Clinical impact of low coverage in whole-exome genetic testing in the assessment of familial arrhythmogenic right ventricular cardiomyopathy: a case report

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Background
Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited condition, with approximately 60% of patients carrying a possibly disease-causing genetic variant. Known desmosomal genes account for about 50% of those variants. We herein report a family with ARVC in which a pathogenic desmosomal variant was missed because of the initial genetic testing method.

Case summary
A 54-year-old man diagnosed with ARVC underwent genetic cascade screening for a heterozygous titin variant (TTN: c.26542C>T), detected in his phenotypically affected sister. He did not harbour this TTN variant. Moreover, reclassification of this variant based on the American College of Medical Genetics (ACMG) 2015 criteria showed it to be likely benign. Upon genetic re-screening with a dedicated cardiomyopathy panel a heterozygous missense variant in desmoglein-2 (DSG2: c.152G>C) was found. His sister’s DNA was re-analysed and the same DSG2 variant was detected, and classified as LP (likely pathogenic) by current literature.

Discussion
The initial genetic screening tool used in the patient’s sister (whole-exome sequencing, WES) failed to detect the likely causative desmosomal variant in our family. While WES represents a good tool in searching for novel genes in Trio Analysis, it has a low DNA coverage in important regions (mean 10×) of known ARVC-associated genes. We therefore propose using smaller panels with better coverage in the clinical setting, such as Trusight-cardio (mean DNA coverage 100–300×) as an initial genetic screening method.

Keywords
Arrhythmogenic cardiomyopathy • Arrhythmogenic right ventricular cardiomyopathy • Desmoglein-2 • Familial • Genetic variant • Case report

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Learning points
• To understand the genetic basis of arrhythmogenic right ventricular cardiomyopathy (ARVC), which is commonly associated with desmosomal variants.
• To highlight the fact that identification of a pathogenic genetic variant associated with ARVC is a major diagnostic task force criterion in the 2010 Task Force Criteria.
• To understand the advantages and pitfalls of different genetic screening tools in the diagnosis of patients with arrhythmogenic cardiomyopathies.
• To highlight the importance of cascade family screening in ARVC: genetic cascade screening is an important tool for close monitoring and early intervention in asymptomatic family members.

Introduction
Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetic disease, characterized by a high propensity for ventricular arrhythmias and fibrofatty infiltration of the right ventricle. It is mainly transmitted as an autosomal dominant trait with variable penetrance. Approximately 60% of patients carry a genetic variant more than 50% of genes currently linked to ARVC are desmosomal, although variants in non-desmosomal genes have been identified. ARVC is complex in its genotype and highly variable in its phenotype. With the availability of next-generation sequencing (NGS), a growing number of hypothetical pathogenic variants can be detected; however, diagnostic sensitivity of the different available genetic screening methods is variable, which must be considered when choosing the appropriate test.

We herein report a family with ARVC in which a pathogenic desmosomal variant was missed because of the initial genetic testing method.

Timeline

| Year     | Event                                                                 |
|----------|-----------------------------------------------------------------------|
| 1991     | Sister definite diagnosis of arrhythmogenic right ventricular cardiomyopathy |
| 2014     | Sister gene test (whole-exome sequencing) Identification of likely pathogenic TTN c.2654C>T variant |
| November 2019 | Patient VT presentation                                            |
| December 2019 | Patient implantable cardioverter-defibrillator implantation         |
| December 2019 | Gene cascade screening                                             |
| January 2020 | Cardiomyopathy panel and identification of DSG2 c.152G>C variant |
| November 2020 | Patient last follow-up                                               |

