Pharmacogenetic analysis of cinacalcet response in secondary hyperparathyroidism patients

Background: Secondary hyperparathyroidism (SHPT) is one of the major risk factors of morbidity and mortality in end-stage renal disease. Cinacalcet effectively controls SHPT without causing hypercalcemia and hyperphosphatemia. However, there is significant inter-individual response variance to cinacalcet treatment. Therefore, we aimed to evaluate the genetic effects related with parathyroid hormone regulation as factors for cinacalcet response variance.

Methods: Patients with a diagnosis of SHPT based on intact parathyroid hormone (iPTH) >300 pg/mL on dialysis were included in this study. They were over 18 years and have been treated by cinacalcet for more than 3 months. Responders and nonresponders were grouped by the serum iPTH changes. Twenty-four single nucleotide polymorphisms of CASR, VDR, FGFR1, KL, ALPL, RGS14, NR4A2, and PTHLH genes were selected for the pharmacogenetic analysis.

Results: After adjusting for age, sex, and calcium level, CASR rs1042636 (odds ratio [OR]: 0.066, P=0.027) and rs1802757 (OR: 10.532, P=0.042) were associated with cinacalcet response. The association of haplotypes of CASR rs1042636, rs10190, and rs1802757; GCC (OR: 0.355, P=0.015); and ATT (OR: 2.769, P=0.014) with cinacalcet response was also significant.

Conclusion: We obtained supporting information of the associations between cinacalcet response and CASR polymorphisms. CASR single nucleotide polymorphisms (SNPs) rs1802757, rs1042636, and haplotypes of rs1042636, rs10190, and rs1802757 were significantly associated with cinacalcet response variance.

Keywords: CASR, calcium sensing receptor, SHPT, genetic polymorphisms, haplotype, single nucleotide polymorphisms

Introduction

Dysregulation of mineral homeostasis due to failing kidney function leads to decreased renal phosphate excretion, elevated fibroblast growth factor 23 level, and reduced synthesis of calcitriol. These changes lead to consistent secretion of parathyroid hormone (PTH) contributing to the development of parathyroid hyperplasia and secondary hyperparathyroidism (SHPT) in end-stage renal disease. SHPT with elevated calcium and phosphate levels contributes to the development of renal osteodystrophy, erythrocytosis, resistance, vascular calcification, and left ventricular hypertrophy. These clinical features are strongly associated with increased morbidity and mortality in dialytic end-stage renal disease population.

It is therefore critical to maintain optimal level of PTH in these patients. However, because of the complicated problems of lowering PTH with simultaneously controlling imbalanced serum levels of calcium and phosphate, traditional therapies of phosphate binders and vitamin D analogues for managing SHPT have all too often led to clinical problems, including hypercalcemia.
hyperparathyroidism, and refractory hyperparathyroidism or over-suppression of PTH.5

One of the newer treatment options for SHPT is cinacalcet which increases the sensitivity of the calcium-sensing receptor (CaSR) on the parathyroid gland cells to extracellular calcium,6 resulting in a decreased calcium set point and circulating PTH levels.6–9 The cinacalcet treatment resulted in 41% of SHPT patients achieving PTH and Ca × P levels recommended by the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines6 whereas fewer than 10% of the patients treated with phosphate binders and vitamin D analogs reached optimal control.10 Moreover, cinacalcet reduced the risk of parathyroidectomy by 91%.11 Cinacalcet treatment is effective to the extent that it can be utilized as an alternative to surgical intervention in some patients.12,13 Despite the overall response to cinacalcet being promising in a number of clinical trials,14–16 the therapeutic efficacy has a significant inter-individual variability of 12.1%–46% PTH reduction, representing drug resistance.17–21 The factors associated with the drug resistance include delayed therapy, persistent hyperphosphatemia, nodular hyperplasia,22 and reduced expression of CaSR and vitamin D receptor (VDR).23–25 In particular, genetic polymorphisms affecting CaSR and VDR expression have been considered as factors for drug resistance. The genetic polymorphism of CASR Arg990Gly (c.2968A > G, rs1042636) was associated with different cinacalcet response.26 Several other studies27–29 on CASR presented the possible association of CASR rs1042636 and rs2221266 polymorphisms with PTH level difference. VDR gene polymorphisms of rs7975232 (Apa I), rs731236 (Taq I), and rs1544410 (Bsm I) were also associated with PTH regulation.30–32 These studies presented the clinical importance of genetic polymorphisms in PTH regulation. However, the results available so far show limited evidence for genetic polymorphisms associated with cinacalcet response involving a small number of patients and not including phosphate and bone regulation-related genes in PTH homeostasis. Thus, we aimed to evaluate the frequency of variants of study genes related to PTH regulation and the association between SNPs and haplotypes of genes and cinacalcet response in Korean SHPT patients on dialysis.

Methods

Study subjects

Study patients were selected from a tertiary university hospital in Seoul, Korea, between June 2011 and July 2014. Patients with a diagnosis of SHPT based on intact parathyroid hormone (iPTH) >300 pg/mL were included in this study. The patients were over 18 years and have been treated by cinacalcet for more than 3 months. All these patients were on either hemodialysis or peritoneal dialysis, receiving optimal medical therapy in the form of dietary restriction, phosphate binders, or vitamin D sterols. The main exclusion criteria were serious concomitant hepatic disease, active cancers, or taking strong CYP3A4 inhibitors, such as itraconazole, clarithromycin, diltiazem, and verapamil.

The study complies with the Helsinki Declaration and was approved by the Ethics Committee of the Seoul National University Hospital (IRB #: H-1408-082-604). Written informed consents were obtained from all patients.

Data collection

Demographic information on the cause of chronic kidney disease (CKD), mode and duration of dialysis, concomitant drugs, and biochemical parameter levels of serum calcium, phosphorus, alkaline phosphatase, iPTH, creatinine, estimated glomerular filtration rate using Modification of Diet in Renal Disease equation, albumin, and hemoglobin at the start and during 3 months of cinacalcet treatment was obtained. Responders and nonresponders were grouped by their serum iPTH changes. Nonresponders were grouped as the patients whose iPTH levels increased even after 3 months of cinacalcet treatment.33,34 Otherwise, those patients whose iPTH values showed any reduction during the period of 3-month cinacalcet treatment were defined as responders.

