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Original

Relationship of calcium-sensing receptor gene polymorphism CASR rs 1042636, CASR rs 1802757, and cinacalcet response among Egyptian hemodialysis patients with secondary hyperparathyroidism

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Abstract

Introduction: Secondary hyperparathyroidism (SHPT) is a common complication associated with morbidity and mortality among hemodialysis (HD) patients.

Objectives: The current study aims to evaluate the frequency of CASR gene polymorphism variants related to parathyroid hormone (PTH) regulation (CASR rs1042636 and CASR rs1802757) and to test the hypothesis that single nucleotide polymorphisms (SNPs) in the CASR gene alter the response to cinacalcet among Egyptian HD patients with SHPT.

Patients and Methods: A case-control study that included 50 HD patients with intact parathyroid hormone (iPTH) ≥300 pg/mL treated with cinacalcet for a 6-month duration and 40 healthy volunteers as a control group. Eligible patients were recruited from Ain Shams university hospitals. Blood samples were collected from patients and controls to assess allele frequencies of CASR gene polymorphism variants using real-time polymerase chain reaction (PCR), corrected calcium (Ca), phosphorus (P), Ca×P product, iPTH level, and alkaline phosphatase before and after treatment. HD patients were categorized into two groups based on the reduction percentage; responders' patients (iPTH ≥20%) and non-responders to cinacalcet treatment.

Results: Of 50 HD patients, 48 (96%) carried the rs1042636 AA wild gene, while only two (4%) carried the rs1042636 AG mutant gene, 42 (87.5%) carried the rs1802757 CC wild genotype, and 6 (12.5%) carried the CT mutant genotype. The minor alleles T and G were (6.3% and 2%) respectively, with no statistically significant difference between the patient and control groups regarding the CASR genotypes or alleles distribution. There was no significant difference between responders and non-responder's patient groups regarding CASR genotypes or allele frequencies. Moreover, no significant correlation between CASR genotypes or alleles to delta change of Ca, P, Ca×P product, or PTH was seen. However, CASR rs1802757 CT mutant genotype was associated with a significant reduction in alkaline phosphatase levels after treatment.

Conclusion: There is no significant association between the gene polymorphism CASR rs1042636 or CASR rs1802757 and the reduction in PTH levels as a response to cinacalcet treatment among Egyptian HD patients with SHPT.

Keywords: CASR, Gene polymorphism, Cinacalcet, Hemodialysis, Secondary hyperparathyroidism

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Introduction

There is a significant increase in the worldwide prevalence of chronic kidney disease (CKD), where 13.4% (11.7-15.1%) of the general population are suffering; moreover, the end-stage kidney disease (ESKD), patients on renal replacement therapy, are ranging from 4.902 to 7.083 million (1). The severe complication known as chronic kidney disease-mineral bone disorder (CKD-MBD) is associated with the higher prevalence of secondary hyperparathyroidism (SHPT) in conjunction with CKD. This condition is accompanied by a higher risk of fracture, cardiomyopathy, anemia, pruritus, vascular calcification, and reduced quality of life. It also affects the overall morbidity and mortality burden in the dialytic ESKD population (2). The pathogenesis of SHPT in ESKD patients on dialysis is related to metabolic disorders of the active vitamin D (calcitriol), calcium (Ca), phosphate (P), and fibroblast growth factor 23. Calcium acts on the Ca-sensing receptor (CASR) of the parathyroid gland; thus, any abnormality of CASR or vitamin D receptor (VDR) is

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Implication for health policy/practice/research/ medical education

Cinacalcet is efficacious in lowering parathyroid hormone (PTH) levels in chronic kidney disease (CKD) patients on regular hemodialysis (HD) with secondary hyperparathyroidism (SHPT), which has been closely associated with morbidity and mortality among those patients, as reported by previous studies. The Evaluation of Cinacalcet HCl Therapy to Lower Cardiovascular Events (EVOLVE) trial demonstrated that some patients did not respond to cinacalcet treatment. Genetic variability could illustrate the altered response to cinacalcet treatment. Some recent studies have revealed that CASR gene single nucleotide polymorphism (SNP) CASR rs1042636 and CASR rs1802757 may be associated with cinacalcet response variability in CKD patients with SHPT. However, research on CASR gene polymorphism and cinacalcet drug response is limited, and results are disparate, particularly among Egyptian patients. The present study aims to evaluate the prevalence of variants of calcium-sensing receptor gene polymorphism related to PTH regulation CASR rs1042636 and CASR rs1802757 and to test the hypothesis that SNPs of CASR alter the response to the calcimimetic (cinacalcet) among Egyptian HD patients with SHPT. To our knowledge, this is the first pilot study to investigate calcium-sensing receptor gene polymorphism CASR rs1042636, CASR rs1802757, and cinacalcet response among Egyptian HD patients.

involved in the pathogenesis of SHPT in CKD (3).

