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Featured Article: Explore cause of death —human and animals—
Invited Review

Molecular autopsy for sudden death in Japan

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Abstract: Japan has various death investigation systems; however, external examinations, postmortem computed tomography, macroscopic examinations, and microscopic examinations are performed regardless of the system used. These examinations can reveal morphological abnormalities, whereas the cause of death in cases with non-morphological abnormalities can be detected through additional examinations. Molecular autopsy and postmortem genetic analyses are important additional examinations. They are capable of detecting inherited arrhythmias or inherited metabolic diseases, which are representative non-morphological disorders that cause sudden death, especially in infants and young people. In this review, we introduce molecular autopsy reports from Japan and describe our experience with representative cases. The relationships between drug-related deaths and genetic variants are also reviewed. Based on the presented information, molecular autopsy is expected to be used as routine examinations in death investigations because they can provide information to save new lives. (DOI: 10.1293/tox.2023-0080; J Toxicol Pathol 2023; 37: 1–10)

Key words: forensic autopsy, metabolic autopsy, molecular autopsy, postmortem genetic analysis, sudden death

Introduction

The Department of Forensic Medicine at Tokyo Imperial University was established in 1889, and the government of Japan introduced the crime procedure law and stipulated the provision of a judicial autopsy system in 1890[1, 2]. Currently, both the criminal and civil justice system, as well as public health and social welfare programs are subjected to forensic medicine in Japan.

The current Japanese death investigation system varies across regions. All unnatural deaths must be reported to the police. Each case is then categorized as either criminal, suspicious, or non-criminal. Criminal cases and some suspicious cases are autopsied as part of a judicial autopsy, whereas non-criminal cases are not subjected to judicial autopsy. The medical examiner system, which covers the death investigation of non-criminal cases, is available mainly in Tokyo, Osaka, and Kobe city (administrative autopsy), while consent autopsy (consent of the bereaved family is necessary), which also targets non-criminal cases, is rarely performed except in a few regions[1, 3]. Recently, the Act on the Investigation of the Cause of Death and Identification of Bodies Handled by the Police was implemented, leading to the establishment of a new type of autopsy[4] wherein some non-criminal cases are autopsied; however, this system remains imperfect. The autopsy rate is low, and in most non-criminal cases, a general clinician prepares a death certificate without an autopsy[1].

Regardless of the applicable autopsy system, external examinations are performed first. Recently, postmortem computed tomography (CT) has been used for this purpose[5]. Macroscopic examination during forensic autopsy involves surveying the organs in the three main cavities (skull, thorax, and abdomen), as well as the subcutaneous soft tissue of the neck, back, and limbs. Microscopic examination is performed in almost all the cases (Fig. 1). This classical autopsy procedure helps to reveal morphological abnormalities, regardless of further macroscopic or microscopic examination; however, the cause of death in cases with non-morphological abnormalities is detected by additional examination. In this review, we introduced the examination of the causes of death in patients with non-morphological abnormalities,

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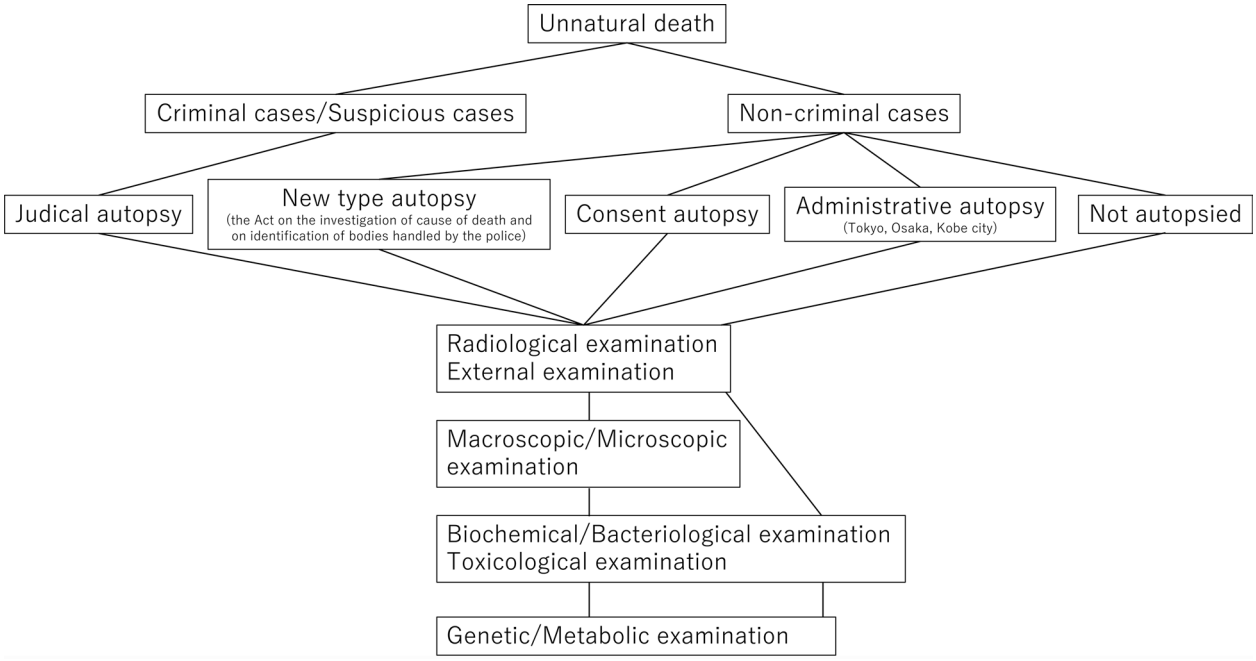

