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Letter to the Editor

Whole Exome Sequencing in a Rare Disease: A Patient with Anomalous Left Coronary Artery from the Pulmonary Artery (Bland-White-Garland Syndrome)

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To the Editor:

Anomalous origin of left coronary artery from pulmonary artery (ALCAPA), also known as the Bland-White-Garland syndrome, is a rare congenital abnormality, with an incidence of 1 in 300,000 live births. ALCAPA was reported in Brooks (1885) but there are vast knowledge gaps on pedigree, epidemiology, and genetic/genomic studies of ALCAPA. Our search of the published literatüre on PUBMED using the keywords ALCAPA and ''mutation'' or ''polymorphism'' identified only one hit, dealing with a single candidate gene analysis (Sawaya et al., 2011). Genomics correlates of this rare disease are particularly missing and much needed for new insights into future novel diagnostics and potential therapeutic interventions. ALCAPA is associated with myocardial cell death, heart failure, and often mortality when it is not treated.

Herein we report the results of the first whole exome sequencing (WES) of an ALCAPA patient who has been followed for 8 years; his clinical course was described previously elsewhere (Türkmen et al., 2014).

Materials and Methods

Case and phenotype description

The patient, 36-year-old athlete, was admitted to the SANKO Hospital in Gaziantep in southeast Turkey, with a complaint of left-sided atypical chest pain, without any known prior disease or drug use history. His physical examination revealed normal vital signs with a pansystolic murmur at the mesocardiac and pulmonary areas. Electrocardiography (ECG) showed sinus rhythm without pathological findings, whereas transthoracic echocardiogram (TTE) revealed mild mitral and tricuspid regurgitation, and a pulmonary artery pressure of 40/18 mmHg. In the parasternal short-axis view, an indefinite turbulent flow was detected in the pulmonary artery by color Doppler. There were no ischemic ST-T ECG changes in the exercise stress test. The left coronary artery (LCA) could not be seated on the left by the Judkins and Amplatz catheters. The right coronary angiography showed the dilated right coronary artery (RCA) and well-developed coronary collaterals filling the LCA, leading to pulmonary artery washing by a reverse flow. Coronary computed tomography showed that LCA was arising from the pulmonary artery and perfused by collaterals directly from the aorta and RCA. Surgical correction was planned, but the patient declined this option. Since then, the patient has been followed up by performing TTE at 6-month intervals along with an annual 24-h ECG Holter analysis and remains asymptomatic.

WES, data analysis, and processing

The analysis was reviewed and approved by the institutional ethics committee of the SANKO University Hospital in Gaziantep, Turkey. A written informed consent was obtained from the subjects before analysis. Peripheral venous blood samples were collected from the patient, his father, mother, and two brothers. DNA was isolated by the salting out protocol after exclusion of cytogenetic abnormality. WES and attendant data analyses were performed on the Ion Proton Platform (Life Technologies, Grand Island, NY, USA), as described previously (Alfadhel et al., 2015).

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In brief, the target regions in the exome were amplified using the Ion AmpliSeq™ Exome RDY Kit (Life Technologies). It consisted of 12 primer pools (294,000 amplicons) that targeted >97% of the coding region, and accounted for a total of 33 MB. Raw sequence data analyses, including base calling, de-multiplexing, alignment to the human reference genome (Genome Reference Consortium GRCh37), and variant calling, were performed using the Torrent Suite Software v.4.0.2 (Life Technologies). In terms of variant annotation and filtering, the coverage analyses evaluated in a two-step-process the coverage on the single base level for the complete design and provided detailed statistics on the average coverage as well the percentage of bases with minimum coverage. The RefSeq coding bases and splice junctions considered confidently callable were determined by a minimum of 10× coverage and no more than 10% MAPQ0 (ambiguously mapped) reads. A 100× mean depth of coverage was aimed for the samples. Variant prioritization was performed with a cascade of filtering steps as described previously (Alfadhel et al., 2015).

We considered all candidate variants that were identified on both sequenced DNA strands and that account for sequenced DNA strands and with a minimum depth of coverage of 10×. Common variants (≥1% in the general population) were discarded by comparison with the 1000G (www.1000genomes.org), the Exome Variant Server (http://evs.gs.washington.edu), the Exome Aggregation Consortium database (ExAC, http://exac.broadinstitute.org), and CentoMD® (www.centomd.com), to filter out both common benign variants and recurrent artifact variant calls. All identified variants were considered *a priori* as variants of unknown significance (VUS). Rare variants predicted to result in a premature truncated protein (nonsense, frameshift mutations, affecting initiation codon, single exon, or multiexon deletions), and other larger genomic rearrangements, as well as canonical splice site mutations (±2 bps) were given the highest priority.

Missense variants and in-frame deletions were evaluated taking into consideration the biophysical and biochemical difference between wild type and changed amino acid, the evolutionary conservation of the nucleotide and amino acid residue in orthologs, a number of *in silico* predictors (SIFT, Polyphen-2, Mutation taster among others), and population frequency data. Putative splicing variants were analyzed using the Alamut version 2.4.5, a software package that uses different splice site prediction programs to compare the normal and variant sequences for differences in potential regulatory signals. Then, prioritized variants were evaluated based on the suspected disease mode of inheritance and compatibility with the clinical phenotype provided for the index based on several databases and sources of information such as the Online Mendelian Inheritance in Man (OMIM®, http://omim.org/), HGMD®, CentoMD, as well as scientific literature searches in PubMed (www.ncbi.nlm.nih.gov/). The selected variants were re-evaluated by two human geneticists to identify those relevant to the patient's phenotype. Selected candidate pathogenic, likely pathogenic, and VUS were sent for confirmation by conventional polymerase chain reaction amplification and Sanger sequencing. Segregation of these variants with the disease was assessed for all available family members.

