Ethnicity of Patients With Germline 
GCM2-Activating Variants and Primary 
Hyperparathyroidism

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Context: Germline gain-of-function variants in the transcription factor GCM2 were found in 18% of 
kindreds with familial isolated hyperparathyroidism (FIHP). These variants [c.1136T>A (p.Leu379Gln) 
and c.1181A>C (p.Tyr394Ser)] were located in a 17-amino acid transcriptional inhibitory domain 
named C-terminal conserved inhibitory domain (CCID).

Objective: We investigated the ethnicity of individuals with germline variants in the 
GCM2 CCID in our primary hyperparathyroidism (PHPT) patient samples and in the Genome Aggregation Database.

Design: Ethnicity information was obtained from an in-house clinical database and genetic counseling. Sanger sequencing of blood DNA was used to determine the genotype of the GCM2 CCID region. Luciferase reporter assays were performed to determine the functional impact of GCM2 variants.

Setting and Patients: National Institute of Diabetes and Digestive and Kidney Diseases endocrine 
clinic is a service that accepts PHPT referral patients.

Results: The GCM2 p.Tyr394Ser variant was found in 41% [95% confidence interval (CI), 22% to 
64%] of Ashkenazi Jewish (AJ) kindreds with FIHP and in 27% (95% CI, 17% to 40%) of AJ patients with 
sporadic PHPT. The p.Tyr394Ser variant was also found in sporadic PHPT patients of European an-
cestry, but at a lower prevalence. The p.Leu379Gln variant was found in 8% (95% CI, 1% to 26%) of 
European kindreds with FIHP and 0.5% (95% CI, 0% to 3.0%) of sporadic PHPT cases of European 
ancestry. The sporadic PHPT patients with GCM2-activating variants often had multigland in-
volvement or postoperative recurrent or persistent disease.

Conclusions: Specific GCM2-activating variants enriched among various ethnic backgrounds could 
contribute to a large number of cases with FIHP or sporadic PHPT.

Freeform/Key Words: Ashkenazi Jewish, familial isolated hyperparathyroidism, sporadic 
primary hyperparathyroidism, GCM2 CCID, familial primary hyperparathyroidism, parathyroid

Primary hyperparathyroidism (PHPT) is a common endocrine disease, characterized by 
hypercalcemia and high or inappropriately elevated parathyroid hormone (PTH) in serum. The majority of PHPT cases occur sporadically, and present with a parathyroid adenoma.
Approximately 5% to 15% of PHPT cases are familial and caused by germline variants in one of several genes, mainly *GCM2*, *MEN1*, *CDC73*, *RET*, and *CASR* [1, 2]. The GCM2 gene, located on human chromosome 6p24.2, encodes a 506-amino acid (AA) transcription factor required for parathyroid development [3]. Inactivating germline variants of GCM2 have been found in kindreds with familial hypoparathyroidism [1, 4–6]. We recently identified activating germline variants in GCM2 associated with familial isolated hyperparathyroidism [FIHP, hyperparathyroidism 4 or HRPT4 (Mendelian Inheritance in Man number: 617343)], accounting for 18% of examined kindreds with FIHP [1]. Germline variants in MEN1, CDC73, RET, and CASR often cause syndromes associated with PHPT, multiple endocrine neoplasia type 1 (MEN1), hyperparathyroidism-jaw tumor syndrome (HPT-JT), multiple endocrine neoplasia type 2A, and familial hypocalciuric hypercalcemia (FHH), respectively. Interestingly, germline variants in MEN1, CDC73, and CASR have also been found in approximately 10% of apparently sporadic and young (<46 years of age) PHPT patients [7, 8].

The GCM2-activating variants found in FIHP cluster in a small domain of 17 AAs that we named C-terminal conserved inhibitory domain (CCID; AA 379 to 395) [1]. Among the 40 kindreds with FIHP, the c.1181A>C (p.Tyr394Ser) variant and the cis variant c.(751C>G; 1136T>A) [p.(Gln251Glu; Leu379Gln)] were found in five and two kindreds with FIHP, respectively [1]. Here, we report that the p.Tyr394Ser variant is highly enriched in PHPT patients of Ashkenazi Jewish (AJ) ancestry, both in familial settings and in sporadic cases. The AJ populations of Central and Eastern European ancestry form a distinct genetic isolate of an even admixture of European and likely Middle Eastern origins [9]. The p.Tyr394Ser variant was also found in sporadic PHPT cases of European ancestry at a lower prevalence. The p.(Gln251Glu; Leu379Gln) variant was found in two familial PHPT cases and in one sporadic PHPT case of European ancestry. In addition, we characterized the functional activities of GCM2 CCID variants recorded in the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/), containing DNA sequence information of approximately 140,000 unrelated individuals of various ancestries. Our data suggest that four GCM2 CCID activating germline variants may predispose PHPT among populations of various ancestries.

