GENETIC ANALYSIS OF HYPERTROPHIC CARDIOMYOPATHY PHENOCOPIES

PhD Thesis

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Szeged

2016

LIST OF PUBLICATIONS

Publications directly related to the thesis:

I. **Csanyi B**, Popoiu A, Hategan L, Hegedus Z, Nagy V, Racz K, Hogye M, Saghy L, Ivanyi B, Csanady M, Forster T, Sepp R. Identification of two novel *LAMP2* gene mutations in Danon disease. *Can J Cardiol* 2016; in press (doi: 10.1016/j.cjca.2016.02.071).

IF: 3,112 (2015)

II. **Csányi B**, Nagy V, Hategan L, Borbás J, Tringer A, Herczeg B, Forster T, Sepp R. Fabry betegség szűrése többszervi érintettséget mutató hipertrófiás cardiomyopathia eseteiben. *Cardiologia Hungarica* 2016; 46: 158-164.

III. **Csányi B**, Hategan L, Nagy V, Obál I, Varga ET, Borbás J, Tringer A, Eichler S, Forster T, Rolfs A, Sepp R. Identification of a novel *GLA* gene mutation, p.Ile239Met, in Fabry disease with a predominant cardiac phenotype. *Int Heart J* 2016, accepted for publication.

IF: 1.938 (2015)

VI. Hategan L, **Csányi B**, Nagy V, Kis O, Kohári M, Ágoston G, Sághy L, Varga A, Iványi B, Forster T, Sepp R. Transthyretin génmutáció azonosítása hipertrófiás cardiomyopathia képében megjelenő amyloidosisban. *Cardiologia Hungarica* 2016, nyomtatásban.

Citable abstracts directly related to the thesis:

I. **Csányi B**, Hategan L, Popoiu A, Rácz K, Hőgye M, Sághy L, Csanády M, Forster T, Sepp R. Danon-betegséget okozó *LAMP2* génmutációk azonosítása hypertrophiás cardiomyopathia fenokópiáiban. Identification of *LAMP2* gene mutations in Danon disease manifested as phenocopies of hypertrophic cardiomyopathy. *Cardiologia Hungarica* 2013; 43:B83.

II. Kanyó É, Popoiu A, Nagy V, Rácz K, **Csányi B**, Hategan L, Hőgye M, Sághy L, Csanády M, Forster T, Sepp R. Magas mortalitás és gyors progresszió Danon betegségben: három Danon betegségben szenvedő család analízise. Clinical course of Danon disease highlights a highly malignant cardiac disease. *Cardiologia Hungarica* 2015; 45:D100.

III. **Csányi B**, Hategan L, Borbás J, Tringer A, Nagy V, Herczeg B, Forster T, Sepp R. Fabry betegség szűrése cardiomyopathia fenotípusában jelentkező kardiális kórképek esetén. Screening for Fabry disease in cardiac disorders manifesting as cardiomyopathy phenotypes. *Cardiologia Hungarica* 2016; 46:F101.

1. INTRODUCTION

1.1. Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is a complex and relatively common genetic cardiac disease characterised primarily by unexplained left ventricular hypertrophy. The disease is more frequent, then it was previously thought as its prevalence was shown to be 1/500-1000. HCM is an important cause of disability and death in patients of all ages, although sudden and unexpected death in young people is perhaps the most devastating component of its natural history.

1.2 Molecular and clinical genetics of hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy is an autosomal dominant inherited genetic disorder with variable expression and penetrance. Specific alterations in genes encoding for mainly sarcomere proteins were found to cause the disease in approximately 60% of individuals with HCM. 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.

1.3. Hypertrophic cardiomyopathy phenocopies

Mutations affecting sarcomere genes are present in 40-60% of HCM patients. In 5-10% of the cases mutations affect genes which may lead to HCM phenocopies, i.e. diseases that mimic HCM but are caused by other etiologies capable of producing myocardial hypertrophy (i.e. Fabry disease, Danon disease, transthyretin amyloidosis, etc.). Some of the inherited syndromes, as well as metabolic and mitochondrial disorders, can present as clinical phenocopies and can be distinguished by their associated cardiac and non-cardiac features and on the basis of their unique molecular genetics. The mode of inheritance, natural history and treatment of phenocopies can differ from those of HCM caused by mutations in sarcomere genes. Detailed clinical evaluation and mutation analysis are, therefore, important in providing an accurate diagnosis in order to enable genetic counseling, prognostic evaluation and appropriate clinical management.

