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Research Article

MUTATIONS IN *ACTA2* GENE DETECTED BY NEXT GENERATION SEQUENCING IN PATIENTS WITH PATHOLOGY OF GREAT VESSELS

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Summary: The *ACTA2* gene provides instructions for making a protein called smooth muscle alpha (α)-2 actin, found in smooth muscle cells that line the layers of the walls of the arteries. It contributes to the ability of these muscles to contract, which allows the arteries to maintain their shape instead of stretching out as blood is pumped through them. The purpose of this study was genetic profiling of patients with phenotype determined by cardio vascular diseases with pathology of great vessels. Clinical diagnosis of patients was done according to standard hospital procedures. Sequencing of the DNA samples was performed on a MiSeq System by targeted next generation sequencing of 174 genes connected to cardiovascular diseases included in TruSight Cardio gene panel (Illumina). Sequencing data were analyzed by SoftgeneticsNextGene Software (2.3.3). Heterozygous variants in gene *ACTA2*(10q23.31) were detected in two patients. In one patient, a 15-years-old boy diagnosed with aortic dissection type III, a heterozygote form of the variant *ACTA2*:p.Arg258Cys was found. The mutation is known and detected in autosomal dominant inheritance of familial form of thoracic aortic aneurysms. The second patient, a 40-years-old woman with aneurysm of abdominal aorta and familial history of father who had died of thoracic aortic aneurysm, a heterozygous *ACTA2*:p.Lys52Glu variant was detected. In conclusion, the results underline the importance of this method in determining mutation status in correlation to clinical phenotype and has large implication in treatment and prognosis of patients and their families in cases with uncertain clinical diagnosis.

Keywords: *ACTA2*; aneurysm; aortic dissection; great vessels; next generation sequencing.

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INTRODUCTION

The pathology of great vessels includes diseases of the aorta, arteries and veins that together with hypertension and peripheral vascular diseases and other disorders as ischemic heart disease or coronary artery disease, cerebrovascular disease, congenital heart disease, rheumatic heart disease, cardiomyopathies and cardiac arrhythmias constitute the complex group of the cardiovascular diseases (CVD) (Mendis et al., 2011). According to World Health Organization, CVD are projected to remain the single leading cause of death causing almost 23.6 million deaths annually by 2030. Although longer morbidity-free rate correlates with personal isolation of risk factors still even for those with optimal parameters, the overall lifetime risk is as high as more than 30% for total CVD (Wilkins, 2012). The scientific recommendation is to characterize genes and genetic variants that are associated with CVD across individuals and populations from multiple ethnic groups as an important step that will lead to new discoveries and optimization of care (Santhi, 2013).

We exploited the use of next generation sequencing by gene panels to possibly reveal a genotype-phenotype relation in patients with CVD disorders. In two patients with pathology of great vessels, namely thoracic aortic aneurysms and aortic dissection and rupture we detected variants in *ACTA2* gene.

ACTA2 (https://www.ncbi.nlm.nih.gov/gene/59) was located by Ueyama et al. (1995) with *in situ* hybridization at the long (q) arm of chromosome 10 at position 23.31 (10q23.31). It consists of least 9 exons that code a protein from the actin protein family called smooth muscle alpha (α)-2 actin found in smooth muscle cells (Ueyama et al., 1984). This protein forms the core of sarcomeres which are necessary for muscles to contract. The walls of internal organs, including blood vessels are composed of smooth muscles that contract without conscious control and in case of blood vessels it prevents excessive stretching or rupture under the pressure of blood that is pumped through.

