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CASE REPORT

Genetic and toxicologic investigation of Sudden Cardiac Death in a patient with Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) under cocaine and alcohol effects

Francesca Cittadini · Nadia De Giovanni · Mireia Alcalde · Sara Partemi · Oscar Campuzano · Ramon Brugada · Antonio Oliva

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Abstract Cocaine and alcohol toxicity is well known, especially when simultaneously abused. These drugs perform both acute and chronic harmfulness, with significant cardiac events such as ventricular arrhythmias, tachycardia, systemic hypertension, acute myocardial infarction, ventricular hypertrophy, and acute coronary syndrome. The present report refers about a patient who died after a documented episode of psychomotor agitation followed by cardiac arrest. At the autopsy investigation, arrhythmogenic right ventricular cardiomyopathy (ARVC) was diagnosed and confirmed by postmortem molecular analysis revealing a mutation in the DSG2 gene. Postmortem toxicological analysis demonstrated a recent intake of cocaine, and the death was attributed to cardiac arrhythmias. The detection of cocaine and cocaethylene in hair samples proved chronic simultaneous intake of cocaine and alcohol at least in the last month. The authors discuss the role of these drugs and genetic predisposition of the ARVC in causing the death of the patient.

Keywords Cocaine · Ethanol · Cocaethylene · Acute/chronic intoxication · Cardiac toxicity

Introduction

Sudden cardiac death is one of the most important mode of death in Western countries; therefore, forensic pathologists have given this problem the appropriate attention it deserves [1, 2]. New methods and techniques in the investigation of sudden death potentially linked to fatal arrhythmias have been developed more recently, especially because the accurate diagnosis of the causes of sudden cardiac death is nowadays of particular importance in forensic field to prevent other fatalities in family members in case of suspected inherited syndromes [3]. Today, pathologists are responsible for determining the precise cause of sudden death, but there is considerable variation in the way in which they approach this increasingly complex task. Autopsy-negative sudden cardiac deaths (SCD) seen in forensic practice are most often thought to be the result of sudden arrhythmic death syndrome. Postmortem genetic analysis is recommended in such cases, but is currently performed in only a few academic centers [4]. The main question that arises in forensic practice is whether it is useful for the pathologist to perform the genetic screening in every case of sudden cardiac death, including those cases where a structural heart disease has been diagnosed through autopsy and histology examinations and after which positive results for psychotropic drugs have been obtained at toxicology investigation. Regarding structural heart diseases, arrhythmogenic right ventricular cardiomyopathy (ARVC) is a rare cardiac disease characterized by a progressive replacement of ventricular myocardium with fatty and fibrous elements, preferentially of the right ventricular (RV) free wall, although up to 50 % of cases also shows a left ventricular (LV) involvement [5]. These structural alterations are responsible for electrical abnormalities, which may cause subsequent ventricular arrhythmias, syncope, and sudden cardiac death (SCD) [6]. The prevalence of ARVC in general population is 1:2500–5000, depending on gender (3.1 in men) [7]. Regarding the age of onset, ARVC especially affects young athletes, being responsible for up to 15 % of SCD in this group [8]. The clinical diagnosis of the disease is established using a set of criteria as proposed by an international Task Force in 1994 and revised in 2010 including genetics [9]. Focus on genetics origin of

F. Cittadini · N. De Giovanni · S. Partemi (✉) · A. Oliva
Institute of Public Health, Section of Legal Medicine, Catholic University, Rome, Italy
e-mail: sarapartemi@gmail.com

M. Alcalde · O. Campuzano · R. Brugada
Cardiovascular Genetics Centre, University of Girona-IDIBGI, Girona, Spain

ARVC, there is a familial occurrence in about 50 % of cases, with autosomal dominant inheritance with variable penetrance and polymorphic phenotypic expression. Nearly 60 % of clinically diagnosed cases showed at least one pathogenic genetic variation (PGV) responsible of the disease after comprehensive genetic screening of current known genes: desmosomal genes, plakophilin-2 (*PKP2*), desmoplakin (*DSP*), desmocolin-2 (*DSC2*), desmoglein-2 (*DSG2*), and plakoglobin (PG) encoded by the *JUP* gene; and non-desmosomal genes, transmembrane protein 43 (*TMEM43*), transforming growth factor beta 3 (*TGFB3*), catenin alpha-3 (*CTNNA3*) desmin (*DES*), lamin A/C (*LMNA*), titin (*TTN*), and phospholamban (*PLN*) [5, 10–12] (Table 1).