Case presentation
A 54-year-old Caucasian man, with no past medical history was referred to our electrophysiology department after an admission to the emergency department because of two syncopes and sustained ventricular tachycardia with superior axis of right bundle branch block morphology (VT, Figure 1A). At presentation, we saw a cardio-pulmonary stable patient with a heart rate of 204 b.p.m., blood pressure of 128/94, and O2 saturation of 97%. Amiodarone at a dose of 300 mg IV did not terminate the VT, but electrical cardioversion (200 J) was successful. His electrocardiogram during sinus rhythm (Figure 1B) showed inferior and lateral T-wave inversions. At admission, the patients’ troponin value was 221 ng/L (<14) and BNP was 8858 ng/L (<121). To exclude an ischaemic cause of the VT, coronary angiography was performed showing a subtotal stenosis of the middle segment of the left circumflex artery, which was resolved with the implantation of two drug-eluting stents. Nevertheless, the presence of an ischaemic coronaryopathy did not exclude the co-presentation of a cardiomyopathy, especially considering his familial predisposition (Figure 2): his younger sister had been diagnosed with ARVC in 1991 based on four major criteria [transthoracic echocardiography (TTE), epsilon wave, T-wave inversions, VT with left bundle branch block morphology and superior axis]. However, he had not wished to be screened at the time of his sister’s diagnosis. A TTE was performed, which showed a non-dilated left ventricle with normal ejection fraction (52%), a dilated right atrium and dilated right ventricle/right ventricular (RV) outflow tract (33 mm) with reduced function (RV fractional area change = 28%, tricuspid annular motion 11 mm), and a akinetic RV free wall. Furthermore, while the cardiac magnetic resonance imaging formally did not fulfill the 2010 Task Force Criteria as the right ventricle was not sufficiently dilated (RV end-diastolic volume index 81 ml/m²) and RV function was preserved (RV ejection fraction 61%), it showed regional dyskinesias and microaneurysms as well as pronounced fibrosis and fatty infiltration of the RV free wall (Figure 3). Cardiac sarcoid was ruled out by 18F-FDG-PET. Since he fulfilled the diagnosis of definite ARVC following the 2010 Task Force Criteria (one major structural, one major family, one minor repolarization and the patient’s sister had already been genetically tested and a pathogenic heterozygous variant in the titin gene (TTN; c.26542C>T, p.His8848Tyr) had been identified 10 years earlier, he was tested for this same variant, which he did not harbour. Moreover, reassessment of this variant following the 2015 ACMG (American College of Medical Genetics) criteria showed it to be likely benign (LB, Class II). Thus, additional genetic testing was performed using a dedicated cardiomyopathy panel covering 176 cardiomyopathy genes by NGS (Trusight cardio-illumina). Herein a likely pathogenic (LP, Class IV) heterozygous variant in desmoglein-2 (DSG2; c.152G>C, p.Trp51Ser) was discovered. The DNA from the sister was re-analysed with Sanger sequencing, and the same DSG2 variant was identified. Assuming the same pathogenic desmosomal variant was missed because of the initial genetic testing method.

Figure 1A: Electrocardiogram during sinus rhythm showing T-wave inversions. Figure 1B: Electrocardiogram during ventricular tachycardia showing inferior and lateral T-wave inversions.
Figure 1  Diagnostic work-up in the 54-year-old man. (A) 12-lead electrocardiogram showing the clinical sustained ventricular tachycardia at a rate of 203 b.p.m. with a superior axis and right bundle branch block. This suggests a basal inferior septal origin. However, invasive mapping of this ventricular tachycardia was never performed, and therefore a right ventricular basal inferoseptal exit of it cannot be excluded. Moreover, septal and left ventricular involvement, particularly in DSG-2 mutation carriers such as our patient, has been previously described. Although our imaging findings including transthoracic echocardiography, cardiac magnetic resonance tomography, and 18F-fluorodeoxy positron emission tomography-PET did not reveal left ventricular involvement and excluded cardiac sarcoid, a small area of scar or fatty infiltration in the basal inferoseptal area that cannot be detected by these imaging modalities may be present in our patient explaining the substrate for this ventricular tachycardia. (B) 12-lead electrocardiogram during sinus rhythm showing inferior and lateral T-wave inversions (minor 2010 arrhythmogenic right ventricular cardiomyopathy Task Force repolarization criterion).

Figure 2  Family tree over three generations (I-III): DSG2: heterozygous (DSG2 +/−; c.294G>A; p.Trp51Ser) missense variant. DSG2 +/+ indicates wild type. TTN: heterozygous (TTN +/−; c.26542C>T; p.His8848Tyr) missense variant. TTN +/+ indicates wild type. Black arrow indicates index patient. All phenotypically affected family members are indicated by filled black circles (female) or black rectangles (male).
Discussion

ARVC is a genetically heterogeneous cardiomyopathy, and disease-causing genetic variants have been identified in several desmosomal and non-desmosomal genes. The importance of genetic screening is exemplified by our case: a correct genetic cascade screening could have helped to diagnose ARVC in this family member at an earlier stage and timely appropriate therapy (e.g. physical exercise restriction, betablockers, eventually ICD implantation) may have avoided his presentation to the emergency department with sustained ventricular tachycardia. While there are different genetic screening methods in the context of ARVC.

