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ZBTB17 Gene rs10927875 Polymorphism in Slovak Patients with Dilated Cardiomyopathy

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Abstract**—**Dilated cardiomyopathy (DCM) is a severe cardiovascular disorder characterized by progressive systolic dysfunction due to cardiac chamber dilatation and inefficient myocardial contractility often leading to chronic heart failure. Recently, a genome-wide association studies (GWASs) on DCM indicate that the ZBTB17 gene rs10927875 single nucleotide polymorphism is associated with DCM. The aim of the study was to identify the distribution of ZBTB17 gene rs10927875 polymorphism in 50 Slovak patients with DCM and 80 healthy control subjects using the Custom Taqman®SNP Genotyping assays. Risk factors detected at baseline in each group included age, sex, body mass index, smoking status, diabetes and blood pressure. The mean age of patients with DCM was 52.9±6.3 years; the mean age of individuals in control group was 50.3±8.9 years. The distribution of investigated genotypes of rs10927875 polymorphism within ZBTB17 gene in the cohort of Slovak patients with DCM was as follows: CC (38.8%), CT (55.1%), TT (6.1%), in controls: CC (43.8%), CT (51.2%), TT (5.0%). The risk allele T was more common among the patients with dilated cardiomyopathy than in normal controls (33.7% versus 30.6%). The differences in genotype or allele frequencies of ZBTB17 gene rs10927875 polymorphism were not statistically significant (p=0.6908; p=0.6098). The results of this study suggest that ZBTB17 gene rs10927875 polymorphism may be a risk factor for susceptibility to DCM in Slovak patients with DCM. Studies of numerous files and additional functional investigations are needed to fully understand the roles of genetic associations.

Keywords**—**Dilated cardiomyopathy, SNP polymorphism, ZBTB17 gene.

I. Introduction

DILATED cardiomyopathy (DCM) is a common form of heart muscle disease, it represents a major cause of cardiovascular morbidity and mortality and is characterized by systolic dysfunction, dilation and impaired contraction of the ventricles, often leading to chronic heart failure and eventually requiring cardiac transplantation [11]. Dilated cardiomyopathy is diseases with estimated prevalence of 37 in 100 000 people. DCM incidence in the general population varies with age and geographical distribution of population [3], [6]. It is the most frequent cause of heart failure and cardiac transplantation in young adults. About one-third of all patients have a suspected familial disease indicating a genetic basis of dilated cardiomyopathies. In 20-50% of cases dilated cardiomyopathy is hereditary disease [1], [2]. Dilated cardiomyopathy may be secondary present in association with systemic disease or syndromes. Mutations in both sarcomeric and cytoskeletal genes have been implicated in dilated cardiomyopathy, but the variable expression and penetrance of each gene that harbors a different mutation result in vast clinical heterogeneity among patients. Detection of etiopathogenetic mutations allows early identification of patients at risk [2]. Many candidate gene studies in humans have tested the association of single nucleotide polymorphisms in various genes coding for proteins with a known cardiovascular function. The ZBTB17 gene encodes protein 17, which contains both zincfinger and BTB domains. Protein 17 is also known as myc-interacting protein 1 (MIZ-1) and is a transcription factor of 87 kDa containing 13 zinc finger domains at its carboxy-terminal end and a BTB / POZ domain at its N-terminus. Recently, a genome-wide association studies (GWASs) on dilated cardiomyopathies indicate that the ZBTB17 gene rs10927875 single nucleotide polymorphism is associated with dilated cardiomyopathy.

In recent years the progress in identifying of genetic causes and acquiring key information regarding the genotype-phenotype correlations in many diseases has been recorded. Clinical genetic testing allows identification of asymptomatic individuals at risk. Knowledge of the cardiomyopathy etiopathogenesis allows implementing clinical examinations of risk individuals, thereby creating opportunities for prevention and treatment interventions. For patients and families members genetic counseling and recommendations for genetic screening with regard to cardiovascular risk is also available.

II. Materials and Methods

The aim of the study was to identify the distribution of ZBTB17 gene rs10927875 polymorphism in the cohort of 130 subjects, 50 patients with dilated cardiomyopathy and 80 healthy control subjects. The mean age of patients with DCM was 52.9±6.3 years, the mean age of control subjects 50.3±8.9 years. Risk factors detected at baseline in each group included age, sex, body mass index, smoking status, diabetes and blood pressure. Blood samples were collected from patients using tubes containing ethylenediaminetetraacetic acid (EDTA). Genomic DNA was extracted from leukocytes by a standard methodology. Genotyping was performed using the Custom Taqman®SNP Genotyping assays (Step One Applied Biosystems).

