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Original article

A pilot study of the association of rs6127099 polymorphism with primary hyperparathyroidism

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Abstract

Primary hyperparathyroidism (PHPT) is the third most common endocrine disorder characterized by autonomous parathyroid hormone (PTH) secretion by one or more parathyroid glands and increased serum calcium concentration. A recent genome wide association study showed that the rs6127099 polymorphism, which is located upstream the CYP24A1 (Cytochrome P450, family 24, subfamily A, polypeptide 1) gene, is associated strongly with elevated serum PTH levels. CYP24A1 gene encodes an enzyme of cytochrome P450, which is responsible for inactivating vitamin D metabolites. As PTH hypersecretion is a common clinical sign of PHPT, the aim of the present study was to investigate the role of the polymorphism rs6127099 as a genetic predisposing factor for PHPT manifestation. Thirty-nine unrelated patients with sporadic PHPT and an equal number of healthy volunteers were enrolled in the study. Polymerase chain reaction and restriction fragment length polymorphism assays were used for rs6127099 genotyping in both groups. No statistically significant difference was observed comparing CYP24A1 rs6127099 A>T genotypes ($p = 0.836$) and A vs T allele ($p = 0.383$) distribution between PHPT patients and controls. In conclusion, rs6127099 polymorphism seems not to be associated with PHPT predisposition. Further independent studies, as the present one, are necessary to evaluate the strong association of rs6127099 polymorphism with PTH levels and its prognostic role in PHPT predisposition.

Key words: Primary Hyperparathyroidism, Parathyroid hormone, PHPT, PTH, CYP24A1, polymorphism.

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Abbreviations

PHPT: Primary Hyperparathyroidism

PTH: Parathyroid hormone

CYP24A1: Cytochrome P450, family 24, subfamily A, polypeptide 1

Introduction

Parathyroid hormone (PTH) is secreted by parathyroid glands and it is the key calcium regulatory hormone in humans. The physiologic role of PTH is to maintain serum calcium concentration in blood by increasing bone resorption, enhancing calcium reabsorption in kidneys and stimulating the synthesis of the active vitamin D –(1,25-dihydroxyvitamin D3) from the inactive form (25-hydroxyvitamin D3). Active vitamin D acts on kidneys and intestines to enhance calcium reabsorption and absorption, respectively. Subsequently, the restored serum Ca^{2+} provides a negative feedback signal to the parathyroid glands, discontinuing the release of PTH (Khundmiri, Murray and Lederer, 2016; Jeon, 2018).

Primary Hyperparathyroidism (PHPT) is an endocrine disorder characterized by autonomous production of parathyroid hormone (PTH) from one or more parathyroid gland(s) and among others is presented with hypercalcemia. It is the third most common endocrine clinical disorder with a prevalence between 0.1-1.0% and an increasing incidence with age which peaks between 50 and 60 year olds (Gopinath and Mihai, 2011; MacKenzie-Feder *et al.*, 2011; Madkhali *et al.*, 2016). The vast majority of cases (90-95%) are sporadic and PHPT is caused from a single parathyroid adenoma in most cases (80-85%) and rarely from multiglandular parathyroid hyperplasia (<15%) and parathyroid carcinoma (<1%) (Pallan, Rahman and Khan, 2012; Madkhali *et al.*, 2016). Approximately 5% of cases of PHPT are familial, associated with genetic syndromes such as multiple endocrine neoplasia (MEN) type 1, 2A or 4 and hyperparathyroidism-jaw tumor syndrome (HPT-JT), while less frequently PHPT cases do not show any syndromic association as happen in familial isolated hyperparathyroidism (FIHPT), familial hypocalciuric hypercalcaemia (FHH), neonatal severe hyperparathyroidism (NSHPT) and autosomal dominant moderate hyperparathyroidism (ADMH) (Gopinath and Mihai, 2011; Iacobone *et al.*, 2015).

