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ORIGINAL ARTICLE

Genetic analysis of sudden cardiac death victims: a survey of current forensic autopsy practices

Katarzyna Michaud · Patrice Mangin · Bernice S. Elger

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Abstract 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. In order to determine actual current practice, an on-line questionnaire was sent by e-mail to members of various forensic medical associations. The questions addressed routine procedures employed in cases of sudden cardiac death (autopsy ordering, macroscopic and microscopic cardiac examination, conduction tissue examination, immunohistochemistry and electron microscopy, biochemical markers, sampling and storage of material for genetic analyses, toxicological analyses, and molecular autopsy). Some questions concerned the legal and ethical aspects of genetic analyses in postmortem examinations, as well as any existing multidisciplinary collaborations in SCD cases. There were 97 respondents, mostly from European countries. Genetic testing in cases of sudden cardiac death is rarely practiced in routine forensic investigation. Approximately 60% of respondents reported not having the means to perform genetic postmortem testing and 40% do not collect adequate material to perform these investigations at a later date, despite working at university hospitals. The survey demonstrated that many of the problems involved in the adequate investigation of SCD cases are often financial in origin, due to the fact that activities in forensic medicine are often paid by and dependent on the judicial authorities. Problems also exist concerning the contact with family members and/or the family doctor, as well as the often-nonexistent collaboration with others clinicians with special expertise beneficial in the investigation of SCD cases, such as cardiologists and geneticists. This study highlights the importance in establishing guidelines for molecular autopsies in forensic medicine.

Keywords Sudden cardiac death · Molecular autopsy · Survey · Forensic medicine

K. Michaud (✉) · P. Mangin
University Center of Legal Medicine Lausanne-Geneva,
Bugnon 21,
1011 Lausanne, Switzerland
e-mail: katarzyna.michaud@chuv.ch

B. S. Elger
University of Geneva, University Center of Legal Medicine
Lausanne-Geneva,
Lausanne, Switzerland

Introduction

The recommendations for the forensic investigation of sudden cardiac deaths (SCD) are numerous, addressing both the autopsy and complementary analyses [1, 2]. In cases of autopsy-negative sudden deaths, often attributed to sudden arrhythmic death syndrome, postmortem genetic testing (a.k.a molecular autopsy) is recommended [3–5]. Recent progress in the fields of molecular biology and human genetics has identified the genetic origin of many cardiac diseases [6–10], resulting in SCD and found in Sudden Infant Death Syndrome (SIDS) [4–6, 8, 10–12]. SCD may be prevented if the appropriate treatment is initiated in affected individuals. As many of these diseases are hereditary, establishing a postmortem diagnosis of a SCD victim is very important for the surviving family members. In 2007, Wedekind stated that postmortem genetic testing should be considered as a part of the comprehensive medicolegal investigation in sudden cardiac death cases without apparent structural heart disease, taking

into consideration the implications for the living family members [13]. Establishing a diagnosis may prevent future cardiac events with the assistance of expert counseling, appropriate lifestyle adjustment, and pharmacological treatment, if available. Recently, Dettmyer and Kandolf suggested that some cases of primary arrythmogenic disorders were misdiagnosed as SIDS [14], and Klintschar reported on the clinical consequences for family members resulting from a medicolegal autopsy in a case of sudden death due to an aortic rupture resulting from Marfan syndrome [15]. Despite the recommendations for and advantages of molecular autopsy, only a few research centers are currently performing it in standard forensic practice. The goal of this project was to assess the current strategies employed by forensic practitioners.

Methods

The members of forensic medical associations (International Academy of Legal Medicine, German, Swiss and, French Societies of Legal Medicine and Mediterranean Academy of Forensic Sciences) were contacted by email and asked to fill out an on-line questionnaire, which was available during a 2-week period. A total of 648 mails were sent; however, the mailing list was not selective and included members not involved in the autopsy of SCD cases, such as toxicologists and psychiatrists. The questionnaire began with the presentation of a typical case of SCD—a 25-year-old man without any known medical history, who died suddenly while playing tennis. The subsequent questions concerned the respondents handling of the case focusing on the forensic autopsy, addressing the judges' orders, macroscopical and microscopical cardiac examination, conduction tissue examination, immunohistochemistry and electron microscopy, biochemical markers, and the sampling and storage of material for genetic and toxicological analyses. Some questions concerned the legal and ethical aspects of genetic analyses in postmortem testing, as well as the existence of any multidisciplinary collaboration.

