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Phospholamban gene mutations are not associated with hypertrophic cardiomyopathy in patients from southern Poland

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Abstract

Background: Hypertrophic cardiomyopathy (HCM) is a genetic disease. The role of phospholamban (PLN) gene mutations is the development of HCM has not been established.

Aim: To screen for *PLN* gene mutations in a group of HCM patients from the southern Poland.

Methods: We included 50 consecutive patients (31 males, mean age 42 ± 14 years) diagnosed with HCM on the basis of typical clinical, echocardiographic, and haemodynamic features. The control group consisted of 50 (sex-, age-matched) healthy subjects with normal echocardiograms.

Results: The genetic analysis was focused on R9C mutation with the ability to block PLN phosphorylation leading to chronic inhibition of SERCA2a activity. Another analysed mutation causing the alteration of PLN level in cells was related to the substitution of a leucine residue at position 39 with a premature stop codon (L39X). The sequence analysis of selected coding regions of the *PLN* gene did not show the presence of mutations in either the patients or the control subpopulations.

Conclusions: Systematic mutation screening did not reveal any mutation in the selected regions of the *PLN* gene. Additionally, no polymorphisms were detected in any patients. Therefore, *PLN* gene mutations were not found to be associated with HCM in the study group.

Key words: hypertrophic cardiomyopathy, phospholamban

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INTRODUCTION

Phospholamban (PLN) is an endogenous sarcoplasmic reticulum calcium ATPase inhibitor and plays a regulatory role in the calcium handling during the process of cardiac contraction/relaxation coupling. Functionally, PLN reversibly inhibits SERCA2a by direct protein-protein interactions and therefore reduces calcium reuptake in sarcoplasmic reticulum [1–3]. Phospholamban mutations have been shown to be associated with elevated cytosol calcium concentration. Thus, although calcium ions are well known regulators of cardiomyocyte function, PLN may be a candidate gene responsible for cardiomyopathies, especially in dilated form. However, mutations in the *PLN* gene have also been associated with hypertrophic cardiomyopathy (HCM). In general, HCM has been defined as a clinically heterogeneous, autosomal-dominant disease of the cardiac sarcomere. Fenotypically, this primary myocardial disease is characterised by unexplained left ventricular (LV) hypertrophy. Mutations

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in the *PLN* gene have not been found to be a frequent cause of HCM in several previous studies. The PLN mutations were rarely detected in Spanish [4], Japanese [5], and Australian [6] populations, and were absent in a group of Greek patients [7].

The aim of this study was to screen for PLN mutations in a group of HCM patients from southern Poland.

METHODS

We included 50 consecutive patients (31 males, mean age 42 ± 14 years) diagnosed with HCM on the basis of typical clinical, echocardiographic, and haemodynamic features. The control group consisted of 50 (sex-, age-matched) healthy subjects with normal echocardiograms.

Transthoracic echocardiographic examination was performed and in each patient M-mode and 2D echocardiograms were obtained, followed by pulsed and continuous-wave Doppler ultrasound. Conventional techniques were used to measure the LV size. The LV contractility was assessed by fractional shortening (as has been recommended previously). The severity and distribution of LV hypertrophy were assessed echocardiographically and maximal LV wall thickness was detected at septum in all patients. Based on the echocardiographic data, no patients with LV cavity dilatation and depressed LV contractility were included in the study.

We performed SSCP mutational screening and DNA sequencing of the *PLN* gene in all participants. Genomic DNA was isolated from leukocytes of peripheral blood using the DNA isolation kit Qiagen. The sequence analysis was focused on two coding regions of the *PLN* gene, where previous studies had confirmed the presence of two mutations impairing the function of the PLN protein. Substitution of arginine by cysteine at amino acid position 9 (R9C) was shown to be related with chronic inhibition of SERCA2a activity. Another analysed PLN region was related with loss-of-function human PLN mutation, the substitution of a leucine residue at position 39 causing a premature stop codon (L39X). The sequence analysis of selected regions (R9C: forward 3′-agactgtggctaaccaatcg-5′, reverse primer 3′-cgaaatctgtttcttaccttgg-5′ the product size 134 bp as well as L39X: forward 3′-tttattatttttacattccaggctacc-5′, reverse primer 3′-gcttttgacgtgcttgttga-5′ the product size 278 bp) was performed using a standard protocols kit BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and a 3130xl Genetic Analyzer (Applied Biosystems, USA). During the sequence analysis, we were screening the whole, selected, coding regions for known mutations as well as searching for new nucleotide variations of the *PLN* gene.

