Selected mutations in the myosin binding protein C gene in the Polish population of patients with hypertrophic cardiomyopathy

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Abstract

Background: Mutations in the gene of myosin binding protein C (MYBPC3) are currently considered the most frequent cause of hypertrophic cardiomyopathy (HCM).

Aim: To assess the frequency of selected mutations in MYBPC3 in the Polish population of HCM patients.

Methods: One hundred eighteen patients with HCM and 118 healthy, age and sex-matched controls were screened for the presence of 14 mutations of MYBPC3 using real time polymerase chain reaction.

Results: Five different mutations were found in six patients in the HCM group whereas no mutations were present in the control group. In three cases the mutations were missense (Arg502Gln, Cys566Arg, Asn755Lys) and in three cases terminal (Gln425ter, Gln1061ter in two unrelated probands).

Conclusion: Mutations in MYBPC3 should be considered a frequent cause of HCM in Poland.

Key words: hypertrophic cardiomyopathy, genetic screening, myosin binding protein C

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Introduction

Hypertrophic cardiomyopathy (HCM) is a primary genetic myocardial disease characterised by asymmetric hypertrophy of the nondilated left ventricle (LV) in the absence of other cardiovascular or systemic disorder which may cause LV hypertrophy. Microscopic abnormalities of myocardial structure include myofibre disarray, abnormal intracellular myofibril architectonics, fibrosis and intimal hypertrophy of arterioles [1]. The leading causes of HCM are single point mutations of genes encoding proteins of contractile apparatus of the cardiomyocyte [2]. So far over 400 various mutations of twelve genes have been reported – and this number is still growing. However, over half of cases of HCM involve mutations in one of three genes: beta-myosin heavy chain (described at the very beginning) [3], myosin binding C protein, and troponin T. Quite recently a gene encoding myosin heavy chain beta isoform, MYH7, was identified as the most frequent cause of these polymorphisms [4]. Recently this point of view has changed following publication of several large genotyping studies in patients with HCM [5, 6]. They indicated that the most common cause of HCM in the European population was mutation in myosin binding protein C gene, MYBPC3.

The aim of this study was to determine the incidence of selected MYBPC3 polymorphisms in the Polish population of HCM patients.

Methods

Study group

The study involved 118 patients with HCM referred to a single university centre. The HCM diagnosis criteria were based on the current literature [7, 8]. The control group consisted of 118 healthy volunteers matched for sex and age. The baseline characteristics of the groups

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Table I. Baseline characteristics of the study and control groups

	HCM (n=118)	Controls (n=118)	p
Age [years]	56.0±15.9	56.0±15.9	NS
Males	56 (48%)	56 (48%)	NS
Medical history			
Family history of HCM	34 (29%)	–	
Family history of SCD	18 (15%)	–	
Age at the time of diagnosis [years]	51.1±15.9	–	
NYHA	1.83±0.77	–	
CCS	1.69±0.82	–	
Syncope	35 (30)	–	
ECG and Holter ECG monitoring			
Atrial fibrillation	12 (10%)	0	
Pathologic Q waves	29 (26%)	0	
ST-T changes	93 (82%)	0	
QRS [ms]	87.9±20.3	72.0±16.7	<0.001
QTc [ms]	411.8±27.9	387.2±27.7	<0.001
Ventricular tachycardia	20 (17%)	0	
VES/hour	17.1±10.6	4.6±6.9	<0.001
Echocardiography			
LVEDD [mm]	41.0±5.8	45.2±2.8	<0.001
Left atrium [mm]	41.6±6.1	34.4±3.3	<0.001
Ejection fraction [%]	55.6±8.8	62.8±5.0	<0.001
Max LV wall thickness [mm]	21.9±4.0	9.7±1.6	<0.001
LV mass [g]	324±98	178±38	<0.001
Hypertrophy location:			
septal	94 (80%)	–	
concentric	18 (15%)	–	
apical	6 (5%)	–	
LVOT obstruction	37 (31%)	–	
Mitral regurgitation [°]	1.11±0.75	–	
SAM	45 (38%)	–	

Abbreviations: SCD – sudden cardiac death, VES – ventricular extrasystole, LVEDD – left ventricular end diastolic diameter, LVOT – left ventricular outflow tract, SAM – systolic anterior motion

