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Receptor gene expressions and their association with biochemical parameters in primary hyperparathyroidism patients

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ABSTRACT

Aims: Primary hyperparathyroidism (PHPT) is recognized by excessive parathyroid hormone (PTH) secretion, leading to hypercalcemia and systemic complications. This study aimed to investigate the gene expression profiles of PTH, calcium-sensing receptor (CaSR), vitamin D receptor (VDR), fibroblast growth factor receptor 1 (FGFR1), and α-Klotho from parathyroid adenomas among PHPT patients and to explore their correlations with biochemical parameters.

Methods: Parathyroid adenoma samples were obtained from 20 PHPT patients who underwent parathyroidectomy. RNA was isolated, and gene expression levels were quantified using one-step reverse transcription quantitative PCR (RT-qPCR). Fold change values were calculated utilizing gene-specific patient average ΔCt values as references. Biochemical data, including serum calcium, phosphorus, PTH, and vitamin D (25-OH), as well as gender, age, and adenoma volumes, were statistically analyzed for correlation with gene expression patterns.

Results: Gene expression analysis revealed significantly lower CaSR expression in small adenomas (<0.2 cm^3) compared to medium and large adenomas, suggesting a possible association between tumor size and early molecular deregulation. Notably, CaSR expression exhibited a positive correlation with the expression levels of both VDR and FGFR1, suggesting the presence of a coordinated regulatory network encompassing calcium and vitamin D pathways. A multiple regression model revealed that serum vitamin D and PTH gene expression serve as positive predictors of CaSR expression, whereas serum PTH levels were negatively associated. A paradoxical positive correlation was observed between serum calcium levels and PTH gene expression, which may reflect impaired feedback control due to CaSR downregulation.

Conclusion: These findings may provide new insights into the pathophysiology of PHPT, highlighting the importance of molecular profiling in understanding disease progression. The established expression patterns and their biochemical correlations may strengthen future efforts toward population-specific diagnosis and targeted therapeutic approaches, particularly in regions with high vitamin D deficiency and distinct disease severity profiles.

Keywords: Primary hyperparathyroidism (PHPT), calcium-sensing receptor (CaSR), PTH, vitamin D receptor (VDR), fibroblast growth factor receptor 1 (FGFR1), α-Klotho

INTRODUCTION

Primary hyperparathyroidism (PHPT) is a prevalent endocrine disorder characterized by the overproduction of parathyroid hormone (PTH), leading to hypercalcemia and its related systemic complications. Globally, PHPT is recognized as the third most common endocrine disease, following diabetes and thyroid-related disorders.[1] Regardless of geographical location, PHPT demonstrates an increased incidence among older populations, particularly affecting postmenopausal women (34–120 women per 100.000 person-years) when compared to men (13–36 men per 100.000 person-years).[2] While typically asymptomatic, the diagnostic criteria for classical PHPT involve serum calcium levels greater than 10.5 mg/dl and PTH levels surpassing 65 pg/ml, with the normal range being 15–65 pg/ml.[3] The pathophysiology of PHPT involves the dysregulation of calcium-regulating receptors, including the calcium-sensing receptor (CaSR), vitamin D receptor (VDR), and the α-Klotho/FGFR1 complex. According to the literature, more studies were conducted regarding the expression levels of VDR and CaSR in parathyroid adenomas.[4-7] Downregulation of CaSR and VDR is frequently observed in PHPT parathyroid adenomas and contributes to dysregulated PTH secretion.[8,9]

Expression of α-Klotho has also been identified in the parathyroid gland, and experimental evidence suggests that fibroblast growth factor 23 (FGF23) modulates PTH secretion via the FGFR1/α-Klotho axis.[10,11] Clinical investigations have reported elevated FGF23 levels in PHPT patients, with correlations to serum calcium, PTH, and

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phosphate concentrations, although findings remain partly inconsistent.[12,13] These observations indicate that the FGFR1/α-Klotho pathway may have a distinct, yet incompletely understood role in PHPT pathophysiology, warranting further investigation at the gene expression level.

