MYOSIN BINDING PROTEIN C GENE *(MYBPC3)* MUTATIONS IN HYPERTROPHIC CARDIOMYOPATHY

Summary of the PhD Thesis

Tímea Tóth
Graduate School of Clinical Medicine
University of Szeged

Supervisor:
Róbert Sepp, MD, PhD

2nd Department of Internal Medicine and Cardiology Center
Faculty of Medicine
University of Szeged

Szeged
2016

LIST OF PUBLICATIONS

Publications directly related to the thesis:

I. **Tóth T,** Sepp R, Orosz A, Nagy V, Pálinkás A, Hőgye M, Csanády M, Forster T. A myozinkötő C-fehérje gén *(MYBPC3)* mutációszűrése magyar hypertrophiás cardiomyopathiás betegekben. *Cardiologia Hungarica* 2009; 39:318-24.

II. **Tóth T,** Sepp R, Orosz A, Nagy V, Pálinkás A, Hőgye M, Csanády M, Forster T. Myozinkötő C fehérje *(MYBPC3)* génmutációt hordozó hypertrophiás cardiomyopathiás családok klinikai és genetikai analízise. *Magy Belorv Arch* 2010; 63: 35-40.

III. Csanády M, Sepp R, **Tóth T,** Orosz A, Nagy V, Hőgye M, Forster T. A myozin kötő C fehérje gén *(MYBPC3)* mutációjának azonosítása veleszületett süketnémasággal társult hypertrophiás cardiomyopathiában. *Orvostudományi Értesítő* 2008; 81:23-25.

IV. **Tóth T,** Nagy V, Faludi R, Csanády M, Nemes A, Simor T, Forster T, Sepp R. The Gln1233ter mutation of the myosin binding protein C gene: causative mutation or innocent polymorphism in patients with hypertrophic cardiomyopathy? *Int J Cardiol* 2011; 153(2):216-9. IF: **7.078**

Citable abstracts directly related to the thesis:

I. Csanády M, **Tóth T,** Orosz A, Nagy V, Hőgye M, Forster T, Sepp R. A myozin kötő C-fehérje gén *(MYBPC3)* splice-site mutációjának azonosítása veleszületett süketnémasággal társult hypertrophiás cardiomyopathiában. *Cardiologia Hungarica* 2008; 38: B46.

II. **Tóth T,** Orosz A, Nagy V, Hőgye M, Csanády M, Forster T, Sepp R. A myozin kötő C-fehérje gén *(MYBPC3)* mutációanalízise hypertrophiás cardiomyopathiában. *Cardiologia Hungarica* 2008; 38: B48.

III. **Tóth T,** Orosz A, Nagy V, Hőgye M, Forster T, Csanády M, Sepp R: Mutation analysis of the myosin binding protein C gene *(MYBPC3)* in hypertrophic cardiomyopathy *Slovenska*

Kardiologija 2008; 5: 34 (FC7-10). Abstracts of 16th Annual Meeting of the Alpe Adria Association of Cardiology.

IV. **Tóth T,** Orosz A, Csanády M, Hőgye M, Forster T, Sepp R: Klinikai és genetikai szűrés veleszületett süketnémasággal társult hypertrophiás cardiomyopathiában. *Orvostudományi Értesítő* 2009; 1:59.

V. **Tóth T,** Orosz A, Nagy V, Hőgye M, Forster T, Csanády M, Sepp R. Myozin kötő C fehérje *(MYBPC3)* génmutációt hordozó családok klinikai és genetikai analízise. *Cardiologia Hungarica* 2009; 39: A54.

VI. Sepp R, Losonczi L, **Tóth T,** Nagy V, Orosz A, Kádár K, Hőgye M, Fekete Gy, Csanády M, Forster T. Kóroki géneloszlás magyar hypertrophiás cardiomyopathiás betegekben. (Disease gene distribution in Hungarian patients with hypertrophic cardiomyopathy). *Cardiologia Hungarica* 2010; 40: G101.