MATERIALS AND METHODS

All patients were treated and clinically diagnosed according to standard procedures for clinical assessment of the hospital where the cardiac evaluation was performed. After signing informed consent by the patients or their parents if minors were tested, blood samples were obtained for DNA extraction by a commercially available QIAamp DNA Blood Mini Kit from Qiagen. Qubit dsDNA BR Assay Kit, Life Technologies and NanoDrop 2.0 was used for quantitative and qualitative assessment of the isolated genomic material. TruSight Cardio panel by Illumina, which includes 174 genes associated to cardio-vascular diseases was used for library preparation. Enrichment and quantification of the enriched DNA libraries in triplicate was done by qPCR on an Illumina qPCR Eco system using KAPA Library Quantification Kit. Accurate concentrations of the dilutions were calculated followed by denaturation and dilution of libraries according to MiSeq System Denature and Dilute Libraries Guide. Samples were loaded for sequencing by use of cartridge as described in MiSeq System User Guide (part # 150276) and sequencing was performed on a MiSeq System using the MiSeq Reagent Kit v2.

SoftgeneticsNextGene Software

(version 2.3.3) was used for analysis of sequencing data. Data aligned to the Human reference sequence - Genome Reference Consortium Human Build 37 (GRCh37/hg19) led to a list of variants that in terms of filtering was annotated by the VariantStudio Software. The final step was validation of the variants in candidate genes by Sanger sequencing.

RESULTS

The genetic profiling of patients with cardio vascular diseases revealed two patients with pathology of great vessels that had heterozygous variants in gene *ACTA2*(10q23.31).

In one patient, a teenager, a heterozygote form of the variant *ACTA2*:p.Arg258Cys was found: Chr10: g.90699300G > A, NM_001613.2:c.772C > T, NP_001604.1:p.Arg258Cys (Table 1). The patient's clinical diagnosis was aortic dissection type III. He was first operated at 6 months, when trans section was performed because of a persisting arterial channel. No cardiovascular deviation was detected until the age of 15, when after a month of active weight lifting and a sudden appearance of strong pain in the back

Table 1. Genetic variant detected in the first patient.

Gene	Variant characteristics*	
***ACTA2** (actin, alpha 2, smooth muscle, aorta)*	Cytogenic location	10q23.3
	Genomic location	Chr10: 90699300
	Variant type	Single nucleotide variant
	Genomic DNA reference sequence accession and version	NG_011541.1:g.56848C>T
	Coding DNA reference sequence accesion and version	NM_001613.2:c.772C>T
	Protein reference sequence accession and version	NP_001135417.1:p.Arg258Cys
	dsSNP ID	rs121434528
	Functional Consequence	Missense
	Clinical significance	Pathogenic/Likely pathogenic
	Genotype	Heterozygous AD
	Conditions	Aortic aneurysm, familial thoracic 6 Moyamoya disease 5 Thoracic aortic aneurysm and aortic dissection

*The coordinates of the genetic variant are according to mapping by the Genome Reference Consortium Human Build 37 (GRCh37)/hg19. Resources of the National Center for Biotechnology Information (NCBI) and databases as ClinVar were used. Variant description nomenclature is according to Human Genome Variation Society (HGVS).

and tingling in his left leg, a dissection of aortae type III and thrombosis of the iliac arteries was diagnosed. Intervention procedures included dilatation and stent on arteria mesentherica, stent on the abdominal aortae and balloon dilatation on left femoral artery. Twenty days later, a stent was placed in left renal artery because of ischemia of the left kidney. A month later, dissection of thoracic aortae was diagnosed and a stent graft was implanted in it. The genetic analysis clarified the diagnosis as a Familial aortic aneurysm, type 6.

In another patient, a 40-years-old woman, a heterozygous novel *ACTA2*:p. Lys52Glu variant was detected: Chr10: g.90707119T > C, NM_001613.2:c.154A > G, NP_001604.1:p.Lys52Glu (Table 2). She had a diagnosis of aneurysm of abdominal aorta that was corrected by an operation. She also had a familial history of the pathology, namely her father died of thoracic aortic aneurysm.