This research seeks to investigate the expression levels of PTH, CaSR, VDR, α-Klotho, and FGFR1 in parathyroid adenomas from Turkish PHPT patients. Furthermore, it aims to establish correlations between these molecular findings and biochemical parameters, including serum PTH, calcium, phosphorus, and vitamin D (25-OH) (D3) measurements, as well as adenoma size, age, and gender. Lastly, through this approach, it is intended to address the limited evidence on α-Klotho/FGFR1 signaling in PHPT. Investigating these population-specific molecular profiles may provide valuable insights for developing future treatment approaches and personalized management strategies in PHPT.

METHODS

Ethical approval was obtained from the University of Health Sciences İstanbul Training and Research Hospital Clinical Researches Ethics Committee (Date: 07.03.2025, Decision No: 52). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients diagnosed with PHPT who met surgical criteria and were admitted to the Endocrinology Clinic at Yeditepe University, İstanbul, Turkiye, underwent parathyroidectomy for the removal of hyperactive parathyroid tissues and were included in this study. Patients were excluded if they were younger than 18 years of age, pregnant, or had serum calcium and PTH levels within the normal reference range. Individuals with known malignancies outside the parathyroid glands were not included. In addition, patients with alternative conditions that could account for hyperparathyroidism, such as chronic renal insufficiency or sarcoidosis, were excluded. Patients with a history of previous parathyroid surgery, parathyroid carcinoma, secondary or tertiary hyperparathyroidism, or incomplete medical records were also excluded. The study included a total of 20 patients (male and female). Information details, including PTH (pg/ml), calcium (mg/dl), phosphorus (mg/dl), D3 (µg/L), gender, preoperative age, and adenoma size, were obtained from the hospital health records. Serum calcium and phosphorus concentrations were measured using photometric methods on the Roche Cobas® c 501 analyzer (Roche Diagnostics, Germany), with Roche Calcium Gen.2 and Phosphate (Inorganic) ver.2 reagent kits, respectively. Serum PTH levels were determined using an electrochemiluminescence immunoassay (ECLIA) on the Roche Cobas® e 601 analyzer (Roche Diagnostics, Germany) with the Elecsys PTH assay kit. Serum 25-hydroxyvitamin D (25-OH D3) levels were quantified using the Elecsys Vitamin D total III assay on the same platform. Adenoma size was determined by pathological assessment following surgical excision. The pathology department recorded tissue dimensions (length, width, and thickness), and tissue volumes were calculated using the ellipsoidal model with the same formula utilized in the literature: length x thickness x width x 0.52.[14,15]

RNA Isolation

All tissue samples were immediately stored at −80°C, followed by RNA isolation. Total RNA isolation was performed according to the PureLink Mini RNA Kit (Ambion-Life Technologies™, USA) instructions. Total RNA was isolated from approximately 50 mg of preserved parathyroid tissues, which were cut into small pieces and subsequently homogenized in RNA lysis buffer, including 1% ß-mercaptoethanol. Individual lysates were transferred to the spin cartridges, followed by the execution of the binding, washing, and elution steps. The RNA yield and purity were measured using a Take3 plate (BioTek instrument) with a BioTek Synergy HTX multimode reader (BioTek, US).

Reverse Transcription-Polymerase Chain Reaction

The expression levels of PTH, CaSR, α-Klotho, FGFR1, and VDR were determined using one-step RT-qPCR with SYBR Green Mastermix (2X) (Nucleogene, Turkiye). The β-actin gene was incorporated for the purposes of normalization and serving as an internal control. The QIAGEN Rotor-Gene Q-2plex device was used to perform one-step RT-qPCR for all tests in this study. Briefly, the composition of the RT-qPCR reagent mix was described in **Table 1**.