VII. **Tóth T,** Nagy V, Faludi R, Hőgye M, Csanády M, Simor T, Forster T, Sepp R. A myozinkötő C fehérje gén *(MYBPC3)* Gln1233ter eltérése hypertrophiás cardiomyopathiában: kóroki mutáció vagy ártatlan polymorfizmus? (The Gln1233ter alteration of the myosin binding protein C gene *(MYBPC3)* in hypertrophic cardiomyopathy: causative mutation or innocent polymorphism?). *Cardiologia Hungarica* 2010; 40: G102.

VIII. Sepp R, Losonczi L, **Tóth T,** Nagy V, Orosz A, Kádár K, Hogye M, Fekete Gy, Csanády M, Forster T. Prevalence of sarcomeric gene mutations in Hungarian patients with hypertrophic cardiomyopathy. *J Kardiol* 2010; 17 (Suppl A): 15.

IX. **Tóth T,** Nagy V, Faludi R, Hogye M, Csanády M, Simor T, Forster T, Sepp R. The Gln1233ter alteration of the myosin binding protein C gene *(MYBPC3)* in hypertrophic cardiomyopathy: causative mutation or innocent polymorphism? *J Kardiol* 2010; 17 (Suppl A): 15.

1. INTRODUCTION

1.1. Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is a primary disease of the myocardium characterised primarily by left ventricular myocardial hypertrophy, predominantly involving the interventricular septum. The cavity of the left ventricle is typically narrow. According to current literature data the disease is more frequent, then it was previously thought as its prevalence was shown to be 1/500-1000. Clinically the patients may be asymptomatic, but the development of symptoms in form of dyspnoea, chest pain, palpitation or syncope is more typical. Arrhythmias are frequent and the risk of sudden cardiac death is also increased.

1.2. Molecular genetics of hypertrophic cardiomyopathy

Genetic studies have shown that in most cases HCM is a hereditary disease with a typical autosomal dominant inheritance, variable penetrance and expression. Using molecular genetic methods specific alterations in genes encoding for mainly sarcomere proteins were found to cause the disease. The most important affected genes implicated in the disease include the beta myosin heavy chain- (*MYH7*), the alpha tropomyosin- (*TPM1*), the troponin T- (*TNNT2*), the myosin binding protein C- (*MYBPC3*), the troponin I- (*TNNI3*), the essential- (*MYL3*) and the regulatory myosin light chain- (*MYL2*), the alpha-cardiac actin- (*ACTC1*) and the titin (*TTN*) genes. Based on the above, HCM can be considered as the disease of the sarcomere.

Although HCM is a genetically heterogeneous disease, the most commonly affected sarcomere gene that can cause HCM is the myosin binding protein C gene (*MYBPC3*). According to literature data on screening different patient populations with HCM the prevalence of mutations affecting the *MYBPC3* gene is 15-25%. Since the identification of the first *MYBPC3* gene mutation nearly 600 other mutations have been identified in the gene. Our team published the first *MYBPC3* gene mutation in a Hungarian patient in 2001.

Approximately two-thirds of the *MYBPC3* mutations result in a truncated protein. This phenomenon can be attributed to either so-called „splice-site” mutations, or nucleotide insertions or deletions, which all can cause a shift in the reading frame. This will lead to incorporation of nonsense sequences which will usually end by an early stop codon activation. Besides, several missense mutations were identified which result in the change of only one amino acid.

2. AIMS

Previous to our work, no information were available with regard to the occurrence, prevalence, or distribution of *MYBPC3* gene mutations in Hungarian patients with hypertrophic cardiomyopathy. Similarly, no data existed on possible specific genotype-phenotype correlations, penetrance rates or specific expression of the disease in a Hungarian HCM patient cohort.

Therefore, in my PhD work I aimed to:

1. Identify mutations affecting the myosin binding protein C gene *(MYBPC3)* in Hungarian patients with hypertrophic cardiomyopathy;
2. Establish the prevalence rate of *MYBPC3* mutations in Hungarian HCM patient population;
3. Conduct clinical and genetic screening of family members of patients with *MYBPC3* gene mutations;
4. Analyze genotype-phenotype correlations of *MYBPC3* mutations identified in Hungarian HCM patients;
5. Characterize specific *MYBPC3* mutations identified in the Hungarian HCM cohort.