To the best of our knowledge, no reports have been published concerning sudden death due to cocaine effects on the heart of individuals affected by ARVC. The effect of cocaine on the cardiac muscle is still under evaluation. In fact, determining the exact effects of cocaine in humans is difficult because of several factors such as routes of administration, different doses, underlying risk factors, and concomitant use/abuse of other substances such as alcohol, caffeine, and amphetamines, which may interact with cocaine. A large variety of cardiovascular diseases has been associated with cocaine use, including acute myocardial ischemia and infarction, arrhythmias and sudden death, myocarditis, cardiomyopthy, hypertension, ruptured aorta, cerebrovascular aneurysm, accelerated aththerosclerosis, and endocarditis [13]. Here, we present the case of a sudden cardiac death in a patient with previous psychomotor agitation followed by cardiac arrest where postmortem investigation, including histology stains, revealed ARVC disease confirmed by genetic analysis. Toxicology analysis showed also acute and chronic intake of cocaine and alcohol. Possible mechanisms of death are discussed.

Case report

The case concerns a 41-year-old man admitted to emergency department after cardiac arrest resuscitated by medical team. Before the hospitalization due to the cardiac event, the man exhibited violent, aggressive, and threatening behavior while he was in a public place. In the emergency room, urine screening test was performed revealing positive results for cocaine and blood alcohol at the following concentrations of 1.99 g/l. During the hospitalization, several ECG traces showed sinus tachycardia with short PR and ST elevation in anterior and ST depression in inferolateral derivations suggesting acute injury (Fig. 1a, b). Pharmacological treatment was performed through the administration of inotropes and rehydration therapy, but the patient died suddenly due to cardiac arrest following a period of 14 h after the admission in the emergency department. The case was referred to the public prosecutor and presented for autopsy investigation.

Materials and methods

Autopsy

Autopsy investigation was performed according to the International Guidelines [8]. Heart weight was compared with expected heart weight in relation to body weight according to Kitzman et al. [14]. Heart was examined according to standardized protocol [15, 16]. Samples were collected for histology, genetics, and toxicology analyses.

Toxicological analysis

Sample collection

Peripheral and central blood, vitreous humor, and hair samples collected before autopsy were processed [17, 18]. Hair strands were about 1.0 cm long and analyzed over the whole length. No urine samples were available. A nasal swab sample was also carried out.

Reagents

All reagents and solvents were of analytic grade. Cocaine, benzoylecgonine, ecgonine methyl ester, and cocaethylene were obtained by LGC Standards (Milano, Italy). Trideuterated analogs of the analytes, obtained by LGC standards as well, were used as internal standards.

Sample analysis

A systematic toxicological analysis (STA) procedure as recommended by TIAFT guidelines [http://www.tiaft.org] was used. The detection of volatile organic compounds, with particular attention to ethanol, was performed on postmortem blood (both central and peripheral) and vitreous humor samples. n-Propanol was used as internal standard, and the samples were analyzed by head-space gas chromatography. Central blood sample was pretreated with acetonitrile for the protein precipitation before the screening technique performed with a TOX/See Drug Screen Test obtained by Bio-Rad. The presence of amphetamine (cutoff 1000 ng/mL), metamphetamine (cutoff 1000 ng/mL), cocaine (cutoff 300 ng/mL), 11-nor-9-carboxy-tetrahydrocannabinol (cutoff 50 ng/mL), opiates (cutoff 300 ng/mL), benzodiazepines (cutoff 300 ng/mL), barbiturates (cutoff 300 ng/mL), and methadone (cutoff 300 ng/mL) metabolites was investigated. Central blood was also subjected to the general unknown analysis using 3 mL aliquot of sample, submitted to solvent extraction from acid and alkaline solutions, and then analyzed by a FOCUS Gas Chromatograph coupled with DSQ (Thermo Electron Corp., Milano, Italy) operating in electron impact mode (70 eV) and collecting data in scan mode. Nasal swab