1. Patients and Methods

A. Patients

Patient blood DNA samples were collected and analyzed with written informed consent according to protocols approved by the Institutional Review Boards of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Human Genome Research Institute. Patients with suspected or confirmed PHPT were enrolled in the NIDDK protocol. Family histories of patients were obtained by genetic counselors or by a research nurse. Sporadic PHPT in patients was diagnosed with hypercalcemia and elevated serum PTH levels, after ruling out secondary causes, and without a known family history of PHPT at the time of preparation of this manuscript. Most of the patients also underwent parathyroidectomy at the National Institutes of Health, and hyperplasia or adenoma was verified histologically. Patients with PHPT were enrolled between 1988 and 2016.

Self-reported Jewish patients with PHPT were considered to be of AJ ethnicity in this study. Ashkenazi ancestry was not specifically inquired for the majority of the Jewish patients with PHPT. Given that approximately 90% of Jews in the United States are Ashkenazi, an observation previously confirmed by whole-genome sequencing and principal component analysis (PCA) [9], the number of AJ patients in our PHPT group is not expected to deviate significantly. In addition, our own PCA analyses of exome data in the ClinSeq® cohort also showed that 88% of self-reported Jews are genetically AJ (Results).
In addition to 40 kindreds with FIHP (12 with AJ ancestry, 25 with European ancestry, two with African ancestry, and one with mixed ancestry) described previously [1], this study included five more AJ probands in unrelated kindreds with FIHP, 52 AJ patients with sporadic PHPT, and 21 AJ patients with a diagnosis of MEN1, HPT-JT, or FHH. Of the 52 AJ sporadic PHPT patients, 29 patients had a single adenoma with 16 of those diagnosed at or older than 46 years of age. Twenty-three patients had multigland, postoperative recurrent, or persistent disease (18 patients with multigland involvement, six with recurrent PHPT, 11 with persistent PHPT, and seven diagnosed younger than 46 years of age). Also included in this study were 42 self-reported European patients with a diagnosis of MEN1, HPT-JT, or FHH and 275 patients with sporadic PHPT constituting non-AJ ancestries (204 self-reported European, 52 African, eight Asian, and 11 Latino patients), of which 59 patients (14 of African descent, two of East Asian descent, 32 of European descent, 11 of Latino descent) were previously reported [1]. Of the 275 non-AJ sporadic PHPT patients, 169 patients had a single adenoma with 92 patients diagnosed at or older than 46 years of age. There were 106 patients with multigland, postoperative recurrent, or persistent disease (83 patients with multigland involvement, 34 with recurrent PHPT, 47 with persistent PHPT, and 62 diagnosed younger than 46 years of age). The characteristics including the sex for all AJ patients and the European patients with GCM2 CCID variants are listed in Supplemental Table 1. The sex of patients was not used in statistical analyses.

B. Principal Component Analysis of ClinSeq® Exome-Sequencing Data

PCA was performed on the whole exome sequencing dataset of 951 individuals enrolled in the ClinSeq® project [10, 11]. ClinSeq® participants were mostly recruited from the Washington, DC, metro area, and were enriched for individuals with cardiovascular diseases. Status of PHPT was not investigated in these participants. All of the ClinSeq® participants were interviewed by a genetic counselor and information was obtained on ethnicity including AJ. Single-nucleotide polymorphism were excluded from PCA if located in long-range linkage disequilibrium regions, as determined by single-nucleotide polymorphism pruning using the PLINK toolset. PCA and stratification correction was performed with the software EIGENSTRAT.

C. GCM2 Genotyping

Blood DNA was isolated using standard methods. The primer pair GCM2_E5.3F and E5.3R was used for polymerase chain reaction (PCR) of blood DNA to amplify the GCM2 DNA encoding AA 361 to 506 followed by Sanger sequencing as described [1].

