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Desmin A213V substitution represents a rare polymorphism but not a mutation and is more prevalent in patients with heart dilation of various origins

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Several desmin mutations have been described in patients with cardiomyopathies and distal myopathies. Among them, A213V substitution has been associated with three completely different clinical phenotypes: restrictive cardiomyopathy, dilated cardiomyopathy and isolated distal myopathy. However, the identification of this substitution also in control subjects has highlighted the question if the A213V shift represents a conditional mutation, giving rise to cardiomyopathy only in the presence of other predisposing factors. The aim of the present work was to study the potential role of this substitution in predisposing to heart dilation.
Methods and results. We screened 108 patients with heart dilation due to ischemic heart disease, alcoholic cardiomyopathy or viral myocarditis, and 300 healthy controls for the presence of A213V substitution by direct sequencing and confirmed the results by site-specific restriction. In the control group A213V substitution was identified in 3 out of 300 patients, representing a rare polymorphism with a frequency of approximately 1%, which corresponds to the earlier reported frequency. In the study group A213V substitution was found in 5 out of 108 cases, corresponding to approximately 4.6% ($p < 0.035$). Therefore we conclude that A213V desmin substitution represents a conditional mutation, i.e. a rare polymorphism that plays a role as a predisposing factor resulting in maladaptive heart remodelling in the presence of other pathological factors.

Key words: Desmin, polymorphisms, heart dilation

Introduction

Desmin is a chief intermediate filament of muscle cells. It is highly expressed postnatally in all types of muscle tissue where it contributes to the structural and mechanical integrity of the skeletal and cardiac myocytes as well as smooth muscle cells (1). It connects to the sarcomere at the level of the Z-discs, and serves as a linker between neighboring myofibrils, thus, providing their simultaneous and effective contraction. It is also involved in proper mitochondrial positioning, ensuring mitochondrial membrane stability and proper mitochondrial function (2). Recently, besides just structural functions, desmin along with some other Z-disk associated proteins has been shown to play an important role in mechanosensing and mechanotransduction (3, 4).

Desmin mutations have been associated with cardiac disorders, such as cardiomyopathies (dilated, restrictive or hypertrophic), and with skeletal myopathy (5). More than 40 different desmin mutations have been described up to date with most of them giving rise to combined cardiac and skeletal muscle phenotype. In terms of the prevalence, desmin mutations have been shown to be a rare cause of dilated cardiomyopathy (1-2%), whereas it is found in a considerably larger proportion of restrictive cardiomyopathies in association with intracellular aggregates and conduction defects (6-9). Recently A213V desmin substitution has been described in seven unrelated patients with three different phenotypes; distal skeletal myopathy, restrictive cardiomyopathy, and dilated cardiomyopathy (8, 10, 11). However, this substitution has been found also in control groups with a frequency of approximately 1%, and has also been described in a familial case of dilated cardiomyopathy where it did not segregate with the disease phenotype (6). Therefore, it was suggested that A213V substitution represents a rare

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polymorphism rather than a true disease-causing mutation. Our group has previously described this substitution in a patient with late onset and rapid progression of heart dilation, no signs of coronary artery disease or previous myocardial infarction, but with a long history of arterial hypertension (8). We therefore hypothesize that A213V substitution can play a predisposing role in heart dilation in presence of other provocative and unfavorable factors. This hypothesis of A213V constituting a conditional mutation was here tested by comparing the frequency of the desmin A213V substitution in a group of patients with heart dilation due to various factors to the frequency in a healthy control group.