Serum calcium and phosphate levels were measured by standard methods; iPTH was measured using a double-antibody immunoradiometric assay for the quantitative determination of intact biological chain of 84 amino acids of PTH in human serum (ELSA PTH, Cisbio Bioassays, Codolet, France). Serum calcium was reported as albumin corrected values throughout this research.

Genotype selection and analysis

Twenty-five SNPs in nine genes which are previously known as related to calcium, phosphate, and bone metabolism were selected from the results of previous literatures and databases. Of these genes, CASR26–29,35–37 and VDR38–40,43,45 involved in calcium regulation; FGFR1,46,47 KL,42 RGSI4,43,44 and SLC34A143 related to phosphate serum concentration; and ALPL40,46 NR4A248,49 and PTHLP40,53 related to bone formation were evaluated. Peripheral blood samples were collected in ethylenediaminetetraacetic acid (EDTA) containing tubes and stored at −80°C before DNA isolation. The SNaPshot assay was performed according to the manufacturer’s instructions (ABI PRISM SNaPshot Multiplex kit, Thermo Fischer

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Statistical analysis

The Hardy–Weinberg Equilibrium of each SNP was tested using the goodness-of-fit chi-square test to compare the expected frequencies of genotypes in controls; SNPs with \( P > 0.05 \) were considered to be in Hardy–Weinberg Equilibrium. The independent-samples \( t \)-test and Mann–Whitney test were used to determine the differences between parametric and nonparametric characteristics of patients and biochemical parameter changes. The chi-square or Fisher’s exact test was performed to assess the difference between categorical variables of the patient characteristics. Unconditional logistic regression analysis and chi-square or Fisher’s exact test were used to evaluate the frequency analysis of polymorphisms of the genes and the relationships between different genotypes and cinacalcet response. Statistical analysis was carried out using IBM SPSS Statistics 21.0 for Windows (SPSS Inc., IBM Corporation, Armonk, NY, USA). Haplotypes and haplotype frequencies were calculated using Haplovew software (version 4.2, Massachusetts Institute of Technology, Cambridge, MA, USA). The haplotype with \( P < 0.05 \) was considered statistically significant.

Results

Patient data

A total of 70 dialysis patients with SHPT were included in the biochemical parameter analysis. Baseline characteristics were well balanced between the responders and nonresponders except for baseline calcium levels (\( P = 0.001 \)). The baseline iPTH for 68 patients was 622 (300–1,493) pg/mL versus 601 (316–1,183) pg/mL (\( P = 0.375 \)) for responders versus nonresponders, respectively. The parathyroid gland hyperplasia or adenomatous state did not differ between two groups (\( P = 0.726 \)). The amount of vitamin D (calcitriol or paricalcitol) taken by the patients (\( P = 0.116 \)) and the number of patients on phosphate binders and vitamin D analogues were not different between the two groups (\( P = 0.116 \) and \( P = 1.000 \), respectively) (Table 1). The maintenance dose of cinacalcet ranged from 12.5 to 100 mg per day, depending on patient’s status. The dose was higher in nonresponders: responders 25 (12.5–75) mg/day versus nonresponders 50 (25–100) mg/day (\( P < 0.001 \)).

Biochemical parameter changes

The biochemical parameter changes between baseline and 3-month treatment are summarized in Table 2. Cinacalcet significantly reduced serum iPTH levels in 53 responders. The percent iPTH changes between baseline and 3-month treatment were \(-42.29 (-97.41 \text{ to } 9.23) \% \) versus 6.16 (0.90 to 45.25) \% in responders versus nonresponders, respectively (\( P < 0.001 \)). At 3-month treatment, 39.6\% (21/53) of responders had an iPTH level below 300 pg/mL, the level recommended by the K/DOQI guidelines.9 The percent change of serum calcium concentration at 3-month treatment was \(-5.75 (-25.67 \text{ to } 15.81) \% \) in responders compared to \(-7.13 (-19.09 \text{ to } 1.67) \% \) in nonresponders (\( P = 0.182 \)). The percent change of serum phosphorus reduction in responders was \(-9.84 (-69.23 \text{ to } 69.43) \% \) compared to \(-8.42 (-33.33 \text{ to } 72.43) \% \) in nonresponders (\( P = 0.170 \)).

Frequency analysis of genetic polymorphisms

The loci, allele, and minor allele frequency (MAF) results are summarized in Table 3. The observed MAFs in \( CASR \) rs1042636 and rs1802757 were 45.8\% and 35.8\%, respectively. \( VDR \) rs7975232 (Apa I) and rs1544410 (bsm I) were 16.0\% and 6.3\%, respectively, whereas rs2228570 (Fok I) was 38.2\%.

Association with cinacalcet response

Association of 24 SNPs with iPTH

The association of 24 SNPs with cinacalcet response was evaluated (Table 4). The chi-square test showed that \( CASR \) rs1042636 had a significant difference in genotype frequencies between responder and nonresponders (\( P = 0.036 \)) but the MAF was not significant (\( P = 0.075 \)). The chi-square test also proved that \( CASR \) rs1042636 had a significant association with cinacalcet response (odds ratio [OR]: 0.267, \( P = 0.035 \)) in the dominant genetic model (Table 5). Unconditional logistic regression analysis showed that \( CASR \) rs1042636 (OR: 0.074, \( P = 0.025 \)) (95\% confidence interval [CI]: 0.008–0.721) and rs1802757 (OR: 0.116, \( P = 0.042 \)) (95\% CI: 1.077–69.075) had an association with the cinacalcet response in the genotype model, indicating that the A to G substitution of rs1042636 decreased the risk of nonresponse by 93\%, whereas the C to T substitution of rs1802757 increased the risk of nonresponse by approximately eight times. After adjusting for age, sex, and baseline calcium level, \( CASR \) rs1042636 (OR: 0.066, \( P = 0.027 \)) and rs1802757 (OR: 10.532, \( P = 0.042 \)) were associated with cinacalcet response.
**Table 1** Demographic characteristics at baseline for included subjects (n=70)