Table 1 Genes associated with ARVC

Gene	Locus	MIM	Gene ID	Protein	Inheritance
CTNNA3	10q21.3	607667	291999	αT-Catenin	AD
DES	2q35	125660	1674	Desmin	AD
DSC2	18q21	125645	1824	Desmocollin-2	AD
DSG2	18q12.1	125671	1829	Desmoglein-2	AD/AR
DSP	6p24	125647	1832	Desmoplakin	AD/AR
JUP	17q21	173325	3728	Junction plakoglobin	AR/AR
LMNA	1q22	150330	4000	Lamin A/C	AD
PKP2	12q11	609040	5318	Plakophilin-2	AD/AR
PLN	6q22.1	172405	5350	Phospholamban	AD
TGFB3	14q24.3	190230	7043	Transforming growth factor β3	AD
TMEM43	3p25.1	612048	79188	Transmembrane protein 43	AD
TTN	2q31.2	188840	7273	Titin	AD

AD autosomal dominant, *AR* autosomal recessive, *MIM* Mendelian Inheritance in Man

coming from both nostrils was eluted with methanol and submitted to gas chromatographic/mass spectrometric analysis after concentration of the eluate using the instrumentation previously described. All the samples collected during autopsy, including hair specimen previously hydrolyzed, were submitted to confirmation analysis for the detection of cocaine and metabolites (benzoylecgonine, ecgonine methyl ester, and cocaethylene) using in-house validated method [19]. Briefly, each sample properly diluted was added with deuterated internal standard analogs of each compound, submitted to a liquid/liquid solvent extraction and pentafluoropropyl derivatization. The gas chromatographic/mass spectrometric

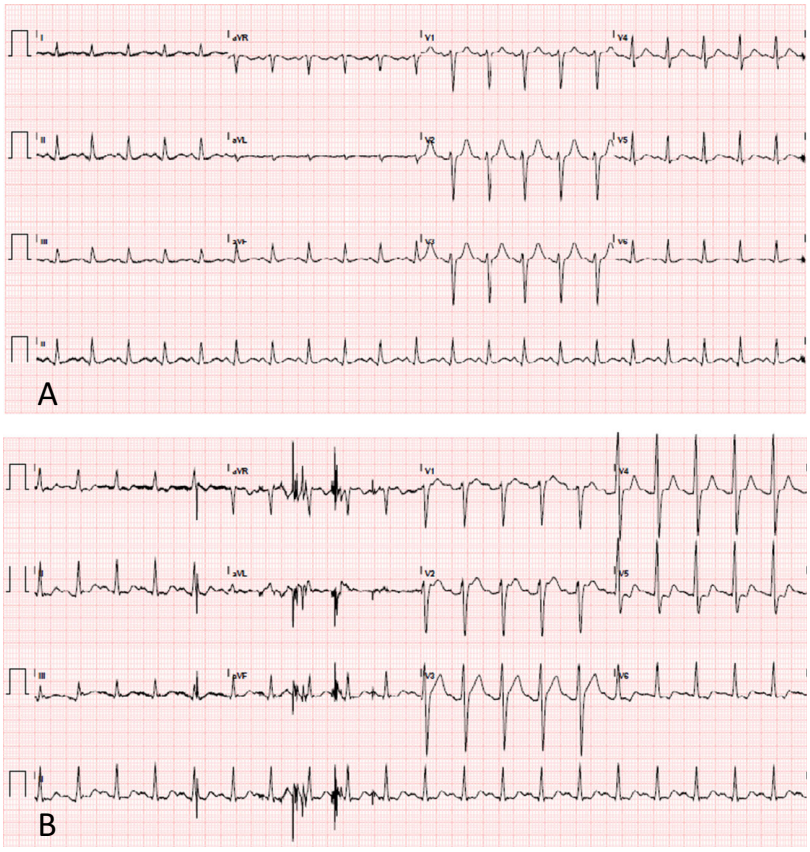

Fig. 1 ECGs before the cardiac arrest showing sinus tachycardia with short PR interval (**a**) and ST elevation in anterior and ST depression in inferolateral derivations (**b**)

detection with the instrumentation previously reported was performed in selected ion monitoring mode by choosing three ions for each compound.

