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Association of a 5′-untranslated region polymorphism in the *CASR* gene with primary hyperparathyroidism and renal dysfunction

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ABSTRACT

Background: Primary hyperparathyroidism (PHPT) is a common endocrine disease characterized by abnormal secretion of parathyroid hormone (PTH) and a broad spectrum of clinical manifestations. Genetic polymorphisms in the calcium-sensing receptor (*CASR*) gene, especially in regulatory regions, might influence the risk and severity of the disease. *Aim:* This study aimed to investigate the association between a 5′-untranslated region (5′-UTR) polymorphism of the *CASR* gene and PHPT, and to evaluate its relationship with key biochemical and renal parameters. *Methodology:* In this case–control study, 60 patients with PHPT and 60 healthy controls were enrolled. Serum biochemical markers, including PTH, albumin-corrected calcium, phosphate, vitamin D, alkaline phosphatase (ALP), creatinine, and estimated glomerular filtration rate (eGFR), were measured. The 5′-UTR polymorphism of the *CASR* gene was analyzed using PCR–single-strand conformation polymorphism (PCR-SSCP), followed by confirmatory DNA sequencing. Associations between SSCP haplotypes and clinical parameters were assessed statistically. *Results:* PHPT patients exhibited significantly higher serum PTH levels (145.5 ± 40.1 *vs.* 32.8 ± 10.5 pg/mL, $p<0.001$), lower vitamin D levels (18.5 ± 5.5 *vs.* 35.2 ± 8.8 ng/mL, $p<0.001$), and elevated ALP levels ($p<0.01$) compared with controls. Renal dysfunction was evident in patients, with significantly higher serum creatinine and lower eGFR ($p<0.05$), despite the absence of significant hypercalcemia. PCR-SSCP analysis identified two distinct haplotypes (two-band and three-band patterns). The three-band variant was significantly more frequent in PHPT patients than in controls (25.0% *vs.* 8.3%; $p=0.019$; OR = 3.6, 95% CI: 1.2–11.4). Sequencing revealed a regulatory A>G substitution (rs7652589) at position 117 in the 5′-UTR of CASR. Among PHPT patients, the three-band variant was associated with higher creatinine levels and lower eGFR ($p<0.05$), suggesting more pronounced renal impairment, while ALP showed a borderline increase ($p=0.051$). *Conclusion:* A polymorphic variant in the 5′-UTR of the *CASR* gene is significantly associated with PHPT and may contribute to disease susceptibility and renal dysfunction. This regulatory variant might influence *CASR* expression and calcium homeostasis, suggesting a possible role for non-coding genetic variation in the pathophysiology and clinical heterogeneity of PHPT.

KEYWORDS

primary hyperparathyroidism, calcium-sensing receptor, *CASR*, 5′-UTR polymorphism, PCR-SSCP, renal function

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1. INTRODUCTION

Primary hyperparathyroidism (PHPT) is the third most frequent endocrine disease after thyroid diseases and diabetes mellitus and is a significant cause of disturbed calcium metabolism worldwide [1]. It is marked by autonomous and excessive secretion of parathyroid hormone (PTH), which leads to persistent disturbances of calcium and phosphate homeostasis. Approximately 80–85% of PHPT cases are due to solitary parathyroid adenoma, whereas 15-20% cases are due to multiglandular hyperplasia. Parathyroid carcinoma is an uncommon etiology, accounting for less than 1% of patients [2].

Biochemically, PHPT is described by hypercalcemia with elevated or inappropriately normal PTH levels, frequently associated with hypophosphatemia and hypercalciuria [3]. The clinical presentation of PHPT ranges from asymptomatic biochemical abnormalities to severe multisystem disease [4]. Reduced bone mineral density, increased risk of osteoporotic fractures and renal complications which includes nephrolithiasis and progressive impairment of renal function are of clinical importance [5–7].

