Summary of the dissertation in the field of medicine and health sciences

Genetically determined dilated cardiomyopathy – clinical characteristics, role of biomarkers, prognosis

Dissertation in the form of a publication cycle

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Promoter:

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Publications constituting the basis of the dissertation

1. Titin Truncating Variants in Dilated Cardiomyopathy - Prevalence and Genotype-Phenotype Correlations.

Franaszczyk M., Chmielewski P., Truszkowska G., Stawiński P., Michalak E., Rydzanicz M., Sobieszczańska-Małek M., Pollak A., Szczygieł J., Kosińska J., Parulski A., Stokłosa T., Tarnowska A., Machnicki M.M., Foss-Nieradko B., Szperl M., Sioma A., Kuśmierczyk M., Grzybowski J., Zieliński T., Płoski R., Bilińska Z.T.

PLoS One. 2017 Jan 3;12(1):e0169007. doi: 10.1371/journal.pone.0169007. eCollection 2017. PMID: 28045975

IF 2,766

2. Can Circulating Cardiac Biomarkers Be Helpful in the Assessment of *LMNA* Mutation Carriers?

Chmielewski P., Michalak E., Kowalik I., Franaszczyk M., Sobieszczańska-Małek M., Truszkowska G., Stępień-Wojno M., Biernacka E.K., Foss-Nieradko B., Lewandowski M., Oręziak A., Bilińska M., Kuśmierczyk M., Tesson F., Grzybowski J, Zieliński T., Płoski R., Bilińska Z.T.

Journal of Clinical Medicine. 2020 May 12;9(5):1443. doi: 10.3390/jcm9051443. PMID: 32408651

IF 5,688

3. Titin-Related Dilated Cardiomyopathy: The Clinical Trajectory and the Role of Circulating Biomarkers in the Clinical Assessment.

Chmielewski P., Truszkowska G., Kowalik I., Rydzanicz M., Michalak E., Sobieszczańska-Małek M., Franaszczyk M., Stawiński P., Stępień-Wojno M., Oręziak A, Lewandowski M., Leszek P., Bilińska M., Zieliński T., Płoski R., Bilińska Z.T.

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Introduction

Dilated cardiomyopathy (DCM) is a disease of great social importance, as it often affects young people, limits their professional and family activity, and may lead to sudden cardiac death or end-stage heart failure. It is characterized by a significant phenotypic diversity, and in the preclinical phase, it can be expressed, inter alia, in the form of isolated left ventricular dilatation or arrhythmias.

The etiology of DCM is complex, but genetic factors are known to play a significant role. The diversity of the clinical course, prognosis and response to treatment is largely due to the etiological heterogeneity, including the complexity of the genetic background with several dozen genes spanning different gene ontologies. Exploring the genetic background and subsequent differences in clinical trajectories may contribute - in line with the postulates of personalized medicine - to the development of new treatment methods, potentially more effective, because targeted at the mechanism of the disease.

In recent years, there have been tremendous knowledge advancements in the field. However, the natural history of these rare diseases is still little known, making clinical decisions difficult.

DCM-causing variants (mutations) are most often found in the *TTN* and *LMNA* genes.

LMNA, encoding the nuclear protein lamin A/C, was the first identified gene whose mutations result in DCM inherited in autosomal dominant mode. They are responsible for approx. 6% of DCM cases.

LMNA-dependent cardiomyopathy differs from most DCM cases by rapid disease progression and is characterized by a high frequency of serious adverse events, both arrhythmic, including sudden cardiac death, and related to heart failure (HF), leading to end-stage disease. Atrioventricular block and atrial arrhythmias are usually found early in cardiolaminopathies, anticipating ventricular arrhythmias and onset of HF symptoms.

In 2002, it was demonstrated for the first time that DCM may also be caused by mutations in the *TTN* gene, which encodes the giant striated muscle protein titin, which is the supporting component of the sarcomere. The adoption of next generation sequencing (NGS) at the end

of the 2000s has facilitated examination of large genes such as *TTN*. In 2012, the first study was published assessing the prevalence of mutations in *TTN* in patients with DCM. Initially, however, their causative role was contested. In the European atlas of the clinical genetics of DCM published in 2015, *TTN* truncating variants (*TTN*tv) were not considered the most frequent. Currently, *TTN*tv are known as the most common genetic cause of DCM, identified in approx. 20% of DCM cases.