Table 1. RT-qPCR components with final concentrations

Component	Final concentration
Reaction mix (2X)	1X
Enzyme mix (40X)	1X
Primer mix (F+R)	300 nM
Nuclease-free water	
RNA template	10 ng/µL

The final volume of the RT-qPCR mixture was 16 µL, and the cycling conditions were programmed as follows: RT incubation and enzyme activation were serially performed at 50°C for 15 min and 95°C for 2 min 30 sec. Afterwards, it was cycled 45 times at 95°C for 15 sec for denaturation and 60°C for 30 sec for annealing/extension. Melt curve analysis was conducted following the instrument's recommendations. All reactions were run in triplicate. Gene expression levels were analyzed using the comparative Ct method (ΔΔCt), with the average delta Ct value of each gene across all patients serving as the reference for fold change calculations. The primer sequences are presented in **Table 2**.

Statistical Analysis

All data analyses were performed using IBM SPSS Statistics (version 27). The Shapiro–Wilk test evaluated the normality of continuous variables (n=20). For normally distributed variables, comparisons between two independent groups used the independent-samples t-test. For comparisons among three or more groups, one-way analysis of variance (ANOVA) was used. Levene's test checked the homogeneity of variances. When the equal variances assumption was met ($p \geq 0.05$),

Table 2. Target genes and the primer sequences used in the study

Target genes	5'–3' primer sequences
β-actin	F: TGATCCACATCTGCTGGAAGGT R: GACAGGATGCAGAAGGAGATTACT
PTH	F: GACATTGTATGTGAAGATGATACC R: GCTTCTTACGCAGCCATTCTA
α-Klotho	F: CCTCCTTTACCTGAAAATCAGCC R: CAGGTCGGTAAACTGAGACAGAG
CaSR	F: GGACAGCGGGAACAGGATTTGAGAG R: CCCAGTTAGTCCCGGTTCCTTCACC
VDR	F: AGCCTCAATGAGGAGCACTCCAAG R: CGGGTGAGGAGGGCTGCTGAGTA
FGFR1	F: CCAACTTAGTGAAACCCCATCT R: CCCAACAA ATACAGTCTGGTCA

PTH: Parathyroid hormone, CaSR: Calcium-sensing receptor, VDR: Vitamin D receptor, FGFR1: Fibroblast growth factor receptor 1

Tukey's honestly significant difference (HSD) post hoc test was applied; otherwise ($p<0.05$), the Games–Howell post hoc test was used to account for heterogeneity in variances. For non-normal variables, the Kruskal–Wallis H test compared group distributions.

Pearson's correlation analysis was performed to explore potential relationships between continuous variables that met the assumption of normality. For variables that were not normally distributed (e.g., PTH, D3, and α-Klotho_FC), Spearman's rank-order correlation was used instead. All statistical tests were two-tailed, and p-values less than 0.05 were regarded as statistically significant.

Categorical variables, such as gender and adenoma size classification, were analyzed using Pearson's Chi-square test of independence. In cases where more than 20% of the expected cell frequencies were less than 5, the Fisher–Freeman–Halton exact test was employed as a more appropriate alternative. Exact p-values and 99% confidence intervals were obtained using a Monte Carlo simulation with 10.000 iterations.