Materials and methods

Patient cohort

The study group included patients with sings of heart failure (NYHA class II-IV) and enlarged left ventricle dimensions (LVEDD > 56 mm) with preserved of decreased contractile function due to various etiologies: ischemic heart disease, arterial hypertension, metabolic syndrome, alcoholic cardiomyopathy and inflammatory cardiomyopathy. They patients with genetic dilated cardiomyopathy (familial or sporadic forms) were excluded from the study. Totally, 108 patients with heart failure and cardiac dilation were included in the study group. Of these 70 (65%) were due to ischemic heart disease, including 19 (17.5%) post MI, 14 (13%) with metabolic syndrome and arterial hypertension, 10 (9%) with alcoholic cardiomyopathy and 14 (13,%) with inflammatory cardiomyopathy. Clinical data and case history were obtained by direct physical examination during patient's visit and from medical records. Echocardiography was performed according to standard protocol. Written informed consent was obtained prior to patient enrollment and was approved by the local ethical committees of Almazov Federal Centre, St.Petersburg and Stockholm. The control group included 300 healthy donors with the same Caucasian background.

Sequencing of the desmin gene

Genomic DNA was extracted from peripheral blood using a phenol-chlorophorm purification method. Exon 2 of the desmin gene (*DES*) including exon-intron boundaries was amplified by PCR followed by direct DNA sequencing (8). In all cases A213V substitution has been confirmed by restriction enzyme analysis, since A213V substitution introduces a restriction site for AccBsI, thus, cutting the original 384 bp PCR product to 103+281 fragments.

Statistical analysis

Echocardiography data were analyzed using a Student's t-test and expressed as mean ± SD. The difference in the distribution of genotypes between the study group and controls was statistically analyzed by means of the Fisher's exact test to obtain a *P* value. A *P* value of less than 0.05 was considered significant. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to express the strength of the association between a polymorphism and the disease.

Results

Direct sequencing of *DES* exon 2 revealed A213V substitution in 5 patients from the study group, and in 3 cases from control group (4.6% and 1% respectively, P < 0.035, Table 1). There was no significant difference in mean echocardiography values between patients with and without A213V substitution in the study group (Table 2). The major cause of heart failure in a patient 1 was ischemic heart disease without arterial hypertension or metabolic syndrome, complicated with ST-myocardial infarction 7 years prior to examination. Patients 2 and 3 both had metabolic syndrome including arterial hypertension, dyslipidemia and diabetes mellitus. Patient 4 was a young man suffering from chronic post-viral myocarditis confirmed by Dallas criteria after endomyocardial biopsy 4 yeas prior to examination. In patient 5 dilated cardiomyopathy was observed in combination with arterial hypertension and Marfan-like connective tissue disorder phenotype.

Discussion

Desmin A213V substitution has previously been described in different cardiac/muscle phenotypes. The first patient, described in Holland, had skeletal distal myopathy and no cardiac phenotype (10). In spite of concomitant double-mutation in the alpha–glucosidase gene, skeletal muscle biopsy with desmin and αB crystalline -positive protein aggregates and with no evidence of glycogen or phosphatase-positive vacuoles strongly suggested the A213V desmin shift to be disease causing. The second A213V substitution was described in a familial case of restrictive cardiomyopathy without signs of skeletal muscle affection, where A213V substitution segregated with

Table 1. Direct sequencing of *DES* exon 2.

Genotype	Study group (n = 108)	Control group (n = 300)
A213A	105 (95.5%)	297 (99%)
A213V	5 (4.6%)	3 (1%)

P < 0.035 CI – 1.1-20.0 OR 4.7

Table 2. Mean echocardiography values observed in patients with and without A213V substitution.

	LVEDD	LVESD	RVEDD	LA	LVPWd	LVPWs	IVSd	IVSs	EF
Pat.1	61	49	37	57.8	12.3	18.2	12.6	15	31.42
Pat.2	63	49	37.3	48	16	17	14	16	44.5
Pat.3	56	29.4	23.1	53.9	10.5	16.4	12.6	15.5	55.7
Pat.4	74	59	34	45	12.3	12.3	9.5	9.9	27.9
Pat.5	62	42.6	22.4	48	8.2	17.1	9.7	13.5	28
Mean A213V group	63.2 ± 5.9	45 ± 10.8	29.2 ± 7.4	48.7 ± 5.1	11.8 ± 2.8	15.7 ± 2.2	11.5 ± 1.9	13.7 ± 2.4	39 ± 12.2
Mean study group	62.2 ± 6.6	49.2 ± 11.9	29.5 ± 6.9	50.5 ± 8.1	10.0 ± 2.0	14.1 ± 3.2	10,7 ± 2.9	12.8 ± 3.1	37.7 ± 14.9