The prognosis in cardiotitinopathy has been shown to be similar to that in other types of DCM. A good response to typical HF treatment has been demonstrated. The risk of arrhythmias in cardiotitinopathy has not been well defined, although the presence of *TTN*tv has been shown to be a risk factor for sustained ventricular arrhythmia in a cohort of DCM patients with implanted cardioverter-defibrillator (ICD).

The phenotypes of *TTN*- and *LMNA*-related cardiomyopathies are among the best characterized. However, the previous studies did not include the assessment of the diagnostic and prognostic usefulness of the biomarker profile. Assessing the risk of sudden cardiac death is particularly significant, as it can guide the decision about prophylactic ICD implantation.

Identification of the DCM-causative variants in probands allows the search for mutation carriers in the probands' families who are at high risk of developing the disease (even if currently asymptomatic) and require periodic screening to detect the disease at an early stage. The optimal screening strategy is not yet established. Therefore, it would be helpful to identify disease markers that appear first in its course. Again, the usefulness of cardiac biomarkers has not been evaluated in this context.

Aims of the studies

1. Verification of the prevalence of *TTN* truncating variants in DCM population and assessment of their clinical significance by comparing the phenotypic characteristics and prognosis of *TTN*tv-positive and *TTN*tv-negative probands (publication No. 1 - 2017).

2. Clinical characteristics of DCM-causative mutation carriers, including the cardiac biomarkers; analyzing the natural history of cardiomyopathy to identify its early markers

a) among LMNA mutation carriers (publication No. 2 - 2020),

b) among TTN mutation carriers (publication No. 3 - 2022).

3. Assessment of the prognostic role of cardiac biomarkers in predicting sudden cardiac death or its equivalents and comparing it with currently used risk factors

a) among LMNA mutation carriers (publication No. 2 - 2020),

b) among TTN mutation carriers (publication No. 3 - 2022).

Material and methods

The study cohorts were recruited from DCM patients and their relatives diagnosed and treated at the Unit for Screening Studies in Inherited Cardiovascular Diseases of the National Institute of Cardiology, Warsaw in 2010-2020. Genetic testing was offered to all DCM probands, and subsequently also to their relatives, in accordance with the principles of family cascade screening. Genetic testing was performed using NGS except when a pathogenic variant in one of the cardiomyopathy-associated genes had been identified earlier using other methods. Variants identified in probands were followed-up in relatives with Sanger sequencing.

The medical records of patients (probands and relatives) were analyzed retrospectively, in particular the data from the first documented visit to the Institute, prior medical records and follow-up data. Baseline characteristics included medical history, physical examination, 12-lead electrocardiography, two-dimensional echocardiography, Holter ECG monitoring, and laboratory tests, including cardiac biomarkers measured in the period of clinical stabilization: high-sensitivity cardiac troponin T (hsTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP). All records were reviewed for the first documented occurrence of disease indicators such as echocardiographic abnormalities (left ventricular systolic dysfunction, left ventricular dilatation), symptoms of HF, arrhythmias, conduction disturbances and increased concentrations of cardiac biomarkers, and on this basis, the penetrance of individual disease symptoms was calculated using the Kaplan-Meier method.

In order to assess the prognosis, major cardiovascular events were documented, in particular: 1. end-stage heart failure (esHF), defined as death due to heart failure, heart transplantation or left ventricular assist device implantation; 2. malignant ventricular arrhythmia (MVA), defined as sudden cardiac death or its equivalents: successful resuscitation due to sudden cardiac arrest, sustained, hemodynamically unstable ventricular tachycardia or adequate ICD intervention (antiarrhythmic pacing or shock).

Results

Publication No. 1: Titin Truncating Variants in Dilated Cardiomyopathy - Prevalence and Genotype-Phenotype Correlations. PLoS One. 2017 Jan 3; 12 (1): e0169007. doi: 10.1371 / journal.pone.0169007. eCollection 2017. PMID: 28045975

In the group of 72 probands diagnosed with DCM who underwent NGS in 2012-2014, 16 *TTN* truncating variants (*TTN*tv) were identified in 17 probands (24% of all DCM probands, including 30% in the familial DCM, and 18% in the sporadic DCM). As a result of cascade screening in relatives, another 29 *TTN*tv carriers were identified.

