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Identification of 4 New Loci Associated With Primary Hyperparathyroidism (PHPT) and a Polygenic Risk Score for PHPT

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Abstract

Context: A hypothesis-free genetic association analysis has not been reported for patients with primary hyperparathyroidism (PHPT).

Objective: We aimed to investigate genetic associations with PHPT using both genome-wide association study (GWAS) and candidate gene approaches.

Methods: A cross-sectional study was conducted among patients of European White ethnicity recruited in Tayside (Scotland, UK). Electronic medical records were used to identify PHPT cases and controls, and linked to genetic biobank data. Genetic associations were performed by logistic regression models and odds ratios (ORs). The combined effect of the genotypes was researched by genetic risk score (GRS) analysis.

Results: We identified 15 622 individuals for the GWAS that yielded 34 top single-nucleotide variations (formerly single-nucleotide polymorphisms), and *LPAR3-rs147672681* reached genome-wide statistical significance (P = 1.2e-08). Using a more restricted PHPT definition, 8722 individuals with data on the GWAS-identified loci were found. Age- and sex-adjusted ORs for the effect alleles of *SOX9-rs11656269*, *SLITRK5-rs185436526*, and *BCDIN3D-AS1-rs2045094* showed statistically significant increased risks (P < 1.5e-03). GRS analysis of 5482 individuals showed an OR of 2.51 (P = 1.6e-04), 3.78 (P = 4.0e-08), and 7.71 (P = 5.3e-17) for the second, third, and fourth quartiles, respectively, compared to the first, and there was a statistically significant linear trend across quartiles (P < 1.0e-04). Results were similar when stratifying by sex.

Conclusion: Using genetic loci discovered in a GWAS of PHPT carried out in a Scottish population, this study suggests new evidence for the involvement of genetic variants at *SOX9*, *SLITRK5*, *LPAR3*, and *BCDIN3D-AS1*. It also suggests that male and female carriers of greater numbers of PHPT-risk alleles both have a statistically significant increased risk of PHPT.

Key Words: primary hyperparathyroidism, genetics, genome-wide association study, polygenic risk score

Abbreviations: *CASR*, calcium-sensing receptor; CCA, corrected calcium; FHH, familial hypocalciuric hypercalcemia; GoDARTS, Genetics of Diabetes and Audit Research Tayside Study; GoSHARE, Genetics of Scottish Health Research register; GRS, genetic risk score; GWAS, genome-wide association study; ICD, International Classification of Diseases; OR, odds ratio; PHPT, primary hyperparathyroidism; PTH, parathyroid hormone; SNV, single-nucleotide variation.

Primary hyperparathyroidism (PHPT) is a commonly encountered endocrine disorder with an estimated prevalence of approximately 0.5% to 1%, which increases with age (1–3). PHPT typically manifests with a raised serum calcium concentration in association with an inappropriate elevation in serum parathyroid hormone (PTH) and usually results from benign parathyroid adenomas (85% of cases) or parathyroid gland hyperplasia (10%-15% of cases), and rarely from parathyroid carcinoma (< 1% of cases). In many instances PHPT is mild and patients are frequently asymptomatic, so many cases can go undiagnosed and untreated, or revealed only incidentally on routine biochemical testing. However, PHPT may be associated with significant morbidity, which includes osteoporosis, renal stones, and nephrocalcinosis (3). Furthermore, previous epidemiological studies of patients with PHPT have indicated there may be an increased all-cause and cardiovascular mortality (3–5).

While the majority of patients presenting with PHPT have a nonfamilial (ie, sporadic) etiology, up to 10% of cases occur as part of a hereditary disorder, either as part of a wider endocrine syndrome or as an isolated endocrinopathy (6). In contrast, familial isolated hyperparathyroidism describes the occurrence of hereditary PHPT as a sole endocrinopathy and 15% to 20% of such kindreds harbor activating mutations in the *GCM2* gene but the majority are genetically undefined (7, 8). Another disorder of calcium metabolism,

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namely familial hypocalciuric hypercalcemia (FHH), is associated with inactivating mutations of the calcium-sensing receptor (*CASR*) in approximately 65% of cases and with variants in either the *GNA11* or *AP2S1* genes accounting for the majority of remaining cases (9).