| Characteristics                      | Total (N=70)* (%) | Grouped by cinacalcet response (N=68)** (%) | P-value |
|--------------------------------------|-------------------|---------------------------------------------|---------|
|                                      | Responders (n=53) | Nonresponders (n=15)                       |         |
| Male sex, n (%)                      | 32 (45.7)         | 27 (45.9)                                   | 4 (26.7) | 0.096 |
| Age, years, median (range)           | 50 (21–75)        | 49 (21–75)                                  | 52 (24–71) | 0.325 |
| BMI, kg/cm², median (range)          | 22.37 (16.01–31.45) | 22.08 (16.01–31.00) | 23.29 (17.76–27.38) | 0.206 |
| Cause of CKD, n (%)                  |                   |                                             |         |
| DM                                   | 12 (17.2)         | 7 (13.2)                                    | 4 (26.7) | 0.680 |
| HTN                                  | 10 (14.3)         | 7 (13.2)                                    | 2 (13.3) | 1.000 |
| GN                                   | 28 (40.0)         | 22 (41.5)                                   | 6 (40.0) | 0.916 |
| Others                               | 20 (28.6)         | 17 (32.1)                                   | 3 (20.0) | 0.762 |
| Mode of dialysis, n (%)              |                   |                                             |         |
| HD                                   | 19 (27.1)         | 11 (20.8)                                   | 7 (46.7) | 0.093 |
| PD                                   | 40 (57.1)         | 33 (62.3)                                   | 6 (40.0) | 0.124 |
| Both                                 | 11 (15.7)         | 9 (17.0)                                    | 2 (13.3) | 1.000 |
| Dialysis duration, median (range)    | 97 (38–195)       | 77.5 (15–300)                               | 97 (38–195) | 0.363 |
| Use of phosphate binder/calcium supplement, n (%) | 62 (88.6) | 48 (90.6) | 14 (93.3) | 1.000 |
| Lab values, median (range)           |                   |                                             |         |
| iPTh (pg/mL)*                        | 620 (300–1,493)   | 622 (300–1,493)                             | 601 (316–1,183) | 0.375 |
| Albumin corrected calcium (mg/dL)    | 9.84 (7.4–13.52)  | 9.72 (7.40–13.52)                           | 10.50 (9.18–11.88) | 0.001 |
| Phosphorus (mg/dL)                   | 5.85 (3.40–12.80) | 5.70 (3.50–12.80)                           | 6.00 (3.40–7.50) | 0.673 |
| Ca × P (mg/dL²)                      | 58.35 (32.41–94.72) | 57.01 (32.41–94.72) | 61.80 (37.38–71.10) | 0.787 |
| ALP (IU/L)                           | 106 (41–393)      | 100 (41–381)                                | 124 (61–762) | 0.252 |
| Hb (g/dL)                            | 10.50 (7.00–14.60)| 10.70 (7.00–14.00)                         | 10.10 (7.70–14.60) | 0.107 |
| Albumin (g/dL)                       | 3.80 (2.40–11.50) | 3.80 (2.40–5.10)                            | 3.90 (3.10–4.40) | 0.953 |
| Serum creatinine (mg/dL)             | 11.69 (4.30–20.66)| 12.03 (4.30–20.66)                        | 10.85 (4.35–18.12) | 0.248 |
| GFR (ml/min/1.73 m²)                 | 3.80 (2.10–15.7)  | 3.80 (2.50–13.30)                           | 3.80 (2.10–15.70) | 0.842 |
| Comorbidities related with CKD-MBD, n (%) | 6 (8.6)         | 5 (9.4)                                    | 1 (6.7) | 1.000 |
| Osteoporosis                         | 6 (8.6)           | 5 (9.4)                                    | 1 (6.7) | 1.000 |
| Osteopenia                           | 22 (31.4)         | 18 (34.0)                                  | 3 (20.0) | 0.361 |
| Fracture                             | 11 (15.7)         | 7 (13.2)                                   | 4 (26.7) | 0.243 |
| PTH hyperplasia or adenoma          | 15 (21.4)         | 12 (22.6)                                  | 3 (20.0) | 0.726 |
| HTN                                  | 51 (72.9)         | 39 (73.6)                                  | 10 (66.7) | 0.745 |
| IHD, HF, AF, LVH                     | 25 (35.7)         | 16 (30.2)                                  | 8 (53.3) | 0.666 |
| Comorbidity related with neoplasm    | 13 (18.6)         | 7 (13.2)                                   | 5 (33.3) | 0.442 |

Notes: *A total of 70 patients were included in the final analysis. **A total of 68 patients who had available iPTh level were included in the cinacalcet response analysis. Significant results are marked in bold.

Abbreviations: AF, atrial fibrillation; ALP, alkaline phosphatase; BMI, body mass index; CKD, chronic kidney disease; CKD-MBD, chronic kidney disease-mineral bone disease; DM, diabetes mellitus; GFR, glomerular filtration rate; Hb, hemoglobin; HF, heart failure; HTN, hypertension; GN, glomerulonephritis; HD, hemodialysis; IHD, ischemic heart disease; iPTh, intact parathyroid hormone; LVH, left ventricular hypertrophy; PD, peritoneal dialysis; PTh, parathyroid hormone.

**Association of CASR haplotypes with iPTh**

The distribution of haplotypes was constructed for three genes and assessed for the association with the cinacalcet response. Linkage disequilibrium structures were designated by the $D'$ value shown in Figure 1. Each of CASR, VDR, and ALPL genes included one haplotype block. The correlation coefficient ($r^2$) between rs1042636 and rs1802757, rs1042636 and rs1802757, and rs10190 was significant.

**Table 2** Comparison of biochemical parameter percent changes between baseline and 3-month treatment with cinacalcet (n=68)

| Parameter                           | Responders Median (range) (%) | Nonresponders Median (range) (%) | P-value* |
|-------------------------------------|-------------------------------|----------------------------------|---------|
| ΔiPTh (pg/mL)                       | −42.29 (−97.41–[−9.23])       | 6.16 (0.90–45.25)                | <0.001  |
| ΔCalcium (mg/dL)                    | −5.75 (−25.67–15.81)          | −7.13 (−19.09–1.67)              | 0.182   |
| ΔPhosphorus (mg/dL)                 | −9.84 (−69.23–69.43)          | −8.42 (−33.33–72.43)             | 0.170   |
| ΔCa × P (mg/dL²)                    | −14.70 (−71.13–68.70)         | −8.84 (−36.99–43.70)             | 0.296   |
| ΔALP (IU/L)                         | 1.60 (−28.48–146.58)          | 10.71 (−29.26–93.89)             | 0.211   |

Notes: The 3-month treatment value for biochemical parameters is defined as the mean value during baseline to 3-month treatment. *Descriptive $P$-values were obtained from the independent-samples t-test or Mann–Whitney U-test. Significant results are marked in bold.