D. DNA Construct, Luciferase Assay, and Western Blot

The GCM2 wild-type (WT), p.Leu379Gln, and p.Tyr394Ser expression constructs and the luciferase reporter construct pGL4-6xGBS-Luc containing six copies of the GCM-binding site were described previously [1]. Additional GCM2 variant expression constructs were made by PCR from the blood DNA of individuals with other variants (p.Thr387dup and p.Ala393_Gln395dup), or by a PCR-ligation-PCR method (p.Lys388Gln and p.Lys388Glu), and followed by subcloning as described [1]. Luciferase assays in HEK293FT cells and western blots were performed as previously described [1].

E. Statistics

Confidence interval (CI) was determined by the modified Wald method. Fisher’s exact test (two-tailed) was used in a 2 × 2 contingency table. Paired Student’s t test (two-tailed) was used for comparing luciferase assays between GCM2 WT and variants. Two-tailed Mann–Whitney test was used to compare clinical features in patient groups. Statistical analyses including the calculation of odds ratios were performed using the Graphpad Prism 5.0 software. P values lower than 0.05 were considered significant.
2. Results

A. A Frequent GCM2-Activating Variant in Ashkenazi Jewish Kindreds With FIHP

Previously, we identified two activating germline variants in the GCM2 CCID in kindreds with FIHP, c.1181A>C (p.Tyr394Ser) in five kindreds and p.Leu379Gln in the cis variant c.(751C>G; 1136T>A) [p.(Gln251Glu; Leu379Gln)] in two kindreds [1]. An examination of the demographic information of the patients in our clinical database showed that the probands in all of the five kindreds with the GCM2 c.1181A>C (p.Tyr394Ser) reported themselves as Jewish. Among the 40 FIHP probands that we previously screened for GCM2 variants, seven probands with WT GCM2 were also self-reported Jewish. Genetic counseling of individuals in these 12 kindreds indicated all of their four grandparents were of AJ ethnicity. We next examined the clinical information of other nonsyndromic PHPT cases in our database who were self-reported AJ patients (n = 57) and identified five additional probands who had a family history of PHPT (Supplemental Table 1). GCM2 genotyping using blood DNA samples revealed two probands with the heterozygous p.Tyr394Ser variant. Thus, among 17 AJ kindreds with FIHP (12 from our previous report and five in the current study), 41% (seven of 17; 95% CI, 22% to 64%) had the GCM2 p.Tyr394Ser variant.

Among 25 kindreds with FIHP of European ancestry (from our previous report), 8% (two of 25; 95% CI, 1% to 26%) had the cis variant c.(751C>G; 1136T>A) [p.(Gln251Glu; Leu379Gln)].

B. A Frequent GCM2-Activating Variant in Ashkenazi Jewish Patients With Sporadic Primary Hyperparathyroidism

To determine whether the germline p.Tyr394Ser variant was also present in patients with sporadic PHPT, we sequenced the GCM2 CCID region in the blood DNA samples of 52 AJ patients diagnosed with sporadic PHPT. We found 14 patients with the p.Tyr394Ser variant. Thus, 27% (14 of 52; 95% CI, 17% to 40%) of these AJ sporadic PHPT patients had the p.Tyr394Ser variant. In addition, we found one patient with a CCID variant, c.1177_1185dupGCCTACCAG (p.Ala393_Gln395dup), and another patient with a variant C-terminal to the CCID, c.1342A>G (p.Met448Val). These two variants are rare with their minor allele frequencies (MAFs) lower than 0.004 among all ethnic populations in the gnomAD (Table 1). All variants found in these AJ patients were heterozygous.

C. GCM2 p.Tyr394Ser Variant in the Ashkenazi Jewish Population Not Selecting for PHPT

To determine whether GCM2 p.Tyr394Ser is a common variant in the AJ, we analyzed the prevalence of this variant in three independent AJ cohorts with unknown PHPT status. In the ClinSeq® cohort with 951 individuals, no individual was observed with the p.Tyr394Ser variant. Because all five GCM2 exons were well-covered in the ClinSeq® exome-sequencing data, we concluded that all of 951 individuals had the WT allele for the variant, GCM2 c.1181A>C (p.Tyr394Ser). We performed PCA of the ClinSeq® dataset to identify AJ admixture in the cohort (Fig. 1). Of the 800 participants of White race, 162 identified themselves as AJ, of which 148 identified all of their four grandparents as AJ. The PCA showed that 142 (88%; 95% CI, 82% to 92%) of 162 self-reported AJ individuals clustered together and well separated from the self-reported European group (Fig. 1). In addition, 11 of 638 (1.7%; 95% CI, 0.9% to 3.1%) whites who were not self-reported as AJ also clustered together with 142 AJ. Thus, 153 individuals were likely of AJ ancestry in the ClinSeq® dataset, and none (0%; 95% CI, 0% to 3.0%) of these individuals had the p.Tyr394Ser variant. This analysis also showed that 88% self-reported Jews were genetically of AJ ancestry, and a small percentage (1.7%) of self-reported Europeans could also be of AJ ancestry.