the disease (11). Further, our group previously described this substitution in a patient with late-onset dilated cardiomyopathy, first degree AV block and left bundle branch block. However, findings from Taylor et al. put a directly causative role of the A213V substitution at question. The substitution was found in one large pedigree with familial DCMP out of 116 families studied, as well as in 6 out of 306 non-familial cases (6). While absence of clinical symptoms in several A213V carriers from this study could be explained by low penetrance, absence of this substitution in one affected family member almost ultimately excludes its exclusive primary disease-causing nature. However the possibility that A213V is a rare allelic variant, predisposing to malign cardiac remodeling as a conditional mutation has not previously been analyzed.

In our study the frequency of A213V substitution in control group correspond to the earlier reports and constitutes approximately 1% (6-8). However, overrepresentation of A213V in the group with cardiac dilation strongly supports that this substitution may have an unfavorable impact on cardiac remodeling under various stress conditions. If so, it is particular interesting to unravel a mechanism, by which structural polymorphism of desmin influences a pattern of cardiac remodeling. In our study we did not see any overt gross alterations of desmin or vimentin filament network in A213V desmin transfected HeLa cells. A213V desmin is able to participate in fine filamentous network and does not appear, at the light microscope level, to interfere with vimentin filaments. This corresponds to the earlier data from our as well as other groups, obtained on different cell types (6, 10). However, it does cause aberrant IF assembly as monitored by viscometer analysis, a very sensitive tool to assess filament aggregation (12). Further, it has been found that pathological effect of mutant desmin does not completely correlate with the ability of aggregate formation (12, 13). Kreplack et. al showed severe alteration of nanomechanical properties of two C-terminal desmin rod domain mutations in spite of their ability to form filamentous network and absence of desmin aggregates in transfected cells (14). If this is true also for A213V substitution, altered biophysical properties of A213V desmin can predispose to cardiac vulnerability under certain stress conditions.

Recently, Z-disks were shown to be an important element of mechanical stretch sensing machine. Several desmin and Z-disk associated proteins as well as desmin itself have been shown to play an important role in development of cardiac hypertrophy induced by various stimuli (15-18). In this context, modified properties of A213V desmin can predispose to altered adaptation of muscle cells to external and internal stress factors, such as pressure overload, ischemia or impaired metabolism. This could be a case, for example, in a patient with alpha-glucosidase gene deficiency in skeletal muscles, in patients with impaired glucose metabolism or in patient with connective tissue disorders. This substitution is likely to be of less importance in familial cases of DCMP, where genetic origin of the disorder due to severe alterations in myocyte proteins by itself is sufficient to cause a clinical phenotype. However, in non-genetic DCMP and in patients with heart dilation due to other acquired causes where many other factors besides genetic play a pivotal role this substitution can make sense as a conditional mutuation. It is interesting to note that another rare polymorphism of cardiomyocyte structural protein nebulette has been shown to associate with non-familial DCMP, but not with familial cases (19). Therefore it could be particular important to study rare gene variants and their impact on cardiac remodeling, since genome-wide approach, generally accepted now for searching new disease-causing and disease-modifying genes, usually does not cover rare polymorphisms with a frequency 5% and less.

In conclusion, we have shown that the A213V substitution represents a rare polymorphism with a population frequency of approximately 1%, that is overrepresented in patients with heart dilation of various origins (4,6%). This makes it most likely that the A213V shift constitutes a conditional mutation predisposing to malign cardiac re-

modeling under other stressful conditions. More studies A213V desmin biomechanical properties can shed light on the detailed mechanisms, involved in cardiac maladaptation in A213V patients.

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