Fig. 1. Regardless of the autopsy system, external examination, postmortem radiological examination, and macroscopic examination are performed. Biochemical, bacteriological, and toxicological examinations are routinely performed in forensic autopsy. Genetic/Metabolic examinations are not routinely performed, but are recommended, even if an autopsy is not performed.

especially in the context of molecular autopsy.

Toxicological Analysis

One of the most important non-morphological abnormalities in forensic science is drug-related death. Toxicological examinations, including those for alcohol, psychotropic drugs, and various other therapeutic drugs, are necessary for the diagnosis of this condition. Suicide, improper use of psychotropic drugs (overdose or criminal use), and illegal drug use have become major social problems. Death investigation systems and forensic autopsies can help uncover such cases because drug-related deaths have no specific findings and are difficult to diagnose without suspicion. Therefore, comprehensive drug screening is important, even if drug use is not suspected[6–8]. In a famous case of serial killing covered by the media, no drug involvement was suspected in the victim's death; however, as another victim was later revealed to have been killed by some kind of drug, police investigations revealed that the first victim had also been administered drugs by the perpetrator. However, in addition to criminal cases, accidental or self-use cases also need to be investigated.

Caffeine is a commonly administered drug in daily use. It is found in coffee and tea, and its proper use is beneficial for humans. However, information on the risk of caffeine exposure has only been uncovered using forensic autopsies. Takayama *et al.* reported the case of a male in his early 20's working a night shift, whose cause of death was diagnosed as caffeine intoxication[9]. He consumed many energy drinks to stay awake and alert, but this resulted in excessive caffeine intake. Energy drinks, unlike most medicines, can be consumed repeatedly; therefore, the authors emphasized the dangers of repeated and chronic consumption of caffeinated products such as energy drinks and caffeine-containing drugs. We also reported a case of caffeine-related death[10]. An 18-year-old female with depression was found dead at home. There was significant vomiting around her mouth, and her room contained a few hundred empty blister packs of sleepiness inhibitors, including caffeine and some vitamins. Toxicological tests revealed a high amount of caffeine in many samples, and caffeine intoxication was diagnosed. At that time, reports of fatal caffeine intoxication were rare in Japan. As caffeine is not an illegal drug and is very easy to obtain, information from death investigations and forensic autopsies notified society regarding the dangers of caffeine overdose. According to the Act on Promotion of Policy about Death Investigation established in 2019, "Knowledge obtained by death investigations should be widely used as information that contributes to the improvement and promotion of public health;"[11] therefore, announcement of these cases is an important aspect of forensic science.

Sudden Natural Death and Molecular Autopsy

Sudden natural death is defined as death primarily attributed to an illness or an internal malfunction of the body and is not directly influenced by external forces[12]. Most cases of sudden death are attributed to cardiac arrest caused by morphological or non-morphological cardiac diseases. The

latter is also an important non-morphological abnormality in forensic science. Although the details of histopathological examination for sudden natural death should be referred using text books and review articles[13], a macroscopic autopsy alone often fails to detect the cause of death; in up to 40% of cases of sudden death, no definite cause of death is identifiable by autopsy[14, 15]. Molecular analysis is used to detect the cause of death in patients without morphological abnormalities.

Because deaths may occur suddenly, unexpectedly, and sometimes outside hospital settings, information regarding the health condition in these cases is not apparent to the relatives of the deceased or the medical staff. Owing to this, these cases are reported to the police as unnatural deaths but are mostly classified as non-criminal; therefore, autopsies are often not performed. However, in our region, the autopsy rate is rather high and cases of sudden death are likely to be autopsied, occasionally resulting in the diagnosis of rare diseases. Notably, the investigation of sudden death is important for medical pathophysiology and social issues. In this section, we focus on molecular autopsy in Japan from the perspective of postmortem genetic analysis.