The Ashkenazi Genome Consortium had previously published a cohort consisting of 128 AJ individuals analyzed by whole genome sequencing and PCA [9]. Five individuals (3.9%; 95% CI, 1.4% to 9.1%) had the heterozygous c.1181A>C (p.Tyr394Ser) variant in this cohort [9].

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The beta release of the gnomAD, which applied PCA on whole exome and genome sequencing datasets, showed that 122 of 5081 AJ were heterozygous for c.1181A>C, and two were homozygous for the c.1181A>C allele. Therefore, 2.4% (95% CI, 2.1% to 2.9%) of AJ had the c.1181A>C (p.Tyr394Ser) variant in the gnomAD dataset. The prevalence of the p.Tyr394Ser variant is markedly lower in non-AJ populations in the gnomAD, with MAFs of 0.021%, 0.005%, and 0.004% observed in populations of non-Finnish European, Latino, and African ancestries, respectively (Table 1). The p.Tyr394Ser variant was not found in the people of Finnish or Asian ancestries in the current release of the gnomAD dataset.

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### Table 1. Allele Frequencies of GCM2 CCID Variants by Ethnic Origin

| Complementary DNAa | Protein | Activating Variantb | gnomAD Browser Allele Frequencyc (%) | All | African | AJ | European (Finnish) | European (Non-Finnish) | Latino | East Asian | South Asian | Other |
|-------------------|---------|---------------------|-------------------------------------|-----|---------|---|-------------------|-----------------------|--------|------------|-------------|-------|
| c.1136T>A         | p.Leu379Gln | Yes                |                                     | 0   | 0       | 0 | 0                 | 0                     | 0      | 0          | 0           | 0     |
| c.1144G>A         | p.Val382Met  | Yes                | 0.007                              | 0.004 | 0       | 0 | 0.008             | 0                     | 0.011 | 0.019      | 0.013        |       |
| c.1158_1160dupCAC | p.Thr387dup  | No                  | 0.083                              | 0.831 | 0       | 0 | 0.047             | 0                     | 0.003 | 0.014      |             |       |
| c.1162A>C         | p.Lys388Gln  | No                  | 0.002                              | 0     | 0       | 0 | 0.005             | 0                     | 0      | 0          |             |       |
| c.1162A>G         | p.Lys388Glu  | Yes                | 0.003                              | 0.006 | 0       | 0 | 0                 | 0                     | 0.035 | 0          |             |       |
| c.1181A>C         | p.Tyr394Ser  | Yes                | 0.058                              | 0.004 | 1.240   | 0 | 0.021             | 0                     | 0.005 | 0          | 0.108       |       |
| c.1177_1185dupGCC | p.Ala393_Gln395dup | No | 0.130                        | 0.023 | 0.010   | 0.011 | 0.122             | 0.356                  | 0      | 0.152      | 0.337       |       |
| c.1217G>A         | p.Arg406Gln  | n.d.               | 0.030                              | 0.301 | 0       | 0 | 0.002             | 0.014                  | 0.005 | 0          |             | 0     |
| c.1342A>G         | p.Met448Val  | n.d.               | 0.021                              | 0.004 | 0.325   | 0 | 0.006             | 0.030                  | 0      | 0.003      | 0.054       | 0     |

Allele frequencies of the four activating variants above:

- 0.068
- 0.013
- 1.240
- 0.029
- 0.005
- 0.045
- 0.019
- 0.121

Abbreviation: n.d., not determined.
aGenbank: NM_004752.3. The c.1217G>A (p.Arg406Gln) and c.1342A>G (p.Met448Val) variants are located C-terminus to the CCID.
bData from the current study and Guan et al. [1].
cApproximate total allele numbers in ancestry populations (gnomAD browser beta release accessed 28 October 2016): All, 252,000; African, 17,000; AJ, 10,000; Finnish, 23,000; non-Finnish European, 112,000; Latino, 36,000; East Asian, 17,000; South Asian, 31,000; other, 7,000.
dBecause these variants were mainly heterozygous, the frequency of variant carrier is the allele frequency shown multiplied by two.