The genetic basis of sporadic PHPT is complicated and a number of genes has been reported to be involved in the disease, including cell regulatory genes (Cyclin D1-CCND1, Menin1- MEN1), genes of the Wnt/β-catenin pathway and, apoptotic and growth factor genes (Mizamtsidi *et al.*, 2018). As parathyroid hormone production and serum calcium levels are increased in PHPT, genes involved in calcium homeostasis could also be genetic candidate factors for the disease. Mutations in CaSR (Calcium Sensing Receptor) and VDR (Vitamin D Receptor) genes have been reported to be associated with PHPT, but in most cases this association is restricted in certain population groups or in certain PHPT symptoms (Carling *et al.*, 1997; Yamauchi *et al.*, 2001; Christensen *et al.*, 2013; Vezzoli *et al.*, 2014; Wang *et al.*, 2016; Matana, Popović, *et al.*, 2018), which means that the role of calcium homeostasis genes in PHPT needs more investigation.

Recently, a genome wide association study (GWAS) showed that rs6127099 polymorphism, that lies 38 kbp upstream of CYP24A1 (Cytochrome P450, family 24, subfamily A, polypeptide 1) gene, is associated with elevated PTH serum levels (Robinson-Cohen *et al.*, 2017). CYP24A1 gene (20q13) encodes the cytochrome P450 component of the 25-hydroxyvitamin D3-24-hydroxylase enzyme which catalyzes the degradation of the vitamin D molecule by converting 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 into 24-hydroxylated products (Jones, Prosser

and Kaufmann, 2012). Taking into account that PTH serum concentration is high in PHPT patients, the aim of the present study was to evaluate the potential role of rs6127099 polymorphism as a genetic risk factor for PHPT.

Materials and Methods

Thirty-nine unrelated patients with PHPT (2 males and 37 females; mean age 52.6 ± 14.3 years; range 29–74 years) were enrolled in the study. The diagnosis of PHPT was made by the elevated PTH and calcium levels in blood serum and was established with at least two imaging methods (sonography, ^{99m}Tc-sestamibi scintigraphy, 4D-CT). In addition, 39 ethnic matching healthy volunteers (2 males and 37 females; mean age 49.1 ± 18.8 years; range 20–85 years) with no personal or family history of chronic autoimmune or neoplastic diseases were studied. The samples sizes were calculated using an online calculator (http://www.pilotsamplesize.com) designed for pilot trials, as the present study, setting an 85% upper confidence level and probability at 0.05 (Viechtbauer *et al.*, 2015).

This study adhered to the tenets of the declaration of Helsinki (version 2002) and approved was by the Ethics Committees of Aristotle University of Thessaloniki (approval number 498/17-7-2019). Written consent was obtained from each participant in the study.

Genomic DNA was extracted from peripheral blood lymphocytes using the PureLink Genomic DNA Kit (Invitrogen). Polymorphism rs6127099 was studied with restriction fragment length polymorphism (RFLP) assay. The primer pair F: 5'-TCCAGAACACCAGACCAGGG-3', R:5'GAGCATCCCTTAGTGGGCAT T-3' was used for the amplification of rs6127099 polymorphism. The restriction assay was performed using the PstI restriction endonuclease (New England Biolabs). All samples were run twice.

SPSS statistical package (SPSS Inc.) was used to test differences in polymorphism distribution between PHPT patients and controls (Pearson's chi-square, Yates' chi-square if any expected frequency was below 1 or if the expected frequency was <5 in more than 20% of cells). Furthermore, the odds ratio (OR) with a confidence interval (CI) of 95% was calculated (reference allele vs variant allele). A difference at $P \leq 0.05$ was considered as statistically significant.

Results

The distribution of genotypes of rs6127099 polymorphism in PHPT patients and controls is shown in Table 1. No statistical significant difference was observed in the distribution of rs6127099 A>T genotypes distribution between PHPT patients and controls ($p = 0.836$). Similarly, no statistical significant difference was observed comparing rs6127099 A allele vs. T allele distribution between the studied groups ($p = 0.383$).