The calcium-sensing receptor (*CASR*) is involved in calcium homeostasis and is a strong candidate gene for susceptibility, clinical severity and phenotypic variability in PHPT [8]. *CASR* is ubiquitously expressed and is the main extracellular calcium sensor to regulate PTH secretion in parathyroid glands and renal tubular calcium reabsorption in response to changes in circulating calcium levels [8,9]. Impaired function of *CASR* shifts the calcium–PTH set point to higher serum calcium concentrations, which are required to suppress PTH secretion and thereby contribute to the characteristic biochemical profile of PHPT [9].

The *CASR* gene is located on chromosome 3q13.3–21 and encodes the calcium-sensing receptor, a 1078–amino acid, dimeric, G-protein–coupled receptor [10,11]. *CASR* has a large extracellular calcium-sensing domain, a seven-transmembrane domain, and an intracellular C-terminal tail. The intracellular domain plays a crucial role in downstream signal transduction by interacting with G-proteins and cytoskeletal scaffold proteins, including filamin A [12,13]. Genetic variations in any of these domains or in the regulatory elements controlling *CASR* expression may affect receptor function and downstream calcium signaling [13].

Increasing evidence indicates that polymorphisms of regulatory regions of the *CASR* gene, such as the 5′-untranslated region (5′-UTR), may affect the efficiency of gene transcription, mRNA stability and, ultimately, the level of CASR expression [14,15]. Downregulation of *CASR* expression or activity leads to decreased parathyroid cell sensitivity to extracellular calcium, which results in inappropriate PTH secretion and increased severity of disease. Interest in genetic variability of *CASR* is increasing, but data in Middle Eastern populations are scarce and the clinical significance of 5′-UTR variations in PHPT is not well understood [15].

Therefore, the present study was designed to investigate a frequent genetic variation in the 5'-UTR of the *CASR* gene in the Iraqi population. Specifically, we aimed to determine its association with the risk of PHPT and whether this variant is associated with established markers of disease severity, including serum PTH levels, albumin-corrected calcium levels and measures of renal function. Recognizing the genetic contributors to PHPT in this population may provide insights into disease pathophysiology and promote more individualized risk stratification and management strategies.

2. METHODOLOGY

2.1. Study design and sampling

A case–control study was carried out in Babylon Province, Iraq, between January and September 2024 to investigate the genetic and biochemical characteristics associated with PHPT. The study population included 120 participants, 60 patients with a diagnosis of PHPT and 60 age- and sex-matched healthy controls.

Patients were selected from Hillah Teaching Hospital based on established clinical, biochemical and laboratory criteria for PHPT. Healthy controls were selected from healthy individuals attending routine health checkups at the same institution, and they had no history of endocrine, renal or metabolic bone disorders. All participants provided written informed consent before enrollment.

2.2. Clinical and biochemical assessment

All patients with PHPT were subjected to a detailed clinical examination by a trained health professional prior to the commencement of any therapeutic intervention. Demographic data and medical history were obtained for all subjects.
Venous blood samples (5 mL) were aseptically obtained from each participant. Of this, 2 mL was transferred to EDTA tubes for genomic DNA extraction. The remaining blood was allowed to clot and centrifuged at 3000 rpm for 15 min for serum separation. Serum was divided into aliquots and stored at −20°C in sterile Eppendorf tubes until biochemical analyses were conducted.

In both groups, serum parathyroid hormone (PTH), creatinine, phosphate and estimated glomerular filtration rate (eGFR) were assessed using standardised laboratory assays. Albumin-corrected calcium, a key parameter for an accurate diagnosis of hypercalcemia, was calculated using total serum calcium concentrations corrected for serum albumin. Serum 25-hydroxyvitamin D and alkaline phosphatase (ALP) were also measured to evaluate the status of bone and mineral metabolism. To exclude secondary hyperparathyroidism (SHPT), vitamin D deficiency was defined as serum 25-hydroxyvitamin D [25(OH)D] level <20 ng/mL (<50 nmol/L) according to the Endocrine Society Clinical Practice Guidelines and the diagnostic criteria for normocalcemic primary hyperparathyroidism (NPHPT) proposed by the Fifth International Workshop on PHPT. Patients with 25(OH)D levels below this cut-off and in whom vitamin D deficiency was deemed the main reason for PTH elevation (clinical assessment and lack of the other criteria for PHPT) were excluded from the study. Chronic kidney disease (eGFR <60 mL/min/1.73 m²), malabsorption syndromes, hypocalcemia, and use of medications known to interfere with calcium or PTH metabolism were additional exclusion criteria for SHPT. Diagnosis of PHPT was based on persistently elevated or inappropriately normal PTH levels on at least two separate measurements with normal albumin-corrected serum calcium and after exclusion of all identifiable secondary causes.