Differences in the distribution of genotypes and alleles of rs10927875 polymorphisms in ZBTB17 gene between the cases and controls were evaluated using the chi-square (χ^2) test. Hardy-Weinberg equilibrium was tested by a goodness-of

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fit χ^2 test to compare the observed genotype frequencies with those expected among control subjects. All statistical analyses were performed with SPSS 16.0.

III. Results and Discussion

In our study we present the results of ZBTB17 gene rs10927875 polymorphism genotyping in relation to dilated cardiomyopathy in Slovak population. The distribution of investigated genotypes of rs10927875 polymorphism within ZBTB17 gene in the cohort of Slovak patients with dilated cardiomyopathy was as follows: CC (38.8%), CT (55.1%), TT (6.1%), the distribution in controls: CC (43.8%), CT (51.2%), TT (5.0%) (Table I).

TABLE I
Genotype Distribution of RS10927875 Polymorphism within ZBTB17 Gene in the Cohort of Slovak Patients with Dilated Cardiomyopathy and Controls

Patients (n=50)			Controls (n=80)			χ2	p
genotype	n	%	genotype	n	%		
CC	19	38.8	CC	35	43.8		
CT	27	55.1	CT	41	51.2	0.16	0.6908
TT	4	6.1	TT	4	5		

Detected allele distribution of rs10927875 polymorphism in ZBTB17 gene in Slovak patients with dilated cardiomyopathy and control subjects are shown in Table II.

TABLE II
Allele Distribution of RS10927875 Polymorphism within ZBTB17 Gene in the Cohort of Slovak Patients with Dilated Cardiomyopathy and Controls

Patients (n=50)			Controls (n=80)			χ2	p
allele	n	%	allele	n	%		
C	65	66.3	C	111	69.4	0.26	0.6098
T	35	33.7	T	49	30.6		

The risk allele T was more common among the Slovak patients with dilated cardiomyopathy than in normal controls (33.7% versus 30.6%). Hardy-Weinberg equilibrium was tested for each group of participants using χ^2 test. Using the chi-square (χ^2) test we compared genotype and allele frequencies between patients with dilated cardiomyopathy and controls. The differences in genotype and allele frequencies of ZBTB17 gene rs10927875 polymorphism between patients with diagnosed dilated cardiomyopathy and control subjects were not statistically significant (p=0.6908; p=0.6098). No differences in genotype or allele frequencies in ZBTB17 gene rs10927875 polymorphism between patients with dilated cardiomyopathy and control subjects were found (χ^2=0.16, p=0.6908; χ^2=0.26, p=0.6098). Further studies are necessary for obtaining of more reliable results on the larger population for clarification molecular mechanisms with practical use in everyday practice.

Dilated cardiomyopathy is a common form of heart muscle disease with a prevalence of 1/2500 in the general population. It represents a major cause of cardiovascular morbidity and mortality, eventually requiring cardiac transplantation [5]. Dilated cardiomyopathy is characterized by systolic dysfunction, dilation and impaired contraction of the ventricles often leading to chronic heart failure. In approximately 35% of cases, dilated cardiomyopathy is a familial disease. Sarcomeric and cytoskeletal genes mutation have been implicated in etiology of dilated cardiomyopathies. The variable expression and penetrance of sarcomeric and cytoskeletal genes different mutations results in clinical heterogeneity among patients. Many studies have been conducted to identify the molecular basis of dilated cardiomyopahy. At least 15 different causal genes have been identified with variable individual prevalence ranging from 1% to 10% [8], [9]. These genes are related not only to sarcomeric proteins but also to proteins of the nuclear membrane, lamin A and lamin C (lamin A/C), cytoskeletal (desmin, dystrophin, dystrophinsarcoglycan complex) and phospholamban. Moreover, 7 loci genetically linked to dilated cardiomyopathy have been reported but without gene identification. Preliminary data suggest that MIZ-1 (a transcription factor encoded by ZBTB17) may play a role in the transcriptional activation of numerous other genes [4], [9]. To date, only a few common susceptibility alleles for sporadic dilated cyrdiomyopathy have been identified from candidate-gene approaches. As it is well known that both immunity and apoptosis play an important role in the pathology of dilated cyrdiomyopathy, the polymorphism in the ZBTB17 gene might be associated with dilated cardiomyopathy. Clearly, functional studies are required to support these hypotheses.