1.3.1. Danon disease

Danon disease (OMIM# 300257) is a rare X-linked dominant disorder characterized by cardiomyopathy, skeletal myopathy, and mental retardation. While skeletal myopathy is generally mild and the mental retardation variable, it is hypertrophic cardiomyopathy which

dominates the clinical picture and determines the outcome. Women are less severely affected than men, with disease onset in late adulthood and with slower progression.

Danon disease is caused by the primary deficiency of lysosome-associated membrane protein-2 (LAMP-2). Inheritance of Danon disease has been considered to be X-linked dominant because in most familial cases males are affected predominantly, affected mothers usually have milder and later-onset cardiac symptoms, and no male-to-male transmission has been described. The *LAMP2* gene maps to chromosome region Xq24. The *LAMP2* open reading frame consists of 1,233 nucleotides and encodes 410 amino acids. The molecular diagnosis of Danon disease has so far been based on the demonstration of LAMP-2 protein deficiency in skeletal or cardiac muscle and/or the identification for *LAMP2* gene mutations.

1.3.2. Fabry disease

Fabry disease (FD, OMIM# 301500) is a rare X-linked recessive disorder caused by mutations in the *GLA* gene (OMIM# 300644), encoding a lysosomal hydrolase enzyme, α-galactosidase A enzyme (α-gal A; GLA; EC 3.2.1.22). Mutations affecting the *GLA* gene and enzyme will result in the accumulation of complex sphingolipids, mainly globotriaosyl-ceramide (Gb3) in the lysosome. In the hemizygous patients, symptoms are typically first experienced in early childhood. During adolescence, the affected subjects may exhibit angiokeratomas, hypohidrosis, proteinuria, progressive renal insufficiency and cornea verticillata. Progressing with age, patients may manifest cardiomyopathy, arrhythmia and cerebrovascular complications in the fourth decade. Cardiac involvement as left ventricular hypertrophy, hypertrophic cardiomyopathy and conduction disturbances are detected in the 60% of the Fabry patients. The most common causes of death are renal failure, heart failure and/or heart attack, myocardial infarction and stroke.

The human lysosomal α-galactosidase A enzyme is encoded by an unique gene, *GLA* (OMIM#300644)**,** located on the long arm of chromosome X (Xq21.3-q22). The major transcript of *GLA* gene consists of six introns and seven exons comprising 1318 base pairs (bp). It encodes a homodimeric glycoprotein composed of 429 amino acids. Currently 664 *GLA* gene mutations are known in the literature, which may be associated with the development of Fabry disease.

1.3.3. Tranthyretin amyloidosis

Amyloidoses are a group of diseases which are caused by extracellular deposition of a similarly appearing morphologically indistinguishable material called amyloid. Amyloid deposition can affect variety of tissues, organs, most commonly the kidneys, liver, heart,

autonomic nervous system, either together multiple organs or isolated the organs of the body. The involvement of the heart is the most common in three form of amyloidosis. Immunoglobulin light chain deposition occurs in case of AL amyloidosis. Wild-type transthyretin protein accumulates in SSA (senile systemic amyloidosis) amyloidosis, while mutant transthyretin protein is deposited in ATTR amyloidosis.

Familial TTR-linked amyloidosis (ATTR) is an autosomal dominant genetic disorder with incomplete penetrance, caused by mutations in the transthyretin gene (*TTR*) encoding transthyretin protein. The gene for human transthyretin (*TTR*; MIM# 176300) maps to chromosome 18 (18q12.1). The major transcript of *TTR* gene consists 3 introns and 4 exons comprising 957 base pairs (bp). It encodes a homotetrameric transthyretin protein of 147 amino acids. Transthyretin amyloidosis typically affects two organ systems, therefore the disease leads to two main phenotypes: in familial amyloid polyneuropathy the phenotype is dominated by neuropathy, while in familial cardiac amyloidosis cardiomyopathy predominates. However, considerable overlap exists between the two major phenotypes. Besides the two main forms, oculo-meningeal forms of the disease are also known.