RESULTS

The study cohort consisted of 20 patients diagnosed with PHPT, exhibiting a mean age of 50.4±10.5 years. The biochemical analysis revealed elevated serum calcium levels (mean 11.38±0.70 mg/dl) and PTH levels (mean 132.7±59.7 pg/ml) consistent with classical PHPT. It is noteworthy that the levels of D3 were observed to be deficient, with a mean concentration of 19.8±11.5 µg/L. This finding corroborates previous reports indicating a widespread vitamin D insufficiency within the Turkish population.[16] The mean phosphorus level was recorded as 2.68±0.49 mg/dl. The mean adenoma volume was 0.51 cm^3 (range: 0.12–1.95 cm^3) (**Table 3**). Gender-based comparisons (**Table 4**) utilizing an independent-samples t-test indicated that male patients exhibited significantly higher calcium levels (11.95±0.84 mg/dl) compared to females (11.20±0.57 mg/dl; $t(18)=2.313$, $p=0.032$). The effect size was moderate (Cohen's $d=0.64$), signifying a practically meaningful difference. While no significant differences were identified in phosphorus levels or the expression of key genes (PTH_FC, FGFR1_FC, VDR_FC, CaSR_FC) across genders ($p>0.05$), there were small effect sizes observed for PTH_FC ($d=0.37$) and CaSR_FC ($d=0.32$). These findings are considered preliminary and should be interpreted with caution. To confirm their potential biological importance, further studies with larger sample sizes and greater statistical power are needed. For the variables that violated the normality assumption (PTH, D3, and α-Klotho_FC), the Mann–Whitney U test revealed no significant gender-based differences: PTH ($U=33.000$, $p=0.563$), D3 ($U=34.500$, $p=0.649$), and α-Klotho_FC ($U=34.000$, $p=0.620$).

Table 3. Descriptive clinical and biochemical parameters of the study cohort (n=20). Values presented include the mean, median, minimum, and maximum for pre-operative age, serum PTH (pg/ml), serum calcium (mg/dl), serum phosphorus (mg/dl), serum D3 (µg/L), and adenoma volume (cm^3)

	Age	PTH (pg/ml)	Calcium (mg/dl)	Phosphorus (mg/dl)	D3 (µg/L)	Adenoma volume (cm^3)
Mean	50	132.72	11.38	2.69	19.79	0.51
Median	50	118.00	11.29	2.70	18.00	0.27
Minimum	26	65.30	10.22	1.93	3.00	0.12
Maximum	69	335.30	13.10	3.53	58.00	1.95

PTH: Parathyroid hormone

The analysis of gene expression patterns, utilizing a one-way ANOVA, revealed significant variation in CaSR_FC levels across adenoma size categories ($F(2, 18)=3.664$, $p=0.046$) (**Table 5**). The post-hoc analysis using the Games–Howell test demonstrated that small adenoma groups (<0.2 cm^3) displayed significantly lower CaSR_FC expression compared to medium (0.2–0.5 cm^3; mean difference=–3.81909, $p=0.048$) and large (>0.5 cm^3; mean difference=–4.69167, $p=0.014$) adenoma groups, suggesting a potential relationship between adenoma size and CaSR dysregulation. Nevertheless, no statistically significant difference was observed between the medium and large adenoma groups ($p=0.756$).

Correlation analysis between biochemical and molecular variables was shown in **Table 6**. Pearson's correlation analysis identified several significant associations, including a positive relationship between calcium levels and PTH_FC ($r=0.520$, $p=0.016$), suggesting that increased calcium levels are correlated with higher PTH gene expression. Additionally, FGFR1_FC was strongly correlated with VDR_FC ($r=0.616$, $p=0.003$), and CaSR_FC showed positive correlations with both FGFR1_FC ($r=0.434$, $p=0.049$) and VDR_FC ($r=0.605$, $p=0.004$), implying potential regulatory or functional relationships. No other significant Pearson correlations were identified, and age did not exhibit a significant correlation with any continuous variables (all $p>0.05$). Spearman's rank-order correlations among non-normally distributed variables revealed no significant associations. Specifically, PTH was not significantly correlated with D3 ($\rho=-0.064$, $p=0.783$) or α-Klotho_FC ($\rho=-0.134$, $p=0.563$), and no association was observed between D3 and α-Klotho_FC ($\rho=-0.071$, $p=0.759$).