The calcium receptor (CaR) belongs to the G protein-coupled transmembrane receptor superfamily; it is composed of 3 domains: an extracellular 612-amino acid (aa) ligand-binding portion, a membrane-spanning section that is hydrophobic (250 aa), and a cytosolic COOH-terminal tail (250 aa). CASRs are a dimeric family of C-class G-protein-coupled receptors, found on the surface of parathyroid cells, and involved in the regulation of parathyroid hormone (PTH) secretion, PTH gene expression as well as parathyroid cell proliferation (4). Thus, it is an essential player in bone and mineral metabolism by regulating PTH secretion, skeletal development, and urinary calcium excretion; CASR downregulation contributes to parathyroid hyperplasia, and the progression of SHPT. The human CASR gene is located on chromosome 3q13.3-21 (5).

Traditional treatment options include administration of 1, 25 dihydroxy cholecalciferol (calcitriol) or vitamin D receptor activator (VDRAs) analogs. However, VDRAs have calcemic and phosphatemic effects. Hyperphosphatemia also contributes to SHPT progression and must be managed by phosphate binders. The Calcimimetic cinacalcet is considered an alternative drug for SHPT treatment. Nowadays, combining VDRA and calcimimetics is the treatment of choice for SHPT, with a beneficial effect in reducing cardiovascular disease in ESKD patients. Parathyroidectomy also may be required in refractory hyperparathyroidism cases (6).

Cinacalcet is a calcimimetic agent used in treatment of SHPT in CKD. The calcimimetics enhance the CASR sensitivity, and lead to a conformational change in the CaR, thus, reducing the stimulation threshold by extracellular calcium, which consequently reduces PTH levels. Cinacalcet effectively reduces PTH, serum phosphate, and serum calcium levels in dialysis patients with SHPT. It also minimizes the risk of fractures, parathyroidectomy, and cardiovascular hospitalization (7,8). Some individuals did not respond to cinacalcet medication, as shown by the Evaluation of Cinacalcet HCl Therapy to Lower Cardiovascular Events (EVOLVE) experiment. Furthermore, Blacks with CKD showed higher PTH levels than other racial groups, according to earlier studies (9). Genetic variability could explain the altered response to cinacalcet treatment. CASR gene single nucleotide polymorphisms (SNPs), CASR rs1042636 and CASR rs1802757 have been linked to cinacalcet response variability in CKD patients with SHPT, according to some studies (10). Nevertheless, research on CASR gene polymorphism and cinacalcet drug response is still limited, and the results are disparate, especially among Egyptian patients.

Objectives

The current study aims to evaluate the frequency of variants of calcium-sensing receptor gene polymorphisms related to PTH regulation CASR rs1042636 and CASR rs1802757 and to test the hypothesis that SNPs of CASR alter the response to the calcimimetic (cinacalcet) among Egyptian hemodialysis (HD) patients with SHPT.

Patients and Methods

Study design

This case-control study included 50 prevalent HD patients and 40 healthy volunteers, matched for age and gender as a control group. Eligible clinically stable HD patients (Age >18 years old) were recruited from Ain Shams university hospital. Prevalent HD patients were maintained on adequate dialysis, three sessions per week for ≥6 months, using bicarbonate dialysate with a calcium concentration of 1.5 mmol/L delivered by central dialysis fluid delivery system in HD units, high flux dialyzer surface area, and heparin as an anticoagulant. HD patients with SHPT [intact parathyroid hormone (iPTH) ≥300 pg/mL] were treated with cinacalcet for six months' duration. Patients with active infections, chronic inflammatory disease, decompensated liver disease, malignancy, patients with a history of parathyroidectomy, or patients taking strong CYP3A4 inhibitors, such as azole antifungal agents, macrolide antibiotics, amiodarone, a tricyclic antidepressant, bupropion, calcitonin, bisphosphonate, steroid hormones, or benzodiazepine drugs were excluded from the study. Patients underwent detailed history taking and thorough clinical examination, demographic data on age, gender, body mass index, duration of HD, etiology of renal failure, comorbidities, history of cardiovascular disease, bone fractures, and medication. For 6 months, study patients were divided into responder and non-responder groups for cinacalcet treatment.