Somatic inactivation and/or loss of heterozygosity of the *MEN1* locus is observed in 20% to 40% of sporadic tumors, while whole-exome sequencing studies of parathyroid adenomas reveal recurrent somatic *MEN1* mutations in 20% to 30% of tumors (10, 11). Loss of function in *CDC73* and *CDKN* have been implicated in sporadic tumors, while other candidate driver genes have also been implicated in parathyroid tumorigenesis such as the *EZH2* and *ZFX* genes, although variants in such genes are observed at low frequency (12).

Despite progress in understanding the hereditary basis of familial PHPT and knowledge of some of the somatic alterations present in parathyroid tumors, there is a paucity of larger studies investigating whether additional germline genetic variations may contribute to the risk of sporadic PHPT at a population level. Prior GWAS studies evaluating determinants of serum PTH concentration have been reported and identified several potential associated loci, which include those in the vicinity of the *CYP24A1*, *CASR RGS14*, *CLDN14*, *RTDR1*, *RASGEF1*, and *DPP10* genes (13, 14). Notably, of these genes, *CYP24A1*, a member of the cytochrome P450 family responsible for inactivation of vitamin D, and *CASR*, are directly implicated in calcium/PTH homeostasis. However, previous candidate association studies evaluating variants in genes relevant to calcium homeostasis (eg, vitamin D metabolism, *CASR*), have demonstrated no consistent genetic contribution to PHPT disease risk. To our knowledge a hypothesis-free genome-wide analysis has not been reported for a population PHPT cohort. Thus, in this study, we aimed to investigate genetic associations with PHPT using both GWAS and candidate gene approaches.

Materials and Methods

Discovery Cohort

A cross-sectional study was conducted among individuals older than 18 years from the Genetics of Scottish Health Research register (GoSHARE) and the Genetics of Diabetes and Audit Research Tayside Study (GoDARTS) recruited in Tayside, Scotland (UK). All individuals in these populations are of White ethnicity and have been described previously (15, 16). Electronic medical records (biochemistry, prescribing, hospital admissions, and demographics) were used to ascertain people with PHPT, and were anonymously linked to genetic biobank data by the Health Informatics Centre of the University of Dundee (http://www.dundee.ac.uk/hic). The same data linkage procedure and phenotype definition criteria were applied to these datasets.

Phenotype Definition

Patients with at least 2 recordings of serum-corrected calcium (CCA) greater than 2.55 mmol/L at least 1 year apart, who had a record of PHPT from the International Classification of Diseases (ICD) 10th revision codes (ICD10: E21.0, E21.2, E21.3), and/or who had parathyroid surgery from the Operations and Procedures Codes 4 (OPCS-4: B14, B16, Z13.5) during the study period (1994-2018) were defined as being PHPT case patients. Patients with FHH from ICD codes (ICD10: E83.5) and/or tertiary hyperparathyroidism identified from having an estimated glomerular filtration rate less than or equal to 30 mL/min 36 months before or within 6 months after first raised serum-CCA were excluded. The median serum CCA recorded throughout the study period for each patient was estimated, and control individuals had an average serum CCA concentration of 2.1 to 2.55 mmol/L and never had a record of low or high CCA. A locally determined formula for serum CCA was used: CCA mmol/L = total calcium mmol/L + (0.012 × [39.9 – albumin g/L]), where 39.9 was the Tayside mean serum albumin.

Genetic Data

Genotype data was available from 4 platforms: Illumina HumanOmni Express -12VI platform (Illumina), Affymetrix 6.0 platform (Affymetrix), Illumina Infinium custom GWAS chip (Illumina), and the Global Screening Arrays (GSA) version 2 (Illumina). Imputation of nongenotyped single-nucleotide variations (SNVs, formerly known as single-nucleotide polymorphisms [SNP]) was performed using IMPUTE2 and MINIMAC4 against the haplotype reference consortium (17); calls made with an imputation score of less than 95% were discarded.

