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Clinical Research Article

Variant Tyr 394Ser in the *GCM2* Gene Is Rare in a Cohort of Ashkenazi Jews With Primary Hyperparathyroidism

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Abstract

Context: Various genes have been associated with familial and sporadic primary hyperparathyroidism (PHPT), including activating mutations of the glial cells missing transcription factor 2 (*GCM2*) gene.

Objective: The aim of this study was to assess the prevalence of the *GCM2* p.Tyr394Ser variant in the Jerusalem Ashkenazi Jewish (AJ) population with PHPT, and to conclude whether routine genetic testing is justified.

Methods: The blood of 40 self-reported AJ patients with PHPT and 200 AJ controls was tested for the *GCM2* p.Tyr394Ser variant. Demographic and medical information was extracted from the patients' charts and evaluated accordingly.

Results: Two (5%) PHPT patients and 3 (1.5%) controls were heterozygotes for the tested variant. Our patients were mostly (87.5%) sporadic cases. One of the heterozygote patients had familial PHPT; the other had 2 parathyroid adenomas, and the levels of his blood and urinary calcium were extremely high.

Conclusion: Our results suggest that in AJ patients with sporadic, single-gland PHPT, the likelihood of the tested variant is low and genetic testing should be limited to those with familial PHPT or multiglandular disease.

Key Words: activating mutation, Jewish, heterozygotes, parathyroid adenoma, genetic

Abbreviations: AJ, Ashkenazi Jewish; Ca, calcium; CCID, C-terminal conserved inhibitory domain; *GCM2*, glial cells missing transcription factor 2; PHPT, primary hyperparathyroidism; PTH, parathyroid hormone; ULN, upper limit of normal.

Primary hyperparathyroidism (PHPT) is a common disease characterized by elevated levels of calcium (Ca) and parathyroid hormone (PTH) in the serum [1-3]. This condition may be asymptomatic; however, weakness, fatigue, gastrointestinal symptoms, and depression may be present. The complications of this condition include nephrolithiasis, renal damage, and osteoporosis [3]. Peptic ulcer [4] and pancreatitis [5] have also been associated with PHPT. The etiology is usually a parathyroid adenoma, and the treatment is surgical [6]. Most cases are sporadic; however, familial syndromes that are well documented include multiple endocrine neoplasia 1 and 2, jaw tumor syndrome, and others [7].

The glial cells missing transcription factor 2 (*GCM2*) gene, located on chromosome 6, encodes a transcription factor that is essential for the development of the parathyroid glands [8]. Inactivating mutations of this gene have been found in patients with familial hypoparathyroidism [9, 10]. In recent years, several activating mutations of the *GCM2* gene have been found in patients with sporadic or familial PHPT [11]. These variants are located in the 17 amino acid transcriptional domain named C-terminal conserved inhibitory domain (CCID).

One study assessed sporadic solitary parathyroid adenomas and found a *GCM2*-activating variant overall frequency of 6.57%, approximately 3-fold greater than its frequency in the general population [12]. In another study investigating the ethnicity of PHPT patients with germline variants in the *GCM2* CCID, the *GCM2* p.Tyr394Ser variant was found in 41% and 27% of Ashkenazi Jewish (AJ) kindreds with familial, isolated, and sporadic PHPT, respectively. The prevalence in the general (non-PHPT selected) Ashkenazi population was 2.4%, and the prevalence in the non-Jewish population was much lower [13]. These findings suggest an association between variants in the *GCM2* gene and familial/sporadic PHPT in the AJ population.

AJs constitute one of the largest Jewish ethnic divisions in Israel. In 2018, 31.8% of Israeli Jews self-identified as Ashkenazi [14]. This makes it highly interesting to assess the prevalence of the *GCM2* p.Tyr394Ser variant among the Israeli PHPT population.

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The aim of this study was to assess the prevalence of the *GCM2* p.Tyr394Ser variant in the Jerusalem AJ population with PHPT. We hypothesized that the prevalence would be high, thus justifying routine genetic testing in this group.

