3.9)	1.7 (0.8-3.4)	30 (2-84)
	TT	619.2 (199.7-625.6)	90.3 (39-154.3)	1.455 (0.03-14.1)	676.5 (75-1585)	43 (22-212)	2.125 (1.6-2.35)	1.9 (0.8-2.1)	37 (31-69)
rs2221266	CC	314.8 (62.6-625.6)	65.7 (5-350)	1.44 (0.3-14.1)	715 (88-2237)	64.3 (1.9-220)	2.1 (0.4-3.1)	1.7 (0.4-3.5)	33.5 (2.9-107)
	CT	314.8 (62-625.6)	72.5 (3-385.3)	1.375 (0.03-9.3)	522 (75-3189)	51.9 (8.7-257)	2.02 (1.4-3.9)	1.8 (0.8-3.2)	33 (2-84)
	TT	314.8 (63.2-625.6)	67.3 (8.5-209.7)	1.58 (0.75-7.02)	668 (169-2447)	55.8 (27-148)	2.01 (1.6-2.3)	1.4 (0.8-2.4)	39 (20-141)

PTH: parathyroid hormone, BALP: bone-specific alkaline phosphatase.

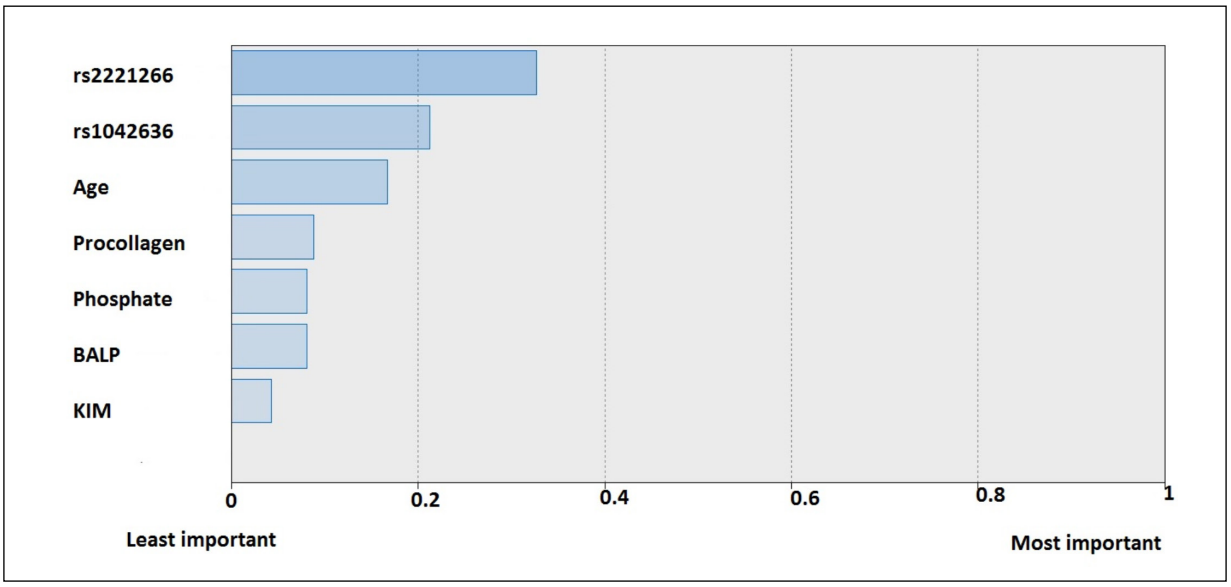

Figure 3. Predictors of importance for vitamin D deficiency. BALP: bone-specific alkaline phosphatase; KIM: kidney injury molecule. The horizontal bars represent the predictor variables of importance in determining vitamin D deficiency. The top variables in the figure hold greater significance and are organized hierarchically, indicating that those below are of lesser importance.