Genome-wide Association Study

Standard pre–genome-wide association study (GWAS) quality control was performed (18). Filters were applied to include variants with minor allele frequency greater than 1%. Linkage disequilibrium pruning and concordance between clinically recorded and genetically determined sex was checked. A Hardy-Weinberg equilibrium threshold of *P* greater than 10e-10 was used for cases and *P* greater than 10e-6 for controls. GWAS were performed using Plink 2.0. Binary logit models of the outcome of PHPT were compared to population/unselected controls. The model used was PHPT (case vs control) ~ SNVs + age + age^2 + sex + principal components (1–10). GWAS were run on samples of the platforms described previously, and a meta-GWAS across the platforms was also performed using Plink 2.0.

Genetic Association Study on Selected Single-Nucleotide Variations

This study was carried out using the top single SNVs at *P* less than 5.0e-06 identified from the GWAS (ie, candidate genes). This analysis was performed using a more restricted phenotype definition by which patients had 2 raised CCAs at least 1 year apart with a maximum serum PTH greater than 3 pmol/L and/or a 24-hour urinary excretion greater than 7 mmol/day to be considered as cases by biochemistry. Genetic tests of association were performed by logistic regression models. The combined effect of the genotypes was researched by genetic risk score (GRS) analysis using an unweighted sum of PHPT increasing alleles across the selected SNVs available in our cohort. Participants missing more than 2 of these SNVs were excluded from the analyses. Odds ratios (ORs) from logistic models were also adjusted for age at first CCA recording and sex. Linking correlated alleles were annotated using the LDlink database with the default search options (19). All statistical analyses were conducted using STATA/MP, version 15.1 software (StataCorp).

Results

We identified 88 752 individuals as being eligible for the GWAS after excluding those younger than 18 years and those with FHH or tertiary hyperparathyroidism, of whom 15 622 (778 case patients and 14 844 controls) had available genetic data in the GoDARTS/GoSHARE biobank. PHPT cases were more likely to be female (64.4 vs 45.8%), older (77.1 vs 72.1 years), with diabetes mellitus (80.1 vs 55.8%), and had a higher average serum CCA (2.6 vs 2.3 mmol/L) concentrations than controls ($P < 1.0e-03$). The GWAS included age, sex, and 2 principal components as covariates to adjust for possible population stratification, which provided an acceptable control; the genomic inflation factor lambda (λ) was 1.03. It yielded 34 top single SNVs at P less than 5.0e-06, but only *LPAR3-rs147672681* at chromosome 1 reached genome-wide statistical significance ($P < 5.0e-08$) (Supplementary Table 1) (20). Supplementary Fig. 1 shows a Manhattan plot of the tested SNVs, and the quantile-quantile plot is shown in Supplementary Fig. 2 (20). All SNVs were in Hardy-Weinberg equilibrium ($P < 10e-04$).

The use of a more restricted phenotype definition identified a study cohort of 8722 individuals (307 case patients and 8415 controls) with available genetic data on the selected SNVs from the GWAS (Fig. 1). PHPT case patients were more likely to be female (65.2 vs 44.2%; $P < 1.0e-03$), had a higher average serum PTH (9.4 vs 6.5 pmol/L; $P < 1.0e-03$) and CCA (2.6 vs 2.3 mmol/L; $P < 1.0e-03$) concentrations than controls (Table 1).

Table 2 shows the results of the genetic association study of the selected 34 genetic variants. An association was found for 19 noncoding SNVs, with 4 reaching the Bonferroni statistical significance threshold at 1.5e-03 (ie, 0.05/34). The nearest genes to these 4 loci were *SOX9* (SRY-box transcription factor 9), *SLITRK5* (SLIT and NTRK-like family member 5), *LPAR3* (lysophosphatidic acid receptor 3), and *BCDIN3D-AS1* (BCDIN3D antisense RNA 1). Further adjustment of logistic regression models for age and sex did not change the size and direction of the effect estimates. Age- and sex-adjusted ORs for the effect alleles of *SOX9-rs11656269* (OR = 2.12; 95% CI, 1.56-4.04; P = 8.7e-05), *SLITRK5-rs185436526* (OR = 3.09; 95% CI, 1.73-5.53; P = 1.4e-04), *LPAR3-rs147672681* (OR = 2.05; 95% CI, 1.35-3.11; P = 7.0e-04), and *BCDIN3D-AS1-rs2045094* (OR = 1.51; 95% CI, 1.18-1.94; P = 8.5e-04) showed increased risks for PHPT.