Calibration and validation parameters

Specificity, linearity, lower limit of quantification, limit of detection, accuracy, and repeatability were evaluated for the gas chromatography/mass spectrometric analysis of cocaine and metabolites according to literature suggestions. Specificity was found to be satisfactory as the chromatograms were free of coeluting peaks. Linearity was evaluated by means of calibration curves constructed for each analyte, finding a linear response from 10 to 500 ng/ml for liquid samples and from 0.1 to 5.0 ng/mg for hair specimens. The lower limit of quantification and the limit of detection estimated as the lowest amount of analyte were found to be as low as 10 and 20 ng/ml for liquid samples and 0.2 and 0.1 ng/mg for hair, respectively, for all the substances. Accuracy and repeatability showed satisfactory results. Intraday and interday coefficient of variation values were found to range below 15 and 20 %, respectively.

Genetic analysis

Whole blood samples collected during autopsy were processed, and DNA was extracted by the automatic extractor Chemagic MSM I (Chemagic human blood). DNA was amplificated by polymerase chain reaction (PCR), purified by ExoSAP-IT (Isogen), and directly sequenced by dideoxy chain termination method in ABI PRISM BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sequencing process was processed in a 3130xl Genetic Analyzer (Applied Biosystems) and analyzed by means of SeqScape Software v2.5 (Life Technologies) comparing obtained results with the reference sequence from hg19. Patient's DNA was screened for the most common genes associated with ARVC: *PKP2* (ENST00000070846), *DSP* (ENST00000379802), *DSC2* (ENST00000280904), *DSG2* (ENST00000261590), and *JUP* (ENST00000310706). Genetic analysis included all exonic and flanking intronic regions (−7 to +10) that respect the 3′ and 5′ extremes of the exons as potentially related to erroneous splicing processes [20]. To name and analyze each identified variation and to consider their potential relation with ARVC, we consulted public genetic databases as 1000 Genomes Browser [21]; NHLBI GO Exome Sequencing Project (ESP), Seattle, WA; and dbSNP, NCBI [22]. Since new exome data are questioning the pathogenicity of previously ARVC-associated genetic variants [23], we selected and analyze all identified variants with a minor allele frequency lower than 1 % minor frequency allele (MAF <0.01). From all these low frequency variants, we studied their previous association with ARVC using Human Gene Mutation Database (HGMD) [24]. All these low frequency variants and missense novel variants were accurately analyzed by Condel (CONsensus DELeteriousness score of missense SNVs database) in silico platforms to predict their potential pathogenicity.

Results

Autopsy and histology findings

The victim was 180 cm in height and weighed 88 kg. No significant injuries were observed on external examination. The main findings were noticed on the heart. The heart was 635 g in weight (normal weight; 349±67.2 g) [14]. The right ventricular (RV) wall was 30 mm thick (normal thickness; 4.2 ±1.52 mm) (Fig. 2a). The transillumination documented structural changes localized to the apex of the right ventricular and fatty replacement of the right ventricular wall was observed, right ventricular myocardial thickness was on average 3 mm, and the thickness of fibro-fatty tissue was 4 mm (Fig. 2b). Significant abnormalities of subepicardial and intramural coronary arteries and of valves were absent; the inner surface of the coronary arteries was smooth with no signs of stenosis. Microscopic examination showed diffuse and segmental lack of myocardium in the RV free wall replaced by fatty and some residual myocyte with perinuclear vacuolization and myofibril loss. Only subendocardial layers were preserved, in which some myocardium appears to be interspersed with fibrosis. Persistent strands of cardiomyocytes bordered by or embedded in a variable area of fibrosis were observed also in the epicardial and mediomural layers inside the fat (Fig. 2c, d). No inflammatory infiltrates were documented. Examination of the other organs was negative except for pulmonary edema and a remarkable polyvisceral stasis.