A multiple linear regression was performed to identify predictors of CaSR_FC expression. Predictor variables included adenoma volume, age, calcium, phosphorus, PTH, D3, PTH_FC, VDR_FC, FGFR1_FC, and α-Klotho_FC. The model was statistically significant, $F(10, 10)=4.288$, $p=0.015$, explaining 81.1% of the variance in CaSR_FC expression ($R^2=0.811$; adjusted $R^2=0.622$). Model diagnostics confirmed that the residuals were approximately normally

Table 4. Gender-based comparisons of biochemical and molecular parameters

Variable	Male mean±SD	Female mean±SD	Test statistic (df)	p-value	Effect size (Cohen's d)
Calcium (mg/dl)	11.96±0.84	11.20±0.57	t (19)=2.313	0.032*	0.64 (moderate)
Phosphorus (mg/dl)	2.69±0.64	2.68±0.45	t (19)=0.289	0.776	0.09 (trivial)
PTH (pg/ml)	161.52±102.65	123.73±39.81	U=33.000	0.563	0.49 (medium)
D3 (µg/L)	18.72±8.43	10.13±12.57	U=34.500	0.649	0.80 (large)
PTH_FC	2.38±2.45	−0.74±12.57	t (18)=1.392	0.179	0.37 (small)
CaSR_FC	0.81±3.84	−0.25±3.03	t (18)=0.785	0.445	0.32 (small)
α-Klotho_FC	−0.12±4.22	0.04±2.72	U=34.000	0.620	0.05 (trivial)

Independent-samples t-test for normally distributed variables; Mann–Whitney U test for non-normally distributed variables. * p<0.05 was considered statistically significant. SD: Standard deviation, PTH: Parathyroid hormone, CaSR: Calcium-sensing receptor

Table 5. Comparison of CaSR_FC expression across adenoma size categories

Comparison	Mean difference	p-value
Small vs. medium	−3.81909	0.048*
Small vs. large	−4.69167	0.014*
Medium vs. large	0.87258	0.756

CaSR: Calcium-sensing receptor, Adenoma size: small (<0.2 cm^3), medium (0.2–0.5 cm^3), large (>0.5 cm^3), Post-hoc comparisons were conducted using the Games–Howell test. * p<0.05 was considered statistically significant

Table 6. Correlation analysis between biochemical and molecular variables

Variable pair	Correlation coefficient	p-value
Calcium vs. PTH_FC	r=0.520	0.016*
FGFR1_FC vs. VDR_FC	r=0.616	0.003*
CaSR_FC vs. FGFR1_FC	r=0.434	0.049*
CaSR_FC vs. VDR_FC	r=0.605	0.004*
PTH vs. D3	ρ=−0.064	0.783
PTH vs. α-Klotho_FC	ρ=−0.134	0.563
D3 vs. α-Klotho_FC	ρ=−0.071	0.759

PTH: Parathyroid hormone, CaSR: Calcium-sensing receptor, VDR: Vitamin D receptor, FGFR1: Fibroblast growth factor receptor 1. Pearson's correlation-for normally distributed variables; Spearman's correlation-for non-normally distributed variables. * p<0.05 was considered statistically significant.

distributed, homoscedastic, and that no multicollinearity issues were present (VIFs<2.55). Three variables emerged as significant predictors: PTH levels showed a negative association (B=−0.029, β=−0.546, p=0.017), while D3 (B=0.114, β=0.414, p=0.034) and PTH_FC (B=0.585, β=0.715, p=0.007) demonstrated positive relationships with CaSR_FC expression. The remaining variables, including adenoma volume, age, calcium, phosphorus, VDR_FC, FGFR1_FC, and α-Klotho_FC, did not reach statistical significance but contributed to the overall model. To assess model adequacy, a post-hoc power analysis was conducted using G*power (version 3.1.9.7). Based on the observed effect size (f^2=4.29), 10 predictors, and a sample size of 20, the achieved power was 0.997, indicating a very high probability of detecting a true effect.