2.3. DNA extraction and polymerase chain reaction (PCR)

Genomic DNA was extracted from EDTA-anticoagulated whole blood samples using a commercial DNA extraction kit (Geneaid, Taiwan) according to the manufacturer's protocol. The concentration and purity of extracted DNA were assessed spectrophotometrically.

The 5′-untranslated region (5′-UTR) of the calcium-sensing receptor (*CASR*) gene was amplified by polymerase chain reaction (PCR) using sequence-specific primers synthesized by Macrogen (South Korea):

- Forward primer: 5′-ACTGCCCTCATCATTCCTTC-3′
- Reverse primer: 5′-ATCATCCTCCCTGCAAGAAC-3′

PCR amplification was carried out in a 20 µL reaction volume containing 3 µL of genomic DNA, 12.5 µL of Green Master Mix, 1 µL of each primer, and 2.5 µL of nuclease-free water. The thermal cycling conditions consisted of an initial denaturation at 94°C for 5 min, followed by 33 cycles of denaturation at 94°C for 45 sec, annealing at 60°C for 60 sec, and extension at 72°C for 45 sec, with a final extension at 72°C for 4 min. PCR reactions were performed using a Biometra thermocycler (Germany).

2.4. PCR-Single strand conformation polymorphism (PCR-SSCP) analysis

PCR products were initially verified by electrophoresis on a 1% agarose gel stained with ethidium bromide and visualized under ultraviolet light using a gel documentation system (Cleaver Scientific, UK). Single-strand conformation polymorphism (SSCP) was used to detect genetic variations in the amplicons with clear and specific bands.

For SSCP analysis, 10 µL denaturing loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol, pH 8) was added to each PCR product. The mixtures were denatured at 95°C for 8 min and immediately cooled on ice for 10 min to prevent reannealing of the DNA.

The denatured samples were then loaded on an 8% nondenaturing polyacrylamide gel and electrophoresed for 160 min at 200 V and 90 mA. After electrophoresis, a sensitive silver staining method was used to visualize the DNA bands [16]. The mobility shifts were analysed and the banding patterns were consistent with the single-stranded (ssDNA) and double-stranded (dsDNA) conformations. The ssDNA migration differences were

interpreted to represent polymorphisms in the amplified genomic region.

2.5. Statistical analysis

The data were coded, entered and analysed through SPSS version 26 (IBM Corp., Armonk, NY, USA). The normality of continuous variables was tested using the Shapiro–Wilk test and graphically using histograms and Q–Q plots. Data that were normally distributed are expressed as mean ± standard deviation (SD) and data that were not normally distributed as median (interquartile range, IQR). Categorical variables were expressed as frequencies and percentages [17].

Baseline characteristics and biochemical parameters were compared between PHPT patients and controls by independent samples t-test for normally distributed data. Categorical variables have been compared using the Chi-square test.

2.6. Ethical approval and consent to participate

The study was performed in accordance with ethical standards of the University of Babylon Ethical Committee (Ethical Code:17560-SN663). All volunteers gave written informed consent prior to sample collection.