Laboratory tests

Serum total calcium (Ca), phosphorus (P), Ca×P product, iPTH level, alkaline phosphatase, albumin, creatinine, and urea were done using Roche/Hitachi Cobas® c501 system (Roche Diagnostics International Ltd., Switzerland); in addition to blood hemoglobin levels at the start and after six months of cinacalcet treatment.

Percentage of change (Δ%) = ($\frac{\text{pretreatment-post treatment}}{\text{pre-treatment}} \times 100$) was calculated to evaluate changes in serum PTH, alkaline phosphatase, total calcium, and phosphorus after cinacalcet treatment. Study HD patients were grouped into responders' patients whose iPTH values showed reduction during the period of 6-month cinacalcet treatment with a percentage of PTH reduction ≥ 20%, and non-responders were grouped as patients with PTH levels increased even after six months of cinacalcet treatment. The KDIGO recommended that PTH be maintained between two to nine times the upper limit of normal (130 to 600 pg/mL) for ESKD patients on dialysis. Consequently, we analyzed the results also according to the KDIGO PTH target, patients with (iPTH ≤600 pg/mL) post-cinacalcet treatment for a 6-month duration (responders) were compared to non-responders not maintained on KDIGO target (iPTH >600 pg/mL) post cinacalcet treatment in this study.

Blood samples were collected from study patients and control groups to assess the allele frequencies of variant CASR gene polymorphisms (CASR rs1042636 and CASR rs1802757) using real-time PCR. Genomic DNA was extracted from peripheral blood using a GeneJet whole-blood genomic DNA purification kit (Applied Biosystems, USA). The CASR genes rs1802757 (C/T) and rs1042636 (A/G) genotyping SNPs were done on a 7500 Fast System (Applied Biosystems, Foster City, CA, USA) thermal cycler according to the manufacturer's protocol, using TaqMan® genotyping master mix (Applied Biosystems, USA). In addition, the readymade genotyping assay kit supplied by Applied Biosystems, USA, includes sequence-specific forward and reverse primers and two fluorescent (VIC/FAM) labeled TaqMan probes for distinguishing between the two alleles for each SNP:

- Two TaqMan probes with minor groove binder for distinguishing between the two alleles of rs1802757 with context sequence: GGAGCCAGAGAGACAGACCGGGGTT (C/T) AAGCCATGGCTTCGTCATTTGCAAG Polymorphism: C/T, transversion substitution
- Two TaqMan probes with minor groove binder for distinguishing between the two alleles of rs1042636 with context sequence: GCCTCAGAAGAA CGCCATGGCCCAC (A/G) GGAATTCTACGCA CCAGAACTCCCT Polymorphism: A/G, transversion substitution

Statistical analysis

The IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp) was conducted for statistical analysis. We used numbers and percentages for qualitative data description. We also conducted the Kolmogorov-Smirnov test to verify the normality of distribution. Regarding the quantitative data, it is described using range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR). The results' significance was established at the 5% level. Comparing categorical variables between groups was performed by the chi-square test, and when more than 20% of the cells had an anticipated count of less than five, Fisher's exact correction for chi-square was applied. The Mann-Whitney U test was conducted to compare the two studied groups in case of abnormally distributed quantitative variables. For the comparison of two periods of normally distributed quantitative variables, we applied the paired t-test. In the case of comparing two periods with abnormally distributed quantitative variables, the Wilcoxon signed ranks test was applied. Regarding the correlation between two abnormally distributed quantitative variables, the Spearman's coefficient was employed. Univariate and multivariate logistic regression analyses were used to analyze the parameters affecting the responder group. The goodness of fit for Hardy-Weinberg equilibrium was tested using the chi-square test. Finally, a *P* value less than 0.05 was considered statistically significant.