Materials and Methods

Patients

We recruited patients by screening the charts of patients admitted to the Shaare Zedek Medical Center between 2000 and 2019 with a clinical diagnosis of PHPT. We contacted patients during their hospitalization or by a telephone call. A total of 58 patients with Ashkenazi last names were contacted. Of these, 40 patients were eventually genetically tested (Fig. 1).

Inclusion Criteria

Inclusion criteria were the following: AJ patients older than 18 years, with a diagnosis of PHPT comprising elevated levels of serum Ca at least twice and an inappropriately high level of PTH.

Exclusion Criteria

Exclusion criteria were the following: a family history of multiple endocrine neoplasia, pregnancy, or dialysis patients.

Sample Size

The study comprised 40 patients. Based on the literature [13], we speculated that this number would yield approximately 10 positive tests.

Blood Tests

Blood tests were performed at our hospital or at each patient's home by a qualified physician (V.L.).

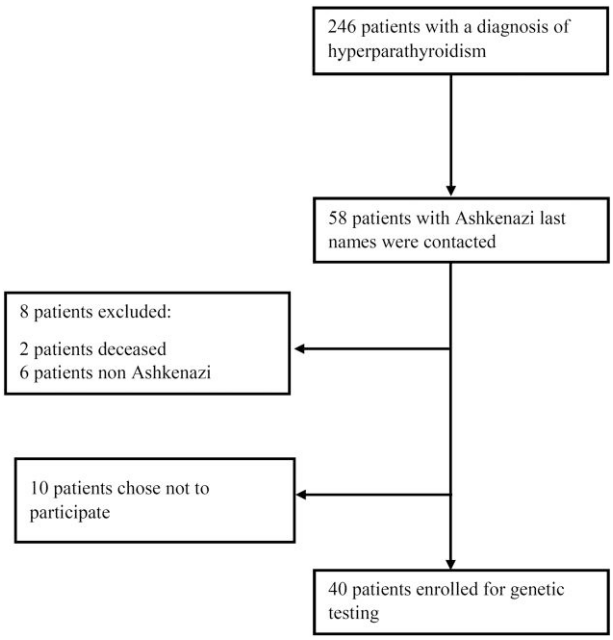

Figure 1. Flowchart of the primary hyperparathyroidism (PHPT) participants in the study. The charts of 246 patients with a diagnosis of PHPT were detected. Forty patients were genetically tested.

Controls

A total of 200 unselected Ashkenazi individuals from our genetic institution database served as controls.

Genetics

Genetic testing was performed at the genetic institute at the Shaare Zedek Medical Center. DNA was extracted from each blood sample by a standard method using the Invitrogen iprep Purelink gDNA Blood Kit according to the manufacturer's instructions. TaqMan allele discrimination assay was used to test all participants for the *GCM2* (GenBank: NM_004752.4) c.1181A > C p.Y394S variant (rs142287570 in dbSNP) (forward primer: GATCACCACCACCACT AAAG, reverse primer: CGCACACTGTCACTGTATTTC, WT probe: CAGGCCUACCAGCC, MUT probe: ACCAGG CCUCCCAG).

Statistics

Dispersion and correlation analyses of the patients' demographic and clinical variables were performed using Excel.

Ethics Considerations

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the ethics committee of the Shaare Zedek Medical Center. Informed consent obtained from all of our hyperparathyroidism patients included in the study. The control group was composed

Table 1. Patient characteristics

Age, y	66 (42-87)
Female sex, No. (%)	32 (80%)
Mean maximum Ca(s), mg/dL (range)	11.2 (10.5-14)
Mean PTH × ULN	2.8 (0.6-7.3)
Mean Ca(u), mg/24 h (range)	388 (216-831)
Familial PHPT, No. (%)	5 (12.5)
Hypertension, No. (%)	28 (70)
Surgery indication, No. (%)	
0	6 (15)
1	9 (22.5)
2	18 (45)
3	5 (12.5)
4	2 (5)

Abbreviations: Ca(s), serum calcium; Ca(u), urinary calcium; PHPT, primary hyperparathyroidism; PTH, parathyroid hormone; ULN, upper limit of normal.