3. RESULTS

Table 1 displays a comparison of selected biochemical parameters between the two groups. Groups Patients with PHPT Controls Serum parathyroid hormone (PTH) levels were significantly higher in the patient group (mean, 145.5 ± 40.1 pg/mL) than in the control group (mean, 32.8 ± 10.5 pg/mL; $p<0.001$). Likewise, patients had a significant deficiency in vitamin D, as mean serum 25-hydroxyvitamin D levels were significantly lower than controls (18.5 ± 5.5 ng/mL *vs.* 35.2 ± 8.8 ng/mL, $p<0.001$). Serum alkaline phosphatase (ALP) levels were significantly higher in the patient group (115.0 ± 35.0 U/L) compared with controls (70.0 ± 15.0 U/L, $p<0.01$). This is in keeping with the increased bone turnover. In contrast, albumin-corrected serum calcium concentrations did not differ significantly between patients (2.21 ± 0.17 mmol/L) and controls (2.34 ± 0.09 mmol/L, $p=0.122$). Serum phosphate levels did not differ between the two groups ($p=0.105$). Importantly, patients with PHPT had significant renal dysfunction, with higher mean serum creatinine and significantly lower estimated glomerular filtration rate (eGFR) compared to healthy controls ($p<0.05$) despite the absence of overt hypercalcemia.

Table 1. Renal function indicators and serum biochemical parameters in patients with PHPT and in healthy controls.

Variable	Patients N=60	Control N=60	*p*-value
Parathyroid Hormone (PTH) (pg/mL)	145.5 ± 40.1	32.8 ± 10.5	***0.001**
Vitamin D (ng/mL)	18.5 ± 5.5	35.2 ± 8.8	*0.001
Albumin-Corrected Serum Calcium (mmol/L)	2.21 **±** 0.17	2.34 **±** 0.09	0.122
Alkaline Phosphatase (U/L)	115.0 ± 35.0	70.0 ± 15.0	***0.01**
Serum Phosphorus (mmol/L)	1.22 **±**0.25	1.11 **±** 0.11	0.105
Serum Creatinine (µmol/L)	119.13**±** 11.41	83.11 **±** 9.01	* **0.001**
eGFR (ml/min /1.73 m^2)	79.44±5.37	102.33**±**4.66	***0.001**

* Statistically significant ($p<0.05$).

The 5′-UTR polymorphism of the *CASR* gene was detected using PCR-SSCP analysis. Agarose gel electrophoresis confirmed the successful amplification of the target region, revealing a distinct 178-bp amplicon corresponding to the *CASR* 5′-UTR (Figure 1).

After amplification of the target region, PCR–SSCP analysis was used to investigate the

conformational polymorphisms in the 5′-UTR of the *CASR* gene. All SSCP gel profiles were systematically aligned and compared for the identification of discrete haplotypes, as previously described [18].

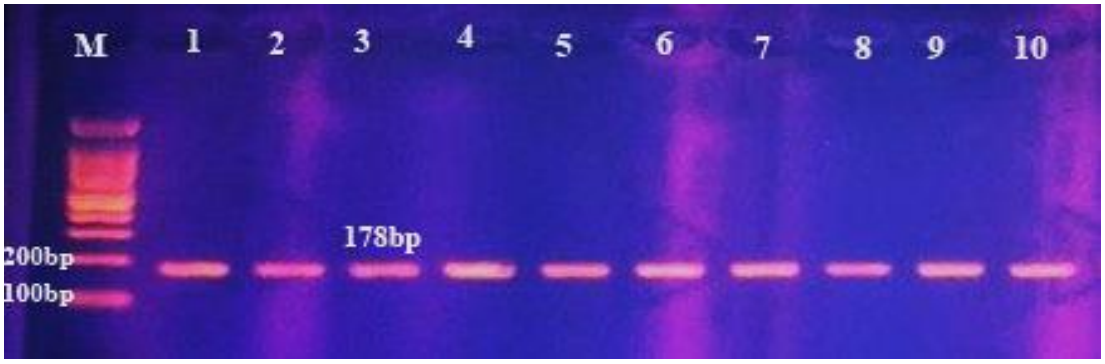

Figure 1. Agarose gel electrophoresis of PCR-amplified 5'-UTR region of the *CASR* gene from patients with PHPT and healthy control groups. M: DNA marker. Electrophoresis was performed on a 1% agarose gel at 75 V for 1 h and visualized with ethidium bromide staining.