The beta release of the gnomAD, which applied PCA on whole exome and genome sequencing datasets, showed that 122 of 5081 AJ were heterozygous for c.1181A>C, and two were homozygous for the c.1181A>C allele. Therefore, 2.4% (95% CI, 2.1% to 2.9%) of AJ had the c.1181A>C (p.Tyr394Ser) variant in the gnomAD dataset. The prevalence of the p.Tyr394Ser variant is markedly lower in non-AJ populations in the gnomAD, with MAFs of 0.021%, 0.005%, and 0.004% observed in populations of non-Finnish European, Latino, and African ancestries, respectively (Table 1). The p.Tyr394Ser variant was not found in the people of Finnish or Asian ancestries in the current release of the gnomAD dataset.

**Figure 1.** PCA of exome data of White individuals in the ClinSeq® project. Circles and plus signs represent self-reported European and AJ individuals, respectively. PCA reduces the dimensionality of the variant information in exome data to principle components. Principal components 2 and 3 (PC2 and PC3) are shown which identifies individuals of European ancestry (cluster on the left) and individuals of AJ ancestry (cluster on the right).
Given that the gnomAD dataset has the largest AJ sample size among these three independent datasets of unknown status for PHPT, it is likely that about 2.4% of AJ have the GCM2 c.1181A>C (p.Tyr394Ser) variant. To determine whether the GCM2 p.Tyr394Ser variant was enriched in AJ individuals with PHPT, we applied Fisher's exact tests on the variant distributions observed in AJ with FIHP, AJ with sporadic PHPT, ClinSeq®, Ashkenazi Genome Consortium, and the gnomAD. The p.Tyr394Ser variant was significantly enriched (P < 0.0001) in our AJ patient groups with FIHP or sporadic PHPT, as compared with the ClinSeq®, the Ashkenazi Genome Consortium, or the gnomAD groups (Table 2). As compared with the AJ group in the gnomAD dataset, the odds ratios of the p.Tyr394Ser variant in AJ FIHP group and AJ sporadic PHPT group were 28.0 (95% CI, 10.5 to 74.7) and 14.7 (95% CI, 7.8 to 27.9), respectively. We also sequenced and found no p.Tyr394Ser variant in 21 AJ patients diagnosed with MEN1, HPT-JT, or FHH.

D. Germline GCM2 Variants in PHPT Patients of Other Ethnicities

To determine whether GCM2 CCID variants were also present in patients with sporadic PHPT of other ancestries, we sequenced the GCM2 CCID region in our patient samples of other ethnicities (Table 3, Supplemental Table 1). Among 204 sporadic PHPT patients of European ancestry, we found two patients with the heterozygous p.Tyr394Ser variant and one (patient SP-17) with the heterozygous p.Leu379Gln variant. No variant was found in 42 patients of European ancestry diagnosed with MEN1, HPT-JT, or FHH.

The c.1136T>A (p.Leu379Gln) variant was previously found to be in the same haplotype with c.751C>G (p.Gln251Glu) and the GCM2 intronic variant c.456+16A>C in two probands in kindreds with FIHP [1]. We therefore sequenced these two regions in the patient SP-17 who had sporadic PHPT and found that the patient had both c.751C>G (p.Gln251Glu) and c.456+16A>C variants. Neither c.751C>G (p.Gln251Glu) nor c.1136T>A (p.Leu379Gln) variants were found in the ~140,000 individuals in the gnomAD. Therefore, it is likely that the GCM2 haplotype with c.(751C>G; 1136T>A) [p.(Gln251Glu; Leu379Gln)] shared a common ancestor in these three individuals.

The GCM2 CCID sequencing in eight patients with sporadic PHPT of Asian ancestry, and 10 patients of Latino ancestry revealed no variants. Among 52 patients with PHPT of African ancestry, we found one patient with the c.1158_1160dupCAC (p.Thr387dup) variant (MAF = 0.831% in people of African ancestry) and another patient with the c.1217G>A (p.Arg406Gln) variant (MAF = 0.030% in people of African ancestry).