Molecular autopsy involves postmortem genetic analyses[16, 17]. The sudden death of young individuals is associated with congenital channelopathy[17, 18]. Inherited arrhythmias are a cause of sudden death and include Brugada syndrome, long QT syndrome (LQTS), short QT syndrome, and catecholaminergic polymorphic ventricular tachycardia. Cardiomyopathies such as arrhythmogenic right ventricular cardiomyopathy (ARVC), dilated cardiomyopathy (DCM), and hypertrophic cardiomyopathy can also cause sudden death. Molecular autopsy revealed potentially pathogenic variants of the genes associated with these conditions in up to 25% of sudden death cases in a young population[19].

Ackerman *et al.* reported the first molecular autopsy by identifying a novel LQTS pathogenic variant (*KCNQ1*) in a 19-year-old woman. They also performed postmortem *SCN5A*-variant analysis in 93 cases of sudden infant death and detected causative genetic variants in two sudden death cases[20, 21]. Initially, molecular autopsy targeted only one or a few genes[22]. However, similar to toxicological screening, genetic analysis is expected to be conducted in a comprehensive manner, because there are numerous candidate genes for these arrhythmias. Thus, next-generation sequencing (NGS) has been utilized for molecular autopsy since its development[23–25]. Currently, postmortem genetic analysis is performed in a comprehensive manner, including whole-genome and exome sequencing[16, 26, 27].

Metabolic Autopsy

Inherited metabolic diseases are congenital diseases caused by genetic variants, sometimes resulting in sudden infant deaths. Metabolic autopsy is a death investigation method that focuses on metabolic diseases[28]. Metabolic autopsy was first reported in 1984 when an 18-month-old infant with non-specific malaise and mild infection of the upper respiratory tract developed a grand malconvulsion and died suddenly. The cause of death was initially considered sudden infant death syndrome (SIDS); however, diffuse fatty changes in the viscera and metabolic analysis revealed that the patient had medium-chain acyl-coenzyme A dehydrogenase (MCAD) deficiency[29, 30]. Metabolic autopsy includes the analysis of metabolic products in the blood, urine, and other fluids, enzyme analysis, and genetic analysis. Nagao reported that no 985A-to-G variant in the MCAD deficiency gene was detected in two Japanese patients who experienced SIDS[31]. Semba *et al.* reported an autopsy case of the neonatal form of carnitine palmitoyltransferase II (CPT II) deficiency in a 2-day-old Japanese boy[32]. We also performed metabolic autopsy in 30 cases of sudden infant death[33]. Although not a routine procedure, metabolic autopsy is advocated in pediatric guidelines[34] and has been prevalent in Japan, not only in autopsy[33, 35–41], but also in the emergency room[42–44].

Molecular Autopsy in Japan

Molecular autopsy targets metabolic diseases, as well as other diseases. Table 1 presents the representative reports of postmortem genetic analyses conducted in Japan.

In 2001, Narita *et al.* reported for the first time a relationship between serotonin transporter gene variation and SIDS[45]. In 2004, Kijima *et al.* analyzed the *PHOX2B* gene in 23 cases of sudden infant death and did not find any variants, except for three polymorphic nucleotide substitutions[46]. The *PHOX2B* gene was recently analyzed from the perspective of a non-polyalanine repeat variant[47]. In 2008, Otagiri *et al.* analyzed arrhythmia related genes and revealed that close to 10% of patients who experienced SIDS carried variants of cardiac ion channel genes[48]. Osawa *et al.* showed that variations in the novel QT interval determinant *NOS1AP* may be involved in the occurrence of SIDS[49]. Similarly, we previously performed molecular autopsy to investigate sudden infant death[33, 35, 37, 39, 50, 51].

For sudden deaths in young people, postmortem genetic analysis was introduced into the field of forensic science in the middle of the 2000's. In studies conducted in 2006 and 2008, our group examined the *RYR2* gene in several autopsy cases of sudden unexplained death and found that three cases carried the variant[52, 53]. They also found that one case among 17 sudden unexplained death autopsy cases carried a *KCNQ1* variant, which was revealed to be pathogenic using knock-in mouse analysis[54]. Sato *et al.* reported four cases of ARVC-related variants[55, 56] and Matsusue *et al.* reported a case of an *SCN5*A variant[57]. Takahashi *et al.* also reported a case of sudden death due to Marfan syndrome caused by an *FBN1* gene variant[58]. These studies were performed using Sanger sequencing, and currently, NGS-based studies are being reported in Japan[37, 39, 51, 59–70].