Table 2. Surgical indications[a]

Age < 50 y, No. (%)	6 (15)
Ca(s) + > 1 mg/dL above ULN	10 (25)
Ca(u) > 400 mg/24 h, No. (%)	10 (25)
Renal stones, No. (%)	10 (25%)
Osteoporosis, No. (%)	26 (65%)
Osteoporotic fractures, No. (%)	6 (15%)

Abbreviations: Ca(s), serum calcium; Ca(u), urinary calcium; ULN, upper limit of normal.
[a]Surgical indications defined in accordance with an international workshop consensus statement [6].

Table 3. p. Tyr394Ser variant patients

Patient	Sex	Ca(s)	PTH × ULN	Ca(u), mg/24 h	Familial PHPT	Renal stones	Osteoporosis	No. of adenomas	Adenoma size, cm
1	Male	10.8	0.97	375	Positive	No	Yes	1	2
2	Male	12.3	2.8	831	Negative	Yes	Yes	2	2

Abbreviations: Ca, calcium; PHPT, primary hyperparathyroidism; PTH, parathyroid; ULN, upper limit of normal.

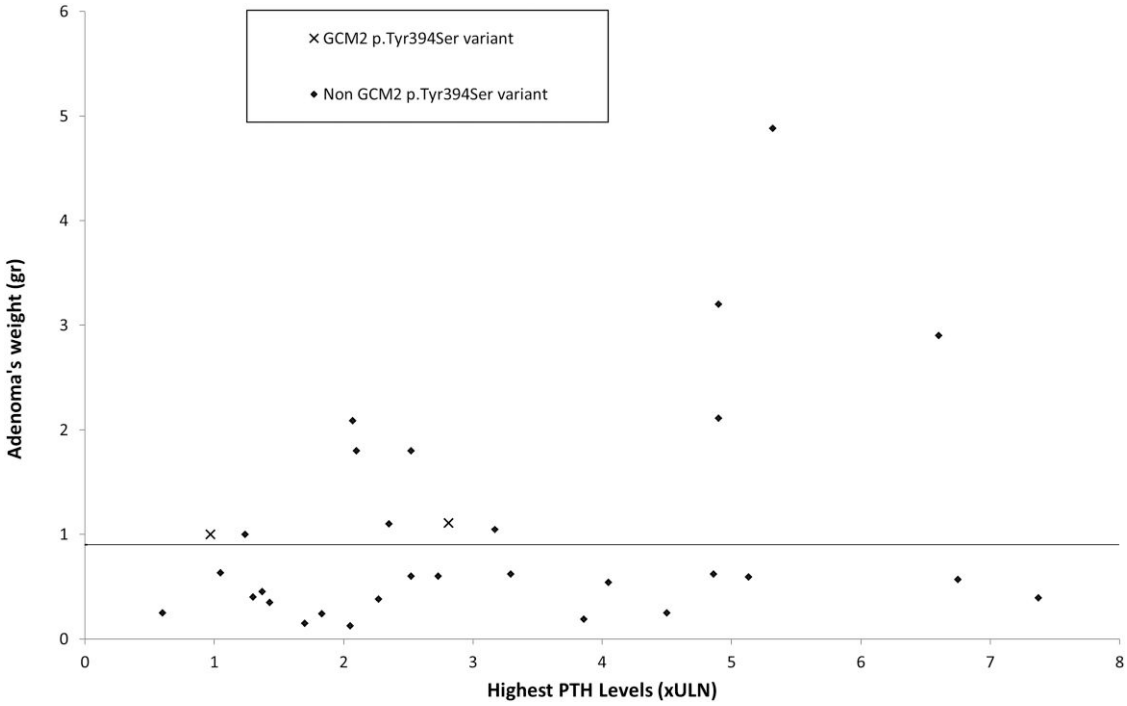

Figure 2. The distribution of 40 primary hyperparathyroidism (PHPT) patients by parathyroid hormone (PTH) levels and size of extracted adenomas.

of anonymous individuals who volunteered to be part of a genetic database.

Results

Forty AJ patients with PHPT and 200 AJ controls underwent the detection of the variant p.Tyr394Ser in the *GCM2* gene. Our genetic testing identified 2 (5%) heterozygotes with the *GCM2* p.Tyr394Ser variant among the PHPT patients; all the other participants carried the wild type. Among the 200 controls, 3 individuals (1.5%) were heterozygotes.