Table 2. The GCM2 p.Tyr394Ser Variant Distribution in AJ Groups

| Group                                               | Total Number | c.1181A>C (p.Tyr394Ser) Allele | Reference at c.1181 | Variant Carrier (%) | Fisher’s Exact Test vs Ashkenazi Genome Consortium | vs AJ in ClinSeq | vs AJ in gnomAD |
|-----------------------------------------------------|--------------|--------------------------------|---------------------|---------------------|--------------------------------------------------|-----------------|-----------------|
| Self-reported AJ probands in kindreds with FIHP     | 17           | 7                              | 10                  | 41.2                | <0.0001                                          | <0.0001         | <0.0001         |
| Self-reported AJ with sporadic PHPT                 | 52           | 14                             | 38                  | 26.9                | <0.0001                                          | <0.0001         | <0.0001         |
| Self-reported AJ with MEN1, FHH, or HPT-JT syndromes| 21           | 0                              | 21                  | 0                   | 1                                                | 1               | 1               |
| Ashkenazi Genome Consortium                          | 128          | 5                              | 123                 | 3.9                 | 0.0188                                           | 0.4437          |                  |
| AJ in ClinSeq                                       | 153          | 0                              | 153                 | 0                   | 0.0188                                           | 0.0918          |                  |
| AJ in gnomAD                                        | 5081         | 124                            | 4957                | 2.4                 | 0.4437                                           | 0.0918          |                  |
Thus, the two GCM2 CCID variants, p.Leu379Gln and p.Tyr394Ser, were also present in patients with sporadic PHPT of European ancestry, although their prevalence is low at 0.5% (95% CI, 0% to 3.0%) and 1.0% (95% CI, 0.04% to 3.7%), respectively. The prevalence of the p.Tyr394Ser variant in our PHPT patients of European ancestry (two of 204 patients) is significantly higher as compared with the variant’s prevalence in people of non-Finnish European ancestry in the gnomAD dataset (27 variant carriers among 63,402 total) as demonstrated by the Fisher’s exact test (P < 0.0001).

E. Transcriptional Activities of GCM2 CCID Variants Found in Patients With PHPT and in the gnomAD Dataset

Previously, we used a luciferase reporter with six copies of a consensus GCM-binding site to measure the transcriptional activities of GCM2 variants in HEK293FT cells, and showed that three GCM2 variants had increased transcriptional activity, p.Leu379Gln, p.Met382Val, and p.Tyr394Ser [1]. The p.Met382Val mutation was initially reported in a parathyroid adenoma sample [12]. We applied the same assay to determine the transcriptional activities of the GCM2 CCID variants found in patients with sporadic PHPT, c.1158_1160dupCAC (p.Thr387dup) and c.1177_1185dupGCCTACCAG (p.Ala393_Gln395dup), as well as the other two CCID missense variants found in the gnomAD, c.1162A>G (p.Lys388Glu) and c.1162A>C (p.Lys388Gln) [Fig. 2, Table 1].

Similar to our previous findings, the p.Leu379Gln and p.Tyr394Ser variants increased the luciferase activities 2.7 and 1.9 fold as compared with the WT GCM2, respectively. The p.Lys388Glu variant also showed increased transcriptional activity 2.6 fold over the WT GCM2. Variants p.Thr387dup and p.Ala393_Gln395dup showed similar activities to WT, and the p.Lys388Gln variant showed lower but statistically significant 10% reduction of transcriptional activity as compared with the WT GCM2 [Fig. 2(a)]. The protein amounts of the transfected GCM2 in cells were similar [Fig. 2(b)].

F. Clinical Presentation of Patients With GCM2 Variants

The 18 patients with the p.Tyr394Ser variant (nine males and nine females) and the one male patient with the p.Gln251Glu; Leu379Gln) variant were diagnosed with PHPT between age 13 and 78 (median 55) years (Supplemental Table 1, Fig. 3). Among these 19 patients, 13 patients had hypercalcemia-related symptoms such as kidney stones (nephrolithiasis) and/or bone loss (osteoporosis or osteopenia). Other nonparathyroid neoplasms were not common in these patients at the time of PHPT diagnosis or follow up. These phenotypes were similar to the patients in FIHP kindreds and with GCM2 CCID variants [1].