Results

Characteristics of the study population

Our study included 50 patients suffering from ESRD and SHPT; they consisted of 35 males and 15 females whose age range was 22-74 years old, and the mean of their body mass index (BMI) was 26.55 kg/m^2. All the included cases are on adequate regular HD with a HD vintage of 8.12 ± 5.90 years, with a mean urea reduction ratio of 60.3 ± 13.09% and Kt/V of 1.34 ± 0.6.

The etiology of kidney disease was variable among the cases, where hypertensive glomerulosclerosis accounted for 11 (22.0%) cases, chronic glomerulonephritis four cases, 6 (12%) autosomal dominant polycystic kidney disease, 3 (6.0%) vesicoureteral reflux, 5 (10%) obstructive uropathy, 10 (20%) unknown etiology and 10 (20%) other causes. All the cases involved in the study took cinacalcet for six months using a mean dose of 40.80 ± 14 mg, while 74% were on oral calcium, calcium acetate, or carbonate, where the mean doses were 2024.3 ± 1068.3 mg/daily; at the same time, only eight cases (16%) used phosphate binders (sevelamer), and 52.0% of the cases were on alfacalcidol drug with a dose range of 1.51- 2.45 mg/wk. The studied cases had a variety of associated comorbidities; 20 were hypertensive, four were diabetic, and 37 had an evident history of cardiovascular disease; moreover, we found a history of bone fracture in 7 cases (14%).

Frequency and correlation analysis of genetic polymorphisms

We assessed the distribution of the two groups under study for the CASR gene SNPs, rs1042636 and rs1802757, as

well as their respective alleles. There were no statistically significant differences between the cases group and the healthy control group, as shown in Table 1. Furthermore, no statistically significant correlation was found between the investigated SNPs and any of the case traits, such as gender, age, vintage of HD, etiology of kidney disease, the existence of cardiovascular disease comorbidity, and laboratory results. Additionally, no significant correlation was found between the studied SNPs or alleles of the CASR gene and a known history of bone fracture.

Classification of the cases

We evaluated the response to cinacalcet using two methods; the percentage of change of iPTH and the KDIGO recommendations; accordingly, we subdivided the cases. Both calcifications, as well as the different responses, are discussed below.

Response according to the percentage of change

Using the percentage of change of iPTH, we distributed the studied cases into two subgroups, percentage of change responders and percentage of change non-responders. Percentage of change responders accounted for 21 (42%) of studied cases. It showed ≥20% reduction in iPTH after cinacalcet treatment, while 29 cases (58%) were percentage of change non-responders with a percentage of reduction <20% or even with increased iPTH level post-treatment.

Comparison between the percentage of change responders and percentage of change non-responders regarding the clinical or demographic data did not show any statistically significant difference (Tables 2 and 3). The measured laboratory parameters showed the same significance, except for the percentage of change reduction of alkaline phosphatase level ($P < 0.01$), as depicted in Table 3.

Statistical analysis revealed that baseline laboratory data, cinacalcet dose, and use of vitamin D did not correlate significantly with the percentage of reduction of iPTH as a response to cinacalcet ($P > 0.05$).

In the context of the studied CASR rs1042636 (A/G) and rs1802757(C/T) gene/SNP expressions; we listed their distribution in the responders and non-responders subgroups, as shown in Table 4. This comparison failed to show any statistically significant difference between both subgroups regarding CASR genotype expression and different alleles' expression.

Response according to the KDIGO recommendations

We redistributed the cases according to KIDGO recommendations. Thus, 9 cases (18%) whose iPTH ≤600 pg/mL post-cinacalcet treatment for a 6-month duration were KIDGO responders, while the rest of the remaining cases, 41 (82%), those with iPTH >600 pg/mL were KIDGO non-responders. Comparison between the latest two groups showed no statistically significant difference regarding demographics and baseline parameters, calcium supplementation dose, calcium supplementation form, phosphorus binders, and cinacalcet dose.

The distribution of studied SNPs among the KIDGO responders and KIDGO non-responders, along with their alleles, are shown in Table 5. Although minor alleles (T, G) and heterozygous mutant genotypes (CT, AG) were expressed more in KIDGO non-responders, there was no statistically significant difference between both groups regarding CASR genotype and different allele expression.