Two different haplotypes were identified by analysis: two-band and three-band SSCP patterns. As shown in Figure 2, ssDNA and dsDNA conformations were clearly resolved in the upper and lower parts of the gel, respectively, allowing a reliable discrimination of the polymorphic patterns identified.

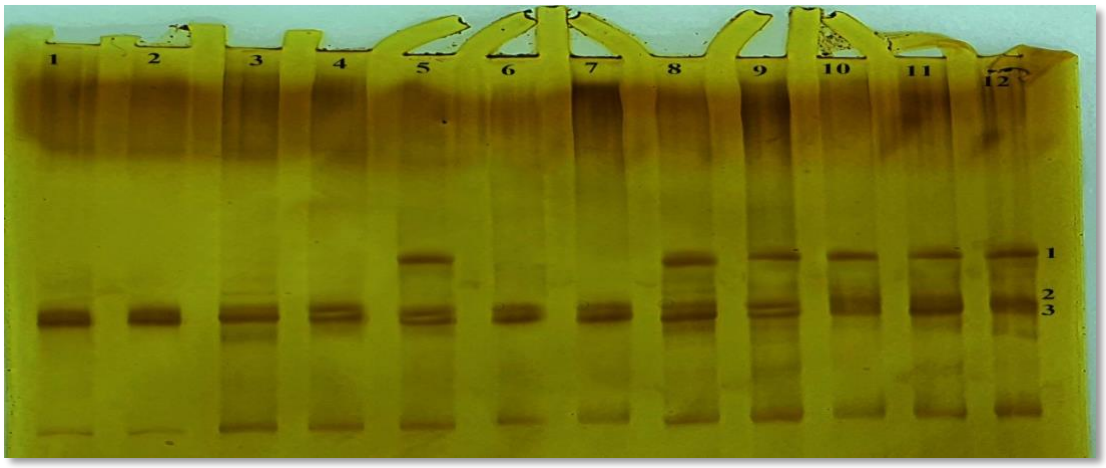

Figure 2. PCR–SSCP analysis of DNA polymorphisms in the 5′-UTR of the *CASR* gene. Lanes 1–12 show representative SSCP profiles of the amplified target region. Lanes 1, 2, and 4 represent the two-band haplotype observed in healthy controls, while lanes 3, 6, and 7 show the two-band haplotype detected in patients. Lanes 5, 8, 9, 10, 11, and 12 display the three-band haplotype, identified exclusively in patients. Electrophoresis was performed on an 8% non-denaturing polyacrylamide gel at 200 V and 100 mA for 120 min, followed by silver nitrate staining. The positions of distinct DNA conformers are indicated (bands 1–3).

Analysis of the PCR-SSCP profiles revealed a significant difference in the conformational DNA polymorphism in the 5′-UTR of the *CASR* gene between PHPT patients and healthy controls based on the banding patterns (Table 2). The three-band SSCP pattern was found to be significantly more common in PHPT patients (25.0%, n=15) compared to controls (8.3%, n=5). Statistical analysis revealed a significant association between three-band variant and primary hyperparathyroidism (p=0.019) with an odds ratio (OR) of 3.6 (95% confidence interval [CI]: 1.2–11.4).

Therefore, the subjects with the three-band SSCP pattern were approximately 3.6 times more likely to have PHPT than those with the two-band haplotype, suggesting that this conformational variant may be a genetic susceptibility marker for PHPT.

