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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 ancestry, 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 involvement 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.

Abbreviations: AA, amino acid; AJ, Ashkenazi Jewish; CCID, C-terminal conserved inhibitory domain; CI, confidence interval; FHH, familial hypocalciuric hypercalcemia; FIHP, familial isolated hyperparathyroidism; gnomAD, Genome Aggregation Database; HPT-JT, hyperparathyroidism-jaw tumor syndrome; MAF, minor allele frequency; MEN1, multiple endocrine neoplasia type 1; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases; PCA, principal component analysis; PCR, polymerase chain reaction; PHPT, primary hyperparathyroidism; PTH, parathyroid hormone; WT, wild-type.

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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 mulitgland, 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].

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

			gnomAD Browser Allele Frequency[c] (%)								
Complementary DNA[a]	Protein	Activating Variant?[b]	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	0.047	0	0.003	0.014
c.1162A>C	p.Lys388Gln	No	0.002	0	0	0	0.005	0	0	0	0
c.1162A>G	p.Lys388Glu	Yes	0.003	0.006	0	0	0	0	0.035	0	0
c.1181A>C	p.Tyr394Ser	Yes	0.058	0.004	1.240	0	0.021	0.005	0	0	0.108
c.1177_1185dup GCCTACCAG	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
Allele frequencies of the four activating variants above[d]			0.068	0.013	1.240	0	0.029	0.005	0.045	0.019	0.121

Abbreviation: n.d., not determined.
[a]Genbank: NM_004752.3. The c.1217G>A (p.Arg406Gln) and c.1342A>G (p.Met448Val) variants are located C-terminus to the CCID.
[b]Data from the current study and Guan *et al.* [1].
[c]Approximate 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.
[d]Because 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.

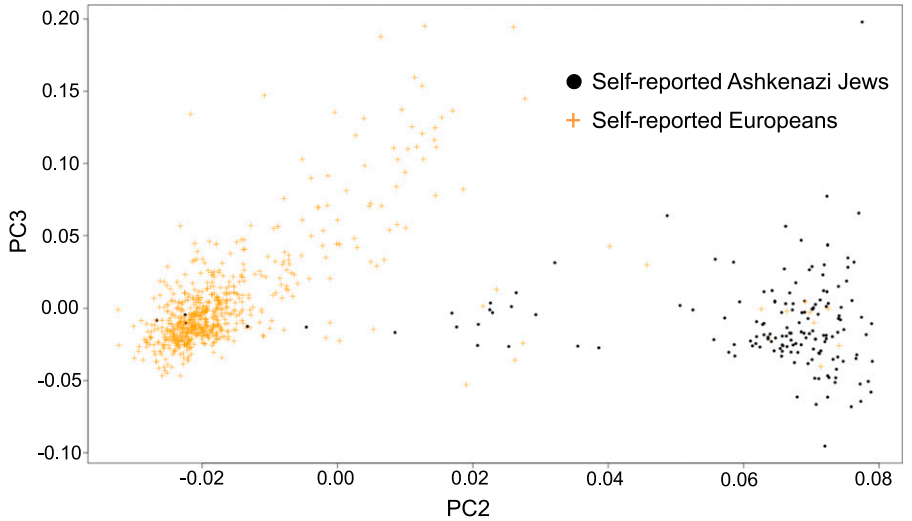

Figure 1. PCA of exome data of White individuals in the ClinSeq® project. Circles and plus signs represent self-reported European and AJ individuals, respectively. PCA reduces the dimensionality of the variant information in exome data to principle components. Principal components 2 and 3 (PC2 and PC3) are shown which identifies individuals of European ancestry (cluster on the left) and individuals of AJ ancestry (cluster on the right).

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

Group	Total Number	c.1181A>C (p.Tyr394Ser)	Reference Allele 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	

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 3. Summary of Patients With Sporadic PHPT Screened for *GCM2*

Ethnicity	Total Number	WT[a]	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

[a]Region encoding GCM2 AA 361 to 506.

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>C (p.Lys388Gln) and c.1162A>G (p.Lys388Glu; 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.