The most common form of genetic variation is the single nucleotide polymorphism defined according to the variation of a single nucleotide occurring in more than 1% of the population. The majority of SNPs are likely to be allelic variants that do not affect the expression or function of a protein. Single nucleotide polymorphisms that directly influence phenotype may be located within coding or regulatory regions of genes and can result in disease. In contrast, single nucleotide polymorphisms within regulatory regions tend to have more quantitative effects; for example, they may alter the expression level of a receptor or signaling protein, resulting in a more subtle variation in the associated phenotype [7]. Polymorhism rs10927875 is located in an intron of ZBTB17 gene on chromosome 1p36.2-p36.1. The locus of interest covers approximately 210 kb in a genomic region; exhibits strong LD; and spans several other genes, including SPEN (spen homolog, transcriptional regulator), HSPB7, CLCNKA (chloride channel Ka), and CLCNKB (chloride channel Kb). Our data, together with the results from previous GWAS on dilated cardiomyopathy, substantiate the importance of rs10927875 and related polymorphisms in the *ZBTB17* locus for DCM susceptibility [10]. However, the biological mechanism explaining the association between the polymorphism rs10927875 and dilated cyrdiomyopathy risk remains unclear. Preliminary data suggest that MIZ-1 (a transcription factor encoded by *ZBTB17*) may play a role in the transcriptional activation of numerous other genes [5], [9], [10].

MIZ-1 is composed of 13 zinc finger domains at its C terminus and a BTB/POZ (Broad-complex, Tramtrack, and Bric-a-brac/pox virus zinc finger) domain at its N-terminus [10]. . Whether MIZ-1 activates or represses the transcription of its target genes depends on its interacting partner. The genes that encode the negative cell cycle regulators Cdkn2b or Cdkn1a have been validated as direct MIZ-1 targets, and c-Myc has been shown to be recruited to the Cdkn1a promoter by MIZ-1; this interaction blocks Cdkn1a induction by p53 and other activators in cancer cells [10].

Studies screened approximately 2000 candidate genes previously implicated in cardiovascular disease in more than 1900 sporadic dilated cardiomyopathy cases in German and French populations. These studies showed that the single nucleotide polymorphisms rs10927875 in ZBTB17 was associated with dilated cardiomyopathy [10].

The pathophysiology of DCM is multifactorial with a possible implication of environmental factors and the existence of a strong genetic component attested by a high rate of familial aggregation. 20–35% of dilated cardiomyopathy cases having an affected first-degree relative. Mutations in more than 30 genes have been identified in monogenic forms of DCM, most of them encoding proteins of the cytoskeleton or the sarcomere. Over the last few years, genome-wide association studies (GWASs) exploiting the power of high density genotyping arrays have led to the discovery of numerous loci implicated in cardiovascular diseases. However, no GWAS of dilated cardiomyopathy has been reported so far, probably because the relatively low prevalence of the disease makes the assembly of large clinically homogeneous cohorts of patients difficult.

Numerous genes encoding cytoskeletal, sarcomeric, and nuclear proteins have been linked to the pathogenesis of dilated cardiomyopathy, and most of the respective mutants disrupt the structural integrity of sarcomeres in cardiac myocytes. Frequently, point mutations in cytoskeletal proteins critically diminish force generation and interfere with mechanical transduction within the contractile apparatus of the myocardium, thereby ultimately leading to impaired systolic function. Since one of the major factors in dilated cardiomyopathy pathogenesis involves autoimmune-mediated damage to cardiac tissue, candidate genes that are involved in controlling immune reactions have currently come into focus in genetic research.

The penetrance of the identified mutations is highly variable and age-dependent. Many relatives of patients with dilated cardiomyopathy show only minor cardiac abnormalities, and it is unknown whether they progress to full cardiomyopathy in later life. Symptoms due to heart rhythm problems (or arrhythmias, which means irregular, fast or slow heart rates) can also be either the first symptom or a symptom that appears after other symptoms have led to a diagnosis of dilated cardiomyopathy. Symptoms of rhythm problems include palpitations (feeling of funny or fast heart beats), syncope (fainting), seizures (convulsions), or even sudden cardiac arrest (heart stops beating effectively requiring resuscitation). These symptoms can occur at any age and with any stage of cardiomyopathy, even if other more severe symptoms of congestive heart failure have not yet appeared. Once there is clinical suspicion based on the patient history and physical exam, the diagnosis of DCM is primarily based on echocardiography. With this test, your physician will be using ultrasound beams to evaluate the heart looking for dilated chambers and decreased pump function. Along with the echocardiogram, there are other tests that will likely be done to confirm the diagnosis or provide clues as to the cause.