We compared several features among the nine probands in FIHP kindreds with GCM2 p.Leu379Gln or p.Tyr394Ser variants (two probands presented in this study and seven probands in our previous study [1]), the 17 sporadic PHPT patients with one of the two variants, and 38 AJ sporadic PHPT patients with WT GCM2. We selected for comparison the 38 AJ sporadic PHPT patients instead of non-AJ sporadic PHPT patients because the groups with GCM2 variants were overwhelmingly of AJ ancestry.

| Ethnicity | Total Number | WT* | Variant (Number of Patient) |
|-----------|--------------|-----|-----------------------------|
| European  | 204          | 201 | p.Leu379Gln (1); p.Tyr394Ser (2) |
| AJ        | 52           | 36  | p.Tyr394Ser (14); p.Ala393_Gln395dup (1); p.Met448Val (1) |
| African   | 52           | 50  | p.Thr387dup (1); p.Arg406Gln (1) |
| Asian     | 8            | 8   | 0                           |
| Latino    | 11           | 11  | 0                           |

*Region encoding GCM2 AA 361 to 506.
As compared with the FIHP GCM2 variant group, the sporadic PHPT GCM2 variant group appeared to have less severe phenotypes (Fig. 3). In the sporadic PHPT GCM2 variant group, the median serum calcium and PTH levels were lower (\(P = 0.071\) and 0.14 respectively), and the maximum dimensions of the largest parathyroid glands resected in the sporadic GCM2 variant group were significantly smaller (\(P = 0.004\)). Compared with the sporadic PHPT cases with WT GCM2, the numbers of parathyroid glands resected were significantly higher in the sporadic PHPT group with GCM2 variant (\(P = 0.004\), Fig. 3). Among the 16 sporadic PHPT patients with GCM2-activating variants who had parathyroidectomy(s) removing enlarged gland(s), there were 13 (81%) patients who either had multiple parathyroid tumors resected (11 patients, 69%) or experienced postoperative recurrent or persistent disease (11 patients, 69%). This supported the germline cause of PHPT in these patients, and contrasted with a single adenoma as the cause for PHPT in over 80% cases [13]. It is noteworthy that NIDDK endocrine clinic accepts referral patients with PHPT, and thus, our patient cohort may be enriched with PHPT patients with familial disease or with more severe phenotypes. Nevertheless, among the 32 evaluable sporadic PHPT cases with WT GCM2 who had parathyroidectomy(s) removing enlarged gland(s), there were 13 (41%) patients who either had multiple parathyroid tumors resected (10 patients, 31%) or experienced recurrent or persistent disease (10 patients, 31%). Thus, the proportion of sporadic PHPT patients who had multiple parathyroid tumors excised or experienced recurrent or persistent disease was significantly higher (\(P = 0.013\), Fisher’s exact test) among the group with GCM2-activating variants as compared with the group without.

3. Discussion

PHPT is a common disease, however, the estimates of prevalence of PHPT in adults vary widely, from 0.1% to 9.4%, as discussed previously [1]. The diagnostic criteria, age, sex, and geography may have contributed to the differences of PHPT prevalence among studies.
For example, autopsy examination indicated that 9.4% of subjects had abnormal parathyroid glands histologically (2.4% had adenoma and 7% had hyperplasia) [15]. It was unknown whether these subjects with abnormal histology also had PHPT biochemically, because PTH levels were not reported and data for serum calcium were not complete [15]. Considering abnormal parathyroid histology and hypercalcemia to be indicative of PHPT, approximately 4.3% of these autopsy subjects, who were mostly older than 50 years old, likely had PHPT biochemically. This estimate is based on the data provided showing that all four cases with an adenoma and with serum calcium levels available were hypercalcemic, and three of 11 cases with serum calcium levels available and with hyperplasia also had hypercalcemia \(4.3\% = (2.4\% \times 4/4) + (7\% \times 3/11)\) [15]. Race is also a factor influencing the PHPT prevalence, with African Americans having the highest prevalence, followed by European Americans, Asian Americans, and Hispanic Americans [18]. We studied the ethnicities of GCM2 variants in PHPT patient samples and in the gnomAD dataset, and identified specific