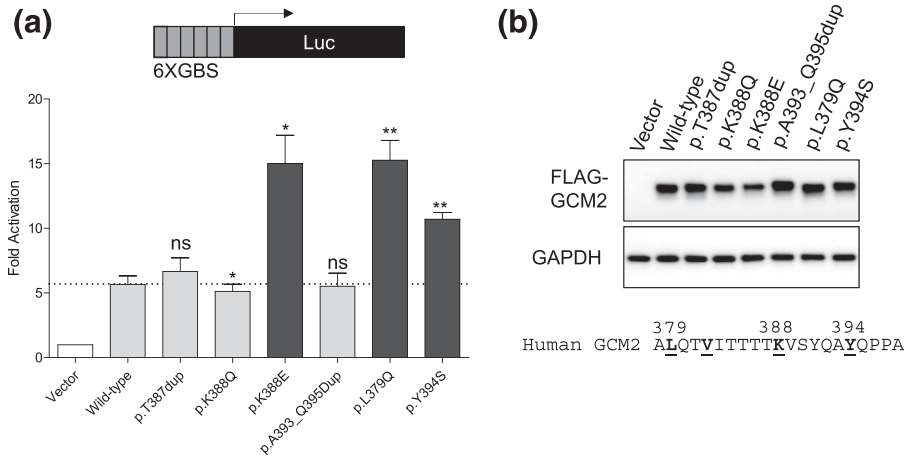

Figure 2. Transcriptional activity assays of GCM2 CCID variants. (a) The transcriptional activities of WT GCM2 and variants were tested in HEK293FT cells cotransfected with a luciferase reporter plasmid containing six copies of a consensus GCM-binding site. Twenty-five nanograms of empty vector, WT GCM2 expressing plasmid, or plasmids expressing the indicated GCM2 variants were cotransfected with 375 ng of 6× GCM-binding site luciferase reporter plasmid. Plotted are the ratios of luciferase activities of GCM2 expressing constructs over empty vector. Means are shown from three independent experiments performed in triplicate. Error bars denote standard error of the mean. *P* values of paired two-tailed *t* tests were used for comparisons between variants and WT GCM2. ns, not significant. * $P < 0.05$; ** $P < 0.01$. (b) Representative western blots of lysates used in luciferase reporter assays. Equal volume of luciferase lysates was analyzed with anti-FLAG to detect transfected GCM2. GAPDH was used as the loading control. The amino acid sequence of the human GCM2 CCID region is shown at the bottom of panel (b).