2. AIMS

Previous to our work, no information was available with regard to the occurrence of HCM phenocopies in Hungarian patients with hypertrophic cardiomyopathy. As HCM phenocopies substantially differ from those of HCM caused by sarcomeric mutations with regard to genetic counseling, prognostic evaluation and appropriate clinical management, their accurate diagnosis by detailed clinical evaluation and mutation analysis is of great clinical importance. Therefore, our aim was to screen HCM patients with suspected multisystem symptoms suggesting HCM phenocopies.

In my PhD work I aimed to: 1. Identify mutations affecting the lysosome-associated membrane protein-2 (*LAMP2*) gene in patients with suspected Danon disease; 2. Identify mutations affecting the α-galactosidase A (*GLA*) gene in patients with suspected Fabry disease; 3. Identify mutations affecting the transthyretin (*TTR*) gene in patients with suspected transthyretin amyloidosis; 4. Conduct clinical and genetic screening of family members of patients with *LAMP2*, *GLA* and *TTR* gene mutations.

3. PATIENTS AND METHODS

3.1. Patients

Patients with the suspicion of HCM phenocopies were analyzed. 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.

3.1.1. Screening for mutations in the *LAMP2* gene in patients with suspected Danon disease

Two young male patients with hypertrophic cardiomyopathy, characterized by marked, concentric left ventricular hypertrophy, elevated levels of creatine kinase, and manifest limb-girdle muscular dystrophy in one case, were investigated.

3.1.2. Screening for mutations in the *GLA* gene in patients with suspected Fabry disease

A total of 21 patients (14 women, 7 men; mean age 52±13), with suspected Fabry disease, underwent screening. Cardiac involvement was present in 18 cases as hypertrophic cardiomyopathy (9 women, 4 men; mean age 46±14) or left ventricular hypertrophy (1 woman, 4 men; mean age 60±7); while restrictive and dilated cardiomyopathy, one case each, was also included. In one case the diagnosis of cornea verticillata indicated the screening. Non-cardiac signs included neurological, renal, ocular or dermatological symptoms. During the screening protocol genetic analysis of the coding regions of the *GLA* gene was performed. In all cases the diagnosis of cardiomyopathies was based on internationally accepted diagnostic criteria. Non-cardiac manifestation included neurological symptoms in 9 cases (cerebrovascular insult, transient ischaemic attack, acroparaesthesia and white matter damage confirmed by CT), renal symptoms in 6 cases (proteinuria, nephropathy, renal failure), ocular symptoms in 2 cases (cornea verticillata, retinal dystrophy), dermatological symptoms in 2 cases and other, not HCM-specific cardiac symptoms in 3 cases (3rd degree AV block, marked restrictive physiology with the exclusion of amyloidosis). Family screening was available in two cases.

3.1.3. Screening for mutations in the *TTR* gene in patients with suspected transthyretin amyloidosis

We analyzed two unrelated patients with HCM morphology and the suspicion of transthyretin amyloidosis. The first patient, 60-years-old, was admitted because of intermittent second- and third- degree AV block; while the other patient, 70-years-old, was hospitalized because of heart failure. Imaging modalities revealed hypertrophic cardiomyopathy phenotypes in both cases, with pronounced diastolic dysfunction and restrictive left ventricular filling. The histopathological examination of a myocardial biopsy confirmed the diagnosis of amyloidosis with an immune classification of transthyretin amyloidosis. Both patients had a history of carpal tunnel syndrome.