Respondents

A total of 97 surveys were completed, the majority from central and southern Europe and the Mediterranean. The numbers of respondents by countries are listed in Table 1. Of the respondents, 74.7% male and 25.3% were female.

Of the respondents, 69.1% worked in a university setting, 6.2% in peripheral hospitals, 4.1% in private practice, and 18.6% in miscellaneous places (mostly judicial administration). Of the respondents, 70.1% worked in forensic pathology, 6.2% in forensic genetics, 4.1% in forensic toxicology, and the remainder in clinical forensic medicine. Of the respondents, 58.9% were involved in teaching forensic medicine.

The professional experience of respondents was the following: 44.3% had between 1 and 10 years of experience, 34% between 11 and 20 years, 11.3% between 21 and 30 years, and 4.1% greater than 30 years.

The estimated mean number of autopsies and sudden cardiac death autopsies performed annually were 180 and 20, respectively.

Autopsy ordering

Of the respondents, 90.8% reported that in the presented case, the police officer (or investigating magistrate) would order a forensic autopsy. Of the respondents, 72.2% noted that the forensic autopsy did not require the consent of the next of kin, while 18.6% said that it did. Ten percent of the respondents reported that a postmortem investigation would not be performed for the presented case.

For the respondents who said that an autopsy would not be performed for the presented case, or would be performed in less than 50% of cases, 28.9% noted it was mainly due to the lack of suspicion of third party intervention, while 11.3% noted it was due to insufficient resources. Other reasons were given for 59.8% of respondents, some of which were detailed in the free-text comments. The differences between countries are shown in Table 2.

Complementary exams performed in the autopsies of SCD cases

Of the forensic pathologists, 56.7% perform the cardiac examination alone or with the help of a pathologist who has a deeper knowledge of cardiovascular pathology. Thirty four percent of the respondents fix the whole heart and referee the case to an expert on cardiovascular pathology. For 9.3% of the respondents, their practice varies depending on the pathology found, but that most frequently the forensic pathologist performs the initial examination and then fixes the entire heart before sending it to a specialized pathologist. Differences between countries are shown in Table 2.

Histological examination of the myocardium using haematoxylin-eosin stain is systematically performed by 71.7% of respondents. This examination is never practiced, or practiced in less than 50% of cases, by 18.4% of forensic pathologists. Examination of the conduction tissue is systematically performed by 20.6% of respondents, while 62.9% of respondents never perform it or do so in less than 50% of cases.

Immunohistochemical examination of the myocardium is systematically performed or performed in more than

Table 1 The respondents and their ability to perform genetic postmortem analyses in cases of SCD listed by country (number of respondents who answered that analysis is possible)

Country	Numbers of respondents/ questionnaires	Possibility to perform genetic testing	Analysis of SCN5A	Analysis of KCNQ1	Analysis of KCNH2	Analysis of RyR2	Analysis of genes implicated in HCM	Analysis of genes implicated in ARDV/C
Argentina	1/4	1	1	-	-	-	-	-
Australia	2/2	2	-	1	1	1	1	1
Austria	1/14	-	-	-	-	-	-	-
Belgium	1/16	-	-	-	-	-	-	-
Canada	2/2	1	-	-	-	-	-	
Colombia	1/3	-	-	-	-	-	-	-
Croatia	1/3	1	1	1	1	1	1	1
Denmark	2/5	2	1	1	1	1	2	2
France	12/156	4	2	2	2	2	3	2
Germany	10/148	6	5	5	5	4	4	4
Iceland	1/3	-	-	-	-	-	-	-
India	3/3	1	-	-	-	-	-	-
Italy	4/24	3	1	1	1		1	2
Japan	1/19	1	1	1	1	1	-	-
Lebanon	2/2	-	-	-	-	-	-	-
Nigeria	1/1	-	-	-	-	-	-	-
Portugal	4/12	-	-	-	-	-	-	-
Romania	2/3	2	-	-	-	-	1	1
Senegal	1/2	-	-	-	-	-	-	-
Serbia	1/1	1	1	1	1	1	-	-
Singapore	1/1	1	1	1	1	1	1	1
Slovakia	1/1	-	-	-	-	-	-	-
Spain	14/20	6	3	2	3	3	5	6
Switzerland	9/119	3	3	3	3	0	2	-
The Nederland	1/2	1	-	-	-	-	-	-
Turkey	13/13	-	-	-	-	-	-	
United Kingdom	1/4	-	-	-	-	-	-	-
Unites States	2/4	1	-	-	-	-	-	-
Others countries	0/16							