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

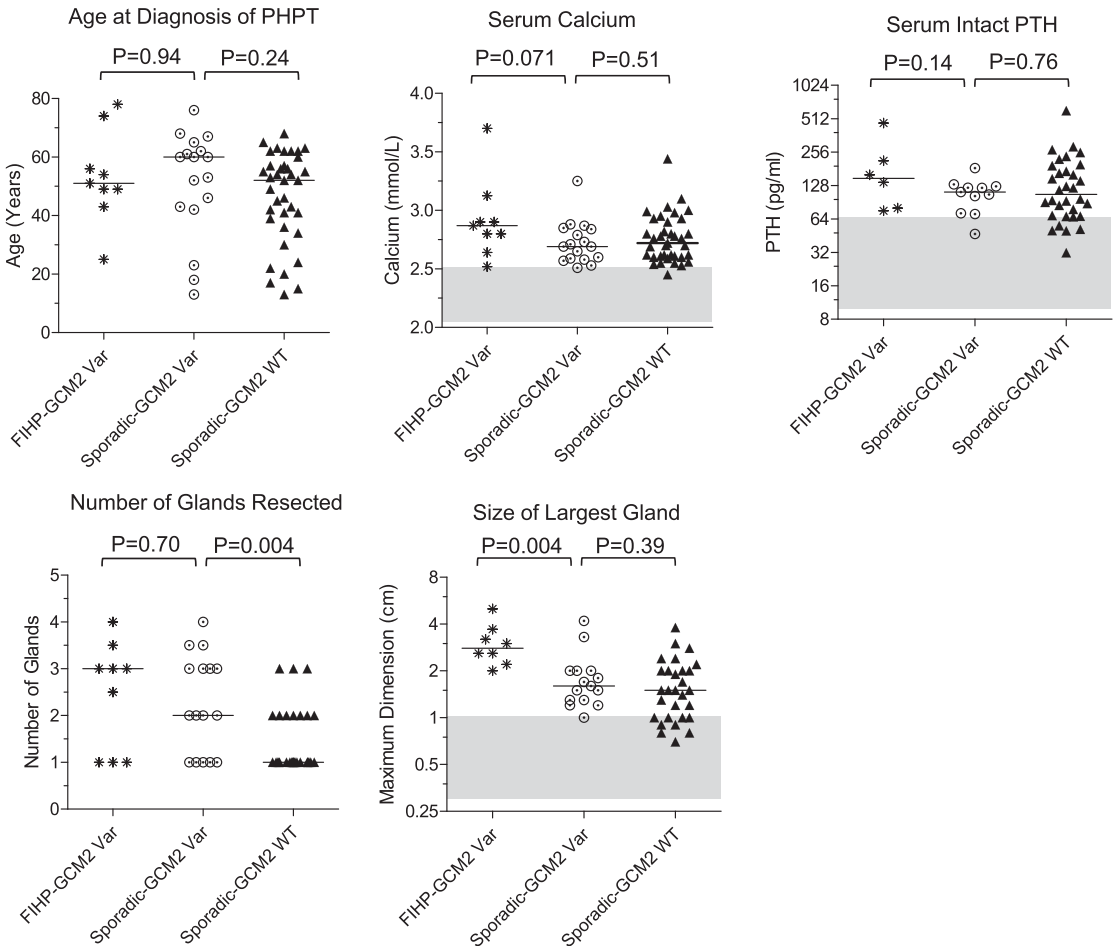

Figure 3. Phenotype comparison among individuals with PHPT. Two-tailed Mann–Whitney test was used for comparisons among three groups with or without *GCM2* CCID activating variants [p.Tyr394Ser or p.(Gln251Glu; Leu379Gln)]: probands in FIHP-affected kindreds and with *GCM2* CCID activating variants (FIHP-*GCM2* 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) and/or 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.

[14–17]. 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% × 4/4) + (7% × 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

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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References and Notes