Toxicology

During the autopsy, no urine was available, and the screening test performed on the pretreated blood sample showed positivity for cocaine metabolites. The alcohol concentration of 1.99 g/l at the admission revealed a drunkenness condition. The analysis of nasal swab did not reveal any other drugs; this result is, however, ineffectual being a possible different way of intake or simply due to the cleaning maneuvers during the stay in hospital. The confirmation analysis demonstrated the presence of cocaine metabolites, benzoylecgonine, and ecgonine ethyl ester, in all the samples examined (Table 2). Cocaine and cocaethylene were also detected in hair sample (Fig. 3) demonstrating the chronic use/abuse of cocaine and alcohol.

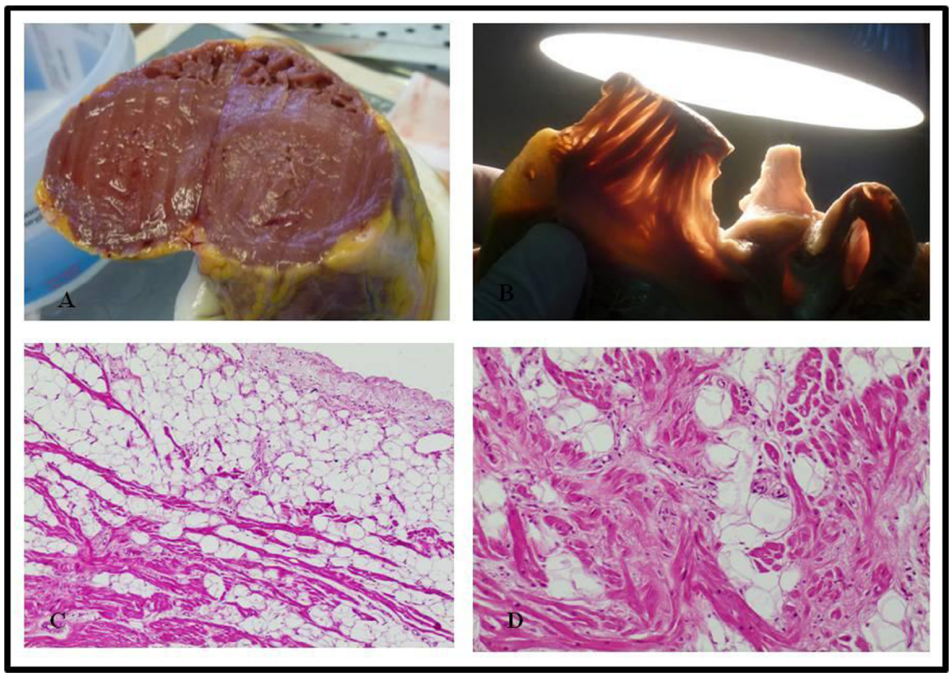

Fig. 2 Macroscopic examination of the heart: marked left and right ventricular hypertrophy (**a**). Transillumination test showing wall's thinning to the apex of the right ventricular (**b**). Microscopic examination: diffuse and segmental lack of myocardium in the RV free wall replaced by fatty and some residual myocyte with perinuclear vacuolization and myofibril loss. Strands of cardiomyocytes bordered by or embedded in a variable area of fibrosis. Disorganized appearance of the ventricular myocardium subpericardial with isolated myocytes (hematoxylin-eosin, ×200) (**c**). At higher magnification are appreciable phenomena of fibrosis with initial myocytes trapping. Myocytes are irregular with branching shapes (hematoxylin-eosin, ×400) (**d**)

Genetics

Genetic study performed in our patient revealed one single pathogenic mutation reported in HGMD as disease associated. This is a missense pathogenic mutation identified in the *DSG2* gene c.2759T>G p.V920G–p.Val920Gly. It was previously associated with the pathology (CM070920) [25, 26]. Pathogenicity of the variant was established using allele frequency from public databases, conservation analysis, and in silico predictors: this is a rare variation with minor allele frequency (MAF) in general population <0.01 producing an amino acid change in a highly conserved position (Fig. 4). In silico, platforms consulted predicted a possibly damaging effect (SIFT 0.18; PPH2 0.157; MA 1.1; FATHMM −1.13, CONDEL 0.528113912633). In addition, the nucleotide alteration supposes a change of Valine (apolar) to Glycine (polar without charge).