DISCUSSION

Aortic pathologies as thoracic aortic aneurysms, dissection and rupture are an important cause of cardiovascular morbidity and mortality. Heterozygous mutations in gene *ACTA2* are associated with vascular diseases as Coronary Artery Disease, Stroke, Moyamoya Disease and Thoracic Aortic Disease (Guo et al., 2009). More precisely they are underlined as a cause for familial thoracic aortic aneurysm-6 (AAT6), Moyamoya disease 5 and Multisystemic smooth muscle dysfunction syndrome (http://omim.org/entry/102620). Missense mutations in *ACTA2* account for 14% of inherited ascending thoracic aortic aneurysms and dissections (TAAD) and in such heterozygous patients, improper contraction occurs because of poorly organized actin filaments in the smooth muscle cells, tissue of the aorta that is medially degenerated with hyperplasia and disarray and the presence of stenosis (Guo

Table 2. Genetic variant detected in the second patient.

Gene	Variant characteristics*	
ACTA2 *(actin, alpha 2, smooth muscle, aorta)*	Genomic location	Chr10:g.90707119
	Variant type	Single nucleotide variant
	Coding DNA reference sequence accesion and version	NM_001613.2:c.154A > G
	Protein reference sequence accession and version	NP_001604.1:p.Lys52Glu
	Functional Consequence	Missense
	Clinical significance	Uncertain significance **NEW**
	Genotype	Heterozygous

*The coordinates of the genetic variant are according to mapping by the Genome Reference Consortium Human Build 37 (GRCh37)/hg19. Resources of the National Center for Biotechnology Information (NCBI) and databases as ClinVar were used. Variant description nomenclature is according to Human Genome Variation Society (HGVS).

et al., 2007). A study of 277 individuals with 41 various *ACTA2* mutations led to the conclusion that aortic events occurred in 48% of the group, the majority presenting with thoracic aortic dissections (88%) associated with 25% mortality (Regalado et al., 2015).

The variant found in the first patient, a heterozygote *ACTA2*:p.Arg258Cys, is a known pathogenic variant and it leads to familial form of thoracic aortic aneurysms inherited in autosomal dominant manner. In some patients that are carriers of this variant, a phenotype of the disease Moyamoya type 5 (MYMY5) occurs displayed as a cerebro-vascular disorder due to stenosis in the terminal parts of the inner carotidal arteries (Guo et al., 2009). The functional tests that this mutation in *ACTA2* leads to defective contractile function of the smooth muscle cells contributing to thoracic aortic disease were performed by Lu et al. (2015) by expression in baculovirus. The mutation allosterically affected multiple regions of the monomer actin leading to functional defects compared with the wild-type. Mutant filaments were less stable than wild-type actin and more affected by cofilin, even in the presence of protective tropomyosin. The motility assay showed that smooth muscle myosin moved mutated filaments more slowly than the wild type ones.

The variant found in the second patient, a heterozygous novel *ACTA2*:p.Lys52Glu has not been described so far and it is of uncertain significance. However, the aneurysm of abdominal aorta in this patient with familial history of thoracic aortic aneurysmis was likely due to the fact that this patient was a carrier of the mutation. Variants with uncertain significance (VUS) are continuously found in this gene in patients with such abnormality as shown in a study of 51 unrelated patients with thoracic aortic aneurysms and dissections examined by whole exone sequencing or TruSight. One sequencing panel was established where two novel *ACTA2* mutations (p.D27G and p.N117S) were detected (Poninska et al., 2016). In a similar study of 70 patients with thoracic aortic aneurysms and dissections again two novel VUS in *ACTA2* were found (Fang et al., 2017). Point mutations of gene *ACTA2* are the most prevalent cause of familial thoracic aortic aneurysms and dissections (Lu et al., 2015)

In conclusion, the clinical diagnosis was clarified in both cases, thus emphasizing the importance of this method in determining mutation status in correlation with clinical phenotype, and it can have large implication in treatment and prognosis of patients and their families in cases with uncertain clinical diagnosis.

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