Figure 3 Diagnostic work-up in the 54-year-old man. Cardiac magnetic resonance imaging showing regional dyskinesia as well as pronounced fibrosis of the right ventricular free wall (red arrow).

variant was found in her, underscoring its pathogenicity due to cosegregation. The patient has been managed by implanting a single-chamber implantable cardioverter-defibrillator (ICD) for secondary prevention and bisoprolol 2.5 mg bid and genetic counselling has been provided to both him and his sister. There is no evidence for non-compliance in medication intake. The last clinical follow-up has been in November 2020: whilst taking beta blocking medication, only non-sustained VT has been documented on ICD interrogation.

in our family, could only be identified at genetic re-screening through a targeted NGS panel providing a higher coverage in guanine-cytosine rich regions of desmosomal genes. Our case well illustrates the many advantages and pitfalls of the different genetic screening methods in the context of ARVC.

Smaller panels focus on a restricted set of genes: given the indirect relation between number of genes and the coverage, focusing on a certain set of genes, allows for increased coverage. Insufficiently covered DNA regions can be filled in with supplemental Sanger sequencing, as well as other technologies [e.g. deletion/duplication assays to identify large copy number variants (CNV)]. Smaller panels have therefore a higher exon coverage and a better clinical sensitivity when compared to WES.

WES allows the simultaneous analysis of all protein-coding regions of the genome and thus does not require a priori knowledge of genes responsible for the disease. Instead of evaluating all 20 000+ human genes, WES can also be filtered against recognized disease-causing genes in order to reduce the chance of incidental findings. The major advantage of WES is its flexibility: analysis can initially focus on the genes with a strong contribution to the patient’s phenotype and extend to a broader gene set as new knowledge emerges. However, clinical adoption of WES as a genetic screening tool in ARVC presents several challenges. A major drawback is incomplete coverage of exons and insufficient detection of larger CNV, leading to lower sensitivity and clinically relevant variants being missed. It is important to mention that if the raw data is systematically reviewed, this low coverage can be detected: regions which are shown to have insufficient coverage need to be re-tested using Sanger sequencing, in order to cover these blind spots. However, this is very time consuming and expensive. In our case, WES failed to identify the causative DSG2 variant and the data re-analysis revealed that the region of the variant had a low DNA coverage (10%). There is also a problem of positive predictive value, because huge amounts of variants can be identified. Correctly evaluating their pathogenicity and distinguishing them from background genetic variation can be challenging.

This is exemplified by the initially reported variant in the TTN gene, initially defined as LP, but then reclassified as LB. Furthermore, current guidelines on genetic testing in cardiomyopathies recommend cautious use of WES. In hypertrophic cardiomyopathy (HCM) for example, the eight sarcomeric gene–disease associations classified as definitive by ClinGen (MYBPC3, MYH7, TNNI3, TNNT2, MYL3, MYL2, ACTC1, and TPM1, reported to explain 30–50% of HCM cases) were all originally based on strong segregation evidence in large pedigrees. Since 2000, seven additional genetic associations (CSRP3, PLN, ACTN2, FLNC, ALPK3, JPH2, and FHOD3) with robust evidence of variant co-segregation with disease, were proposed. Nonetheless, these genes are estimated to be responsible at best for 1% of HCM cases. This being said, while the existence of unknown Mendelian HCM genes is plausible (and thus justifies WES as a gene discovery tool), it is unlikely that such genes will be major contributors to the disease burden. With this regard, ARVC seems to behave in a similar fashion as HCM, since in familial forms up to 60% of cases are genotype...
positive, most of them harbouring pathogenic variants in desmosomal genes.

Conclusions

The negative yield of WES to detect the causative desmosomal variant in our family urges for caution when applying this genetic method as a screening tool in ARVC. While WES represents a good tool in searching for novel genes, it has a low DNA coverage (mean 10×) of known genes. We therefore propose using smaller panels, such as the dedicated cardiomyopathy panel (mean DNA coverage 100–300×) as an initial genetic screening method.

Lead author biography

Sarah Costa was born in Milan (Italy) in 1993. She studied Medicine and Surgery at the University of Pavia, where she graduated in 2018 Summa cum Laude. During her studies, she spent a semester abroad at the University of Tuebingen (DE) and a month at NYU Langone Electrophysiology Department. She returned to NYU Langone as a post-doc in the cardiovascular research lab of Prof. Mario Delmar and Dr Marina Cerrone in 2019. She is currently working as a research fellow at the University Hospital Zurich in the ARVC team.

Supplementary material

Supplementary material is available at European Heart Journal - Case Reports online.

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Slide sets: A fully edited slide set detailing this case and suitable for local presentation is available online as Supplementary data.

Consent: The authors confirm that written consent for submission and publication of this case report including images and associated text has been obtained from the patient in line with COPE guidance.

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