3. PATIENTS AND METHODS

3.1. Patients

3.1.1. Hungarian patients with hypertrophic cardiomyopathy screened for mutations in the *MYBPC3* gene

We analyzed forty-five unrelated patients with hypertrophic cardiomyopathy. In all cases collection of case history data, physical examination, overview of available clinical documentation, 12-lead ECG and transthoracic echocardiography were carried out. In selected cases patients were hospitalized for detailed in-hospital cardiology assessment (24-hour Holter monitoring, stress test, semi-supine bicycle stress echocardiography, cardiac MRI, coronarography, haemodynamic study). In all cases the diagnosis of HCM was based on internationally accepted diagnostic criteria.

3.1.2. Family members of patients with hypertrophic cardiomyopathy carrying *MYBPC3* gene mutations for clinical and genetic investigations

Family members of 5 index patients were investigated in whom 5 different, specific *MYBPC3* gene mutations were identified by our previous investigations [c.3697C>T (p.Gln1233Ter); c.821+1G>A; c.2864_2865delCT (p.Pro955ArgfsTer95); c.1776_1777delGT

(p.Ser593ProfsTer11); c.3407_3409delACT (p.Tyr1136del)]. Family members of the index patient carrying the c.431_432delGT (p.Gly144AlafsTer8) mutation were not available for family screening.

Altogether, 62 family members (30 male, 32 female, age: 40±18 years) were investigated in the 5 families. In all cases clinical assessment were done as described in 3.1.1. In all cases diagnosis of HCM were based on internationally accepted diagnostic criteria, in case of the family members the McKenna criteria were considered.

3.2. Methods

3.2.1. Genetic analysis

In accordance with legal regulations all investigated patients and family members gave written informed consent to molecular genetic analyses. The complete coding sequence and exon-intron junctions of the *MYBPC3* gene (1-35 exons) was amplified by polymerase chain reaction (PCR) from the sample DNAs (Mastercycler Gradient, Eppendorf AG, Hamburg, Germany) with specific primer pairs described in the literature. The PCR products were analysed by 'single strand conformation polymorphism' (SSCP) or 'denaturing high performance liquid chromatography" (DHPLC) chromatography mutation analysis methods, which were based on the different temperature-dependent separation of the DNA sample carrying a different nucleotide (variant). Every abnormal chromatogram was sequenced using the ABI PRISM 310 automated sequencer.

4. RESULTS

4.1. Identification of *MYBPC3* mutations in Hungarian patients with hypertrophic cardiomyopathy

4.1.1. Mutation data

In the 45 analyzed patients with HCM we identified 6 (13%) different causative *MYBPC3* mutations. One of the mutations was a nonsense (stop codon) mutation in exon 33 of the gene (c.3697C>T, p.Gln1233Ter). Another mutation was a splice site mutation at the junction of exon and intron 7 (c.821+1G>A). In other three cases three 2 base-pair microdeletions, affecting exons 27, 18 and 4 were found, which resulted in 'frame-shift' [exon 27: c.2864_2865delCT (p.Pro955ArgfsTer95; exon 18: c.1776_1777delGT (p.Ser593ProfsTer11); exon 4: c.431_432delGT, (p.Gly144AlafsTer8)]. The sixth identified

mutation was a 3 base-pair microdeletion affecting exon 31, which did not cause ‘frame shift’ but led to the deletion of a single amino acid (c.3407_3409delACT, p.Tyr1136del). Each mutation was present in heterozygous form. Three of the mutations were previously reported in the literature (p.Gln1233ter, c.821+1G>A, c.2864_2865delCT) while the other three were novel mutations.