Figure 3. Phenotype comparison among individuals with PHPT. Two-tailed Mann–Whitney test was used for comparisons among three groups with or without GCM2 CCID activating variants [p.Tyr394Ser or p.(Gln251Glu; Leu379Gln)]; probands in FIHP-affected kindreds and with GCM2 CCID activating variants (FIHP-GCM2 Var), sporadic PHPT patients with GCM2 CCID activating variants (Sporadic-GCM2 Var), and sporadic PHPT patients with WT GCM2 (Sporadic-GCM2 WT). Gray areas in the panels for serum calcium, serum intact PTH, and size of largest gland represent the normal ranges of 2.05 to 2.50 mmol/L, 10 to 65 pg/mL, and 0.3 to 1.0 cm, respectively. The number of glands resected was total number of enlarged (≥1 cm) hypercellular glands excised at one or more surgeries. The normal range (0.3 to 1.0 cm) for the maximum dimension of parathyroid glands was from Yao et al. [21], and represents a conservative criterion for identifying enlarged glands. Graphs for serum intact PTH and size of largest gland were plotted in log2 scale for better visualization.
$GCM2$ gain-of-function variants in various ethnic populations including AJ. Currently the AJ population of approximately 10 million mostly live in the United States of America and Israel, and constitute a distinct genetic isolate of an even admixture of European and Middle East origins [9, 19]. The AJ population is highly enriched for a number of autosomal recessive diseases and for alleles that confer a strong risk of common diseases such as breast and ovarian cancers.

We found the $GCM2$ p.Tyr394Ser variant in 41% of AJ kindreds with FIHP and in 27% of AJ patients with sporadic PHPT. Whether the variant arose from a founder mutation remains to be determined. In AJ population not selected for PHPT, the prevalence of the p.Tyr394Ser variant was 2.4% in the gnomAD dataset. It is unknown whether the individuals in the gnomAD dataset were affected by PHPT. To the best of our knowledge, there is no prevalence study reported for PHPT specifically in AJ. The prevalence of both PHPT and the p.Tyr394Ser variant in AJ, as well as the penetrance of the variant in AJ, requires further investigation. Animal knock-in models could help delineate the roles of this variant in parathyroid disease.

The prevalence of the $GCM2$ p.Tyr394Ser variant in other populations was markedly lower; the highest prevalence after AJ was 0.04% for non-Finnish Europeans (Table 1). In our previous study, we suggested that up to 0.2% of European ancestry were susceptible to PHPT due to the p.Tyr394Ser variant, using the data in the ExAC release version 0.3.1 which pooled people of AJ ancestry into European ancestry [1]. The new information in the gnomAD dataset, which includes exome data of individuals in the ExAC dataset as well as additional genome datasets, suggests that the p.Tyr394Ser variant could predispose 2.4% and 0.04% of population of AJ and European ancestries to develop PHPT, respectively. The relatively high prevalence of the $GCM2$ p.Tyr394Ser variant in AJ in gnomAD dataset, suggests that this variant is probably a major risk allele for PHPT in the AJ population.

Of the 52 AJ sporadic PHPT cases, 16 cases belonged to “typical” sporadic cases, which had no multigland, recurrent or persistent disease and PHPT diagnosed at or older than 46 years of age. Two (12.5%) of these 16 cases had the p.Tyr394Ser variant (Supplemental Table 1). In contrast, 11 (48%) of 23 AJ sporadic PHPT cases with multigland, recurrent or persistent disease had the p.Tyr394Ser variant. It is not unusual to encounter sporadic PHPT cases that present with germline variants due to various reasons: (1) lack of family history of PHPT because the relatives did not have severe symptoms related to PHPT or had asymptomatic PHPT, (2) the variant was a de novo mutation, or (3) the penetrance of the variant differed among members in the same family.

Recent genome analyses have shown that rare variants are often found in specific populations [20]. Because of modest number of patients from other ethnicities, we took advantage of the gnomAD dataset with genome data of ~140,000 individuals, and tested the transcriptional activities of all of the missense variants in the $GCM2$ CCID found in the gnomAD dataset. We found that the $GCM2$ p.Lys388Glu variant found in East Asian and African ethnicities, was also an activating variant. Thus, we postulate that this variant may contribute to PHPT and FIHP in people of East Asian and African ethnicities. Together, the four $GCM2$ CCID activating variants, p.Leu379Gln, p.Met382Val, p.Lys388Glu, and p.Tyr394Ser could probably put 2.4% AJ, 0.026% African, 0.058% non-Finnish European, 0.011% Latino, 0.090% East Asian, and 0.039% South Asian patients at risk for developing PHPT (Table 1). We expect that the widespread application of whole-exome or whole-genome sequencing in general populations or during the investigations for other diseases, would uncover a large number of carriers with one of the four $GCM2$ CCID activating variants. Our results suggest that such individuals should undergo close monitoring of calcium and PTH levels in serum.

In summary, our results provide human genetic evidence showing specific activating germline variants in $GCM2$ CCID as a contributor to FIHP and sporadic PHPT in various ethnicities.

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