When these 19 SNVs were combined into a GRS using an unweighted sum of PHPT-risk alleles across the selected SNVs available (ie, PHPT-GRS), there was a statistically significant association with the risk of PHPT in 5482 individuals (238 case patients and 5244 controls) after excluding those individuals missing more than 2 of these SNVs. An OR of 2.51 (95% CI, 1.56-4.04; P = 1.6e-04), 3.78 (95% CI, 2.35-6.07; P = 4.0e-08), and 7.71 (95% CI, 4.78-12.43; P = 5.3e-17) for the second, third, and fourth quartiles, respectively, compared to the first quartile of PHPT-GRS scores were found. There was a highly significant linear trend across quartiles of PHPT-GRS scores (chi-square for linear trend extended Mantel-Haenszel test = 98.9; $P < 1.0e-04$) as shown in Fig. 2. Stratifying by sex, these results were similar for men (n = 3089) and women (n = 2393) (see Supplementary Table 2) (20). Carriers of greater numbers of PHPT-risk alleles were associated with a statistically significant increased risk of PHPT.

Discussion

This record-linkage study used electronic databases to investigate genetic associations with PHPT using genetic loci discovered in a GWAS within a Scottish population. We identified 4 loci located in chromosomes 1, 12, 13, and 17 (mapped genes *LPAR3*, *BCDIN3D-AS1*, *SLITRK5*, and *SOX9*, respectively), and by using a GRS this study showed that carriers of greater numbers of PHPT-risk alleles were associated with a statistically significant increased risk of PHPT.

GoDARTS/GoSHARE databases are longitudinal cohorts and thus more than one biochemical measurement (ie, serum CCA, PTH, 24-hour urinary excretion) was available for the majority of participants. This allowed the use of biochemistry records to identify patients with at least 2 raised serum CCAs apart from each other for more than 1 year but exclude those having an estimated glomerular filtration rate less than or equal to 30 mL/min within 36 months before or 6 months after the first raised serum CCA (ie, tertiary hyperparathyroidism). The application of these criteria confirmed the identification of cases. Unfortunately, data on serum vitamin D were not available at the time of this study.

Population stratification is always a threat to the validity of a GWAS. The best way to avoid this bias is ensuring the study cohort is derived from a genetically homogeneous population. A genomic inflation factor value close to unity (ie, λ = 1.03) reflects no evidence of population stratification in our GWAS (21). GWAS is an approach used to investigate genetic variants spanning the entire genome and has several limitations such as the small effect sizes of most associations (22). By further limiting the analysis to a few numbers of selected SNVs (ie, candidate genes), it is possible to increase the statistical power to detect statistically significant effects. Thus, after running the GWAS, we performed a genetic association study with selected top single SNVs from the GWAS and a more restricted PHPT definition by considering serum PTH concentration and urine calcium excretion in addition to serum CCA to identify cases. This analysis contributed also to assessing the robustness of our GWAS findings by examining the extent to which results are affected by changes in phenotype definition. This approach confirmed the association of *LPAR3* and revealed 3 additional loci that had been suggestive in the GWAS (ie, *BCDIN3D-AS1*, *SLITRK5*, and *SOX9*).

Complications of PHPT are represented by skeletal, kidney, and/or gastrointestinal involvement (23). The present study identified 4 loci mainly related to the skeleton (ie, calcium mobilization, osteoblasts, chondrocytes, and cranial vault traits), and thus relevant to calcium metabolism. *SOX9* encodes a transcription factor that plays key roles during embryonic development both in skeletal development and sex determination. In particular, *SOX9* is required for chondrocyte differentiation and cartilage formation. It negatively regulates maturation and calcification of chondrocytes through upregulation of PTH-related protein (24, 25). More recently, *SOX9* has been demonstrated to be required postnatally to prevent growth-plate closure (26). A GWAS meta-analysis on vault measures in a sample of 4419 healthy individuals of European ancestry found a significant association at *SOX9* (27). Thus, *SOX9* is implicated in many functions related to bone and cartilage physiology, although how this might influence serum calcium and/or parathyroid function remains to be determined. Little is known about the *rs2045094* variant that occurs in the antisense transcript of *BCDIN3D*, a highly