4.2. Clinical and genetic investigations of families of patients with hypertrophic cardiomyopathy carrying *MYBPC3* gene mutations

4.2.1. Mutation data

Mutation carrier status was verified in 30 of the 62 (48%) investigated family members (p.Gln1233Ter: 3/7, c.821+1G>A: 7/20, p.Pro955ArgfsTer95: 15/30, p.Ser593ProfsTer11: 2/2, p.Tyr1136del: 3/3). The mutation occurred in heterozygous form in every family member.

4.2.2. Clinical data

Clinical diagnosis of HCM could be verified in 10 of the 30 (33%) mutation carrier family members, including the index patients. HCM was verified in 5 of the 7 mutation carrier family members in family H 11, and in 2 of the 15 mutation carrier family members in family H 65. In the remaining three families (H 16, H 76, H 92) there was no clinically manifest family member with HCM, except for the index cases. In clinically affected mutation carriers the diagnosis of HCM was established at the age of 27 the earliest, and at the age of 76 the latest. Nine patients were diagnosed with HCM above the age of 40, and 6 patients above the age of 50 years. In case of the clinically affected mutation carriers the average age at the diagnosis was significantly higher than in case of the clinically not affected family members (51±13 vs. 38±17 years, p=0.028). During the 10±8 years (median: 8 years) follow-up period 6 patients died out of the 10 clinically affected mutation carriers (60%) at the average age of 58±10 years, 9±4 years after the diagnosis. In 4 cases the cause of the death was sudden cardiac death, in one case it was stroke and in the last case the death was unrelated to HCM (gastric cancer). In case of the 20 clinically unaffected mutation carriers no death occurred.

4.3. Observation of extreme phenotypic variability in a HCM family carrying the *MYBPC3* c.821+1G>A gene mutation

In family H 11 five patients were diagnosed with manifest HCM. In the family members we observed an extremely variable phenotype with regard to the *MYBPC3* c.821+1G>A gene

mutation, including symptom-free mutation carrier, sudden cardiac death, progression into dilated phase and complications of infective endocarditis.

4.4. Analysis of the p.Gln1233Ter mutation of the *MYBPC3* gene in three carrier families

Further studies identified the *MYBPC3* p.Gln1233Ter mutation in three, apparently not-related index patients. Clinical and genetic family screening of the three families indicated 8 mutation carriers (including the index patients) among the 19 family members screened in the three families. Of the 8 mutation carriers the clinical diagnosis of HCM was established in 5 family members. Importantly, affected-to-affected transmission of the mutation was seen in two of the three families. Haplotype analysis did not prove a founder effect. The mutation was not identified in 149 apparently normal healthy controls (neither in 218 patients with dilated cardiomyopathy, nor in 97 other patients with hypertrophic cardiomyopathy; 928 chromosomes in total).

5. DISCUSSION

5.1. Identification of *MYBPC3* mutations in Hungarian patients with hypertrophic cardiomyopathy

According to literature data, the most frequently affected gene causing HCM is the *MYBPC3* gene. Initial studies reported a prevalence rate of 13-26% for the occurrence of *MYBPC3* gene mutations in HCM patient cohorts. Further studies on disease gene distribution in HCM indicated a 10-35% occurrence rate of *MYBPC3* gene mutations in HCM. In the 45 analyzed Hungarian patients with HCM we identified 6 different causative *MYBPC3* mutations establishing a 13% prevalence rate of the *MYBPC3* gene mutations in the Hungarian HCM patient population. This occurrence rate is very well in agreement with literature data. Considering our previous data, where we found that the mutation rate of the beta myosin heavy chain gene (*MYH7*) in the Hungarian patient population was 5%, and in 100 Hungarian patients with HCM we found no troponin I (*TNNI3*) or troponin T (*TNNT2*) mutations, the *MYBPC3* gene seems to be the most commonly affected gene in the Hungarian patient population with HCM.