Relation between rs1042636 and rs1802757 with different parameters

We compared the different laboratory analyses as regards their basal levels, after treatment and the percentage of

Table 1. Comparison between Hemodialysis patients and the control group according to the frequency analysis of genetic polymorphisms CASR rs1042636 and CASR rs180275

Gene/SNP	HD patients group (n= 50)		Control (n= 40)		χ^2	*P* value[a]
	No.	%	No.	%		
rs1042636						
AA	48	96.0	36	90.0	1.286	0.400
AG	2	4.0	4	10.0		
GG	0	0	0	0		
Allele						
A	98	98.0	76	95.0	1.241	0.409
G	2	2.0	4	5.0		
	HWp1 = 0.885		HWp1 = 0.739			
rs1802757[a]						
CC	42	87.5	34	85.0	0.116	0.734
CT	6	12.5	6	15.0		
TT	0	0	0	0		
	HWp1 = 0.644		HWp1 = 0.608			
Allele						
C	90	93.8	74	92.5	0.107	0.743
T	6	6.3	6	7.5		

HWp: *P* value for chi-square for the goodness of fit for Hardy-Weinberg equilibrium. [a] rs1802757 assay: 2 patients were undetermined.
[a] Fisher's exact test.

Table 2. Comparison between responder and non-responder patient groups according to demographic data (n = 50)

Studied parameter	% Of reduction PTH				Test of Sig.	*P* value
	Responder (≥20%) (n = 21)		Non-responder (<20%) (n = 29)			
	No.	%	No.	%		
Gender						
Male	13	61.9	22	75.9	χ^2= 1.130	0.288
Female	8	38.1	7	24.1		
Age (y), Mean ± SD	50.0 ± 11.81		47.52 ± 14.08		t = 0.65	0.514
BMI (kg/m^2), Mean ± SD	28.19 ± 5.03		25.37 ± 5.39		t = 1.87	0.067
HD vintage (y), Mean ± SD	7.86 ± 6.68		8.31 ± 5.39		U = 252.50	0.664
Aetiology of kidney disease						
Obstructive uropathy	2	9.5	3	10.3	χ^2= 3.382	0.926[a]
Chronic glomerulonephritis	2	9.5	2	6.9		
Hypertensive glomerulosclerosis	5	23.8	6	20.7		
Diabetes	1	4.8	0	0.0		
Polycystic kidney disease	2	9.5	4	13.8		
Vesicoureteric reflux	2	9.5	1	3.4		
Unknown	3	14.3	7	24.1		
Others	4	19.0	6	20.7		
Hypertension	12	57.1	8	27.6	χ^2= 4.433	0. 35
Diabetes mellitus	2	9.5	2	6.9	χ^2= 0.114	1.000[b]
Cardiovascular disease	5	23.8	13	31.7	χ^2= 3.217	0.177
Use of calcium supplements	7	77.8	30	73.	χ^2= 1.230	0.775
Use of phosphorus binders	1	11.1	7	17.1	χ^2= 1.06	0.659
Use of vitamin D dose/week (mg)	14	66.7	12	41.4	χ^2= 3.120	0.077
Cinacalcet dose (mg/d), Mean ± SD	41.43 ± 14.93		40.34 ± 14.51		U= 293.50	0.795

SD, Standard deviation; U, Mann Whitney test; BMI, body mass index, PTH, parathyroid hormone
[a] Fisher Exact; [b] Monte Carlo.

change, among both studied SNPs (Table 6). We found that the percentage of change (reduction) of serum alkaline phosphatase (1.34 ± 6.33) in studied cases with rs1802757 (CT, heterozygous mutant genotype) was significantly higher than the percentage of change (reduction) of serum alkaline phosphatase in patients with (CC, wild genotype) expression (-0.23 ± 22.96, *P* = 0.017). There was also a statistically significant relationship regarding alkaline phosphatase between the expression of the minor allele (T) as a percentage of change (reduction) which was 18.57 ± 18.44 compared to patients who carry the C allele with a mean percentage of change (reduction) of alkaline phosphatase was -0.11 ± 21.74 (*P* = 0.023). This finding means that T substitution of the C allele is significantly associated with a better response to cinacalcet in terms of the percentage of change (reduction) of alkaline

Table 3. Comparison between responder and non-responder HD patients according to various parameters (n=50)