Knowledge of the dilated cardiomyopathy disease genes led to the new hypothesis that dilated cardiomyopathy is a disease of myocardial generation or transmission of force. Better understanding of the expression and function of disease genes may lead to new diagnostic and therapeutic tools.

In our study we identify the distribution of ZBTB17 gene rs10927875 polymorphism in Slovak patients with dilated cardiomyopathy. The results of our study confirm the differences in distribution of investigated genotypes and alleles of rs10927875 polymorphism within ZBTB17 gene in the cohort of Slovak patients with dilated cardiomyopathy and controls. The risk allele T was more common among the Slovak patients with dilated cardiomyopathy than in normal controls (33.7% versus 30.6%). However, detected differences were not statistically significant (p=0.6908; p=0.6098). The results of this study suggest that ZBTB17 gene rs10927875 polymorphism may be a risk factor for susceptibility to dilated cardiomyopathy in Slovak patients. Our study is the first study testing the differences in genotype and allele distribution of rs10927875 polymorphism in ZBTB17 gene in Slovak population. Our findings further highlighted the importance of rs10927875 polymorphism within ZBTB17 gene and provide more information for understanding of the dilated cardiomyopathies genetic architecture. Clearly, functional studies are required to support these hypotheses.

Dilated cardiomyopathy has been extensively investigated for many years, but its pathogenesis remains uncertain. The role of genetic factors in the pathogenesis of cardiomyopathies has received growing attention. One of the many achievements in medical genetics over the past decades has been the ability to visualize sequence differences directly in DNA. However, the biological mechanism explaining the association between the polymorphism rs10927875 within ZBTB17 gene and dilated cardiomyopathy risk remains unclear.

Understanding the genetic heterogeneity of complex polygenic diseases like dilated cardiomyopathy is challenging. One of the many achievements in medical genetics over the past decades has been the ability to visualize sequence differences directly in DNA. However, the biological mechanism explaining the association between the polymorphism rs10927875 and the risk of dilated cardiomyopathy remains unclear.

Current development and application of high performance genotyping methods in genome-wide analysis opens a new era in discovering the nature of complex genetic diseases, which include cardiovascular disease. Cardiomyopathies are become the subject of interest to cardiologists, internists, surgeons, paediatricians as well as genetics. The issue of

cardiomyopathies is considered one of the most current health and socio-economic problems, despite the lack of effective practical solution the basic data and studies on basic research. Advances in molecular biology in the last decade is expanding opportunities for early diagnosis, pathophysiological understanding, treatment, and overall monitoring of many serious diseases. In clinical practice, in Slovakia, however, are not yet sufficiently exploited.

The growing knowledge of human genome research in the last ten years has created the assumptions for explanation the genetic nature of many inherited diseases. With the rapid development of molecular genetic testing technology numerous dilated cardiomyopathies susceptibility genes have been reported by GWASs (genome-wide association studies) allowing for the exploration of the underlying genetic mechanisms for dilated cardiomyopathies. Dilated cardiomyopathy causes considerable morbidity and mortality. Better knowledge of the genetic background and disease-causing mechanisms would probably help in focusing early treatment on right subjects and potentially also developing new treatment modalities and improving cardiac outcome in the affected patients.

IV. Conclusion

The results of the present study suggest that ZBTB17 gene rs10927875 polymorphism may be a risk factor for susceptibility to DCM in Slovak patients with dilated cardiomyopathy. Further studies of ZBTB17 gene polymorphisms of numerous files are needed to fully understand the importance of genetic markers. With the rapid development in human and model organism genome sequence, and the progress in molecular technologies, analytical tools, bioinformatics, and functional genomic, one can expect it will be possible to define the genes and mutations and their functions in the predisposition or the resistance to dilated cardiomyopathies. Our results represent an initial study, it can be considered as preliminary and first of its kind in Slovak population. Studies of numerous files and additional functional investigations are needed to fully understand the roles of genetic associations. Identification of genetic disease markers in at-risk persons could play an important role in preventive health care.

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