The c.821+1G>A point mutation that affects the junction site of exon/intron 7 is a classical splice-site mutation which also results in the activation of a hidden stop codon and the formation of a truncated protein, similarly to the p.Gln1233Ter point mutation which is a stop

codon mutation. Due to the stop codon activation the translation of the protein stops, and exons located distally from the stop codon will be not transcribed and the C terminal part of the protein will be truncated. Except the p.Tyr1136del mutation all the mutations' probable effect is the deletion of the distal part of the protein, which part is responsible for the myosin and titin binding function of the protein. Direct evidence for the above could have been provided from the analysis of mRNA extracted from the heart muscle, but as no native myocardium was available it was not possible to directly prove it. The fourth microdeletion is an "in-frame" deletion which causes the deletion of only one amino acid (p.Tyr1136del). At positions 1135-1136 of the cMyBP-C protein, which is part of the C9 motif, two Tyr locates. Both are evolutionally conserved amino acids that highlights a functionally important role.

Three of the mutations we identified have already been published in the literature (p.Gln1233Ter, c.821+1G>A, p.Pro955ArgfsTer95). The three other mutations out of the six mutations we detected previously were not published (p.Ser593ProfsTer11, p.Gly144AlafsTer8, p.Tyr1136del). Although the p.Ser593ProfsTer11 mutation was not previously published, there is a report in the literature on the p.Val592fs mutation which is almost identical with the one we identified. The latter was described in a couple of Japanese families and was proven that it is a "founder" mutation. Interestingly, 23% of the mutation carrier 30 manifest patients with HCM transformed into end-stage dilated form. In case of our p.Ser593ProfsTer11 mutation carrier patient we also observed the progression into dilated phase, the development of atrial fibrillation and sudden cardiac death.

5.2. Clinical and genetic investigations of families of patients with hypertrophic cardiomyopathy carrying *MYBPC3* gene mutations

Analysing families of index patients with *MYBPC3* gene mutations we found that 30 of the 62 (48%) screened family members were mutation carriers, which ratio is in agreement with autosomal dominant nature of inheritance. Among the 30 mutation carrier patients, we observed the development of HCM in 10 patients, including the index patients (33%).

With regard to the clinical presentation of the *MYBPC3* gene mutations earlier publications reported about non-significant left ventricular hypertrophy, mild symptoms and good prognosis. Subsequent publications have found no difference between patients carrying *MYBPC3* mutations and patients carrying other HCM mutations. In a recent meta-analysis, looking at genotype-phenotype correlations, Lopés et al. pooled patients from eighteen publications (corresponding to a total of 2459 patients). They reported that the presence of any sarcomere gene mutation was associated with a significantly younger age at presentation

(38.4 vs. 46.0 years), a family history of HCM (50.6% vs. 23.1%), family history of SCD (27.0% vs. 14.9%). and greater maximal LV wall thickness (21.0 vs. 19.3 mm). There were no differences when the two most frequently affected genes, *MYBPC3* and *MYH7*, were compared.

In our patient population we observed a malignant appearance in the 10 clinically affected patients carrying *MYBPC3* mutations, 6 of them deceased at average age of 58 years. Five of the 6 above-mentioned deaths were probably HCM-related, including 4 cases of sudden cardiac death, and one case of stroke. In two additional patients, serious, HCM-related progressive disease were observed with a transition into dilated phase and development of endocarditis. The 20 clinically not manifest family members carrying the mutation showed no signs or symptoms of the disease, seemed to be asymptomatic and no death occurred. The latter observation also suggest that not the mutation carrier status itself, but the clinical manifestation and characteristics of HCM determine prognosis.

Interestingly, in patients carrying *MYBPC3* gene mutation, HCM was possible to be diagnosed above 50 years which means prolonged clinical manifestation or long asymptomatic stage. That delayed clinical manifestation of the *MYBPC3* gene mutations is well documented in the literature. Niimura et al. investigating 212 mutation carrier members of 16 families found that it was possible to verify HCM in 58% of mutation carriers under the age of 50, and the penetrance of the mutation also did not reach 100% above the age of 60. It is in sharp contrast with the HCM caused by mutations of the beta myosin heavy chain gene or the troponin T gene, in which the disease was almost fully penetrant by the age of 30.

