Forensic Science International 261 (2016) e1–e4

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Case Report

Postmortem diagnosis of Marfan syndrome in a case of sudden death due to aortic rupture: Detection of a novel *FBN1* frameshift mutation

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ARTICLE INFO

Article history:
Received 21 December 2015
Received in revised form 4 February 2016
Accepted 7 February 2016

Keywords:
Marfan syndrome
Frameshift mutation
FBN1
Autopsy

ABSTRACT

To investigate the sudden death of a 36-year-old Chinese man, a medicolegal autopsy was performed, combining forensic pathological examinations and genetic sequencing analysis to diagnose the cause of death. Genomic DNA samples were extracted from blood and subjected to high-throughput sequencing. Major findings included a dilated aortic root with a ruptured and dissected aorta and consequent tamponade of the pericardial sac. Moreover, arachnodactyly and other skeletal deformities were noted. By sequencing the fibrillin-1 gene (*FBN1*), five genetic variations were found, including four previously known single nucleotide polymorphisms (SNPs) and a novel frameshift mutation, leading to the diagnosis of Marfan syndrome. The frameshift mutation (c.4921delG, p.glu1641llysFsX9) detected in exon 40 led to a stop codon after the next 8 amino acids. The four SNPs included a splice site mutation (c.3464-5 G > A, rs11853943), a synonymous mutation (p.Asn625Asn, rs25458), and two missense mutations (p.Pro1148Ala, rs140598; p.Cys472Tyr, rs4775765). Genetic screening was recommended for the relatives as it was reported that the father and brother of the deceased had died at the ages of 40 and 25, respectively, from sudden cardiac failure. The son of the deceased lacked the relevant mutations. This report emphasizes the important contribution of medicolegal postmortem analysis on the molecular pathogenesis study of Marfan syndrome and early diagnosis of at-risk relatives.

1. Introduction

Marfan syndrome (MFS), an inherited autosomal dominant systemic disorder of the connective tissue, affects the cardiovascular (aorta: aortic aneurysm/dissection; the heart: mitral/aortic valve insufficiency), musculoskeletal (overgrowth), ocular (ectopia lentis), and pulmonary systems, as well as the skin and dura [1,2]. The estimated prevalence of MFS was 1 case per 5000 individuals in general population and 1–2 cases per 10,000 individuals in Chinese populations [2–4]. Latent, preceding states of MFS have often gone undiagnosed. In such cases, MFS patients usually remain asymptomatic until an acute dissection or rupture leads to a catastrophic complication, which ultimately causes unexpected death; the average life expectancy is about 35 years and the fatality rate is 90% [5–7]. Thus, undiagnosed cases of MFS contribute to the amount of sudden unresolved deaths that result in suspicions of medical malpractice, prompting relatives or public prosecutors to request medicolegal autopsies. Cause of death in these unexpected cases can sometimes be attributed to MFS when a spontaneous thoracic aortic dissection is diagnosed.

However, in many other cases, the morphological evidence is less straightforward, leading to inconclusive or overly simplistic autopsy results. Because MFS is heritable, relatives of the deceased are at a high risk for the same disease, and cause of death based solely on morphological pathologies may mean that potential MFS patients are not warned of their risk and remain undiagnosed. Thus, in conjunction with a traditional autopsy, a postmortem genetic analysis is important for determining whether a potentially heritable condition is the underlying cause of death. With regard to MFS specifically, previous studies have reported a link with mutations in the fibrillin-1 gene (*FBN1*) [8]. More than 3000 mutations have been clinically identified in the 235 kb, 65-exon-containing gene, but few reports exist on the postmortem genetic analysis of sudden death cases in MFS patients (*Marfan mutation database*, http://www.umd.be/FBN1/). In fact, heritable factors in disease etiology frequently go unconsidered in China,

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http://dx.doi.org/10.1016/j.forsciint.2016.02.013
0379-0738/

because access to laboratories conducting such genetic analyses is limited and the associated cost is high.

Here, we report the case of a Chinese man who died from acute aortic rupture and was diagnosed with MFS postmortem via autopsy and genetic analysis. Through this report, we hope to emphasize that a medicolegal autopsy is essential both for understanding the molecular pathogenesis of heritable diseases and for informing at-risk relatives.