HCM hypertrophic cardiomyopathy, *ARDV/C* arrhythmogenic right ventricular dysplasia/cardiomyopathy

50% of cases by 7.3% of respondents, and never performed by 57.7% of respondents. Of the respondents, 7.2% did not know if immunohistochemical examination is performed or not.

Electron microscopy of the myocardium is systematically performed or performed in more than 50% of cases by 5.1% of respondents, and never performed by 86.6% of respondents. Of the respondents, 5.5% did not know if electron microscopy is performed or not.

Measurement of biomarkers (such as troponine, BNP, NT-proBNP) is practiced systematically or in more than 50% of cases by only 10.3% of respondents, never by 59.8%, and in less than 50% of cases by 23.7%.

Toxicological analyses are practiced systematically by 73.2% of respondents, and never or in less than in 50% of cases by 13.4% (see Table 3).

No significant statistical differences were observed in the responses between individuals working in a university setting and other institutions, namely judicial centers.

EDTA and frozen myocardium sampling

EDTA blood is collected systematically by 49% of respondents, and never collected by 38.1%. The sampling of frozen myocardium, useful not only for molecular autopsy but also for the detection of viruses in cases suspicious for myocarditis, is systematically performed by 15.5% of respondents, and never or in less than 50% of cases by 79.4% of respondents (see Table 3).

Postmortem genetic testing

Of the respondents, 40.2% report having the ability to perform a molecular autopsy, while 59.8% are not able to. Only a minority of respondents have the possibility to analyze the three genes currently implicated in cardiac channelopathies along with some other genes implicated in hypertrophic cardiomyopathy, arrhythmogenic right ventricular dysplasia/

Table 2 Reasons given by respondents for why autopsies are performed in less than 50% of cases and answers to the question "*n your experience, if a forensic autopsy is performed in a case of a sudden cardiac death, who would perform the examination of the heart?*" for the total numbers of respondents (97) and for some countries. For statistical analysis, only metropolitan France was included (two answers were from overseas regions)

	Number of answers (Percentage)	All respondents 97 (100)	Spain 14 (100)	Turkey 13 (100)	France 10 (100)	Germany 10 (100)	Switzerland 9 (100)	Portugal 4 (100)	Italy 4 (100)
Reason of a low autopsy rate	Insufficient resources	11 (11.3)	2 (14.3)	2 (15.4)	0 (0)	0 (0)	1 (11.1)	0 (0)	0 (0)
	No suspicion of a third party intervention	28 (28.9)	1 (7.1)	3 (23.1)	7 (70.0)	5 (50.0)	3 (33.3)	1 (25.0)	2 (50.0)
	Others	58 (59.8)	11 (78.6)	8 (61.5)	4 (30.0)	5 (50.0)	5 (55.6)	2 (50.0)	2 (50.0)
Who perform the heart examination?	Forensic pathologist	34 (35.1)	1 (7.1)	2 (15.4)	2 (20.0)	9 (90.0)	4 (44.4)	1 (25.0)	0 (0)
	Forensic pathologist helped by a specialist	21 (21.6)	2 (14.3)	3 (23.1)	1 (10.0)	1 (10.0)	4 (44.4)	2 (50.0)	3 (75.0)
	The whole heart is fixed and send to a specialist	33 (34.0)	10 (71.4)	8 (61.5)	4 (40.0)	0 (0)	1 (11.1)	1 (25.0)	0 (0)
	Others	9 (9.0)	1 (7.1)	0 (0)	3 (30)	0 (0)	0 (0)	0 (0)	1 (25.0)

cardiomyopathy, and polymorphic ventricular tachycardia (see Table 1). No significant differences were observed between pathologists working in the university setting and other institutions.