3.2 Methods

3.2.1. Molecular genetic analysis of *LAMP2*, *GLA* and *TTR* genes

The family members and patient's care-givers gave informed consent to molecular genetic investigations. Genomic DNA was isolated from peripheral blood samples according to standard methods (GeneJET Whole Blood Genomic DNA Purification Kit, Thermo Scientific). All the coding exons and flanking intronic regions of the *LAMP2* (9 exons), *GLA* (7 exons) and *TTR* (4 exons) genes, comprising the whole coding sequence, were amplified by polymerase chain reaction with primers published in the literature. PCR products were directly cycle sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Electropherograms were analyzed by Sequencing Analyzer v5.4 Software provided by the supplier.

3.2.2. Restriction fragment analysis of the *LAMP2* mutations

As both of the identified *LAMP2* mutations affected restriction sites for a commercially available restriction enzyme; both mutations were also analysed by restriction fragment analysis. In Family A, the mutation abolished the restriction site of the enzyme AlwNI, while in Family B, the mutation created an extra restriction site for enzyme BslI. Restriction analysis was done according to manufacturers' recommendations.

3.2.3. Bioinformatics

Nucleotide changes are reported according to the database of the European Molecular Biology Laboratory- European Bioinformatics Institute (Ensembl database, www.ensemble.org) using

LAMP2-001 (ENST00000200639), GLA-001 (ENST00000218516), and TTR-001 (ENST00000237014.7) as a reference sequence. The annotation of the *TTR* variants was performed by the new nomenclature (taking into account the length of signal peptide, which consists of 20 amino acids).

3.2.4. Linkage analysis

The size of the family and the presence of affected family members in all three generations carrying the *GLA* p.Ile239Met mutation allowed us to conduct linkage analysis. Linkage analysis was done with the FASTLINK program. Linkage between the affection status and the mutation was modelled with the following parameters: disease allele frequency: 1:10.000, disease penetrance: 90%.

4. RESULTS

4.1. Identification of *LAMP2* mutations in patients with Danon disease

Two new mutations in the *LAMP2* gene were identified in the two index cases. In the index patient of Family A, a G-A transition was detected (c.962G>A) in exon 8 of the gene, which changes the tryptophan coding TGG codon to a stop codon TAG, at codon 321 (p.Trp321Stop, nonsense mutation). In the proband of Family B, a 1 bp insertion in exon 8 (c.973insC) was found, leading to a frame-shift mutation. Prediction analysis indicates the inclusion of 24 extra amino acids and a premature stop codon after the last normal amino acid proline at codon 324 (p.Pro324fs+24X). Corresponding base changes were present in the same position in the reverse strand. The two mutations were not present in 200 chromosomes of normal control subjects coming from the same geographical region.

Both mutations altered restriction sites for a commercially available restriction enzyme; therefore, the two mutations were confirmed by restriction analysis. In Family A, the mutation abolished the restriction site (5'-CAGNNCTG-3') of the enzyme AlwNI, while in Family B, the mutation created an extra restriction site (5'-CCNNNNNNNGG-3') for enzyme BslI. Restriction analysis pattern of amplified PCR products of mutation carriers were in full agreement with predicted effects of restriction site loss and gain, respectively.

Both mutations were predicted to lead to a truncated LAMP-2 protein with a complete loss of the transmembrane domain and the short cytoplasmic tail of the protein. This part of the protein is well conserved among different species and among human splice variants of the *LAMP2* gene and is presumed to be deleterious.

In Family A, DNA was available from the grandmother, mother, two sisters and a cousin. They all proved to be carriers of the mutation. In Family B, DNA was available from the mother and the brother The proband's mother carried the mutation, while the brother did not.

Altogether, 11 family members were screened in the two families, while genetic analysis was possible in 9 family members. Eight family members proved to be carriers of either *LAMP2* gene mutations. Out of the eight mutation carrier family members, four proved to be clinically not affected (in terms of development of cardiomyopathy at last follow up). In addition to the four penetrant cases with DNA diagnosis we identified two additional family members with a suggestive manifestation of Danon disease. This made up 6 patients in the two families with proven or likely diagnosis of the disease.