1. Guan B, Welch JM, Sapp JC, Ling H, Li Y, Johnston JJ, Kebebew E, Biesecker LG, Simonds WF, Marx SJ, Agarwal SK. GCM2-activating mutations in familial isolated hyperparathyroidism. *Am J Hum Genet*. 2016;**99**(5):1034–1044.
2. Marx SJ. Hyperparathyroid genes: sequences reveal answers and questions. *Endocr Pract*. 2011; **17**(Suppl 3):18–27.
3. Günther T, Chen ZF, Kim J, Priemel M, Rueger JM, Amling M, Moseley JM, Martin TJ, Anderson DJ, Karsenty G. Genetic ablation of parathyroid glands reveals another source of parathyroid hormone. *Nature*. 2000;**406**(6792):199–203.
4. Ding C, Buckingham B, Levine MA. Familial isolated hypoparathyroidism caused by a mutation in the gene for the transcription factor GCMB. *J Clin Invest*. 2001;**108**(8):1215–1220.
5. Mitsui T, Narumi S, Inokuchi M, Nagasaki K, Nakazawa M, Sasaki G, Hasegawa T. Comprehensive next-generation sequencing analyses of hypoparathyroidism: identification of novel GCM2 mutations. *J Clin Endocrinol Metab*. 2014;**99**(11):E2421–E2428.
6. Shoback D. Clinical practice: hypoparathyroidism. *N Engl J Med*. 2008;**359**(4):391–403.
7. Starker LF, Akerström T, Long WD, Delgado-Verdugo A, Donovan P, Udelsman R, Lifton RP, Carling T. Frequent germ-line mutations of the MEN1, CASR, and HRPT2/CDC73 genes in young patients with clinically non-familial primary hyperparathyroidism. *Horm Cancer*. 2012;**3**(1–2):44–51.
8. Frank-Raue K, Leidig-Bruckner G, Haag C, Schulze E, Lorenz A, Schmitz-Winnenthal H, Raue F. Inactivating calcium-sensing receptor mutations in patients with primary hyperparathyroidism. *Clin Endocrinol (Oxf)*. 2011;**75**(1):50–55.
9. Carmi S, Hui KY, Kochav E, Liu X, Xue J, Grady F, Guha S, Upadhyay K, Ben-Avraham D, Mukherjee S, Bowen BM, Thomas T, Vijai J, Cruts M, Froyen G, Lambrechts D, Plaisance S, Van Broeckhoven C, Van Damme P, Van Marck H, Barzilai N, Darvasi A, Offit K, Bressman S, Ozelius LJ, Peter I, Cho JH, Ostrer H, Atzmon G, Clark LN, Lencz T, Pe'er I. Sequencing an Ashkenazi reference panel supports population-targeted personal genomics and illuminates Jewish and European origins. *Nat Commun*. 2014;**5**:4835.
10. Johnston JJ, Sanchez-Contreras MY, Keppler-Noreuil KM, Sapp J, Crenshaw M, Finch NA, Cormier-Daire V, Rademakers R, Sybert VP, Biesecker LG. A point mutation in PDGFRB causes autosomal-dominant penttinen syndrome. *Am J Hum Genet*. 2015;**97**(3):465–474.
11. Johnston JJ, Lewis KL, Ng D, Singh LN, Wynter J, Brewer C, Brooks BP, Brownell I, Candotti F, Gonsalves SG, Hart SP, Kong HH, Rother KI, Sokolic R, Solomon BD, Zein WM, Cooper DN, Stenson PD, Mullikin JC, Biesecker LG. Individualized iterative phenotyping for genome-wide analysis of loss-of-function mutations. *Am J Hum Genet*. 2015;**96**(6):913–925.
12. Mannstadt M, Holick E, Zhao W, Jüppner H. Mutational analysis of GCMB, a parathyroid-specific transcription factor, in parathyroid adenoma of primary hyperparathyroidism. *J Endocrinol*. 2011; **210**(2):165–171.
13. Marcocci C, Cetani F. Clinical practice. Primary hyperparathyroidism. *N Engl J Med*. 2011;**365**(25): 2389–2397.
14. Lundgren E, Hagström EG, Lundin J, Winnerbäck K, Roos J, Ljunghall S, Rastad J. Primary hyperparathyroidism revisited in menopausal women with serum calcium in the upper normal range at population-based screening 8 years ago. *World J Surg*. 2002;**26**(8):931–936.
15. Akerström G, Rudberg C, Grimelius L, Bergström R, Johansson H, Ljunghall S, Rastad J. Histologic parathyroid abnormalities in an autopsy series. *Hum Pathol*. 1986;**17**(5):520–527.

16. Boonstra CE, Jackson CE. Serum calcium survey for hyperparathyroidism: results in 50,000 clinic patients. *Am J Clin Pathol*. 1971;**55**(5):523–526.
17. Yu N, Donnan PT, Murphy MJ, Leese GP. Epidemiology of primary hyperparathyroidism in Tayside, Scotland, UK. *Clin Endocrinol (Oxf)*. 2009;**71**(4):485–493.
18. Yeh MW, Ituarte PH, Zhou HC, Nishimoto S, Liu IL, Harari A, Haigh PI, Adams AL. Incidence and prevalence of primary hyperparathyroidism in a racially mixed population. *J Clin Endocrinol Metab*. 2013;**98**(3):1122–1129.
19. Atzmon G, Hao L, Pe'er I, Velez C, Pearlman A, Palamara PF, Morrow B, Friedman E, Oddoux C, Burns E, Ostrer H. Abraham's children in the genome era: major Jewish diaspora populations comprise distinct genetic clusters with shared Middle Eastern Ancestry. *Am J Hum Genet*. 2010;**86**(6):850–859.
20. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG, Exome Aggregation C; Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;**536**(7616):285–291.
21. Yao K, Singer FR, Roth SI, Sassoon A, Ye C, Giuliano AE. Weight of normal parathyroid glands in patients with parathyroid adenomas. *J Clin Endocrinol Metab*. 2004;**89**(7):3208–3213.