	Studied Parameter	% Of reduction PTH		Test of Sig.	*P* value
		Responder (n = 21)	Non-responder (n = 29)		
Corrected Ca (mg/dL)	Before treatment	8.63 ± 1.0	8.97 ± 1.06	t = 1.121	0.268
	After treatment	8.93 ± 0.51	8.90 ± 0.98	t = 0.136	0.893
	Percentage of change	-4.64 ± 12.43	0.20 ± 8.95	U = 260.50	0.387
Phosphorous (mg/dL)	Before treatment	5.35 ± 1.45	5.52 ± 2.02	t = 0.336	0.738
	After treatment	4.57 ± 1.14	5.37 ± 1.63	t = 1.919	0.061
	Percentage of change (reduction)	9.68 ± 26.73	-6.12 ± 44.50	U = 259.50	0.376
Ca×PO_4 product	Before treatment	46.12 ± 13.21	49.35 ± 18.64	U = 275.50	0.569
	After treatment	40.55 ± 10.22	47.86 ± 15.58	U = 219.0	0.093
	Percentage of change (reduction)	6.79 ± 27.52	-7.27 ± 53.18	U = 283.0	0.673
Alkaline phosphate	Before treatment	295.57 ± 228.96	376.52 ± 285.91	U = 290.50	0.783
	After treatment	299.0 ± 223	364.93 ± 289.71	U = 272.0	0.523
	Percentage of change (reduction)	-3.32 ± 19.04	3.84 ± 23.44	U = 168.0*	0.007*

Data are expressed as mean ± SD.
SD, Standard deviation; U, Mann Whitney test; PTH, parathyroid hormone
* Statistically significant at $P \leq 0.05$

Table 4. Comparison between responder and non-responder HD patients to cinacalcet treatment according to CASR rs1042636 (A/G) and rs1802757(C/T) gene /SNP expression

Gene/SNP	% Of reduction PTH				χ^2	*P* value[a]
	Responder (≥20%) (n = 21)		Non-responder (<20%)(n = 29)			
	No.	%	No.	%		
rs1042636						
AA	20	95.2	28	96.6	0.055	1.000
AG	1	4.8	1	3.4		
Allele						
A	41	97.6	57	98.3	0.054	1.000
G	1	2.4	1	1.7		
rs1802757						
CC	17	81.0	25	86.2	0.615	0.842[b]
CT	3	14.3	3	10.3		
Undetermined	1	4.8	1	3.4		
Allele						
C	37	92.5	53	94.6	0.183	0.691
T	3	7.5	3	5.4		

[a] Fisher Exact; [b] Monte Carlo.

Table 5. Comparison between KIDGO responders and non-responders regarding rs1042636 and rs1802757

Gene/SNP	PTH post-treatment				χ^2	*P* value[a]
	Responder (≤600) (n = 9)		Non-responder (>600) (n = 41)			
	No.	%	No.	%		
rs1042636						
AA	9	100.0	39	95.1	0.457	1.000
AG	0	0.0	2	4.9		
Allele						
A	18	100.0	80	97.6	0.448	1.000
G	0	0.0	2	2.4		
rs1802757						
CC	7	77.8	35	85.4	1.933	0.377[b]
CT	1	11.1	5	12.2		
Undetermined	1	11.1	1	2.4		
Allele						
C	15	93.8	75	93.8	0.0	1.000
T	1	6.3	5	6.3		

[a] Fisher Exact; [b] Monte Carlo.

phosphatase, and the presence of the C allele increases the risk of non-response reduction of alkaline phosphatase to treatment (Table 6). However, the mean percentage of change (reduction) of serum PTH in a patient with rs1802757 CT heterozygous mutant genotype was higher than patients with rs1802757 CC wild genotype which were (11.42 ± 42.88, 1.26 ± 44.03), respectively but there was no statistically significant difference ($P = 0.573$). The presence of a minor T allele was associated with a higher mean percentage of change PTH than patients carrying the C allele (11.42 ± 42.88 pg/mL, 1.94 ± 43.55 pg/mL, respectively; $P = 0.565$), with no statistical significance. In addition, no significant relationship was found between rs1802757 genotype CT or CC and the percentage of change regarding serum calcium, serum phosphate, and Ca×PO_4 product (Table 6).