4.2. Identification of *GLA* mutations in patients with Fabry disease

We identified 4 *GLA* mutations in 4 patients (4/21, 19%) out of 21 patients we screened (4 women, average age 49±15 years) [p.Ile239Met (c.717A>G); p.Tyr397Stop (c.1191T>G), c.548-57_-56dupTA; p.Glu358Lys (c.1072G>A)]. Three mutations out of the 4 identified mutations were found in patients with the phenotype of left ventricular hypertrophy or hypertrophic cardiomyopathy comprising 18 cases, therefore the prevalence of *GLA* mutation in this sub-group was 17% (3/18). The fourth mutation (p.Glu358Lys) caused ocular symptoms (cornea verticillata), which indicated the screening, but without substantial cardiac alterations in a female patient.

In particular, a p.Ile239Met *GLA* mutation was identified in an index patient belonging to a three-generation family. Three family members, including the index patient manifested the cardiac phenotype of hypertrophic cardiomyopathy, while two other family members were diagnosed with LV hypertrophy. Taken affection status as the presence of hypertrophic cardiomyopathy, LV hypertrophy or elevated lyso-Gb3 levels, all affected family members carried the mutation while all non-affected family members were non-carriers. Linkage analysis of the family gave a two-point LOD score of 2.01 between the affection status and the presence of the p.Ile239Met *GLA* mutation, strongly supporting linkage. Lyso-Gb3 levels were elevated in all carrier family members (range: 2.4-13.8 ng/ml; upper limit of normal+2 STD: ≤1.8 ng/ml). The GLA enzyme level was markedly reduced in the affected male family member (<0.2 μmol/l/h; upper limit of normal±2 STD): ≥2.6 μmol/l/h).

4.3. Identification of *TTR* mutations in patients with transthyretin amyloidosis

Two non-synonymous *TTR* gene variants were identified in the two patients with TTR amyloidosis. In patient A, an A-G transition was detected (c.323A>G) in exon 3 of the gene,

which changes the histidine coding CAT codon to an arginine coding CGT codon, at codon 108 (p.His108Arg, missense mutation). Among the first-generation relatives of the index patient, the genetic analysis of his mother's blood sample was possible, who died because of heart failure at age of 85 years. Her mutation carrier status was positive.

In patient B, a G-A transition in exon 2 (c.76 G>A) was found, which changes the glycine coding GGT codon to a serine coding codon AGT (p.Gly26Ser). Corresponding base changes were present in the same position in the reverse strand. A synonymous polymorphism, not changing the amino acid sequence, was also detected in the second proband (c.57G>A, p.Glu19Glu).

5. DISCUSSION

5.1. Identification of *LAMP2* mutations in patients with Danon disease

By screening two HCM patients with marked concentric hypertrophy and pre-excitation on the ECG we identified two novel *LAMP2* gene mutations in two families with Danon disease. Both mutations were predicted to lead to a truncated LAMP-2 protein lacking the transmembrane and cytoplasmic domains. We observed a highly malignant phenotype in both families characterized by a large proportion of disease related death.

To the best of our knowledge, one of our families, Family A, is one of the largest family with Danon disease reported to date, in terms of number of affected family members with a proven DNA diagnosis. There are a small number of large families reported in the literature, beside numerous small families. However, in the majority of large families a very significant number of the family members, supposed to be affected, were already deceased at the time of the report, with no material available for DNA investigation and therefore no chance to have a definite DNA diagnosis of the disease. In these cases, although the affection status is suggestive, cannot be taken as proven, which leaves some uncertainty about the clinical phenotype described. In our two families, we identified altogether 8 gene mutation carriers (six in Family A and two in Family B) which clearly helped to draw conclusions about the clinical course of the disease in our families.

In both index cases, the clinical manifestation of the disease was typical for Danon disease, with extreme concentric LV hypertrophy, pre-excitation on the ECG, muscle dystrophy or CK rise, and variable mental retardation. The cardiac phenotype in the affected family members, including female family members was hypertrophic cardiomyopathy, and a high prevalence of

arrhythmias or bradyarrhythmias, necessitating PM implantations. Four disease related deaths occurred in the families, at an average age of 35±18 years, which was clearly lower in males than in females (27±10 vs. 42±25 years).