ther vitamin D insufficient or deficient. Notably, serum levels of procollagen 1 alpha 1 were significantly reduced in individuals possessing the CC genotype of rs10190 (related to calcium-sensing receptors) compared to those with the TT genotype, and in those with the TT genotype of rs739837 (associated with the vitamin D receptor) when compared to the GG genotype, as shown in Figure 2. In a similar vein, serum phosphate levels were significantly lower in participants with the TT genotype of rs10190 compared to those with CC and CT genotypes, and serum

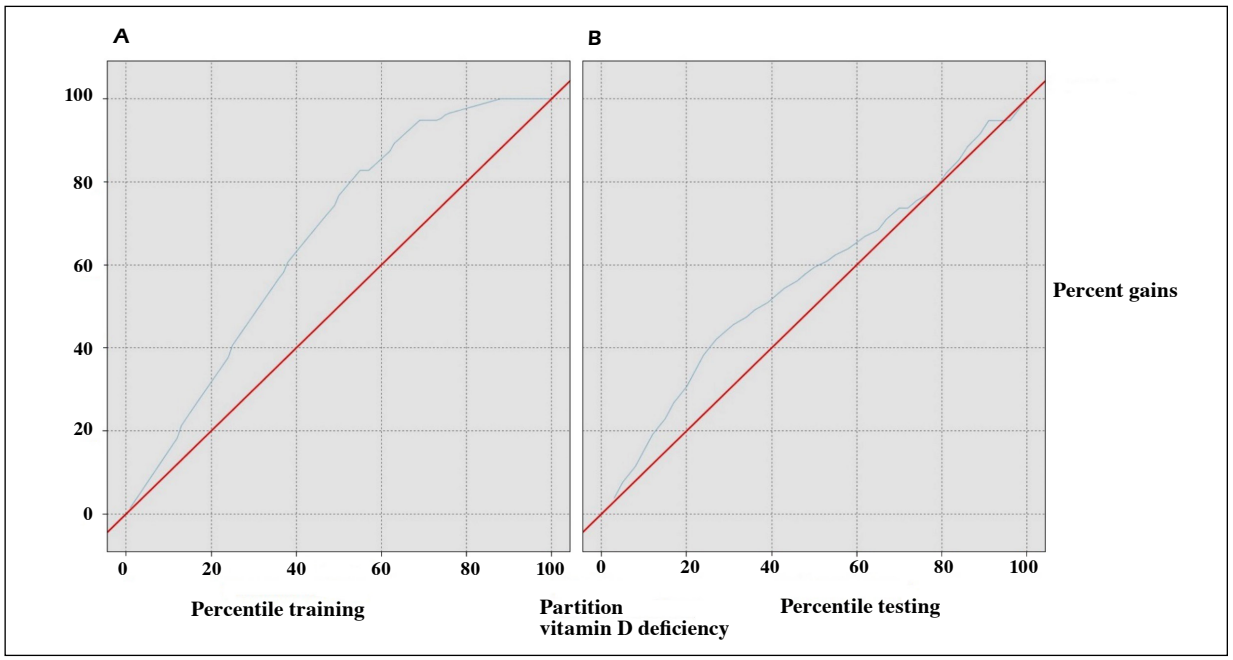

Figure 4. Gains plot between training and testing cohorts. The red diagonal line serves as a randomly generated reference, while the blue lines indicate the model's gains, offering valuable insights for both the training cohort (**A**) and the testing cohort (**B**).

parathyroid hormone (PTH) levels were lower in individuals with the CT genotype of rs1802757 compared to those with the CC genotype. Analysis using machine learning algorithms pinpointed the rs2221266 and rs1042636 SNPs as the most significant predictors of vitamin D deficiency, demonstrating a high level of accuracy in these predictions.