In our patient population we also found 5 mutation carrier family members above the age of 50, and 2 family members above the age of 60, in whom HCM had not been developed yet. The oldest age when HCM was diagnosed was 76 years. The clinical relevance of these observations is that in young adults it cannot be stated that a family member did not inherit the disease, as in *MYBPC3* gene mutation carriers the disease manifests subsequently, maybe at an older age. In case of the above family members cardiology screening is recommended every 3-5 years or in case of complaints.

5.3. Observation of extreme phenotypic variability in a HCM family carrying the *MYBPC3* c.821+1G>A gene mutation

In the large Hungarian HCM family we investigated with the *MYBPC3* c.821+1G>A gene mutation we observed an extremely variable phenotype, including symptom-free mutation

carriers, sudden cardiac death, progression into dilated phase and complications of infective endocarditis. The same *MYBPC3* c.821+1G>A mutation was first published by Niimura et al. (due to the different exon numbering in the form of Int8DSG+1A) in a two-generation family where 9 mutation carriers were observed and 5 of them were clinically affected. There was no death in the family related to HCM. In the families analysed by Erdmann et al. that mutation was also described in two unrelated German families where 5 mutation carriers were found. The disease occurred at the age of 24-59, the left ventricular wall thickness was 19-24 mm, and there was 1 myomectomy, 1 ICD implantation and 2 septal alcohol ablations among the family members. By the analysis of the patients' mRNA 2 aberrant transcriptions were detected in one case with the loss of the exon 7 and in the other case the exon 7/8, furthermore, with the activation of a hidden stop codon in the exon 9. About the two apparently unrelated German families a common 'founder' haplotype was identified. That mutation was also noticed in the report from the Mayo Clinic.

Early investigations looking at phenotype–genotype correlations in HCM observed a high degree of phenotypic variability which appeared to be partly explained by genetic heterogeneity (i.e. involvement of different disease genes). Some genes, or some particular mutations, were reported to be associated with a high risk for sudden cardiac death (e.g. *TNNT2* mutations or *MYH7* p.Arg403Gln mutation), modest LV hypertrophy (*TNNT2* mutations), or delayed cardiac expression (*MYBPC3* mutations). Subsequent studies, based primarily on individual patients rather than families, have reported exceptions and more heterogeneous cardiac expression within a given gene or mutation. With currently available methods for phenotyping, no clear and consistent correlations are detected for most of mutations. Our ability to draw more accurate genotype–phenotype correlations over the spectrum of disease will improve in the future as more robust and quantitative methods to assess phenotype are identified, and as we are able to follow larger genotyped cohorts over time. At a broader level, patients with complex genotype and multiple simultaneous mutations may have more severe or early disease expression, related to a gene dosage effect. This was clearly described in small families and recently suggested in a larger study. Finally, some studies have observed that patients with a pathogenic sarcomere mutation had an increased risk of cardiovascular events, especially heart failure, compared with patients with negative genetic testing and no sarcomere mutation.

5.4. Analysis of the *MYBPC3* p.Gln1233Ter mutation in three carrier families

The *MYBPC3* p.Gln1233Ter mutation had a debated consequence in the literature. The importance of the mutation has been questioned based on the arguments that no affected-to-affected transmission has ever been demonstrated and the mutation was found to be present in apparently normal controls in some populations. In our study we were able to detect the p.Gln1233Ter mutation in three HCM families, and affected-to-affected transmission of the mutation was demonstrated in two of the three families. We also proved that the mutation was absent in a large cohort of control samples (928 chromosomes in total). Based on the above findings we suggested that the p.Gln1233Ter mutation of the myosin binding protein C gene is a true causative mutation for hypertrophic cardiomyopathy, because affected-to-affected transmission of the mutation has been shown for the first time in two families, and it was not found in a large number of control samples. In addition, the characteristics of the mutation, being a stop codon mutation, also suggested a causative role. The former identification of the mutation in some control population in a previous report may have indicated more the presence of silent mutation carriers without signs and symptoms of the disease. The latter was also demonstrated in our families, as three of the 8 mutation carriers did not manifest the disease by the age of 18–37 years.