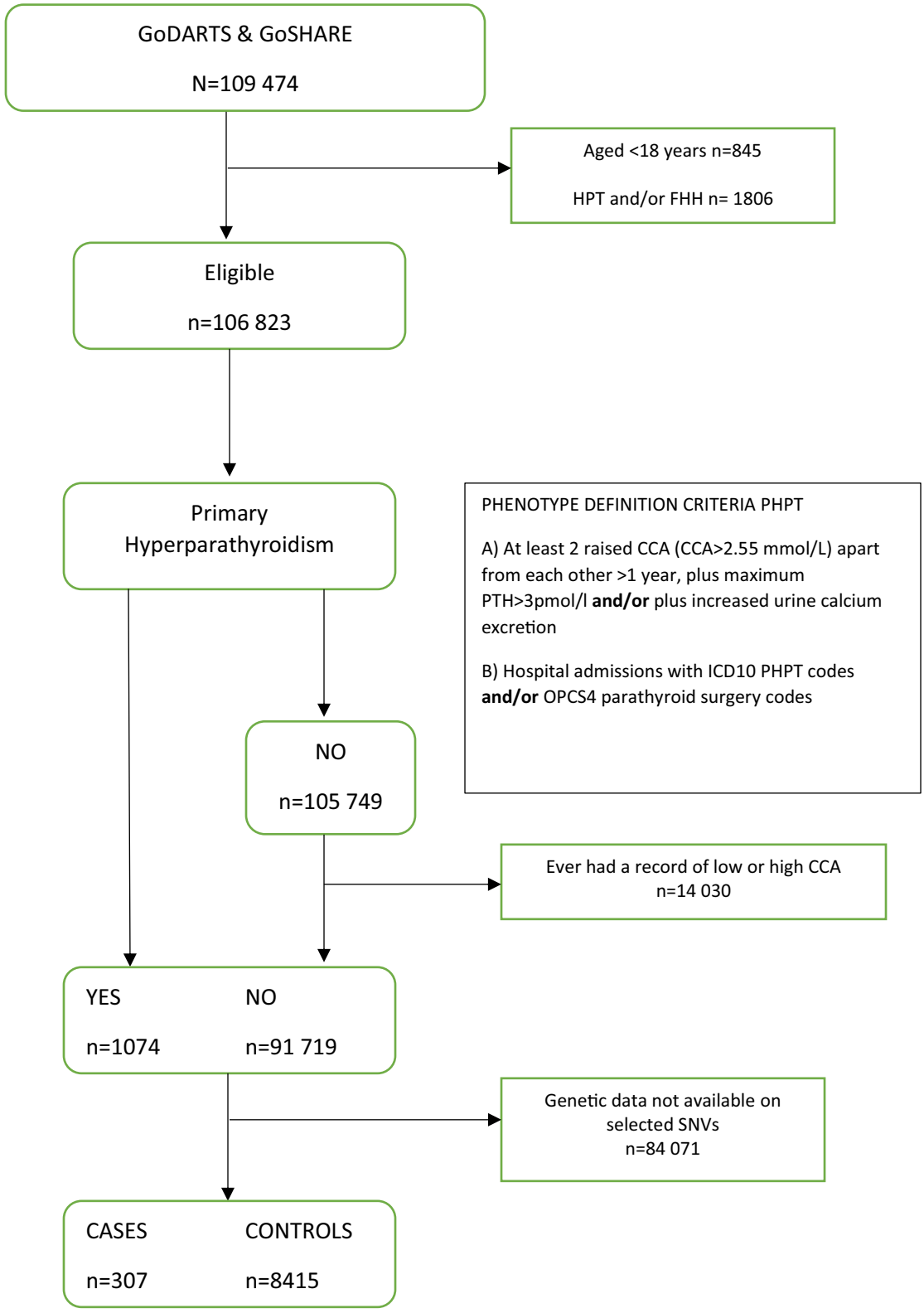

Figure 1. Flowchart describing the study cohort's generation process and the patients included in the genetic association study of the selected genome-wide association study–identified loci. CCA, serum corrected calcium; FHH, familial hypocalciuric hypercalcemia; HPT, tertiary hyperparathyroidism; ICD10, International Classification of Diseases, 10th revision; OPCS4, Classification of Surgical Operations and Procedures version 4; PHPT, primary hyperparathyroidism; PTH, parathyroid hormone.