2. Material and methods

2.1. Clinical summary of patient

A 36-year-old previously "healthy" man presented with abdominal pain and nausea for about 3 days. His surgical history included gastric perforation repair, and he had no other history of drug use. The primary diagnosis of the abdominal pain (either possible acute appendicitis or intestinal obstruction) was uncertain; hence, this patient was made to undergo infusion, anti-inflammatory, and spasmolysis therapy to alleviate the symptoms. The following afternoon, the patient, who had been treated with a second enema, collapsed in the bathroom and quickly died. The relatives suspected medical malpractice and reported the incident to the district court. A medicolegal autopsy was subsequently performed to investigate the cause of death.

2.2. Genetic analysis

With the consent of the deceased's relatives, mutation screening was performed via direct sequencing of the fibrillin-1 gene (*FBN1*) from postmortem blood.

Genomic DNA was extracted from 2-mL venous blood using a *Qiamp Blood Kit (Qiagen, Hilden, Germany)*. All procedures were performed following manufacturer protocol. Qualified DNA samples were submitted to Covaris, Inc. for random fragmentation, and the size of the resultant fragments was mainly distributed between 200 and 250 bp. Next, the 5′ overhangs were filled and the 3′ overhangs were removed by treating purified DNA fragments with T4 DNA polymerase, T4 phosphonucleotide kinase, and the Klenow fragment of *Escherichia coli* DNA polymerase. Terminal A residues were added after a brief incubation with dATP and the Klenow 3′–5′ exo-enzyme, according to standard Illumina protocol. Adapter ligation was then performed and the resulting templates were purified using the Agencourt AMPure SPRI beads, amplified with ligation-mediated PCR (LM-PCR), and hybridized with a customized 2.1 M *Human Array from Roche NimbleGen* (*Madison, USA*) for 68–72 h. The captured fragments were bound to streptavidin beads and isolated to generate libraries for further analysis, while non-hybridized fragments were washed out.

The generated library was loaded on the *HiSeq2500* platform for high-throughput sequencing; the average sequencing depth was approximately 265.4×. Data analysis was performed using the *Illumina Pipeline*. Raw image files were processed using *Illumina Basecalling Software 1.7* with default parameters, and the sequences of each individual file were generated as 90-bp pair-end reads. Sequence data were compared pairwise with published *FBN1* sequences. Mutations were named according to the nomenclature recommended by the Human Genomic Variation Society. The evaluation of mutations consisted of (1) examing the variant type and its location in the functional domain of the protein or the splice site of the gene, (2) the minor allele frequency reported in the large population databases (e.g., *dbSNP website*, http://www.ncbi.nlm.nih.gov/SNP/; *Marfan mutation database*, http://www.umd.be/FBN1/; *ExAC* http://exac.broadinstitute.org/; *EVS*, http://evs.gs.washington.edu/EVS/), (3) the function prediction reported from computation tools (e.g., Mutation Taster, Polyphen-2, and SIFT) to a protein (Table 1).

Table 1
Mutations detected in the FBN1 gene of the deceased.

Mutation type	dbSNP ID	Location	cDNA nomenclature	Variations	Structure	Allele frequency					Pathogenic prediction
						dbSNP	Hapmap	1000 genomes	Local genomes	EVS	ExAC	
Frameshift	Novel	Exon 40	c.4921delG	p.Glu1641LysfsX9	cb EGF-like #23	NA	NA	NA	NA	NA	NA	Disease causing
Splice site	rs11853943	Intron 28	c.3464 -5 G>A	/	cb EGF-like #13	0.329	0.482	0.3068	0.5093	0.1746	0.1709	NA
Missense	rs140598	Exon 28	c.3442 C>G	p.Pro1148Ala	cb EGF-like #13	0.143	0.429	0.1108	0.4154	0.0036	0.0288	Benign
Missense	rs4775765	Exon 11	c.1415 G>A	p.Cys472Tyr	EGF-like #04	1	1	1	1	1	1	Benign
Synonymous	rs25458	Exon 15	c.1875 T>C	p.Asn625Asn	cb EGF-like #06	0.413	0	0.3599	0.5062	0.2175	0.2006	Benign

dbSNP ID = single nucleotide polymorphism (SNP) database identification number; Hapmap = The International HapMap Project; 1000 genomes = 1000 Genomes Project; EVS = Exome Variant Server; ExAC = Exome Aggregation Consortium; NA = not available. The pathogenic prediction were based on three custom Online Software: Mutation Taster (http://www.mutationtaster.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/index.shtml) and SIFT (http://provean.jcvi.org/protein_batch_submit.php?species=human).