Statistical analysis revealed no significant relationship between the presence of AA (wild genotype), AG heterozygous mutant genotype of the CASR gene rs1042636 or alleles (A, G) and the mean percentage of change of iPTH, corrected calcium, phosphate, or alkaline phosphatase after cinacalcet treatment over the 6-month duration of the study ($P > 0.05$) among studied HD patients (Table 6). In the current study, univariate and multivariate logistic regression analysis revealed a non-significant correlation between the CASR gene SNPs, rs1042636 and rs1802757, expression in response to cinacalcet treatment.

Discussion

In the current study, we tested the hypothesis that SNPs in the CASR rs1042636 and rs1802757alter the response to calcimimetic cinacalcet treatment in Egyptian ESKD patients on regular HD with SHPT in actual clinical practice. The frequencies of CASR gene SNPs were assessed in 90 participants, of whom 50 were HD patients, and 40 were healthy volunteers.

We used the percentage of reduction of PTH to classify the studied patients. Our results showed that 58%

Table 6. Relation between rs1042636 and rs1802757 with different parameters (n = 50)

	Studied parameter	rs1042636		rs1802757	
		AA (n = 48)	AG (n = 2)	CC (n = 42)	CT (n = 6)
Corrected Ca (mg/dl)	Before treatment, Mean ± SD	8.81 ± 1.04	9.20 ± 1.27	8.80 ± 1.08	9.12 ± 0.80
	t (*P*)	0.515 (0.609)		0.685 (0.497)	
	After treatment, Mean ± SD	8.91 ± 0.81	9.05 ± 1.20	8.87 ± 0.79	9.12 ± 1.08
	t (*P*)	0.237 (0.813)		0.704 (0.485)	
	Percentage of change, Mean ± SD	-1.98±10.91	1.59±0.55	-1.71±11.33	0.11±4.51
	U (*P*)	40.0 (0.720)		120.0 (0.867)	
Phosphorous (mg/dLl)	Before treatment, Mean ± SD	5.49 ± 1.81	4.40 ± 0.85	5.40 ± 1.89	5.70 ± 1.11
	t (*P*)	0.844 (0.403)		0.383 (0.703)	
	After treatment, Mean ± SD	5.04 ± 1.48	4.90 ± 2.12	4.90 ± 1.46	5.83 ± 0.77
	t (*P*)	0.127 (0.899)		1.525 (0.134)	
	Percentage of change (reduction), Mean ± SD	0.90±39.10	-8.74±27.24	0.55±41.54	-3.61±9.17
	U (*P*)	36.500 (0.589)		87.500 (0.237)	
$Ca \times PO_4$ product	Before treatment, Mean ± SD	48.29±16.67	41.02±13.41	47.36±17.30	51.76±10.17
	U (*P*)	37.0 (0.620)		102.0 (0.474)	
	After treatment, Mean ± SD	44.75±13.76	45.62±25.09	43.41±13.42	52.44±8.88
	U (*P*)	46.0 (0.940)		68.0 (0.073)	
	Percentage of change, Mean ± SD	-1.13±45.20	-6.93±26.21	-1.57±48.25	2.03±7.78
	U (*P*)	39.0 (0.686)		95.0 (0.351)	
PTH (pg/mL)	Before treatment, Mean ± SD	1417±844.12	934±25.46	1428±878.40	1412.67±510.58
	U (*P*)	30.0 (0.418)		118.0 (0.820)	
	After treatment, Mean ± SD	273.4±738.95	882.00±236.17	1291.45±751.16	177.67±446.83
	U (*P*)	33.0 (0.500)		124.0 (0.964)	
	Percentage of change (reduction), Mean ± SD	3.12±44.00	5.19±27.87	1.26±44.03	11.42±42.88
	U (*P*)	43.0 (0.826)		107.0 (0.573)	
Alkaline phosphate	Before treatment, Mean ± SD	346.40±268.51	249.50±102.53	350±272.19	327.83±258.54
	U (*P*)	35.0 (0.558)		103.0 (0.493)	
	After treatment, Mean ± SD	340.50±268.02	259.00±93.34	346.62±270	322.50±253.75
	U (*P*)	35.0 (0.558)		92.500 (0.305)	
	Percentage of change (reduction), Mean ± SD	1.07±22.19	-4.99±5.73	-0.23±22.96	1.34±6.33
	U (*P*)	42.0 (0.790)		51.0* (0.017*)	

SD: Standard Deviation, t: Student t-test, U: Mann Whitney test, * Statistically significant at $P \leq 0.05$.