Because SSCP analysis is based on variations in electrophoretic mobility and does not directly reveal nucleotide changes, a sequence-based validation was performed to confirm the genetic variation underlying the SSCP shifts (Figure 3). Representative samples were sequenced directly and identified single nucleotide polymorphisms (SNPs) that distinguish the identified haplotypes from the reference *CASR* sequence (NM_000388.4 / NP_000379.3). In particular, a substitution mutation (A>G; rs7652589) was detected in the 5′-UTR of the *CASR* gene at position 117 in several patient samples.

This polymorphism is situated in a regulatory region and may affect the affinity of the transcription factor binding and thus may affect the expression of the *CASR* gene. Such regulatory effects suggest a plausible biological mechanism by which the identified 5'-UTR variant might be associated with altered calcium sensing and increased susceptibility to PHPT.

Table 2. Distribution of 5´-UTR *CASR* gene conformational haplotype polymorphisms by number of bands and correlation between patient and control groups.

Conformational haplotypes	Patients N=60	Controls N=60	*p*-value	Odd ratio	95% Confidence interval
2-bands [a]	45 (75 %)	55 (91.7%)			
3-bands	15 (25 %)	5 (8.3%)	0.019*	3.66	0.123-10.863
Total number	60	60			

[a] Reference genotype; * Statistically significant (p<0.05).

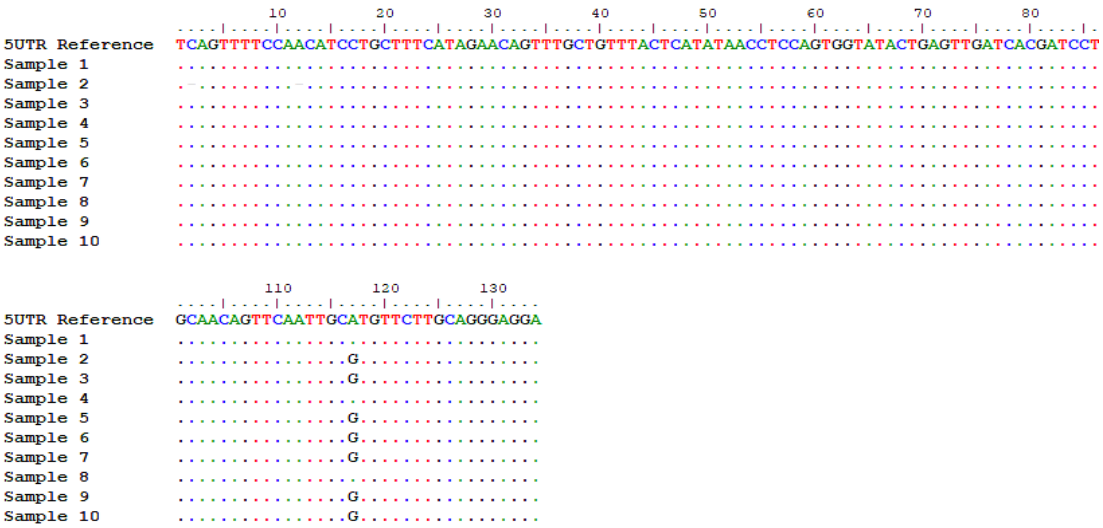

Figure 3. Sequence alignment results of the Homo sapiens 5′-UTR *CASR* gene fragment using BioEdit software (version 7.2.5). Sample 1 represents the control, while samples 2–10 represent the patient group.

Table 3 presents the mean values of selected clinical and biochemical markers in PHPT patients, stratified according to PCR-SSCP haplotypes (two-band *vs.* three-band variant patterns). No statistically significant differences were observed between the two haplotype groups with respect to albumin-corrected serum calcium (p=0.290), serum phosphate (p=0.710), parathyroid hormone (PTH) (p=0.150), or 25-hydroxyvitamin D levels (p=0.600).

In contrast, renal function indices differed significantly between haplotypes. Patients carrying the three-band variant pattern exhibited a significantly higher mean serum creatinine level compared with those harboring the two-band haplotype, indicating a statistically significant

difference (p<0.05). Consistently, the mean estimated glomerular filtration rate (eGFR) was significantly higher in the two-band group than in the three-band variant group (p<0.05).