DISCUSSION

This study provides new perspectives on the biochemical and molecular characteristics of PHPT in a Turkish patient population. Our cohort demonstrated elevated mean PTH and calcium levels, consistent with previous reports from Turkiye. For instance, a 20-year single-center study reported a mean serum calcium of 11.9±2.2 mg/dl and a mean PTH of 467±78 pg/ml among 190 PHPT patients, of whom 72% were asymptomatic at diagnosis.[17] Similarly, a comparative study between Turkiye (Bursa) and the United States cohorts demonstrated significantly higher PTH concentrations (546±75 pg/ml vs. 146±75 pg/ml) and larger adenoma sizes (25.2±1.18 mm vs. 17.5±1.18 mm) among Turkish patients.[18] Furthermore, vitamin D deficiency has been demonstrated to be notably common in Turkish nationals (75%; <50 nmol/l), contributing to more severe biochemical profiles and secondary HPT.[19] These observations support our findings and highlight that Turkish PHPT patients may present with more pronounced biochemical alterations compared to Western populations.

The findings showed complex regulation of PTH, CaSR, VDR, FGFR1, and α-Klotho gene expressions in PHPT adenomas, consistent with previous literature. In this study, CaSR expression (CaSR_FC) was determined to be significantly downregulated in small adenomas (<0.2 cm^3) in comparison to medium and large adenomas, suggesting a potential correlation between the size of adenomas and CaSR dysregulation. This finding is consistent with previous research identifying CaSR downregulation as a hallmark of PHPT. Yano et al.[4] demonstrated that a reduction in CaSR and VDR expression was associated with higher proliferation markers and increased adenoma mass, indicating that CaSR may play an important role in the dynamics of tumor growth. Yet, other studies, such as Varshney et al.,[20] did not establish a direct correlation with tumor weight; instead, they confirmed reduced CaSR mRNA levels in adenomas among the Asian Indian populations. The observation that the lowest CaSR expression was detected in the smallest tumors may tentatively suggest that the loss of CaSR could represent an early alteration in the progression of tumor development, occurring before mass expansion. However, given the cross-sectional nature of this study and the limited number of samples, this interpretation should be considered exploratory and requires validation in larger, longitudinal studies. This observation is also corroborated by immunohistochemical verification from an autopsy-based study, which showed that reduced membrane CaSR expression occurs not only in adenomas but also in the normal parathyroid rim tissue, implying an early and diffuse CaSR suppression in PHPT pathogenesis.[21]

Notably, CaSR_FC exhibited a positive correlation with both VDR_FC and FGFR1_FC. Our results demonstrating downregulation of CaSR and its significant associations with VDR and FGFR1 expression are in line with the immunohistochemical findings of Latus et al.,[22] who reported markedly reduced protein expression of CaSR and VDR in parathyroid glands of PHPT patients compared with controls, while FGFR expression remained unchanged. Interestingly, while Latus et al.[22] did not observe significant correlations of Klotho with serum calcium levels, our dataset revealed interrelationships between CaSR_FC, VDR_FC, and FGFR1_FC at the transcriptional level, suggesting that the molecular cross-talk between these pathways may manifest differently at the mRNA versus protein expression level. This may suggest the presence of a coordinated regulatory network involving calcium sensing, vitamin D signaling, and the FGF23/FGFR1/Klotho pathways in parathyroid adenomas. While the existing literature regarding FGFR1 and α-Klotho expression in PHPT is limited, the data presented indicate a possible interconnected cross-talk across these pathways, aligning with studies that highlight a reduction in CaSR, VDR, and FGFR1-Klotho receptors in chronic hyperplasia.[23] In another SHPT study, decreased α-Klotho and FGFR1 expression in hyperplastic parathyroids were related, inversely, to parathyroid volume,[11] supporting the observation of lower CaSR_FC in smaller adenomas and its interplay with FGFR1_FC. Additionally, low vitamin D status has been associated with increased adenoma weight and serum PTH levels in Turkish patients,[24] Similarly, our cohort demonstrated biochemical severity, featuring elevated levels of calcium and PTH, which corresponds with the national characteristics. The finding of a positive association between D3 and CaSR_FC is consistent with the literature, suggesting that active vitamin D promotes CaSR transcription through vitamin D response elements in the CaSR promoter.[25]