Table 2 Toxicological results

	EME	BEG	Cocaine	Cocaethylene
Central blood (ng/ml)	170	540	Neg	Neg
Peripheral blood (mg/ml)	160	545	Neg	Neg
Vitreous humor (ng/ml)	50	250	Neg	Neg
Serum (ng/ml)	75	450	Neg	Neg
Hair (ng/mg)	1.0	< LOQ	2.7	0.8

EME ecgonine methyl ester, *BEG* benzoylecgonine, *Neg* negative results, *LOQ* limit of quantitation

Discussion

In this study, we have reported a unique case of sudden cardiac death due to the interaction of cocaine and ethanol on an anatomic substrate of ARVC confirmed by histology examination and genetic analysis. What appears truly intriguing in this case is the final underlying mechanisms of death. In fact, the combination of cocaine and ethanol is considered to be more lethal than either substance alone as it increases myocardial oxygen demand. The synergic effect of ethanol and cocaine tends to have greater-than-additive effects on heart rate, concomitant with up to 30 % increased blood cocaine levels [27, 28]. Moreover, cocaethylene may have negative effects on the brain as compared to the use of only one of these two substances. Despite its relatively similar pharmacological and psychomotor stimulant effects, cocaethylene has a longer half-life than cocaine itself, and it is considered to be more effective in producing lethality [29]. Indeed, cocaethylene is associated with a 40-fold increase in risk for acute cardiac events and 25-fold increase risk in sudden death [30]. Pilgrim

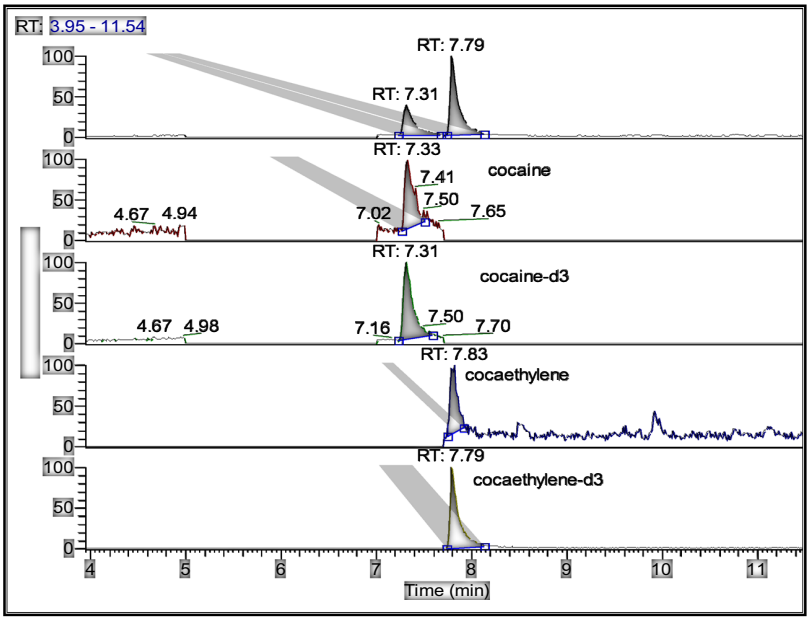

Fig. 3 Cocaine and cocaethylene detected in hair sample by gas chromatography/mass spectrometry in SIM mode

et al. recently reported three different cases involving simultaneous intake of cocaine and ethanol with significant heart disease [30]. The authors postulated that the administration of cocaine concurrently with alcohol in these cases increased the risk of cardiotoxicity and ultimately, sudden death.