Sanger sequencing analysis

The mutations identified in the three genes, *CFTR* (Cystic Fibrosis Transmembrane Conductance Regulator), *MEN1* (Multiple Endocrine Neoplasia Type 1), and *PKP2* (Plakophilin-2), were further confirmed by sequencing of both DNA strands of the coding region, in which the mutations were detected by WES. The reference sequences of the *CFTR*, *MEN1*, and *PKP2* genes are NM_000492.3, NM_130803.2, and NM_004572.3.

Results

The WES analysis identified four mutations that are disease causing or possibly disease causing. Accordingly, in the *CFTR* gene (7q31.2), we observed two mutations (c.202A>G, p.Lys68Glu; c.2173G>A, p.Glu725Lys), one in the *PKP2* gene (12p11.21) (c.1093A>G, p.Met365Val) and the other in the *MEN1* gene (11q13.1) (c.527G>A; p.Arg176Gln) in connection with the ALCAPA phenotype in this report. We confirmed these four mutations in the patient by the Sanger sequencing analysis (Table 1).

Discussion

To the best of our knowledge, this is the first attempt to decipher a putative genomics and broader molecular etiology

Table 1. Heterozygous Alterations Detected in the Anomalous Left Coronary Artery From The Pulmonary Artery Patient by Whole Exome Sequencing

Gene	*c-Position*	*p-Position*	*Zygosity*	*Biological relevance*	*Disease (according to OMIM)*	*Inheritance*
CFTR	c.202A>G	p.Lys68Glu	Heterozygous	Disease-causing mutation	Cystic fibrosis	Autosomal recessive
CFTR	c.2173G>A	p.Glu725Lys	Heterozygous	Disease-causing mutation	Cystic fibrosis	Autosomal recessive
PKP2	c.1093A>G	p.Met365Val	Heterozygous	Disease-causing mutation	Arrhythmogenic right ventricular cardiomyopathy/dysplasia	Autosomal dominant
MEN1	c.527G>A	p.Arg176Gln	Heterozygous	Possibly disease-causing mutation	Multiple endocrine neoplasia 1	Autosomal dominant

CFTR, Cystic Fibrosis Transmembrane Conductance Regulator; *MEN1*, Multiple Endocrine Neoplasia Type 1; OMIM, Online Mendelian Inheritance in Man; *PKP2*, Plakophilin-2.

of ALCAPA using a WES approach. The patient's both parents and his two brothers were healthy without clincal sympotoms of ALCAPA. The four reported mutations have only been detected in the patient.

A missense mutation (c.1093A>G p.Met365Val) was identified at the exon 4 of *PKP2*. The latter gene was previeously linked to the arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D). *PKP2* encodes Plakophilin-2 belonging to the Armadillo-related proteins family. Plakophilin-2 participates in intercellular signaling through the Wnt/β-catenin pathway, desmosome assembly, and is involved in a variety of developmental processes. The majority of mutations in *PKP2* detected in ARVC/D patients are frameshift mutations leading to truncated proteins, which results in haploinsufficiency. It has been thought that the frameshift mutations in *PKP2* lead to a decrease in truncated *Plakophilin-2* by the nonsense-mediated messenger RNA decay mechanism. Only about 10% of the mutations in *PKP2* detected in the ARVC/D cases are missense mutations and their effect on protein structure and desmosome assembly is not well understood hitherto.

The *PKP2* mutation detected in this ALCAPA patient, the missense mutation (Met365Val), has not been reported in any cases with ARVC/D syndrome previously. We speculate that Met365Val substitution might presumably result in altered roles of Plakophilin-2 in intercellular signaling through Wnt/β-catenin pathway, desmosome assembly, and a variety of developmental processes, which, by extension, can contribute to the observed ALCAPA phenotype.

Phenotypic heterogeneity denotes that different mutations in the same gene may result in different diseases or severity of symptoms. For example, in the present case description, the patient was an athlete and ostensibly healthy. The molecular mechanisms leading to mutations in *PKP2* might be responsible for the development of different phenotypic findings such as the ARVC/D syndrome, the Brugada syndrome, and the ALCAPA syndrome.

However, and in contrast to *PKP2*, *MEN1* and *CFTR* have not been hitherto established as salient genes in cardiac development. Yet, the presence of these mutations in combination with *PKP2* mutation might potentially contribute to phenotypic heterogeneity of ALCAPA.

Conclusions

By combining pedigree information with the next WES technology, we were able to narrow down four mutations in three candidate genes potentially related to the ALCAPA phenotype. This is the first study, to our knowledge, reporting the coexistance of mutations in *CFTR, MEN1*, and *PKP2* in an ALCAPA case. Further WES analyses on other ALCAPA cases and functional assessments at protein level are warranted and would contribute to be better delineation of the genomics correlates of this rare disease.

In addition, this letter raises the awareness about this rare disease and the need for innovative molecular diagnostics and broadly framed multiomics (genomics, proteomics, and metabolomics) integrative biology studies of ALCAPA in the future. This shall hopefully enable and guide the health personnel for early diagnosis and surgical interventions and ultimately help achieve improved prognosis for persons and families with ALCAPA.

Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

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Abbreviations Used

ALCAPA = anomalous left coronary artery from pulmonary artery
ARVC/D = arrhythmogenic right ventricular cardiomyopathy/dysplasia
CFTR = Cystic Fibrosis Transmembrane Conductance Regulator
ECG = electrocardiography
LCA = left coronary artery
MEN1 = Multiple Endocrine Neoplasia Type 1
OMIM = Online Mendelian Inheritance in Man
PKP2 = Plakophilin-2
RCA = right coronary artery
TTE = transthoracic echocardiogram
VUS = variants of unknown significance
WES = whole exome sequencing