*TTN*tv-positive probands did not differ significantly from the *TTN*tv-negative ones in age or sex. Similar in both groups were the NYHA functional class, left ventricular ejection fraction (LVEF), the prevalence of atrial fibrillation, and the percentage of subjects with an ICD. Atrioventricular conduction disturbances or left bundle branch block (LBBB) were significantly less common in *TTN*tv-positive probands.

During the median follow-up of 5.3 years, no significant differences were found in the incidence of esHF between probands with and without *TTN*tv (29% vs 31%, p=0.91).

The disease penetrance was incomplete among *TTN*tv carriers, higher in men than in women (p=0.004). During the follow-up period, 17% of *TTN*tv carriers developed esHF, significantly more often among men than among women (p=0.018).

Publication No. 2: Can Circulating Cardiac Biomarkers Be Helpful in the Assessment of LMNA Mutation Carriers? J Clin Med. 2020 May 12; 9 (5): 1443. doi: 10.3390 / jcm9051443. PMID: 32408651

As a result of genetic testing of DCM patients, 18 different pathogenic LMNA variants were identified in 21 probands. As a result of cascade screening in probands' relatives, another 32 LMNA mutation carriers were identified.

At baseline, 18 probands were diagnosed with DCM or non-dilated hypokinetic cardiomyopathy, and 3 with indeterminate cardiomyopathy. Among relatives, the diagnosis of DCM was made only in 4 patients, indeterminate cardiomyopathy in 18 patients, and in 10 patients there were no significant abnormalities in imaging tests and electrocardiography. The probands were on average 11 years older than their relatives (40 vs 29 years, respectively, p=0.002) and were characterized by more severe HF symptoms, lower LVEF, more frequent arrhythmia, and a higher concentration of NT-proBNP. Atrioventricular block was present in almost half of the relatives (44%), while LBBB was found only in the probands. Elevated levels of hsTnT and NT-proBNP were often detected in both groups (67% vs 37%, p=0.065 and 82% vs 36%, p=0.003, respectively).

Penetrance of cardiac abnormalities in the course of cardiolaminopathy was age-dependent. The earliest abnormality was elevated hsTnT level, present in the 2nd and 3rd decades of life in 12% and 27% of LMNA mutation carriers, respectively, preceding atrioventricular block and HF (5% each in the 2nd and 15% each in the 3rd decade of life), and MVA (2% and 13%, respectively). In the 7th decade of life, the penetrance of cardiolaminopathy indicators was almost complete: 98% of patients developed atrioventricular block, 100% had atrial arrhythmias, 90% had HF symptoms, 92% elevated hsTnT, and 100% elevated NT-proBNP levels.

During a median follow-up of 4.2 years, 14 (26%) patients developed esHF: three of them died of HF and eleven underwent heart transplantation. There was one sudden cardiac death, another patient had sudden cardiac arrest with successful resuscitation. 10 (29%) of 34 patients with implanted ICD experienced its adequate discharge.

The univariable analysis of MVA events during follow-up showed no impact of sex and mutation type on MVA occurrence and confirmed the involvement of established risk factors, such as the presence of atrioventricular block, non-sustained ventricular tachycardia (nsVT) or decreased LVEF. Moreover, NT-proBNP concentration ≥150 pg/mL and hsTnT ≥20 ng/L could be even more potent risk factors of MVA (HR> 13, $p \leq 0.02$ in both cases). In multivariable analysis, elevated NT-proBNP level was the only indicator of the occurrence of MVA.

Publication No. 3: Titin-Related Dilated Cardiomyopathy: The Clinical Trajectory and the Role of Circulating Biomarkers in the Clinical Assessment. Diagnostics (Basel). 2022; 12 (1): 13. doi: 10.3390 / diagnostics12010013. PMID: 35054181

NGS testing in DCM patients from our Unit, which resulted in publication No. 1 in 2017, was continued in the following years. As a result, 41 TTN truncating variants were identified in 46 probands. Three of them were excluded from the study due to the presence of likely pathogenic variants in other cardiomyopathy-associated genes. As a result of cascade screening in relatives, another 65 TTNtv carriers were identified.