Table II. Examined mutations of myosin binding protein C gene – MYBPC3

Exon	Nucleotide location	Amino acid exchange	References
3	2326	Thr59Ala	[12]
16	10550	Gln425ter	[12]
	10628	Glu451Gln	[13]
	10713	IVS16-1g>a	[14]
18	10931	Arg495Gln	[15]
	10952	Arg502Gln	[15]
	10966	Gly507Arg	[16]
	11071	Glu542Gln	[17]
19	11564	Cys566Arg	[16]
22	13893	Arg654His	[18]
24	15087	Asn755Lys	[19]
28	18607	Gln969ter	[19]
30	20082	Gln1061ter	[20]
32	20687	Val1115Ile	[16]

are summarised in Table I. The study protocol was approved by the university Ethics Committee and all participants gave their written informed consent to participate in it.

Polymorphism testing

The presence of polymorphisms was determined in DNA isolated from venous blood leukocytes using real time polymerase chain reaction (RT-PCR). This method provides fast and reliable identification of polymorphisms, which was repeatedly confirmed by direct DNA sequencing [9, 10]. Three millilitre samples of venous blood were aseptically collected from each participant using an EDTA test tube. Blood samples were immediately frozen and stored at -25°C. Subsequently they were used to purify DNA with the ionic membrane exchange method using Genomic Midi AX columns (A&A Biotechnology, Poland). DNA concentration was measured in all samples with spectrophotometry. Following isolation DNA was stored at -90°C.

Real time polymerase chain reaction was performed using the 7500 Real-Time PCR System (Applied Biosystems, USA). The following reagents were used (Applied Biosystems, USA):

- TaqMan SNP Genotyping Assays 20X, single nucleotide polymorphism (SNP) specific, including: specific primers, two specific probes each labelled with fluorescent dye, VIC and FAM;
- Taqman Universal PCR Master Mix 2X, including: DNA polymerase (Taqman AmpliGold), deoxynucleotides (dNTP), and buffering components.

Reactions were performed on sterile 96-well plates of 25 µl each. Under aseptic conditions, to each well was added mixed 12.5 µl PCR Master Mix and 1.25 µl SNP Genotyping Assay. Subsequently, 10 ng of individual patients' DNA was dispensed with a pipette in each well and diluted with 13.75 µl of distilled water (target DNA concentration about 0.4 ng/µl). Distilled water was added to four wells in the plate instead of DNA to serve as a negative control. The plate was inserted into the analyser and baseline fluorescence was read, then 10-minute incubation up to 95°C to activate DNA polymerase was applied. Subsequently 40 DNA amplification cycles were repeated at 92°C for 15 seconds, then at 60°C for 60 seconds in each cycle. After amplification the final fluorescence was read. Based on the fluorescence index change during amplification each sample genotype was classified as: homozygotic XX, homozygotic YY or heterozygotic XY, where X and Y stand for specific gene alleles.

We investigated 14 known mutations of myosin binding protein C gene, MYBPC3 (Table II). Mutation selection criteria were: documented association with HCM [11-19] and availability of primers and genetic probes.

Table III. Mutations of myosin binding protein C gene (MYBPC3) detected in the HCM group. One mutation was present in two non-relatives

Mutation	Patient							
	gender	age [years]	age at diagnosis [years]	family history	symptoms	max diastolic LV wall thickness [mm]	LV mass index [g/m^2]	max LVOT gradient [mmHg]
Gln425ter	F	50	50	no	syncope not exercise induced	28	207	no
Arg502Gln	M	76	70	no	NYHA II, CCS II	19	180	30
Cys566Arg	F	34	27	no	no	24	148	no
Asn755Lys	F	55	47	no	no	16	121	no
Gln1061ter	F	27	23	no	no	17	102	no
Gln1061ter	M	57	57	no	no	17	182	64

Abbreviations: LV –left ventricular, LVOT – left ventricular outflow tract, F – female, M – male, NYHA – New York Heart Association, CCS – Canadian Cardiac Society

Results

In the HCM group five mutations of myosin binding protein C gene were detected (MYBPC3) in six subjects (6/118=5.1%). Mutations detected and patients' characteristics are listed in Table III. They included nonsense mutations in three cases (Arg502Gln, Cys566Arg, Asn755Lys) and three terminal mutations (Gln425ter, Gln1061ter in two non-relatives). No mutations were found in the control group.