conserved member of the Bin3 methyltransferase family involved in methylation of cytoplasmic transfer RNA (28). The physiological role of this protein is not well defined although overexpression of *BCDIN3D* is reported in breast cancer, where it is associated with a tumorigenic phenotype and poor prognosis. The further 2 variants occur in proximity

Table 1. Description of genotyped patients with primary hyperparathyroidism (cases) and their comparison controls (n = 8722)

Characteristic	Cases (n = 307)	Controls (n = 8415)	*P*
n (%)			
Female sex	200 (65.2)	3722 (44.2)	< .001
Diabetes mellitus	252 (82.1)	4960 (58.9)	< .001
Mean (SD)			
Age, y	77.5 (10.1)	74.3 (10.7)	< .001
Serum-corrected calcium, mmol/L[a]	2.6 (2.5-2.8)	2.3 (2.2-2.4)	< .001
Serum PTH, pmol/L[a,b]	9.4 (5.6-15.8)	6.5 (3.7-11.6)	< .001

Abbreviation: PTH, parathyroid hormone.
[a]Median (interquartile range) of measurements recorded throughout the study period.
[b]Sample size for serum PTH (n = 873; 299 case patients and 574 comparison cohort).

to *SLITRK5* and *LPAR3*, respectively. *SLITRK5* encodes a transmembrane protein selectively expressed in osteoblasts and acts as a negative regulator of hedgehog signaling that may act to inhibit osteoblast differentiation (29). The *LPAR3* gene encodes a G protein–coupled receptor that functions as a receptor for lysophosphatidic acid and mediates lysophosphatidic acid-evoked calcium mobilization, although any relationship to parathyroid biology is unknown. However, *LPAR3* is also reported to harbor potential tumorigenic properties (30).

There is a longstanding controversy as to whether serum measurements of total calcium should be adjusted for albumin concentration, and if so which formula is the most appropriate. Locally determined formulas (ie, like ours) usually perform better than formulas taken from the literature. Pekar et al (31) reported that when serum total calcium and CCA were compared to ionized calcium results, the outcomes were very similar for those with normal albumin levels, which relates to the vast majority of our patient cohort. The authors also indicated that, compared to total calcium, CCA tended to overestimate the calcium state for patients with hypoalbuminemia. If so, it is possible that CCA misclassified some patients with low albumin levels, but there is no reason to believe that the probability of patients being misclassified occurred differently across case patients and controls in this study (ie, nondifferential misclassification). Nondifferential misclassification will generally bias an effect estimate toward the null. In other words, our genetic effects of association with PHPT might be closer to the null than it would be were there no misclassification at all. In addition, because our algorithm for patient identification made use of longitudinal data (ie, > one biochemical measurement for each patient over time), the effect of any potential misclassification of the outcome due to the calcium state would be much lower than using cross-sectional data (ie, just one measurement for each patient). Although this is unlikely to affect the findings of this study as most patients had normal serum albumin, we recognize that ionized calcium is the best test to performed, but that in most clinical settings it is not practical yet.

We acknowledge that an independent cohort was not included in this study for replication because longitudinal biochemistry data (ie, > one biochemical measurement for individuals over time) from a different population was not available to apply our criteria for identification of case

Table 2. Association of single-nucleotide variations with primary hyperparathyroidism at a statistical significance level of *P* less than 5.0e-02