One of the mutations we identified, p.Trp321Stop, is a nonsense point mutation, presumably leading to premature stop codon at codon 321. The other mutation we described, p.Pro324fs+24X, is a one-base pair insertion which is predicted to lead a frame-shift and incorporation of 24 amino acids before activation of a hidden stop codon. Neither mutation has been described previously. Of note, the latter mutation has been found in the COSMIC database, in melanoma cells, perhaps delineating a higher mutation rate of this region.

5.2. Identification of *GLA* mutations in patients with Fabry disease

In our present work, screening for Fabry disease in patients, who had cardiac, but at the same time other organ manifestations, we identified 4 *GLA* mutations in 4 patients (19%) out of 21 patients we screened (4 women, average age 49±15). The prevalence of *GLA* mutations in Fabry disease considering the subgroup of patients with left ventricular hypertrophy or hypertrophic cardiomyopathy was 17%. The explanation for the much higher prevalence in the present study as compared to previous ones is that we selected the patients not just with isolated cardiac involvement, but with other organ manifestations (neurological, nephrological, dermatological, etc.). These data underlines the fact, that in the case of a "typical" HCM the possible prevalence of Fabry disease is low (approx. 0.5-1%), but if other possible organ manifestation is present in addition to HCM, the chance for the presence of Fabry disease is significantly increased.

One mutation out of four *GLA* mutations we identified is an already known mutation, while the other three are novel gene mutations. The reported *GLA* p.Ile239Met mutation is a novel mutation, although an amino acid change, p.Ile239Thr, at this position has already been described as disease-causing for Fabry disease The third detected *GLA* mutation, p.Tyr397Stop, is a previously unpublished, novel mutation. The mutation presumably caused the interruption of the reading frame, consequently a shorter, truncated protein can be produced, which cannot function properly. The biomarker of Fabry disease, the lyso-Gb3 level was mildly increased in the mutation carrier patient. The fourth identified *GLA* variant, c.548-57_-56dupTA, is a previously unpublished heterozygous variant in intron 3 of *GLA* gene, too.

The *GLA* p.Ile239Met mutation has been observed in a three-generation family. On the basis of the current recommendations of the American College of Medical Genetics and Genomics

(ACMG) and the Association for Molecular Pathology (AMP) there are multiple lines of evidences to characterize the p.Ile239Met mutation as 'pathogenic' for Fabry disease. First and most important, the observation of the markedly decreased GLA enzyme level in the affected male carrier and the increased lyso-Gb3 levels in all the mutation carriers provides evidence for the damaging consequences of the mutation on the gene product. Second, the mutation is absent from controls in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium. Third, the mutation is a novel missense change at an amino acid residue where a different missense change (p.Ile239Thr, see above) determined to be pathogenic has been seen before. Fourth, cosegregation with disease in multiple affected family members in the *GLA* gene, definitively known to cause the disease, has been shown. Fifth, multiple lines of computational evidence support a deleterious effect on the gene or gene product. By applying the rules of the ACMG/AMP guideline for combining criteria to classify sequence variants as 'pathogenic' the p.Ile239Met *GLA* variant that we have identified completely satisfy the criteria of being 'pathogenic'.

5.3. Identification of *TTR* mutations in patients with transthyretin amyloidosis

According to the Human Gene Mutation Database, more than 100 mutations are known to affect the *TTR* gene. Different *TTR* gene mutations will lead to different phenotypes with neuropathic, cardiomyopathic, nephropathic, and ocular forms. Typical forms may be accumulated in specific populations. The clinical manifestation can be highly variable even in the case of the same genetic background: the disease process may remain nearly asymptomatic or it may be severe, may begin at older or at a younger age. Point (missense) mutations are the most typical mutations affecting the gene, but even small deletions and small indel mutations has been also described. In rare cases a „new" (*'de novo'*) mutation can occur, which does not appear in any other family member. Mutations in the *TTR* gene can lead to conformational changes of the encoded protein, which is essential for amyloid fiber formation.