Additionally, patients in the three-band variant group showed a trend toward higher ALP levels, with a borderline difference (p=0.051), potentially indicating greater bone turnover or skeletal involvement, although this observation did not reach conventional statistical significance.

Table 3. Association of the variant pattern with clinical markers in PHPT patients.

Variable	2-band (N=45)	3-band (N=15)	*p*-value
Parathyroid Hormone (PTH) (pg/mL)	113.5 ± 17.2	123.9 ± 14.5	0.150
Vitamin D (ng/mL)	16.6 ± 4.2	14.8 ± 6.9	0.600
Albumin-Corrected Serum Calcium (mmol/L**)**	2.24±0.14	2.29±0.15	0.290
Alkaline Phosphatase (U/L)	89.0 ± 13.6	112.0 ± 9.5	0.051
Serum Phosphorus (mmol/L)	1.19±0.23	1.22±0.25	0.710
Serum Creatinine (µmol/L)	72.84±6.79	112.58±7.24	0.010*
eGFR (ml/min /1.73 m^2)	93.84 ±4.63	71.75±3.53	0.047*

* Statistically significant (p<0.05).

4. DISCUSSION

The present study provides combined biochemical and genetic evidence supporting a contributory role of regulatory variation in the *CASR* gene in both susceptibility to and clinical expression of PHPT. Our results identify a unique biochemical phenotype of high PTH with relatively normal serum calcium levels and a significant association of a 5′-UTR *CASR* polymorphism with increased risk for PHPT and disease severity, particularly renal involvement.

In line with the classical PHPT, our cohort of patients had significantly elevated serum PTH, higher ALP and lower vitamin D levels compared to healthy controls. These findings are consistent with prior studies showing that chronic PTH excess results in increased bone turnover and a compensatory vitamin D depletion through increased conversion to inactive metabolites, or secondary lifestyle and nutritional factors [19,20].

Interestingly, although PTH levels were markedly elevated, albumin-corrected serum calcium levels were not significantly different between patients and controls. This biochemical pattern suggests a large proportion of our cohort to fall within the spectrum of normocalcemic primary hyperparathyroidism (NPHPT). NPHPT has been recognized over the past decade as a common and clinically relevant phenotype of PHPT, often identified during evaluation of osteoporosis, nephrolithiasis, or renal dysfunction rather than frank hypercalcemia [21]. The diagnosis in our study was made after excluding secondary causes including vitamin D deficiency (defined as 25(OH) D <20 ng/mL), renal insufficiency, and malabsorption, based on persistently increased or inappropriately normal PTH with normal albumin-corrected calcium on repeated assessment. However, it is acknowledged that not all patients had a formal standardized protocol of vitamin D repletion and PTH reassessment, as recommended by current NPHPT guidelines [20], prior to enrollment. This is a methodological limitation as residual vitamin D insufficiency may have contributed to PTH rise in a subset of patients. Given the genetic focus of this study and the internally consistent genotype–phenotype associations observed, this is unlikely to have materially affected the main findings. Similar biochemical features have also been reported in European and Asian populations, where normocalcemic disease may be an early presentation of PHPT or a genetically different subtype of altered calcium sensing [22,23].

In this study population overt hypercalcemia was not observed, but PHPT subjects showed significant impairment of renal function, characterized

by increased serum creatinine and decreased eGFR compared to controls. These findings are consistent with emerging evidence that excess PTH per se, independent of serum calcium, may contribute to renal injury by glomerular hyperfiltration, tubular calcium loading and stimulation of interstitial fibrosis [24].

One of the major findings of this study is the significantly higher frequency of the three-band PCR-SSCP haplotype in PHPT patients than in controls. These findings are consistent with previous genetic studies that suggest variants in *CASR* as modifiers of susceptibility and phenotypic heterogeneity of PHPT [24,25]. Most of the previous literature has focused on coding region variants or missense mutations, but regulatory polymorphisms are attracting more attention, especially in non-coding regions affecting gene expression.