Table 1. Statistical analysis of rs6127099 polymorphism between PHPT patients and controls

rs6127099				
Genotypes	**AA**	**AT**	**TT**	
Patients (n=39)	16	20	3	$\chi^2 = 0.888$, df = 2 p-value = 0.642 p-value = 0.836 (yates' correction)
Control group (n=39)	20	17	2	
Alleles	**A**	**T**		
Patients (n=78)	52	26		$\chi^2 = 0.761$, df = 1 p-value = 0.383 OR: 0.739, 95%CI: 0.371-1.465
Control group (n=78)	57	21		

df: degrees of freedom; OR: odds ratio; 95% CI: 95% confidence interval

Discussion

PHPT is an endocrine disorder characterized by increased serum PTH and calcium concentration. Genes involved in calcium homeostasis regulation may have a role in the genetic basis of the disease. CYP24A1 gene, which encodes a monoxygenase of cytochrome P450, catalyzes the inactivation of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 preventing the toxicity of vitamin D (Jones, Prosser and Kaufmann, 2012). Loss of function CYP24A1 mutations cause severe childhood hypercalcaemia, adult hypercalcaemic syndrome and nephrolithiasis, with potent PTH suppression (Schlingmann et al., 2011; Dauber et al., 2012; Nesterova et al., 2013; Molin et al., 2015). Polymorphism rs6127099 lies 38 kbp upstream of CYP24A1 gene. The T allele of rs6127099 was associated with higher serum PTH concentration, suggesting that this allele may provide increased CYP24A1 activity, accelerated catabolism of 1,25-dihydroxyvitamin D3 and increased PTH levels (Robinson-Cohen et al., 2017).

The present study focused on the role of rs6127099 as a candidate genetic risk factor for PHPT. Although serum PTH hypersecretion is a major clinical manifestation of PHPT, no association of rs6127099 polymorphism with the disease was found. Previously, in a GWAS, PTH concentration was strongly associated with polymorphism rs6127099 ($p = 4.2 \times 10\text{-}53$) (Robinson-Cohen et al., 2017). However, the present study for first time validates the reported association and its clinical use in a disorder which is characterized by high PTH levels. The evaluation of GWAS results in random small sample

groups is of great importance when searching for true disease risk factors, as many times the results of GWAS have limited clinical predictive value and other limitations (Kraft, Zeggini and Ioannidis, 2009; Tam et al., 2019). Therefore, even the results of a negative association, as these of the present study, offers to the suggested probable use of a polymorphism as a biomarker in a disease predisposition.

Polymorphism rs6127099 is located in an intergenic DNA region that does not have any known function, i.e. in a coding or regulatory gene sequence according to the national databases. So, it is possible that this polymorphism does not have any effect on CYP24A1 gene. This seems to be consistent with the results of the present study, but as mentioned before it contradicts the results of the genome wide association study (Robinson-Cohen et al., 2017), which argues that the allele T of this polymorphism rs6127099 is related to high PTH serum levels. However, rs6127099 was reported to be in linkage disequilibrium with the variant rs1570669, which has been also associated with high PTH levels, lower circulating calcium and reduced bone mineral density (BMD) at the femoral neck (O'Seaghdha et al., 2013). Therefore, further studies are needed to enlighten the causative variant in PTH levels.

Additionally, it is estimated that 60% of the variation in PTH concentration is genetically determined (Hunter et al., 2001). PTH levels are regulated by many genetic loci. Polymorphisms associated with increased PTH concentration have also been found in other genes, such as in RGS14 (Regulator Of G Protein Signaling 14), CLDN14 (Claudin 14), RTDR1 (Rhabdoid Tumor Deletion Region Gene 1), RASGEF1B (RasGEF Domain Family Member 1B) and DPP10 (Dipeptidyl Peptidase Like 10) (Robinson-Cohen et al., 2017; Matana, Brdar, et al., 2018). Polymorphisms associated with PHPT manifestation have also been found in CaSR and VDR genes (Carling et al., 1997; Yamauchi et al., 2001; Christensen et al., 2013; Vezzoli et al., 2014; Wang et al., 2016; Matana, Popović, et al., 2018), while epigenetic alterations have also been reported to play a role (Sulaiman et al., 2013; Westin, 2016). Therefore, PTH levels in patients with PHPT may represent the interplay of many genes and their variants. Concluding, one limitation of the present study is the small sample size, which reduces the study's statistical power and may lead to a type II statistical error. This means that the association may exist in the population, but the small sample size may not permit it to be detected. However, the low prevalence of the disease may excuse our PHPT patient sample size and give value to our preliminary results. Therefore, the present pilot study of Greek origin, emphasizes the need for additional studies in larger groups of patients of various ethnicities to validate the reported association of rs612099 polymorphism with the high PTH levels and probably with PHPT predisposition.

Conflict of interest

The authors have declared no conflicting interests.

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