(29/50) of our patients were non-responders, while 42% (21/50) were responders. When we used the KIDGO recommendations, nine out of 50 patients (18%) were KIDGO responders, and the remaining 41 patients (82%) were KIDGO non-responders.

These results are in contrast with the study done by Kim et al, which showed that 82.5% were responder patients and 17.5% were non-responder patients (10), as well as Kuczera et al study where 72% were responder patients and 28% were non-responder patients (6), and Jeong et al who showed a similar rate of response recording 78% (53/68) (11). We may explain this discrepancy in response rate by some factors associated with drug resistance, delayed therapy, persistent hyperphosphatemia (12), and reduced expression of CaSR and VDR (13,14).

Regarding the SNP rs1042636, the frequency of genotypes AA, AG, and GG were 96%, 4%, and 0%, respectively, in the patient's group. On the contrary, Ngamkam et al study in Thailand found that the most common genotype was AG genotype at 48.1% (65/135), while AA and GG genotypes were observed in 23.7% (32/135) and 28.1% (38/135) patients, respectively. They identified 103 patients (76.3%) as G carriers and 32 (23.7%) as non-carriers (15).

We found that the allele frequency was 98% for A and 2% for G alleles. The G allele frequency was much less than the frequency observed by both Ngamkam et al, who reported a frequency of 47.8% and 52.2% for A and G, respectively (15), and Jeong et al, who concluded a frequency of 45.8% for the G allele (11).

Regarding the SNP rs1802757, frequencies of genotypes CC, CT, and TT in the patient's group were 87.5%, 12.5%, and 0%, respectively. Allele frequency showed a similar result in which C and T alleles were 93.8% and 6.2%, respectively. The T allele frequency was much less than that observed by Jeong et al, who recorded a frequency of 35.8% (11).

We evaluated the relationship between the response to

cinacalcet and SNPs rs1042636 and rs1802757. We did not detect any statistically significant difference between both cinacalcet responders and non-responders' subgroups with CASR genotypes expression and different alleles expression. However, a significant difference was found in the percentage of change reduction of alkaline phosphate levels between CT and CC genotypes of rs1802757, being higher in CT genotype (1.34 ± 6.33) versus (0.23 ± 22.9; $P = 0.017$) in CC genotype.

On the other hand, The A to G replacement of rs1042636, reduced the risk of nonresponse by 93%, whereas the C to T substitution of rs1802757 raised the risk of nonresponse by about eight times, according to Jeong et al. Patients with the T allele of rs1802757 may therefore be candidates for parathyroidectomy due to their increased likelihood of cinacalcet therapy failure (11).

Jeong et al presented these findings in addition to the earlier research conducted by Rothe et al. According to this study, an Asian dialysis patient with a homozygous (G/G) genotype of CASR rs1042636 showed a significant drop in iPTH following a 2-month course of cinacalcet medication, in contrast to six other patients with distinct genotypes (16). Moreover, Yano and colleagues studied CASR rs1042636 in 122 Japanese HD patients and found that the PTH level was significantly higher in the A/A group than in the G/G group (17). However, the rs1802757 located in 3′ untranslated region has not been well documented.

We compared the percentage of change of several laboratory data between responders and non-responders' patients and found no significant reduction between before and after treatment levels in adjusted calcium, phosphorous, and $Ca \times PO_4$ products except for alkaline phosphatase. This statistically significant reduction of alkaline phosphatase agreed with Bucharles et al (18) and Belozeroff et al (1) studies who observed in their studies on dialysis patients with SHPT a significant decrease in alkaline phosphatase at the end of cinacalcet treatment.

Conclusion

There is no significant association between calcium-sensing receptor gene polymorphism CASR rs1042636 or CASR rs1802757 and reduction of PTH levels in response to cinacalcet treatment among Egyptian HD patients with SHPT. The mutated CASR rs1802757 CT genotype may be associated with a significant decrease in serum alkaline phosphate levels by cinacalcet treatment for further investigations.

Limitations of the study

In a pilot case-control study, data were collected retrospectively, with a relatively small sample size as no fund was received.

Authors' contribution

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Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research conducted in this study adhered to the principles outlined in the Declaration of Helsinki and all procedures performed in the study were by the ethical standards of the Ain-Shams University Hospital research committee (Ethics committee reference number 000017585). All participants provided the consent form before the study. Additionally, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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