Abbreviations: ALP, alkaline phosphatase; PTh, parathyroid hormone.
Table 3 The loci and minor allele frequency (MAF) information of the 24 SNPs in CASR, VDR, FGFR1, KL, RGS14, SLC34A1, ALPL, NR4A2, and PTHLH genes

| Gene/SNP | Chromosome position | Alleles | SNP location | Observed MAF/expected MAF | HWE test (P-value) |
|----------|---------------------|---------|--------------|---------------------------|-------------------|
| CASR     | rs1042636           | 3:122284922 | A/G | Exon | 0.458/0.2065 | 0.59 |
|          | rs2221266           | 3:122277969 | C/T | Intron | 0.410/0.4619 | 0.97 |
|          | rs10190             | 3:122283689 | C/T | 3′UTR | 0.387/0.4669 | 0.81 |
|          | rs1802757           | 3:122286284 | C/T | 3′UTR | 0.359/0.1983 | 0.43 |
| VDR      | rs7975232           | 12:47845054 | A/C | Intron | 0.160/0.4846 | 0.87 |
|          | rs2228570           | 12:47879112 | C/T | Exon | 0.382/0.3285 | 0.80 |
|          | rs1544410           | 12:47846052 | A/G | Intron | 0.063/0.2959 | 0.57 |
|          | rs1802757           | 12:47844285 | C/T | 3′UTR | 0.099/0.0385 | 0.08 |
|          | rs739837            | 12:47844438 | C/A | 3′UTR | 0.176/0.4942 | 0.67 |
|          | rs9729              | 12:47842840 | C/A | 3′UTR | 0.169/0.4902 | 0.71 |
| FGFR1    | rs13317             | 8:38411996 | C/T | 3′UTR | 0.345/0.2250 | 0.06 |
| KL       | rs1207568           | 13:33016046 | C/T | Exon | 0.160/0.1601 | 0.11 |
| RGS14    | rs4074995           | 5:17730742 | A/G | Intron | 0.236/0.1895 | 0.20 |
| SLC34A1* | rs3812035           | 5:177390142 | A/C | 3′UTR | 0.255/0.387  | 0.01 |
| ALPL     | rs1697421           | 1:21496799 | A/G | Exon | 0.458/0.3776 | 0.50 |
|          | rs3200254           | 1:21568242 | C/T | Exon | 0.424/0.2670 | 0.66 |
|          | rs2242420           | 1:21578036 | C/T | 3′UTR | 0.155/0.1172 | 0.47 |
|          | rs2242421           | 1:21578081 | A/G | 3′UTR | 0.345/0.2220 | 0.46 |
|          | rs1697405           | 1:21577713 | G/A | 3′UTR | 0.268/0.3367 | 0.43 |
|          | rs1697406           | 1:21577774 | A/G | 3′UTR | 0.148/0.1318 | 0.49 |
| NR4A2    | rs12080            | 2:15629935 | G/A | 3′UTR | 0.2863/0.169  | 0.45 |
| PTHLH    | rs2796             | 12:27963178 | A/G | 3′UTR | 0.127/0.0727 | 0.76 |
|          | rs6253             | 12:27963008 | A/G | 3′UTR | 0.394/0.3243 | 0.09 |
|          | rs6245             | 12:27963124 | A/G | 3′UTR | 0.085/0.0629 | 0.40 |

Note: *Hardy–Weinberg Equilibrium (HWE) test was not satisfied and SLC34A1 rs3812035 excluded for our analysis. Significant results are marked in bold.

Abbreviations: UTR, untranslated region; SNP, single nucleotide polymorphism; MAF, minor allele frequency.

Discussion

In this study, the genotype frequencies of the SNPs related to PTH regulation and the association with cinacalcet response were evaluated. The observed MAFs in CASR rs1042636 and rs1802757 were double the reported frequency in HapMap whereas VDR rs7975232 (Apa I) and rs1544410 (bsm I) showed a lower frequency than that of HapMap. The rs2228570 (Fok 1) showed a similar frequency. Furthermore, the novel findings of our study are that genetic polymorphisms of CASR rs1802757 besides rs1042636 and haplotypes of rs1042636, rs10190, and rs1802757 were significantly associated with cinacalcet response. The gain of function nonsynonymous SNP of rs1042636, which is
Table 4 Comparisons of genotypes and allele frequencies between responders versus nonresponders group