To the best of our knowledge, this is the first report of sudden cardiac death due to synergic effect of cocaine and ethanol in an individual affected by ARVC confirmed by postmortem molecular analysis. As briefly discussed before, the difficulty in the conclusive forensic report to the public prosecutor was the evaluation of these results especially regarding the ultimate mechanisms of death. In particular, the main question concerns the role played by the anatomical cardiac substrate in comparison with the well-known effect of the drugs (cocaine and ethanol). What is useful in our clinical case are the ECG traces recorded in the emergency department just before the death. The two ECG recordings showed tachycardia with short PR interval, ST elevation in anterior, and ST depression in inferolateral derivations. Is the contribution of acute cocaine effect, together with ethanol, the ultimate cause of mechanism of the death of this patient? Although it has been shown by several studies that cocaine is strongly associated with cardiovascular disease [31, 32], it is possible that in this case, the pre-existing cardiac substrate

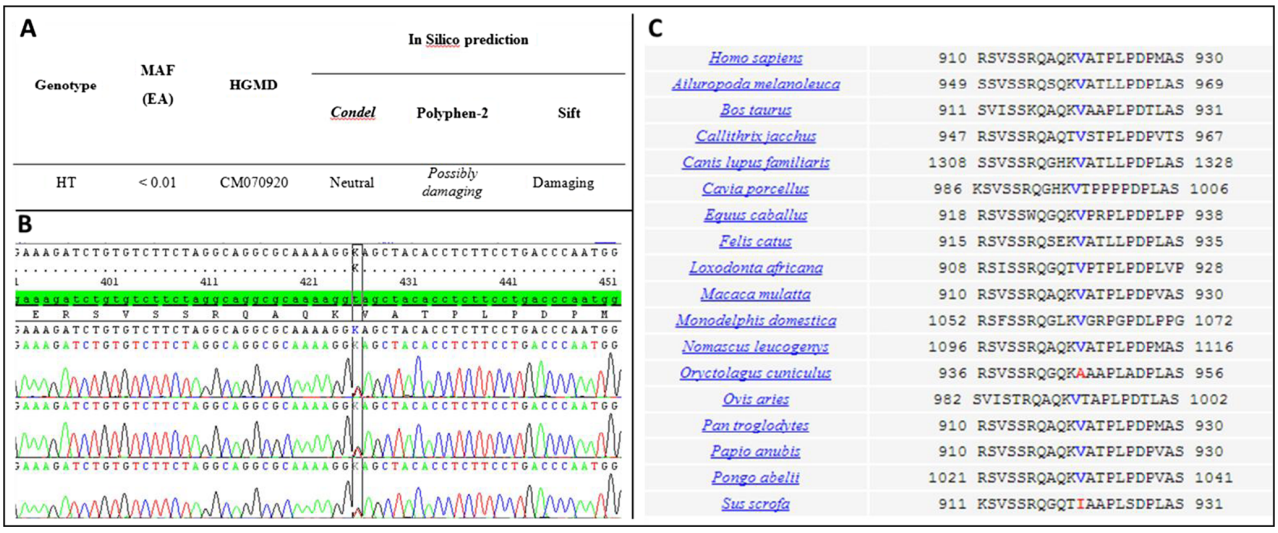

Fig. 4 Missense variation p.V920G_DSG2. **a** In silico predictions for the missense variation. *MAF* minor allele frequency consulted in European American (EA) individuals in exome sequencing project. Genotype: *HT* heterozygous/*HM* homozygous. **b** DSG2 sequence showing nucleotide substitution c.2759T>G. **c** DSG2 protein alignment among species

(ARVC) merely exacerbated the ventricular instability induced by cocaethylene effects. Unfortunately, there are very few information in the literature in order to propose a reliable etiopathogenic hypothesis in this case, especially to discriminate the drug-induced effect alone from the synergic effect of the drug on an anatomical structural disease. Additionally, we need to take into account the contribution of interindividual variability, tolerance, and neuroadaptation, which is almost impossible to predict. Finally, we consider this report as the first case which could be useful in order to reach further insight regarding the underlying mechanism of sudden cardiac death induced by cocaine on a well-known anatomic substrate genetically determined such as ARVC. Moreover, we do consider cases like this in confirming the usefulness in forensic field of postmortem genetic analysis that could reveal inherited cardiac conditions not detected during life especially in patients where medical liability is suspected.

Limitations of the study

Gene-environment interactions are challenging to explore at case report levels. Therefore, we do believe that additional knowledge could be reached only through large-scale studies in sudden cardiac death cases linked to cocaine and alcohol abuse.

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