SNV	CHR	Position	Gene	A1/A	MAF	Cases	Controls	OR (95% CI)[a]	*P*	OR (95% CI)[b]	*P*
rs11656269	**17**	**70125348**	***SOX9***	**C/T**	**0.0371**	**221**	**4814**	**2.13 (1.47-3.10)**	**7.2e-05[c]**	**2.12 (1.46-3.09)**	**8.7e-05[c]**
rs185436526	**13**	**88808800**	***SLITRK5***	**C/T**	**0.0107**	**244**	**5283**	**3.15 (1.77-5.60)**	**8.9e-05[c]**	**3.09 (1.73-5.53)**	**1.4e-04[c]**
rs147672681	**1**	**85353665**	***LPAR3***	**G/A**	**0.0311**	**244**	**5300**	**1.92 (1.27-2.89)**	**1.9e-03**	**2.05 (1.35-3.11)**	**7.0e-04[c]**
rs2045094	**12**	**50229509**	***BCDIN3D-AS1***	**G/T**	**0.1298**	**238**	**5201**	**1.52 (1.19-1.94)**	**7.4e-04[c]**	**1.51 (1.18-1.94)**	**8.5e-04[c]**
rs35928058	6	124221344	*NKAIN2*	T/C	0.0137	237	5202	2.28 (1.29-4.03)	4.2e-03	2.49 (1.41-4.27)	1.7e-03
rs145504087	2	197905320	*ANKRD44*	G/C	0.0258	236	5182	2.02 (1.29-3.17)	2.0e-03	2.00 (1.27-3.15)	2.6e-03
rs62282716	3	150633140	*CLRN1-AS1*	A/G	0.0126	231	5082	2.41 (1.36-4.28)	2.6e-03	2.43 (1.36-4.34)	2.7e-03
rs138472039	4	168206250	*LINC02174*	C/G	0.0145	244	5305	2.38 (1.37-4.12)	1.9e-03	2.29 (1.32-3.99)	3.2e-03
rs140216208	5	75841080	*IQGAP2*	T/G	0.0149	237	5185	2.13 (1.22-3.73)	7.7e-03	2.33 (1.32-4.11)	3.3e-03
rs45618533	1	74847747	*FPGT-TNNI3K*	A/G	0.0711	213	4648	1.60 (1.16-2.19)	3.5e-03	1.57 (1.15-2.16)	4.7e-03
rs345322	1	108412324	*VAV3*	A/G	0.0293	242	5220	1.61 (1.23-2.95)	3.6e-03	1.85 (1.19-2.88)	5.7e-03
rs113075488	2	4501263	*NPM1P48*	T/C	0.0252	240	5257	1.77 (1.12-2.81)	1.4e-02	1.82 (1.15-2.90)	1.1e-02
rs183670739	8	112729353	*RP11-58O3.2*	G/T	0.0114	242	5326	2.13 (1.14-3.98)	1.8e-02	2.24 (1.19-4.21)	1.2e-02
rs11168417	12	48515720	*PFKM*	T/C	0.1463	243	5353	1.33 (1.05-1.69)	1.8e-02	1.34 (1.06-1.71)	1.5e-02
rs182479997	6	147801672	*RP11-15G8.1*	G/T	0.0138	241	5286	2.05 (1.15-3.65)	1.4e-02	2.04 (1.14-3.66)	1.6e-02
rs113622331	8	141520126	*CHRAC1*	A/T	0.0188	234	5041	1.92 (1.12-3.29)	1.8e-02	1.85 (1.07-3.20)	2.6e-02
rs79264750	7	46959622	*AC004901.1*	A/T	0.0159	243	5226	1.93 (1.08-3.47)	2.6e-02	1.88 (1.05-3.38)	3.5e-02
rs148035035	6	21705164	*CASC15*	A/G	0.0165	240	5260	1.86 (1.06-3.27)	2.9e-02	1.82 (1.03-3.21)	3.7e-02
rs77959286	10	55934767	*PCDH15*	C/T	0.0227	246	5353	1.59 (0.97-2.59)	6.4e-02	1.64 (1.01-2.70)	4.9e-02

Abbreviations: A1, coded allele; CHR, chromosome; MAF, minor allele frequency; OR, odds ratio; SNV, single-nucleotide variation.
[a]Univariate logistic regression models.
[b]Age- and sex-adjusted logistic regression models.
[c]SNVs reaching the Bonferroni statistical significance threshold at $\alpha = 0.05/34 = 1.5e\text{-}03$.