| Gene/SNP      | Genotype frequency (%) | Minor allele frequency (%) |
|---------------|------------------------|---------------------------|
|               | Responders (n=53) | Nonresponders (n=15) | P-value | Responders (n=53) | Nonresponders (n=15) | P-value |
|               | AA/AB/BB | AA/AB/BB | B | B |
| CASR          |           |           |           |           |           |           |
| rs1042636 (A/G) | 15.1/50.9/34.0 | 40/53.3/6.7 | 0.036 | 59.4 | 33.3 | 0.075 |
| rs2221266 (C/T) | 41.5/45.3/13.2 | 13.3/53.3/33.3 | 0.086 | 35.8 | 60.0 | 0.111 |
| rs101909 (C/T) | 42.3/44.2/13.5 | 15.4/61.5/23.1 | 0.190 | 35.6 | 53.8 | 0.149 |
| rs1802757 (C/T) | 44.2/48.1/7.7 | 15.4/61.5/23.1 | 0.086 | 31.7 | 53.8 | 0.085 |
| VDR           |           |           |           |           |           |           |
| rs7975323 (G/T) | 66.0/28.3/3.8 | 80/20/0 | 0.336 | 17.9 | 10 | 0.292 |
| rs2228570 (C/T) | 37.7/47.2/15.1 | 33.3/60.0/6.7 | 0.585 | 38.7 | 36.7 | 0.920 |
| rs1544410 (A/G) | 84.9/15.1/0 | 93.3/6.7/0 | 0.672 | 7.5 | 3.3 | 1.000 |
| rs11574129 (T/C) | 82.7/13.5/3.8 | 84.6/15.4/0 | 0.574 | 10.5 | 7.7 | 1.000 |
| rs3847987 (G/T) | 78.8/15.4/3.8 | 84.6/15.4/0 | 0.706 | 11.5 | 7.7 | 0.672 |
| rs7398737 (C/A) | 61.5/32.7/3.8 | 76.9/23.1/0 | 0.762 | 20.2 | 11.5 | 1.000 |
| rs97295 (C/A) | 63.5/30.8/3.8 | 76.9/23.1/0 | 0.769 | 19.2 | 11.5 | 1.000 |
| FGFR3         |           |           |           |           |           |           |
| rs13317 (G/A) | 47.2/39.6/13.2 | 46.7/20.0/33.3 | 0.313 | 33.0 | 43.3 | 0.865 |
| KL            |           |           |           |           |           |           |
| rs1207568 (C/T) | 66.0/32.1/1.9 | 66.7/33.3/0 | 0.865 | 17.0 | 20.0 | 0.852 |
| RGS14         |           |           |           |           |           |           |
| rs4074995 (A/G) | 50.9/30.2/11.3 (F:7.6) | 60.0/33.3/6.7 | 0.828 | 26.4 | 23.3 | 1.000 |
| ALPL          |           |           |           |           |           |           |
| rs1697421 (A/G) | 34.0/41.5/24.5 | 6.7/46.7/26.7 | 0.491 | 45.3 | 50.0 | 0.666 |
| rs3200254 (C/T) | 30.2/45.3/24.5 | 26.7/53.3/6.7 (F:13.3) | 0.837 | 47.2 | 33.3 | 0.818 |
| rs2242420 (C/T) | 65.4/32.7/1.9 | 76.9/23.1/0 | 0.880 | 18.3 | 11.5 | 1.000 |
| rs2242421 (A/G) | 42.3/42.3/15.4 | 46.2/28.5/15.4 | 0.964 | 36.5 | 34.6 | 0.955 |
| rs1697405 (G/A) | 51.9/49.2/3.8 | 46.2/28.5/7.7 | 0.920 | 26.9 | 26.9 | 1.000 |
| rs1697406 (G/A) | 67.3/30.8/1.9 | 69.2/23.1/0 (F:7.7) | 0.858 | 17.3 | 11.5 | 1.000 |
| NR4A2         |           |           |           |           |           |           |
| rs12803 (G/A) | 69.2/28.8/1.9 | 61.5/38.5/0 | 0.721 | 16.3 | 19.2 | 0.704 |
| PTHLH         |           |           |           |           |           |           |
| rs2796 (A/G) | 73.1/25.0/1.9 | 84.6/15.4/0 | 0.589 | 14.4 | 7.7 | 0.672 |
| rs6253 (A/G) | 32.7/51.9/15.4 | 23.1/76.9/0 | 0.296 | 41.3 | 38.5 | 0.656 |
| rs6245 (A/G) | 80.8/19.2/0 | 92.3/7.7/0 | 0.439 | 9.6 | 3.8 | 1.000 |

Notes: Significant result is marked with bold type. F means failure rate (%) to genotype analysis. SNPs are located in 3’ untranslated region and analyzed in 65 patients (responder: 52, nonresponder: 13).

Abbreviations: AA, homozygote major allele; AB, heterozygote allele; BB, homozygote minor allele; SNP, single nucleotide polymorphism.

frequently found in the Asian population (MAF: Han Chinese 52.3%, Japanese 55.8%) and showed the consistent result with the previous study by Rothe et al. The study suggested that an Asian dialysis patient carrying a homozygous (G/G) variant of CASR rs1042636 showed significant iPTH reduction after 2-month cinacalcet treatment compared to another six patients having different genotypes. Several other studies on CASR also presented the possible association of CASR rs1042636 with PTH level difference. However, the functionality of rs1802757 which is located in 3’ untranslated region (UTR) has not been well documented. We can assume that the rs1802757 can be investigated further via similar mechanism. In particular, the SNPs of rs1042636 and rs1802757 and haplotypes of GCC and ATT occurred at high frequencies of 45.8%, 35.9%, 52.9%, and 37.5%, respectively; in our result, the impact of these variants should not be negligible among Asians.