The *MYBPC3* p.Gln1233Ter variant is currently listed with a comment of “with pathogenic allele” in the dbSNP database, and with comment of ‘pathogenic’ in the NCBI ClinVar database. According to the ExAC (ExAc Aggregated Populations) database, which provides data on the genotype of 60,706 unrelated individuals sequenced as part of various disease-specific and population genetic studies, the minor allele frequency of the p.Gln1233Ter variant is 0.000008%, pointing out that this variant is almost non-existent in the general population, which also strengthen the malignant nature of this variant.

6. SUMMARY AND CONCLUSIONS

1. We identified known and novel *MYBPC3* mutations in Hungarian patients with hypertrophic cardiomyopathy.

Six causative myosin binding protein C gene mutations were identified in 45 Hungarian patients with HCM. Similarly to the existing literature knowledge, most of the mutations are predicted to cause shorter-than-normal, truncated proteins. The clinical appearance of the mutations was heterogeneous with high mortality rate in the index patients.

2. We observed a prevalence of 13% of *MYBPC3* gene mutations in the Hungarian HCM patient cohort, which makes the gene the most frequently affected gene in the Hungarian HCM population.

The identification of 6 different causative *MYBPC3* mutations out of 45 Hungarian patients with HCM establish a 13% prevalence rate of the *MYBPC3* gene mutations in the Hungarian HCM patient population. Taking our previous data on disease gene distribution in Hungarian HCM population into consideration, the *MYBPC3* gene seems to be the most commonly affected gene in the Hungarian patient population with HCM.

3. We observed that HCM caused by *MYBPC3* mutations may manifest at older ages in adulthood and its appearance is not typical at young age. Once the disease develops its clinical course is not benign but has a high mortality rate and may carry and increased risk for sudden cardiac death and progression into dilated phase.

According to our data hypertrophic cardiomyopathy caused by the myosin binding protein C manifests especially at older ages, and its appearance is not typical at younger ages. Many patients were diagnosed with HCM above the age of 40, or even above the age of 50 years. The course of HCM caused by *MYBPC3* gene mutations which has already been manifested can be overtly malignant leading to sudden cardiac death or progression into the dilated phase. The latter observation also suggest that not the mutation carrier status itself, but the clinical manifestation and characteristics of HCM determine prognosis.

4. An extremely variable phenotype of the *MYBPC3* c.821+1G>A mutation was observed.

In the large Hungarian HCM family we investigated with the *MYBPC3* c.821+1G>A gene mutation we observed an extremely variable phenotype, including symptom-free mutation

carriers, sudden cardiac death, progression into dilated phase and complications of infective endocarditis.

5. Observing that the *MYBPC3* p.Gln1233Ter mutation was transmitted from affected-to-affected in two carrier families and that it was not present in a large number of controls we provided evidence that the variant is a causative mutation for HCM.

In our work we were able to detect the *MYBPC3* p.Gln1233Ter mutation in three carrier families, and affected-to-affected transmission of the mutation was demonstrated in two of the three families. We also proved that the mutation was absent in a large cohort of control samples (928 chromosomes in total). Based on the above findings we suggested that the p.Gln1233Ter mutation of the myosin binding protein C gene is a true causative mutation for hypertrophic cardiomyopathy. This observation is still holding true in the light of recent genetic data coming from large population studies.

7. ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my supervisor Dr. Robert Sepp for the continuous support of my PhD study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis.

My sincere thanks also goes to Professor Tamás Forster, and Professor Miklós Csanády, current and former Head of Department, who provided me an opportunity to join their team as a PhD student, and who gave access to the laboratory and research facilities. Without they precious support it would not be possible to conduct this research.

I thank my fellow labmates for the stimulating discussions, for the sleepless nights we were working together before deadlines, and for all the fun we have had during my PhD years. In particular, I am especially grateful to Molnár Jánosné Klári for enlightening me the first glance of laboratory work.

Last but not the least, I would like to thank my family, my parents and my grandma for supporting me spiritually throughout writing this thesis and my life in general.