Comparison With the Existing Literature

This investigation stands as the inaugural study to examine the impact of SNPs related to calcium-sensing and vitamin D receptors on calcium metabolism biomarkers in patients with ESRD. Renal osteodystrophy, a prevalent complication, affects nearly three-quarters of individuals with ESRD[20]. It has been demonstrated that variations in procollagen 1 alpha 1 levels can lead to decreased bone mass and heightened bone fragility, underscoring its significance as an indicator of bone health[21]. Procollagen 1 alpha 1 serves as an essential marker for bone formation[22]. In our research, we discovered an association between the SNP rs10190 in calcium-sensing receptors and diminished levels of procollagen 1 alpha 1, suggesting that a loss-of-function mutation in the gene encoding calcium-sensing receptors may trigger hyperparathyroidism, thereby lowering procollagen 1 alpha 1 levels[23].

The effects of rs1802757 on calcium-sensing receptors have remained largely unexplored until now[24]. This polymorphism resides in the untranslated region and is believed to affect the mRNA production of the calcium-sensing receptor, offering a new avenue for understanding its role in calcium homeostasis[24]. Cinacalcet, a calcimimetic agent used to treat secondary hyperparathyroidism in ESRD patients, presents a relevant case for the investigation of SNP influences. Our study's machine learning analysis highlighted rs1042636 as a significant genetic factor contributing to vitamin D deficiency, a finding that aligns with the research by Jeong et al[23], which also indicated an impact of rs1042636 on the therapeutic response to cinacalcet. This suggests the necessity of further research into how SNPs in calcium-sensing receptors might alter the effectiveness of cinacalcet, warranting a broader study cohort. Additionally, studies have shown[25] calcifediol and paricalcitol to be observed with significant suppression of intact PTH. Future studies should explore the relationship between the significant SNPs identified in this study and these drugs.

It is crucial to consider the broader implications of understanding the genetic basis behind the varied responses to treatments in ESRD patients. The interplay between genetics and medication efficacy can significantly influence patient management strategies, emphasizing the importance of personalized medicine in this context. For instance, identifying genetic predispositions that affect drug responsiveness could enable clinicians to tailor treatment plans more effectively, potentially improving outcomes for individuals struggling with complex conditions such as renal osteodystrophy. Moreover, this research underlines the potential for genetic markers to predict complications before they manifest, offering a proactive approach to managing ESRD. Future studies focusing on a wider array of SNPs and their relationship with different aspects of ESRD management could provide invaluable insights, paving the way for advancements in treatment methodologies and the development of novel therapeutic agents tailored to genetic profiles. Such endeavors would not only enhance our understanding of ESRD and its complications but also improve the quality of life for affected individuals through more personalized and effective healthcare solutions.

Strengths and Limitations

The current study provides initial evidence regarding the impact of various SNPs associated with vitamin D and calcium-sensing receptors on calcium metabolism. However, it is important to note that this study does not extend to assessing the clinical implications of these SNPs, particularly in relation to bone fragility and fracture risk. We were unable to gather information on the vitamin D and calcium supplements taken by the study participants because of access limitations. Furthermore, it's important to interpret the decision-tree models carefully, given the small sample size in this study.

Conclusions

Single nucleotide polymorphisms in vitamin D and calcium-sensing receptors significantly affect calcium metabolism biomarkers in patients with ESRD. Therefore, it is crucial to assess the clinical implications of these SNPs, particularly their impact on patient health and treatment outcomes, as this represents a pressing priority in the field.

Conflict of Interest
The authors declare no conflict of interest.

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AI Disclosure
We acknowledge the use of ChatGPT for grammatical correction in this manuscript.

Informed Consent
Written consent was obtained from each study participant before their recruitment.

Ethics Approval
Ethical approvals were obtained from the Research Ethics Committee of the College of Medicine & Medical Sciences, Arabian Gulf University (approval No. E09-PI-11-21, dated 31/01/2022) and Salmaniya Medical Complex (approval No. 150061221, dated 06/12/2021).

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Authors' Contributions
K.S: conceived the idea, wrote the proposal, obtained funding; KS, AMQ, MMQ: data collection, data curation, data interpretation; AJ: genotyping and biomarker estimation; KS: wrote the first draft of the manuscript. All authors are involved in the revision and acceptance of the final draft.

Availability of Data and Materials
The data is available from the corresponding author and will be shared upon reasonable request.

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