The regulation of PTH secretion and synthesis involves a multitude of pathways. However, in our study, the SNPs of genes other than CASR seemed not to correlate with cinacalcet response. Studies of Casr and Vdr knockout mice can explain part of our results. VDR-deficient mice which developed severe SHPT could be corrected by stabilizing serum calcium concentrations, whereas CaSR-deficient mice with SHPT were not corrected by elevated serum calcium and vitamin D levels and normal serum phosphorus.
| Gene/SNP       | Genotype model* | OR (95% CI) | OR (95% CI) | OR | OR (95% CI) |
|---------------|----------------|-------------|-------------|----|-------------|
| CASR          |                |             |             |    |             |
| rs1042636 (A/G) | 0.074 (0.008–0.721) | 0.395 (0.106–1.479) | I   | 0.267 (0.074–0.957) | 0.139 (0.017–1.142) |
|               | P=0.025       | P=0.168     |             |    |             |
| rs2221266 (C/T) | 5.500 (0.839–36.059) | 3.826 (0.730–20.044) | I   | 4.613 (0.945–22.526) | 2.813 (0.758–10.431) |
|               | P=0.076       | P=0.112     |             |    |             |
| rs10190 (C/T)  | 5.500 (0.741–40.803) | 4.304 (0.730–20.044) | I   | 4.033 (0.811–20.056) | 1.929 (0.423–8.784) |
|               | P=0.095       | P=0.112     |             |    |             |
| rs1802757 (C/T) | 8.625 (1.077–69.075) | 3.680 (0.707–19.156) | P<0.042 | 4.362 (0.878–21.669) | 3.600 (0.695–18.646) |
|               | P=0.122       |             |             |    |             |
| VDR           |                |             |             |    |             |
| rs7975232 (G/T) | 0.365 (0.073–1.823) | I   | 0.324 (0.065–1.607) | P=0.200 |
|               | P=0.219       |             |             |    |             |
| rs2228570 (C/T) | 0.500 (0.050–4.978) | 1.440 (0.416–4.983) | I   | 1.212 (0.362–4.059) | 0.402 (0.046–3.497) |
|               | P=0.554       | P=0.565     |             |    |             |
| rs1544410 (A/G) | 0.402 (0.046–3.497) | I   | 0.402 (0.046–3.497) | P=0.672 |
|               | P=0.409       |             |             |    |             |
| rs11574129 (T/C) | 0.438 (0.050–3.853) | I   | 0.350 (0.041–3.015) | P=0.439 |
|               | P=0.456       |             |             |    |             |
| rs3847987 (G/T) | 0.741 (0.141–3.905) | I   | 0.606 (0.118–3.121) | P=0.717 |
|               | P=0.723       |             |             |    |             |
| rs739837 (C/A) | 0.917 (0.245–3.433) | I   | 0.815 (0.220–3.021) | P=1.000 |
|               | P=0.897       |             |             |    |             |
| rs9729 (C/A)  | 1.007 (0.268–3.791) | I   | 0.889 (0.239–3.307) | P=1.000 |
|               | P=0.991       |             |             |    |             |
| FGFR1         |                |             |             |    |             |
| rs13317 (C/T) | 1.500 (0.355–6.347) | 0.429 (0.100–1.828) | I   | 0.724 (0.229–2.826) | 2.045 (0.520–8.044) |
|               | P=0.582       | P=0.252     |             |    |             |
| KL            |                |             |             |    |             |
| rs1207568 (C/T) | 1.029 (0.304–3.486) | I   | 0.972 (0.289–3.276) | P=0.964 |
|               | P=0.963       |             |             |    |             |
| RGS14         |                |             |             |    |             |
| rs4074995 (A/G) | 0.500 (0.053–4.732) | 0.938 (0.267–3.292) | I   | 0.818 (0.252–2.653) | 0.512 (0.057–4.626) |
|               | P=0.546       | P=0.920     |             |    |             |
| ALPL          |                |             |             |    |             |
| rs1697421 (A/G) | 1.821 (0.266–12.473) | 2.705 (0.497–14.718) | I   | 2.361 (0.465–11.979) | 0.929 (0.220–3.928) |
|               | P=0.541       | P=0.250     |             |    |             |
| rs3200254 (C/T) | 0.667 (0.104–4.261) | 1.120 (0.282–4.449) | I   | 0.973 (0.261–3.627) | 0.621 (0.121–3.197) |
|               | P=0.668       | P=0.872     |             |    |             |
| rs2242420 (C/T) | 0.972 (0.260–3.632) | 0.915 (0.246–3.400) | I   | 0.915 (0.246–3.400) | 0.718 (0.178–3.088) |
|               | P=0.967       | P=1.000     |             |    |             |
| rs2242421 (A/G) | 0.917 (0.153–5.508) | 0.833 (0.221–3.138) | I   | 0.856 (0.252–2.902) | 1.000 (0.186–5.390) |
|               | P=0.924       | P=0.788     |             |    |             |
| rs1697405 (G/A) | 1.500 (0.132–17.037) | 1.227 (0.347–4.343) | I   | 1.080 (0.308–3.791) | 1.485 (0.141–15.659) |
|               | P=0.744       | P=0.751     |             |    |             |
| rs1697406 (A/G) | 1.200 (0.313–4.596) | I   | 0.686 (0.164–2.866) | P=0.739 |
|               | P=0.790       |             |             |    |             |
| NR4A2         |                |             |             |    |             |
| rs12803 (G/A) | 1.500 (0.422–5.338) | I   | 1.250 (0.361–4.327) | P=0.724 |
|               | P=0.531       |             |             |    |             |
| PTHHL         |                |             |             |    |             |
| rs2796 (A/G)  | 0.487 (0.096–2.472) | I   | 0.452 (0.090–2.280) | P=0.385 |
|               | P=0.385       |             |             |    |             |
| rs6253 (A/G)  | 1.286 (0.341–4.853) | I   | 1.000 (0.268–3.732) | P=1.000 |
|               | P=0.711       |             |             |    |             |
| rs6245 (A/G)  | 0.350 (0.041–3.015) | I   | 0.350 (0.041–3.015) | P=0.439 |
|               | P=0.339       |             |             |    |             |

Notes: Significant results are marked in bold type. *Logistic regression analysis. **Chi-square analysis.

Abbreviations: AA, homozygote major allele; AB, heterozygote; BB, homozygote minor allele; CI, confidence interval; OR, odds ratio; PTH, parathyroid hormone; SNP, single nucleotide polymorphism.
which demonstrates that CaSR is the major regulator of PTH secretion and bone abnormalities.\(^\text{52}\) Thus, other genes related to phosphate and bone metabolism do not have much effect on the PTH regulation by cinacalcet.

The maximum dose of cinacalcet approved in Korea is 100 mg/day and the higher titration dose did not control the PTH level in nonresponders. Previous studies suggested that early response to cinacalcet determined the effectiveness of cinacalcet treatment.\(^\text{63,64}\)

Therefore, our study can suggest that CKD patients who carry T allele of rs1802757 and/or ATT haplotype of rs1042646, rs10190 and rs1802757 have high risk of cinacalcet treatment failure and might be candidates for the parathyroidectomy. There have been pharmacoeconomic issues about the indefinite long-term cinacalcet treatment versus one-time surgery. The research in the 5 European countries by Iannazzo et al.\(^\text{65}\) proved that cinacalcet treatment was beneficial when compared to the standard therapy of vitamin D analogues in cost-effectiveness and cost utility model compared to standard therapy of vitamin D analogs. Komaba et al.\(^\text{66}\) presented that cinacalcet was cost-effective in 99.9% of the Monte Carlo simulations in parathyroidectomy ineligible patients, but only 0.9% of the simulations in parathyroidectomy eligible patients. Thus, nonresponders to cinacalcet treatment can have minimal cost-effectiveness compared to surgery in economic issues.

SHPT patients are highly vulnerable to cardiovascular morbidity and mortality and neither parathyroidectomy\(^\text{67}\) nor cinacalcet\(^\text{68}\) reduces the risk of death or major cardiovascular events in SHPT patients undergoing dialysis.