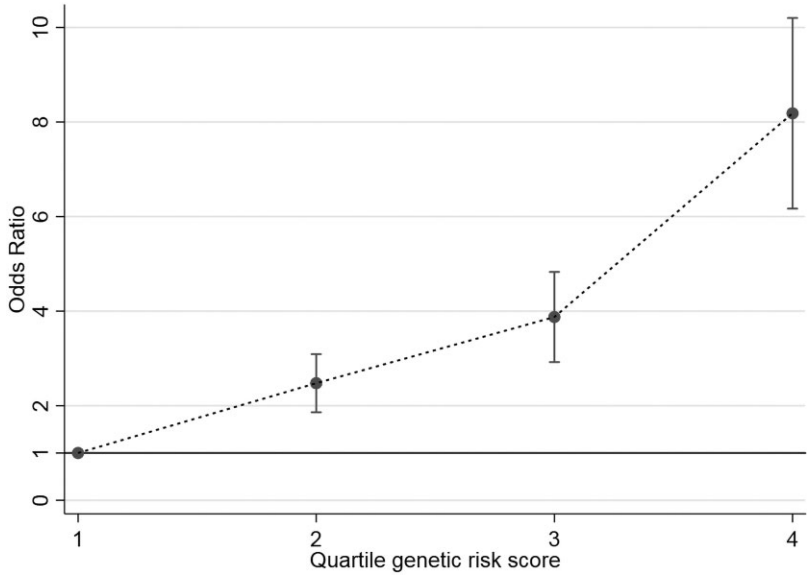

Figure 2. Association of primary hyperparathyroidism (PHPT)-based genetic risk score (GRS) with the risk of developing PHPT (n = 5482). Odds ratios (± SE) for quartiles of the GRS. Chi-square for linear trend (extended Mantel-Haenszel test) = 98.92 ($P < 5.0e-04$).

patients and controls. As expected, a phenome-wide association study on hyperparathyroidism and other disorders of the parathyroid gland (ICD10: E21.0-E21.5) using UK Biobank cross-sectional data (GeneATLAS database, http://geneatlas.roslin.ed.ac.uk) did not replicate any of our top signals (32). Important differences in study design (phenome-wide association study vs GWAS), type of data (cross-sectional vs longitudinal data) and phenotype definition (any hyperparathyroidism and other disorders of parathyroid gland vs PHPT) may have accounted for that (33). Failure to replicate an independent genetic effect may also provide important information about the complexity of the underlying genetic architecture (34).

We also acknowledge that a weighted GRS, defined as weighted sums of risk alleles of SNVs, is a more powerful alternative to a single SNV approach or the use of unweighted GRS for detection of genetic associations. However, appropriate external weights from an independent study must be available. Thus, we made use of an unweighted GRS because no external weights were available because of the novelty of this study. This novel GRS was statistically significantly associated with the risk of PHPT, and there was a highly significant linear trend across quartiles of PHPT-GRS scores that was similar for men and women.

In conclusion, this record-linkage study used electronic databases to investigate hypothesis-free genetic associations with PHPT, using genetic loci discovered in a GWAS carried out in a Scottish population. We used a new approach to define PHPT cases, based on routine biochemistry data and hospital admissions, to achieve a reasonably representative phenotype of this disease. This study suggests new evidence for the involvement of genetic variants in the vicinity of *SOX9*, *SLITRK5*, *LPAR3*, and *BCDIN3D-AS1*. It also suggests that carriers of greater numbers of PHPT-risk alleles are associated with a statistically significant increased risk of PHPT in men and women. Our results need to be verified in an independent cohort and at the molecular level to confirm hypothesized pathways of PHPT mechanisms.

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All analyses were performed on anonymized data sets. This study was approved by the East of Scotland Research Ethics Service–EoSRES (HIC data sets V2, REC ref. 18/ES/0126, IRAS ID 143637), and informed consent was obtained for all participants.

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Author Contributions

E.S.P. researched/analyzed data and wrote the manuscript; P.J.N. planned the study, researched data, and wrote the manuscript; S.S. analyzed data and wrote the manuscript; M.K.S. researched data and wrote the manuscript; C.N.P. contributed to data analysis and to discussion; and G.P.L planned the study, researched data, contributed to the discussion, and reviewed/edited the manuscript.

Disclosures

The authors have nothing to disclose.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Data Availability

These are consented data and because of their sensitive nature are stored in secure computing environments. Data can be shared based on specific requests but as such are not publicly available.

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