The p.His108Arg *TTR* mutation, we identified in Patient A has already been described previously in a Swedish family. They reported a 65-years-old index patient with bi-ventricular heart failure, with diffusely thickened ventricular walls (IVS: 21 mm), left ventricular hypokinesis, and elevated NT-pro-BNP levels. Besides cardiac involvement, gastrointestinal and polyneuropathic symptoms were also present. The patient died at the age of 70 years after 5 years of follow-up. Six additional affected family member were confirmed during family screening, five had disease manifestation in the form of cardiomyopathy, which appeared

dominantly at later ages (>50 years). Genealogical study showed that they all have common ancestors 9-10 generations back into the 17th century originating from Dalarna (Dalecarlia) in Sweden. So this mutation could be designated as a 'founder' mutation in Sweden.

The p.Gly26Ser *TTR* gene variant identified in Patient B is not a rare variant worldwide, according to the literature. The allele frequency of p.Gly26Ser is 6-12% in an average Caucasian population, 4% in North American Ashkenazi Jews, 7% in North American non-Jews, 6% in Portuguese and 1% African-American population. Based on these evidences, the variant is considered to be a benign, non-amyloidogenic TTR variant. Considering the accumulation of the TTR demonstrated by immunohistochemistry assay and the lack of *TTR* gene mutation, the infiltration in the heart of Patient B corresponds to wild-type transthyretin and the case corresponds to senile amyloidosis.

6. SUMMARY AND CONCLUSIONS

1. We identified two novel *LAMP2* gene mutations in patients with Danon disease

By screening two patients with extreme concentric LV hypertrophy, pre-excitation on the ECG, muscle dystrophy/CK rise, and variable mental retardation, we identified two families with two novel *LAMP2* gene mutations, p.Trp321Stop and p.Pro324fs+24X, causing Danon disease. Both mutations were predicted to lead to a truncated LAMP-2 protein that presumably lacks the transmembrane and cytoplasmic domains. We observed a markedly malignant phenotype in both families characterized by a large proportion of disease related death.

2. We identified known and novel *GLA* gene mutations in patients with Fabry disease

Screening patients with suspected Fabry disease, based on the presence of cardiac and extra-cardiac manifestations, we identified known (p.Glu358Lys) and novel (p.Ile239Met, p.Tyr397Stop, c.548-57_-56dupTA) *GLA* gene mutations. In particular, we described a family with a novel p.Ile239Met *GLA* gene mutation where cardiac involvement in the form of hypertrophic cardiomyopathy, LV hypertrophy and ECG changes was the most common manifestation of the disease and severe renal failure occurred in one family member. We concluded that the p.Ile239Met *GLA* mutation is a pathogenic mutation for Fabry disease and obviously associated with a late onset and predominantly a cardiac variant of the disease.

3. We established a 17% prevalence rate of Fabry disease in patients with hypertrophic cardiomyopathy or left ventricular hypertrophy manifesting additional symptoms, indicating multi-organ involvement

Screening patients with suspected Fabry disease, based on cardiac involvement (mostly in the form of hypertrophic cardiomyopathy or left ventricular hypertrophy) and additional signs of non-cardiac manifestation (neurological, renal, ocular or dermatological symptoms) we found a 17% prevalence of *GLA* mutations indicating Fabry disease. Our results suggest, that in case of unexplained left ventricular hypertrophy or hypertrophic cardiomyopathy and additional suspicious organ manifestations the possibility of Fabry disease should be higher.

4. We identified *TTR* gene mutations in patients with TTR amyloidosis

Two non-synonymous transthyretin gene variants were identified in two patients with hypertrophic cardiomyopathy phenotype. In the first case a previously published, malignant missense mutation (p.His108Arg) was found in the index patient and also in her mother, therefore the case corresponded to familial transthyretin amyloidosis. Wild type transthyretin deposition was detected in the second case, thus in this case the patient had senile systemic amyloidosis.

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 friends for supporting me spiritually throughout writing this thesis and my life in general.