Interestingly, the significant positive correlation between serum calcium levels and PTH gene expression may appear paradoxical under normal physiological regulation, though it aligns well with the pathophysiological mechanisms underlying PHPT. Under physiological conditions, raised levels of extracellular calcium inhibit PTH transcription and secretion by activating the CaSR in parathyroid cells. However, in PHPT, CaSR expression is typically downregulated or functionally impaired. This disruption of the negative feedback mechanism permits the ongoing PTH synthesis, despite hypercalcemia.[4] The excessive production of PTH contributes to further bone resorption via osteoclast activation, thereby sustaining a cycle of hypercalcemia and hormonal dysregulation.[26] Therefore, the identified positive correlation possibly indicates the autonomous nature of adenomatous parathyroid tissue, which continues to express PTH mRNA despite elevated serum calcium levels. Lastly, the significantly higher serum calcium levels observed in male patients may represent a preliminary observation of potential gender-related variation in PHPT. Given the small cohort size, this finding should be interpreted with caution and considered exploratory until validated in larger studies.

Limitations

This study presents valuable insights into gene expression dynamics in PHPT patients; however, several limitations must be acknowledged. In light of these limitations, the interpretations presented herein should be regarded as exploratory and hypothesis-generating rather than definitive.

First, the sample size was limited to 20 parathyroid adenoma tissues, which may restrict the generalizability of the findings. Secondly, due to ethical restrictions, normal parathyroid tissues from healthy individuals could not be included as direct controls. While such samples are difficult to obtain, it is also important to recognize that even histologically "normal" parathyroid tissue adjacent to adenomas in PHPT patients or thyroid-related disease patients may not reflect truly unaffected physiology. Therefore, the use of such adjacent tissue as a control could yield misleading results. Instead, the average ΔCt values derived from the entire patient cohort for each gene were used as reference baselines for fold-change calculations. While this method allowed for internal normalization, it may obscure subtle expression variations that could be better highlighted through comparison with truly unaffected tissue. In addition, clinical parameters such as serum creatinine levels, detailed comorbidity data, and medication histories were not consistently available for all patients and were therefore not included. While none of the patients had known chronic renal insufficiency or major comorbidities, the absence of these parameters should be recognized as a limitation.

Lastly, this investigation represents the initial molecular profiling phase of a broader research project titled "Investigating efficacy of computationally repurposed drugs Cucurbitacin I, DG 041, IMD 0354 in in vitro PHPT Model regarding gene and protein pathways". This project is also a continuation of a previously published study.[27] Future studies will build upon this dataset by testing the effects of candidate drugs in functional in vitro PHPT models.

CONCLUSION

This study provides the first comprehensive qPCR-based profiling of PTH, CaSR, VDR, FGFR1, and α-Klotho expression, with a specific focus on PHPT patients from Turkiye. It contributes to the growing body of literature that represents distinct clinical and molecular features of PHPT. The findings highlight the downregulation of CaSR expression in smaller adenomas, suggesting that CaSR suppression may be an early event in parathyroid tumors. The observed correlations among CaSR, VDR, and FGFR1 gene expressions further point to a potentially coordinated regulatory network involving calcium, vitamin D, and FGF23/Klotho signaling pathways in parathyroid adenomas. Additionally, the paradoxical positive correlation between serum calcium and PTH gene expression supports the notion of impaired feedback inhibition attributed to CaSR dysfunction, a hallmark of PHPT pathophysiology. The results presented, along with the spotted gender-related differences in calcium levels and the observed molecular profiles, emphasize the

importance of incorporating both biochemical and gene expression parameters in the investigation of PHPT. Further research involving larger and more diverse populations is warranted to validate these findings and to explore their potential clinical implications, including early diagnosis and personalized treatment strategies.

ETHICAL DECLARATIONS

Ethics Committee Approval

Ethical approval was obtained from the University of Health Sciences İstanbul Training and Research Hospital Clinical Researches Ethics Committee (Date: 07.03.2025, Decision No: 52).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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