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**Table 6** Association of PTH regulation by cinacalcet with CASR, VDR, and ALPL haplotypes

| Gene/SNP | Haplotype | Haplotype frequency (%) | Case/control frequencies (%) | OR (95% CI) | P-value |
|----------|-----------|-------------------------|----------------------------|-------------|---------|
| **CASR** |           |                         |                            |             |         |
| rs1042636 (A/G) | GCC | 0.529 | 33.3/58.5 | 0.355 (0.151–0.832) | 0.015 |
| rs1802757 (C/T) | ATT | 0.375 | 57.3/31.9 | 2.769 (1.208–6.347) | 0.014 |
| rs10190 (C/T) | ACC | 0.064 | 8.5/5.8 | 1.786 (0.420–7.597) | 0.423 |
| | ACT | 0.032 | 0.9/3.8 | – | 0.575 |
| **VDR** |           |                         |                             |            |         |
| rs9729 (C/A) | CTGCG | 0.815 | 88.5/79.8 | 1.940 (0.531–7.081) | 0.404 |
| rs11574129 (T/C) | ACTAT | 0.092 | 7.7/9.6 | 0.783 (0.161–3.814) | 1 |
| rs3847987 (G/T) | ATGAT | 0.068 | 3.8/7.6 | 0.480 (0.057–4.019) | 0.686 |
| rs7975232 (C/A) |         |               |                            |            |         |
| rs1697406 (A/G) | GCC | 0.638 | 65.4/63.5 | 1.088 (0.442–2.678) | 0.855 |
| rs2242420 (C/T) | GCA | 0.192 | 23.1/18.3 | 1.342 (0.475–3.794) | 0.578 |
| rs2242421 (A/G) | ATA | 0.162 | 11.5/17.3 | 0.623 (0.169–2.300) | 0.567 |

**Note:** Significant results are marked in bold type.

**Abbreviations:** CI, confidence interval; OR, odds ratio; PTH, parathyroid hormone; SNP, single nucleotide polymorphism.
timely management of SHPT at the initial stage may offer long-term survival in CKD patients. Several studies have proved the efficacy and safety of cinacalcet in stage 3 and 4 CKD patients with SHPT; however, the noticeable PTH decrease followed by hypocalemia and hyperphosphatemia brought about the avoidance of cinacalcet prescription in CKD predialysis patients. Since our findings in this study provided a potential target for the investigation of cinacalcet nonresponder group who have high possibility of parathyroidectomy or refractory SHPT, CASR SNPs may be useful biomarkers to administer cinacalcet in early stage of CKD patients to attain the individualized medical treatment of SHPT.

The main factors of cinacalcet resistance after several months or years of therapy are severely enlarged parathyroid gland (>1 cm), nodular hyperplasia, and the downregulation of CaSR and VDR by uremic status which is resulted by inadequate dialysis. However, the parathyroid hyperplasia did not affect the cinacalcet nonresponsiveness in our short-term study as in previous studies. The dose of vitamin D was not different between two groups and findings from the study by Block et al presented that cinacalcet reduced PTH levels regardless of whether the vitamin D doses were increased, decreased, or unchanged. We performed multiple testing by Bonferroni correction and false discovery rate test. The result turned out that the association of rs1042636 or rs1802757 with PTH change was not significant. However, neither of these methods take into account the correlation of SNPs due to linkage disequilibrium, which tags the genetic variation across gene regions. Our result showed that the three SNPs of CASR, five SNPs of VDR, and three SNPs of ALPL comprise one haplblock each, representing that the SNPs are not independent and highly correlated. Thus, we rather not reflect the multiple testing in the results.

Our study has several limitations as follows. The first, 70 Korean patients are relatively small for genetic association study and diverse ethnic factors could not be considered. This limitation should warrant further large-scale studies to replicate and confirm our findings. The second, the included patients were on either hemodialysis or peritoneal dialysis; the Kt/V (\(\text{K}_{\text{urea}} \times \text{T} / \text{V}_{\text{urea}}\)) value which indicates the dialysis adequacy is not reciprocally conversed between two modalities, so the influence of dialysis could not be evaluated in our study. The third, we focused on the 3-month treatment of cinacalcet, and the result of long-term follow-up study on the mineral bone disease and cardiovascular disease could not be assessed. The last, most patients were on either phosphate binders or vitamin D analogs and the proportion of patients on either drug was not different between two groups, but the possible impact of different amount of concomitant calcium intake cannot be ruled out.

**Conclusion**

We obtained supporting evidence for an association between cinacalcet response and CASR polymorphisms. We performed a comprehensive search for SNPs within genes associated with PTH regulations to find the precise frequencies and association with cinacalcet response in 70 dialytic SHPT patients for the first time in Korea. CASR SNPs rs1802757, rs1042636, and haplotypes of rs1042636, rs10190, and rs1802757 were significantly associated with cinacalcet response variance.

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**Disclosure**

The authors report no conflicts of interest in this work.