Legal and ethical aspect of retrospective postmortem genetic testing

A few questions concerned the legal and ethical aspects of genetic analyses in the forensic context. One of the questions asked if the forensic pathologist needs the approval of the ethics committee and/or the consent of the next of kin in order to perform a retrospective study. Of the respondents, 18.6% (two from Germany, two from Portugal, two from Romania, one from Austria, one from Croatia, one from Italy, one from Japan, one from Nigeria, one from Serbia, one from Slovakia, four from Spain, and one from the USA) may perform genetic analyses in any case without the consent of the next of kin or the ethics committee. Of the respondents, 25.6% need the approval of the local ethics committee and the consent of the next of kin

Table 3 Complimentary exams and sampling of material for molecular autopsy. The question concerning the toxicological exams was: "*In cases of sudden death mentioned above, if the autopsy is negative, how often do you perform complete toxicological analyses (and not only an immunoassays screening)*"

	Number of answers (Percentage)	All respondents 97 (100)	Spain 14 (100)	Turkey 13 (100)	France 10 (100)	Germany 10 (100)	Switzerland 9 (100)	Portugal 4 (100)	Italy 4 (100)
Performing of toxicological analyses after a negative autopsy	Never	5 (5.2)	0 (0)	1 (7.7)	1 (10)	1 (10)	1 (11.1)	0 (0)	0 (0)
	In less than 50% of cases	8 (8.3)	0 (0)	0 (0)	1 (10.0)	4 (40.0)	0 (0)	0 (0)	0 (0)
	In more than 50% of cases	13 (13.4)	2 (14.3)	0 (0)	2 (20.0)	2 (20.0)	1 (11.1)	1 (25.0)	1 (25.0)
	In every or almost every case	71 (73.2)	12 (85.7)	12 (92.3)	6 (60.0)	3 (30.0)	7 (77.8)	3 (75.0)	3 (75.0)
Sampling of EDTA blood	Never	37 (38.1)	5 (35.7)	4 (30.8)	4 (40.0)	5 (50.0)	1 (11.1)	1 (25.0)	0 (0)
	In less than 50% of cases	7 (7.2)	1 (7.1)	1 (7.7)	0 (0)	2 (20.0)	0 (0)	1 (25.0)	0 (0)
	In more than 50% of cases	5 (5.2)	0 (0)	1 (7.7)	1 (10.0)	0 (0)	0 (0)	0 (0)	0 (0)
	In every or almost every case	48 (49.5)	8 (57.1)	7 (53.9)	5 (50.0)	3 (30.0)	8 (88.9)	2 (50.0)	4 (100)
Sampling of frozen myocardium	Never	61 (62.9)	12 (85.7)	9 (69.2)	7 (70.0)	2 (20.0)	5 (55.6)	4 (100)	2 (50.0)
	In less than 50% of cases	16 (16.5)	0 (0)	2 (15.4)	0 (0)	6 (60.0)	0 (0)	0 (0)	1 (25.0)
	In more than 50% of cases	4 (4.1)	1 (7.1)	0 (0)	1 (10)	1 (10.0)	0 (0)	0 (0)	0 (0)
	In every or almost every case	15 (15.5)	1 (7.1)	2 (15.4)	2 (20.0)	1 (10.0)	4 (44.4)	0 (0)	1 (25.0)

(six from Switzerland, five from Germany, three from France, two from Canada, two from Turkey, one from Iceland, one from Spain, two undeclared). Of the respondents, 26.8% need either the approval of the local ethics committee or that of the next of kin. Twenty-two point seven percent do not have the means of performing retrospective genetic testing.

Juridical authorisation to perform the molecular autopsy

One question asked if the pathologist needs the permission of the investigating magistrate or prosecutor in order to perform genetic testing. Of the respondents, 23.7% reported that permission was required before performing the retrospective postmortem screening. For juridical investigations, 43 out of 88 respondents do not require the permission of the investigating magistrate in order to perform the molecular autopsy, whereas the remaining 45 respondents need the authorisation in order to determine the cause of death.

Interdisciplinary collaboration

Collaborations between the departments of medical genetics and cardiology exist for 19.6% of respondents, and more frequently (p value>0.001) in institutions were genetic testing takes place (i.e., university setting). Of the respondents, 61.9% not collaborate with other departments, which rise to 81.0% in institutions where genetic testing is not performed. Approximately 20% of respondents who work in institutions that perform genetic testing do not have any established collaborations.