Of the 40 PHPT patients, 32 (80%) were female and the average age at evaluation was 66 years. All the patients were of AJ ethnicity by self-report. Thirty-six patients reported having 4 AJ grandparents, 1 patient reported having 2 AJ grandparents, and 3 patients did not give a definite figure. Five (12.5%) patients reported having at least one first-degree relative with diagnosed PHPT. The average maximal serum Ca level was 11.2 mg/dL and the average urinary Ca was 388 mg/24 hours, although this parameter was available for only 23 (57%) patients (Table 1). PTH levels were elevated in the majority of the patients, while in 3 patients they were in the high-normal level. Thirty-four (85%) patients had at least one indication for surgical treatment (Table 2). Thirty-seven parathyroid glands were excised from 34 patients. Six patients did not undergo surgery. Thirty-two patients had adenomas, whereas in 2 patients the diagnosis was hyperplasia. Of the patients with adenomas, 30 patients had a single adenoma and 2 patients had 2 adenomas. The average size (largest dimension) of the excised glands was 1.79 cm (0.3-4 cm) and the median weight was 0.59 g (0.12-4.8 g).

One of the two PHPT heterozygotes had familial PHPT. The other heterozygote had 2 parathyroid adenomas, and the levels of his blood and urinary Ca were extremely high (Table 3). In both cases, the excised glands weighed more than 0.9 g, above the median weight in our cohort. The level of PTH did not differentiate the variant carriers from wild-type cases (Fig. 2).

Discussion

Previous studies found several *GCM2* gene-activating mutations in sporadic and familial PHPT in various ethnicities [11-13]. One study found 2 recurrent *GCM2* variants (p.[Gln251Glu; Leu379Gln] and Tyr394Ser) in 7 (18%) of 40 familial isolated hyperparathyroidism–affected kinders [11]. The aim of this study was to evaluate the prevalence of the p.Tyr394Ser variant among our PHPT AJ patients and to determine whether genetic testing for this variant should be considered.

The incentive for our study was a previous study by Guan et al [13] that found a prevalence of 41% and 27% in familial and sporadic PHPT respectively among AJ patients. Our results differ substantially, with a significantly lower incidence of the variant; only 2 (5%) of our patients were carriers of the variant. Our patients were mostly (87.5%) sporadic cases. However, the prevalence of the genetic variant was much lower than that of the sporadic patients in Guan's study. It should be noted that in Guan's study, 19 of 52 (36.5%) of the sporadic cases had multiglandular disease, whereas in our study only 4 of 34 (11.7%) had multiglandular disease. The difference may be explained by the fact that the previous study was performed at a referral center. Thus, their cohort may be enriched with more aggressive disease, as elucidated by the authors [13]. The authors further note that when assessing the patients with sporadic, single-gland disease without persistent or recurrent disease, only 2 of 16 (12.5%) patients carried the p.Tyr394Ser variant. Our study included 40 individuals and suggests an even lower prevalence. In Guan's study, 7 of 17 (41%) of the AJ with familial PHPT were carriers of the p.Tyr394Ser variant; other studies also suggest an overrepresentation of various variants in the *GCM2* CCID, including the p.Tyr394Ser variant among multiglandular or familial PHPT patients. However, low penetrance has been demonstrated, questioning the clinical significance of genetic testing for these variants [15].

Our study has several limitations. Our cohort included only 5 patients with familial PHPT and only 4 with multiglandular disease; therefore, it is difficult to conclude as to the prevalence of the genetic variant in these groups. Other limitations of our study are that we lack information as to recurrent and persistent disease among the patients and that the only clinical information we have regarding the controls is that they are of AJ origin. The 3 heterozygotes in the control group may or may not have PHPT. However, the low prevalence both in patients and controls supports our conclusion.

In summary, our study suggests that among AJ with sporadic, single-gland PHPT, the prevalence of the *GCM2* p.Tyr394Ser variant is very low and routine genetic testing is not advisable. Further studies focused on PHPT patients with multiglandular or familial disease may assist to clarify the role of this variant.

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Disclosures

The authors have no conflicts of interest to disclose.

Data Availability

Some or all data sets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

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