Discussion

Myosin binding protein C is a structural and regulatory protein forming a sarcomere thick filament complex [20]. The MYBPC3 gene is located on chromosome 11. Chronologically it was the fourth gene associated with HCM [21]. However, the results of large genetic studies suggested that it is the most common gene responsible for the development of HCM in the European population [5, 6].

In our study we determined the presence of 14 selected mutations of myosin binding protein C gene in a group of 118 HCM patients and age- and sex-matched healthy individuals. Five different mutations were found in six HCM patients. No mutation was found in healthy subjects from the control group. As far as we are aware, this is the first study including such a large group of Polish patients with HCM. Previous Polish reports focused on selected mutations of genes modifying disease course [22].

The incidence of MYBPC3 polymorphisms in our group is only seemingly low. It should be noted that the analysis involved only 14 of 150 known mutations of this gene. Mutations were found in 5% of patients, but the examination was sensitive to only about 10% of known polymorphisms. This may suggest a high rate of MYBPC3 mutations in the Polish HCM population, which may be helpful to properly design larger genetic studies in the future. An undoubted advantage of genetic diagnosis in each patient is the possibility to easily analyse their family members' genotypes and make definite confirmation or exclusion of HCM burden.

The relatively low number of patients in whom mutations were confirmed in our study makes it impossible to perform a statistical comparison with the remaining HCM subjects. Subjects with MYBPC3 mutations were noted to have mild symptomatic course and relatively minor LV hypertrophy. This finding is consistent with available published data suggesting relatively mild course of the disease in MYBPC3 mutation carriers compared to carriers of other genetic mutations [23].

Surprisingly, none of the patients with confirmed mutations had a positive family history of HCM. This may be explained by the asymptomatic course of the disease reported by four of six patients in this group. However, it may also indicate low diagnostic sensitivity with respect to HCM in the Polish population. It should be stressed however that no screening of first degree relatives, which could have allowed verification of family background of HCM in each subject, was performed in our study.

In summary, the results of our study indicate frequent presence of myosin binding protein C gene mutation in patients with HCM in Poland.

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Wybrane mutacje genu dla białka C wiążącego miozynę w polskiej populacji chorych na kardiomiopatię przerostową

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Streszczenie

Wstęp: Kardiomiopatia przerostowa (ang. *hypertrophic cardiomyopathy*, HCM) jest pierwotną, uwarunkowaną genetycznie chorobą mięśnia sercowego charakteryzującą się nieprawidłowym przerostem mięśnia nieposzerzonej lewej komory, niewywołanym innym schorzeniem układu krążenia lub chorobą układową. Podstawową przyczyną choroby są pojedyncze mutacje punktowe w obrębie genów dla białek aparatu kurczliwego kardiomiocytu. Za najczęstszy gen odpowiedzialny za wystąpienie kardiomiopatii przerostowej uważany jest obecnie gen dla białka C wiążącego miozynę (*MYBPC3*).

Cel: Celem badania było określenie częstości występowania wybranych mutacji genu dla białka C wiążącego miozynę w polskiej populacji chorych na HCM.

Metodyka: Do badania włączono 118 chorych na HCM oraz 118 zdrowych osób dobranych pod względem płci i wieku. Obie grupy przebadano pod kątem obecności czternastu znanych mutacji genu dla białka C wiążącego miozynę za pomocą reakcji łańcuchowej polimerazy czasu rzeczywistego (RT-PCR) z użyciem swoistych sond znakowanych fluorescencyjnie.

Wyniki: U sześciu osób z rozpoznaną HCM stwierdzono obecność pięciu różnych mutacji genu dla białka C wiążącego miozynę. W trzech przypadkach były to mutacje zmiany sensu (Arg502Gln, Cys566Arg, Asn755Lys), a w trzech mutacje terminalne (Gln425ter, Gln1061ter u dwóch niespokrewnionych osób). Żadna z badanych mutacji nie występowała w grupie kontrolnej.

Wnioski: Mutacje genu dla białka C wiążącego miozynę należy brać pod uwagę jako częstą przyczynę HCM w Polsce.

Słowa kluczowe: kardiomiopatia przerostowa, badania genetyczne, białko C wiążące miozynę

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