Contact with families/family doctors

One questioned asked if there was any contact established with the surviving family members and/or the family doctor. Such contact is more frequent in places where molecular autopsies are performed (p value=0.03, Table 4).

Opinions about genetic testing

Of the respondents, 58.8% think that molecular autopsies should be performed in every case. Of the respondents, 30.9% noted that testing is too expensive. When third party intervention is not suspected, 22.7% of respondents think that the molecular autopsy does not have juridical interest, 16.5% of respondents reported that the interpretation of the genetic results is too complicated, and 6.2% answered that the "molecular autopsy is too complicated from the legal and ethical point of view". Several respondents highlighted the fact that occasionally the forensic pathologist never receives the results of the genetic tests. Others noted that postmortem genetic testing was often performed "illegally", without consent of the deceased or their next of kin. Many respondents suggested that the ethical issues should be more thoroughly discussed.

Others comments and propositions

A total of 29 general free-text comments were received, some of which were very detailed. Many comments concerned the cost of genetic testing, while others referred to the selection of cases and the difficulties in the interpretation of results. Respondents who require juridical authorisation commented on the occasional disinterest of the investigating magistrate regarding the determination of the cause of death in cases without suspicion of third party intervention.

Some respondents suggested that such analyses should only be performed in academic centers that have ethics committees and where approval of the next of kin can be obtained, and that these institutions should perform genetic testing for other pathologies (not just cardiac disease), in the hopes of preventing death in living family members. Several respondents noted that the tests should be covered by public health funds at no additional cost to the forensic pathologist or family (e.g., Denmark) and that the samples should be preserved indefinitely so that relevant investigations can be performed at a later date upon the request of the forensic pathologist or the family doctor. Several respondents also proposed the creation of well-publicized national centers, funded by state money, to which all these cases should be referred.

Discussion

According to the results of this survey, genetic testing is practiced in routine forensic investigations of SCD cases by only a minority of respondents. Approximately 60% of respondents do not have the means to perform genetic postmortem testing and 40% do not collect appropriate samples to perform these investigations, despite working in a university setting. There was no statistical difference in the routine practice of complementary exams, including molecular autopsy, between respondents who work in the university setting versus other institutions. The main reasons why genetic tests are so infrequently used are the elevated costs of such analyses, and the legal restrictions involved with the sampling and storage of DNA.

Table 4 Contact with family members and/or the family doctor in cases of SCD. The responses to two questions (in your practice, do you have contact with family doctors of victims of SCD*? In your practice, do you have contact with families of victims of SCD?*) were compared with the ability to perform the postmortem genetic analyses in cases of SCD shown in Table 1

	Frequency of contact	All respondents (%)	Respondents performing genetic testing (%)	Respondents not performing genetic testing (%)
Contact with families	Never	23.7	20.7	22.8
	In less than 50% of cases	32.9	24.1	42.1
	In more than 50% of cases	12.4	13.8	10.8
	Always	28.9	41.4	24.6
Contact with family doctors	Never	33.0	13.3	41.4
	In less than 50% of cases	34.0	40.0	34.5
	In more than 50% of cases	16.5	16.7	13.8
	Always	16.5	30.0	10.3

Our survey shows that routine practice varies widely with respect to the autopsy ordering, the standard investigations performed, and the collection and storage of samples. Molecular autopsies are widely used for research purposes, but in forensic practice often the most basic investigations are not systematically performed. Some institutions do not even perform an autopsy in cases of SCD, although the extent to which this occurs is very difficult to evaluate. When an autopsy is performed, there is often no concurrent histological examination of the myocardium and frequently the impossibility, due to lack of availability or inexperience, to perform more sophisticated investigations, such as conduction tissue examination, immunohistochemistry, electron microscopy, and biochemical marker measurement. Encouraging institutions to perform routine postmortem genetic testing is inutile if even the basic tests are not done. The results of this survey are in accordance with a recent online survey of current autopsy practices performed in the UK, which showed the discrepancies between the guidelines published by The Royal College of Pathology and what is realistically achieved in daily practice. The reasons suggested by the authors are related to lack of time, financial constraints, and the introduction of the Human Tissue Act. [16].