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## Supplementary materials

### Table S1 The primer sets and melting temperature (Tm) used for the SNaPshot assay

| Gene  | rs number  | Strand | Primer sequence | Tm |
|-------|------------|--------|-----------------|----|
| CASR  | rs1042636  | Forward| Forward primer  | CAGATGCAGCAGAGGT |
|       | rs2221266  | Reverse| Forward primer  | CAAAGCTCTGGAACCTGGA |
|       |            |        | Reverse primer  | GCCTCAGAGAAYKCCATGCSCAC |
|       | rs10190    | Reverse| Forward primer  | CGTTTACCCCCGTGGAT |
|       |            |        | Reverse primer  | TCCATTGGTGTGCTACGTTC  |
|       |            |        | Genotyping primer| GTGTGCTACGTGGTAAAT |
|       | rs1802757  | Forward| Forward primer  | CCAAGAAAGATCCACCTCCTCA |
|       |            |        | Reverse primer  | TGGGCTAGGGCATACATACTTG |
|       |            |        | Genotyping primer| ATCCATAAGCTCTGAGGGGA |
| VDR   | rs7975232  | Forward| Forward primer  | TGGAGCACTGGCAGGTACAGG |
|       | rs2228570  | Reverse| Forward primer  | TGGTGGGGATTAGCAGTGAGG |
|       | rs1544410  | Forward| Forward primer  | AGCCCTCAAGAGTCAAGG |
|       |            |        | Reverse primer  | TGGGCTAGGGCATACATACTTG |
|       | rs11574129 | Forward| Forward primer  | TGCCTGGGCAAGCAGGAGG |
|       |            |        | Reverse primer  | CACAGCTCGAGGATGG |
|       | rs3847987  | Reverse| Forward primer  | GAGTGAATACTCAAGTGAC |
|       | rs739837   | Reverse| Forward primer  | CACCAGGCCATCTCTCTT |
|       | rs9729     | Forward| Forward primer  | GGGAAAAGACCCACCTCA |
|       |            |        | Reverse primer  | AGGGGTTGGGGGTGGAGGCTTG |
|       | rs3200254  | Forward| Forward primer  | TACTCCATAGCCATGCC |
|       | rs2242420  | Reverse| Forward primer  | ACTAGTCCCCCCACGTGAT |
|       | ALPL       | Forward| Forward primer  | TTGAGTGAGTGGTCTGA |
|       | ALPL       | Reverse| Forward primer  | GCAGCTCCAAAGAAGGAGG |
|       | ALPL       | Reverse| Reverse primer  | CCCCTATGAGACCTTTAAGGGA |
|       | ALPL       | Genotyping primer| TCTGTAACCAGCATGCTG |
|       | SLC34A1*   | Forward| Forward primer  | TCCCTTTCACCTGAGGAGG |
|       | rs3812035  | Reverse| Forward primer  | CAGGTTAGGGGAGTTCC |
|       | rs1697421  | Forward| Forward primer  | TCTGTAACCAGCATGCTG |
|       | rs3200254  | Forward| Forward primer  | TCTGTAACCAGCATGCTG |
|       | ALPL       | Reverse| Forward primer  | TCTGTAACCAGCATGCTG |
|       | ALPL       | Reverse| Reverse primer  | TCTGTAACCAGCATGCTG |
|       | ALPL       | Genotyping primer| TCTGTAACCAGCATGCTG |

(Continued)
Table S1  (Continued)

| Gene  | rs number | Strand | Primer sequence                          | Tm  |
|-------|-----------|--------|------------------------------------------|-----|
|       |           |        | Forward primer                           |     |
|       |           |        | Reverse primer                           |     |
|       |           |        | Genotyping primer                         |     |
| rs2242421 | Forward | Forward primer | ACTACGTCCCCCACGTGAT | 60  |
|         |          | Reverse primer | CCTGGTGCTCTGAGTGA |     |
|         |          | Genotyping primer | CCTCTGAAACCACGGGGA |     |
| rs1697405 | Reverse | Forward primer | ACTACGTCCCCCACGTGAT | 60  |
|         |          | Reverse primer | CCTGGTGCTCTGAGTGA |     |
|         |          | Genotyping primer | GCCCCCACCTCCCTGGGCCC |     |
| rs1697406 | Forward | Forward primer | ACTACGTCCCCCACGTGAT | 60  |
|         |          | Reverse primer | CCTGGTGCTCTGAGTGA |     |
|         |          | Genotyping primer | CTTGACTGCAAGAAAAAGGGA |     |
| NR4A2  | rs12803  | Reverse | Forward primer                           |     |
|        |          | Reverse primer | GGCAGAGATAAGCCGGTGA |     |
|        |          | Genotyping primer | TGGGCTAGGGCACTACATTG |     |
| PTHLH  | rs2796   | Forward | Forward primer                           |     |
|        |          | Reverse primer | TGCCTCAGCTGGTTTTC | 55  |
|        |          | Genotyping primer | TGGCAGATTGCTGGGTTTTC |     |
| rs6253 | Forward  | Forward primer | TGCCTCAGCTGGTTTTC | 55  |
|        |          | Reverse primer | CATGATGCTGGCTCTGGT |     |
|        |          | Genotyping primer | TCAGAAATCTGCTCGCTTAAAGCA |     |
| rs6245 | Forward  | Forward primer | TGCCTCAGCTGGTTTTC | 55  |
|        |          | Reverse primer | CATGATGCTGGCTCTGGT |     |
|        |          | Genotyping primer | TCCCAGACCATATAGAGGGGCTGA |     |

Note: *SLC34A4 was not included in the final analysis because it didn’t meet the Hardy Weinberg Equilibrium (HWE).*
Table S2 Association between biochemical parameters and the genotypes* of four SNPs of CASR

| Parameter          | Total Δ% (mean ± SD) | Δ% by genotypes, median (range) |
|--------------------|----------------------|---------------------------------|
|                    | rs1042636            | rs2221266                        | rs10190                        | rs1802757                        |
|                    | AA (n=14)            | GA + GG (n=56)                  | CC (n=24)                      | CT + TT (n=46)                   | CC (n=25)                      | CT + TT (n=40)                   |
| Ca mg/dL           | -6.64 (-19.09–15.81) | -6.64 (-25.67–5.07)             | -6.27 (-25.67–5.07)            | -6.50 (-25.67–5.07)              | -5.75 (-25.67–5.07)            | -6.68 (-25.67–5.07)             |
| P-value             | 0.622                | 0.889                           | 0.863                          | 0.909                            | 0.940                          | 0.989                           |
| P mg/dL            | -8.13 (-43.69–69.43) | -9.35 (-69.23–72.44)            | -9.23 (-69.23–72.44)           | -9.84 (-69.23–72.44)             | -8.87 (-42.08–63.83)           | -9.23 (-69.23–72.44)            |
| P-value             | 0.463                | 0.934                           | 0.714                          | 0.989                            | 0.874                          | 0.989                           |
| Ca × P mg²/dL²     | -12.22 (-44.87–68.70)| -14.80 (-64.98–68.70)           | -14.80 (-64.98–68.70)          | -14.90 (-64.98–68.70)            | -13.68 (-48.81–61.24)          | -14.80 (-71.13–68.70)           |
| P-value             | 0.686                | 0.874                           | 0.647                          | 0.862                            | 0.874                          | 0.862                           |
| ALP IU/l           | 2.36 (-28.48–146.58) | 2.35 (28.48–93.89)              | 2.36 (28.48–93.89)             | 2.36 (28.48–93.89)               | 2.36 (28.48–93.89)             | 2.36 (28.48–93.89)              |
| P-value             | 0.310                | 0.614                           | 0.197                          | 0.373                            | 0.91                           | 5.14                            |

Note: *analyzed by a dominant genetic model.

Abbreviations: ALP, alkaline phosphatase; SD, standard deviation; SNP, single nucleotide polymorphism.