In our study, sequence analysis revealed a uniform A>G substitution (rs7652589) at position 117 in the *CASR* 5′-UTR in carriers of the three-band variant. Similar regulatory variants have been demonstrated to affect transcription factor binding, mRNA stability or translational efficiency in other endocrine genes [26]. Functional studies of *CASR* expression show that the reduced availability of receptors on parathyroid chief cells causes an upward shift of the calcium-PTH set point, which leads to inappropriate PTH secretion at normal serum calcium levels [26,27]. This mechanism provides a biologically plausible explanation for the association we found in our cohort.

Our study's major and clinically relevant finding was the strong genotype-phenotype association in the PHPT cohort. Serum creatinine was significantly higher and the estimated glomerular filtration rate (eGFR) was lower in patients with the three-band variant pattern than in those with the two-band haplotype. In this context, the identified *CASR* 5′-UTR variant may predispose to PHPT and also worsen renal dysfunction even without overt hypercalcemia. Such associations of *CASR* variants with renal outcomes have been previously reported especially in the setting of hypercalciuria and nephrolithiasis both of which contribute to progression of chronic kidney disease in PHPT [28,29].

In addition, the trend for increased ALP with the three-band variant group although not statistically significant, may suggest increased skeletal turnover or bone involvement. Altogether these findings suggest that the 3-band 5'-UTR variant may be a marker of a more aggressive disease phenotype with disproportionate renal and possibly skeletal involvement. Clinically, this raises the possibility that genotyping for regulatory *CASR* variants may help to identify PHPT patients who are at increased risk for end-organ complications and thereby allow earlier intervention and closer surveillance. But there are some limitations to note with these strengths. The small sample size restricts statistical power and may restrict the generalizability of our findings. Furthermore, a standardized pre-enrollment routine of vitamin D repletion followed by serial PTH rechecks was not uniformly used, a known limitation in the diagnostic workup of NPHPT. Current guidelines recommend confirmation of NPHPT by documentation of persistent elevation of PTH after correction of vitamin D deficiency and other secondary causes, which was not formally verified in all participants. In addition, in patients with concomitant vitamin D insufficiency, PTH was not routinely reassessed after vitamin D supplementation, which may have led to a certain degree of diagnostic heterogeneity in the normocalcemic subgroup. Future studies should consider implementing a vitamin D repletion and PTH re-evaluation protocol before enrollment to establish a more rigorous phenotypic classification of NPHPT in this population. this study is also primarily observational and genetic in nature, and aims to establish genotype-phenotype correlations in our cohort. The exact molecular mechanisms that underlie the functional effects of the rs7652589 variant on the transcription and translation of *CASR* are not directly quantified due to the lack of functional validation in the form of *in vitro* or *in vivo* assays (e.g., gene expression analysis). Hence, additional functional and mechanistic studies in larger multicenter cohorts are needed to definitively elucidate the downstream pathways that connect this regulatory 5′-UTR polymorphism with altered calcium sensing and PHPT susceptibility.

5. CONCLUSION

The present study shows that a regulatory polymorphism in the 5′-UTR region of the *CASR* gene, corresponding to the three-band PCR-SSCP haplotype and the rs7652589 (A>G) variant, was significantly associated with both increased susceptibility to PHPT and greater disease severity, particularly reduced renal function. These findings underscore the role of non-coding *CASR* genetic variation in modifying disease risk and clinical heterogeneity and indicate that genetic profiling could be useful for risk stratification and personalized management of PHPT and warrants further validation in larger functional studies.

ADDITIONAL INFORMATION

ACKNOWLEDGMENTS
None.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

ETHICAL STATEMENTS
The study was performed in accordance with ethical standards of the University of Babylon Ethical Committee (Ethical Code:17560-SN663). All volunteers gave written informed consent prior to sample collection

ARTIFICIAL INTELLIGENCE (AI) USE
The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI. No AI tools were used in the preparation of this manuscript.

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