RESULTS

In echocardiographic assessment, in the entire HCM group, both LV cavity sizes were normal, with preserved contractility expressed by normal fractional shortening 43 ± 7%. In all HCM patients, the ventricular septum thickness was markedly increased to 23 ± 5 mm, whereas the thickness of the posterior wall was within a normal range of 10 ± 3 mm. The septum/posterior wall thickness ratio was 2.3 ± 0.4, indicating asymmetric pattern of LV hypertrophy in all patients.

The genetic analysis was focused on R9C mutation with the ability to block PLN phosphorylation leading to chronic inhibition of SERCA2a activity. Another analysed mutation causing the alteration of PLN level in cells was related to the substitution of a leucine residue at position 39 with a premature stop codon (L39X). The sequence analysis of selected coding regions of the *PLN* gene did not show the presence of mutations in either the patient or the control subpopulations.

DISCUSSION

To date, more than 400 HCM-related mutations in ten genes encoding contractile sarcomeric proteins have been identified. However, the mutations identified in these genes only account for the disease in about 60% of HCM patients [6, 8]. Genes encoding Ca(2+) regulatory proteins responsible for Ca(2+) homeostasis have been suggested as possible candidates for HCM. Calcium ions have been shown to play a regulatory role in cardiomyocyte biology and function. PLN is a modest modulator of intracellular Ca2+ homeostasis and may be another candidate gene, apart from the well documented and known genes *MYH7* (beta myosin heavy chain), *TNNT2* (cardiac T troponin) or myosin-binding protein C (*MYBPC3*) responsible for the development of cardiomyopathy [6]. The HCM genes screened for causative mutations encode proteins of the sarcomer and generated positive results in no more than 60% of the patients echocardiographically diagnosed as HCM. Recently, mutations in Z-disc associated proteins (titin, telothonin, muscle LIM and myozenin) responsible for the structural organisation of sarcomer, has been discovered in HCM patients [6]. The mutations of these genes are infrequent (below 5%). Consequently, mutations in other genes account for the remaining one third of HCM patients. Pulling together findings from our study with previous Spanish, Japanese, Australian and Greek studies, it seems that PLN mutations are very rare and do not play a crucial role in HCM development.

CONCLUSIONS

Systematic mutation screening did not reveal any mutation in the selected regions of the *PLN* gene. Additionally, no polymorphisms were detected in any patients. Therefore, *PLN* gene mutations were not found to be associated with HCM in the study group.

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Conflict of interest: *none declared*

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Mutacje genu dla fosfolambanu nie wiążą się z występowaniem kardiomiopatii przerostowej u mieszkańców południowej Polski

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Streszczenie

Wstęp i cel: Celem pracy było badanie przesiewowe w celu poszukiwania mutacji w obrębie odcinków genu kodującego funkcjonalne miejsca białka fosfolambanu (PLN) u pacjentów z kardiomiopatią przerostową (HCM) zamieszkujących rejon południowej Polski.

Metody: Do badania zakwalifikowano kolejnych 50 pacjentów (31 mężczyzn i 19 kobiet w średnim wieku 42 ± 14 lat). Grupę kontrolną stanowiło 50 zdrowych ochotników z prawidłowym echokardiogramem i odpowiednio dobranych pod względem płci i wieku (dla adekwatnego porównania z grupą HCM).

Wyniki: Analiza sekwencji dotyczyła przede wszystkim mutacji R9C (odpowiedzialnej za zablokowanie fosforylacji PLN prowadzącej do całkowitego zablokowania aktywności ATP-azy SERCA2a). Kolejną analizowaną zmianą była mutacja L39X prowadząca do zmiany poziomu PLN w komórkach. Badanie wybranych fragmentów genu dla PLN nie wykazało zarówno nowych, jak i znanych (R9C, L39X) mutacji zarówno w grupie badanej, jak i w grupie kontrolnej.

Wnioski: Na podstawie uzyskanych wyników stwierdzono, że poszukiwane mutacje w obrębie genu dla PLN nie wydają się odgrywać kluczowej roli w etiopatogenezie HCM w przebadanej aktualnie grupie.

Słowa kluczowe: fosfolamban, kardiomiopatia przerostowa

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