The majority of genetic heart diseases that can cause sudden cardiac death follow an autosomal dominant inheritance pattern, meaning that the probability of having additional family members affected is high. Making a diagnosis is very important as it may help prevent sudden deaths in living relatives [17–19]. Unfortunately, the link between the postmortem forensic investigation of a sudden cardiac death victim and the clinical investigation of surviving family members is difficult to establish. This difficulty may result from legal restrictions, such as the impossibility to obtain the consent of the next of kin, or from the inability to contact living family members. The respondents establish contact with the family in less than 30% of cases and with the family doctor in only 16%. Even institutions that routinely perform genetic testing only establish contact with the family members in 20.7% of cases and with the family doctor in 13.3%. This evokes several important ethical questions: *What happens with the results in such cases? Do family members have access to the results?*

Another problem in the management of sudden cardiac death cases is the frequent isolation of the forensic pathologist from other medical fields. This may result from the historical fact that the forensic pathologist largely works in response to requests from magistrates or other judicial authorities. They infrequently contact other specialists, except in cases where medical responsibility is implicated. This isolated approach is not beneficial in SCD cases, especially in regards to genetic testing and the transmission of results to families. Unfortunately, collaboration between the departments of medical genetics and cardiology only exists for 19.6% of respondents. More than 80% of the respondents who do not perform genetic testing declared that they do not collaborate with other departments. If more institutions begin to perform genetic analyses, the collaboration between services will hopefully increase.

Currently, the forensic pathologist acts as an expert and does not have any clear legal obligations toward the family. Legal and ethical obligations do exist in other, somewhat similar cases [20]. The prevailing approach of forensic practitioners must be re-evaluated. Family members of SCD victims have increasing access to information via the Internet; and in their search to find an explanation for the cause of death, they are sure to pose more and more

questions. Guidelines should be established regarding autopsy procedures in cases of SCD, including the responsibilities to inform living family members. These issues go beyond forensic medicine and require a broader discussion among health care providers. The role of the forensic pathologist in determining the cause of death might need to be separated from the public health and ethical issues of addressing the consequences for the family.

The opinion of those experienced with genetic testing is that the best solution, currently in place in a few countries, is the creation of national academic centers to which all cases of SCD can be referred. Such centers should be well publicized, funded by central state money and would require the consent of the next of kin, if available.

A limitation of this study is the low response rate of 15%, which can partially be explained by the fact that the available mailing lists of members of forensic medical associations were non-selective and did not list their professional activities. The questionnaire was, therefore, sent to many individuals who are not implicated in SCD cases, i.e., forensic toxicologists, psychiatrists, specialists in clinical legal medicine, etc. The low response rate of forensic pathologists working in peripheral hospitals or in private practice may indicate that the non-respondents of this survey are either not interested in cases of SCD or do not have the means to appropriately investigate them. The presented data reflects the practices and opinions of people who are most likely interested in the topic of SCD. Taking this into consideration, the percentage of institutions where a full investigation of SCD occurs is likely lower than that reported in this study.

It would be very difficult to selectively contact all individuals who perform SCD autopsies considering the variations of forensic medical structures and practices in different countries. In many countries, the molecular autopsies in cases of SCD are already performed by professionals not trained in forensic medicine (i.e., cardiologist or cardiac pathologists) in order to properly inform living family members. In our opinion, the importance of the genetic origin of many cardiac diseases, which can result in SCD, must be emphasized, in the hopes of establishing multidisciplinary collaborations between forensic pathologists and other experts in the medical field.

Conclusion

This survey shows that many of the problems involved in the adequate investigation of SCD cases are financial in origin, and caused by the fact that activities in forensic medicine are paid by and often dependent on the judicial authorities. Problems also exist concerning the contact with the family members and/or the family doctor, as well as the often-nonexistent collaboration with others clinicians with special expertise, such as cardiologists and geneticists.

It is too soon to draft final guidelines concerning molecular autopsies. As an initial step, we propose that each country should establish a clear legal framework for postmortem genetic analysis in the forensic context. In our opinion, a complete autopsy following the existing recommendations should be performed in all cases of SCD, and a second opinion should be obtained from an expert in the field of cardiovascular pathology. In the near future, the criteria for performing a molecular autopsy should be established in the by a team of international experts. The appropriate sampling and storage of material for genetic analyses is essential in the anticipation of future technical progress. Finally, forensic pathologists should realize the importance of the genetic origin of many cardiac diseases resulting in SCD and attempt to establish multidisciplinary collaborations.

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