The study cohort was composed of 70 patients diagnosed with DCM, 13 patients with indeterminate cardiomyopathy, and 25 healthy relatives with no signs of cardiomyopathy. The DCM patients were young (mean age 40 years), the majority of them were male (79%) with features of mild HF at baseline: 80% were in NYHA class 1–2, the mean LVEF was 36% and the median NT-proBNP concentration was 534 pg/mL. Atrial arrhythmias were found in 31%, and nsVT in 55% of them. There was no significant difference in age between them and their non-DCM relatives, suggesting incomplete penetrance, especially in women who made up the majority (68%) of the non-DCM group. The concentration of hsTnT was significantly higher in the DCM group, but it remained low (median 6.7 vs <3.0 ng/L, respectively).

Penetrance of cardiac abnormalities in the course of cardiotitinopathy was age-dependent. The earliest abnormality was left ventricular dysfunction, defined as left ventricular systolic dysfunction or dilatation, detected in the 2nd, 3rd, and 4th decades of life in 8%, 26%, and 47% of carriers, respectively. It preceded the onset of HF symptoms and the increase in NT-

proBNP concentration by 5-10 years, as well as the onset of severe (often reversible) left ventricular systolic dysfunction (LVEF <35%) and ventricular arrhythmia. Atrial arrhythmias and conduction disturbances appeared late in the course of cardiotitinopathy, and elevated hsTnT level seemed to be an indicator of the end stage.

During the median follow-up of 5.2 years, 13 (12%) of 108 *TTN*tv carriers developed esHF: 5 patients died of HF, and 8 were transplanted. MVA also occurred in 13 (12%) patients, and consisted mostly of adequate ICD interventions, one patient died suddenly, and two had sudden cardiac arrest interrupted by cardiopulmonary resuscitation.

The univariable analysis of MVA events showed the possible influence of risk factors such as the presence of atrial arrhythmias, LBBB, nsVT, or severely reduced LVEF. NT-proBNP concentration was strongly associated with the occurrence of MVA, while the concentration of hsTnT had no significant effect. In multivariable analysis, NT-proBNP concentration ≥650 pg/mL was the best predictor of MVA and outperformed other risk factors such as severely decreased LVEF, LBBB and nsVT.

Summary

1. *TTN* truncating variants are an important cause of hereditary DCM, found in almost one fourth of patients with DCM diagnosed and treated at our Unit. Patients with *TTN*-related DCM do not show significant phenotypic differences compared to other DCM patients, except for a low prevalence of atrioventricular and intraventricular conduction disturbances. The presence of *TTN*tv in patients with DCM does not increase the risk of progression to end-stage HF.

2a. Cardiolaminopathies are characterized by conduction disturbances and arrhythmias preceding the onset of left ventricular systolic dysfunction and symptoms of HF. Increased concentrations of hsTnT and NT-proBNP are detected often at an early stage of the disease. Penetrance of cardiac abnormalities in the course of cardiolaminopathy was age-dependent, with the earliest abnormality being elevated hsTnT levels, preceding the onset of atrioventricular block, ventricular arrhythmia, and HF. Therefore, the assessment of cardiac biomarkers may be useful in the detection of cardiolaminopathy.

2b. In the course of cardiotitinopathy, the earliest detected abnormalities are dilatation and/or systolic dysfunction of the left ventricle, which precede the symptoms of HF, an increase in NT-proBNP concentration and the occurrence of ventricular arrhythmia. Atrial arrhythmias and conduction disturbances appear late in the course of cardiotitinopathy, and the concentration of hsTnT is not elevated in early, but only in the end-stage of the disease. Thus, measurements of cardiac biomarkers cannot replace echocardiography in the detection of an early disease stage in asymptomatic *TTN*tv carriers.

3. Increased NT-proBNP concentration was the strongest risk factor for malignant ventricular arrhythmia in DCM associated with both *TTN*tv and *LMNA* mutations, and outweighed other risk factors such as decreased LVEF and nsVT.

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