Although some global guidelines recommend molecular autopsy for sudden death, this has not yet become a routine procedure in most countries including Japan[19]. In some cases, inherited genetic disease testing is performed as part

Table 1. Postmortem Genetic Analysis in Japan

Subjects	Remarks	Genetic analysis	Gene	Author	Year
SUDI		Risk variant	*ACADM*	Nagao *et al.*[31]	1996
SUDI		Risk variant	*5-HTT*	Narita *et al.*[45]	2001
SUDI		Risk variant	*PHOX2B*	Kijima *et al.*[46]	2004
Coronary heart disease		Risk variant	*eNOS*	Ameno *et al.*[73]	2006
SUDY		Pathogenic variant	*RYR2*	Nishio *et al.*[52]	2006
SUDY		Pathogenic variant	*RYR2*	Nishio *et al.*[53]	2008
SUDI	Patch clamp experiment	Pathogenic variant	Chanelopathy	Otagiri *et al.*[48]	2008
SUDI	Case report	Metabolic autopsy	*CPT2*	Semba *et al.*[32]	2008
SUDY	Animal experiment	Pathogenic variant	*KCNQ1*	Nishio *et al.*[54]	2009
SUDI		Risk variant	*SCN5A*	Nakatome *et al.*[74]	2009
SUDI		Risk variant	*NOS1AP*	Osawa *et al.*[49]	2009
Cardiomyopathy		Risk variant	*TNNI3*	Murakami *et al.*[75]	2010
Marfan syndrome	Case report	Pathogenic variant	*FBN1*	Takahashi *et al.*[58]	2010
Rhabdomyolysis by Methamphetamine		Pathogenic variant	*RYR1, CPT2, VLCAD, CYP2D6*	Matsusue *et al.*[89]	2011
SUDI		Metabolic autopsy	*CPT2*	Yamamoto *et al.*[33]	2011
SUDY	Case report	Pathogenic variant	*DSG2*	Sato *et al.*[55]	2011
SUDY	Case report	Pathogenic variant	*SCN5A*	Matsusue *et al.*[57]	2012
SUDI		Metabolic autopsy		Yamamoto *et al.*[35]	2012
SUDY		Risk variant	*KCNQ1*	Kamei *et al.*[91]	2014
SUDI	Case report	Metabolic autopsy	*ACADVL*	Takahashi *et al.*[36]	2014
SUDI		Risk variant	*CPT2*	Yamamoto *et al.*[50]	2014
SUDI	NGS	Metabolic autopsy	Comprehensive	Yamamoto *et al.*[37]	2015
SUDI		Metabolic autopsy	*CPT2, ACADM*	Takahashi *et al.*[42]	2015
SUDY		Pathogenic variant	*DSP*	Sato *et al.*[56]	2015
SUDI		Pathogenic variant	*CPT2, ACADM*	Takahashi *et al.*[43]	2016
SUDI	Case report	Metabolic autopsy	*CPT2*	Takahashi *et al.*[38]	2016
SUDY	NGS	Pathogenic variant	Comprehensive	Hata *et al.*[59]	2016
SUDY in hot bath	Case report, NGS	Pathogenic variant	Comprehensive	Hata *et al.*[60]	2017
SUDY with epilepsy	NGS	Pathogenic variant	Comprehensive	Hata *et al.*[61]	2017
SUDI	NGS	Chanelopathy and metabolic autopsy	Comprehensive	Oshima *et al.*[39]	2017
SUDY		Risk variant	*KCNQ1*	Nagasawa *et al.*[92]	2018
SUDI	NGS	Risk variant	Comprehensive	Yamamoto *et al.*[76]	2018
SUDI		Metabolic autopsy	*ACADM*	Kaku *et al.*[44]	2018
SUDY	Case report, NGS	Pathogenic variant	*PRICKLE1*	Hata *et al.*[62]	2019
SUDI	Case report	Metabolic autopsy		Kozawa *et al.*[40]	2019
SUDY	Case report, NGS	Pathogenic variant	*RBM20*	Yamamoto *et al.*[63]	2019
SUDY with epilepsy	Case report, NGS, iPS	Pathogenic variant	*STXBP1*	Yamamoto *et al.*[64]	2019
SUDI	Case report, NGS	Pathogenic variant	*JPH2, PKD1*	Miura *et al.*[83]	2020
Dravet syndrome	NGS	Pathogenic variant	*SCN1A*	Hata *et al.*[65]	2020
SUDI and relatives	NGS	Chanelopathy and metabolic autopsy	Comprehensive	Shingu *et al.*[51]	2021
SUDY	Case report	Pathogenic variant	*MT-TL1*	Yoshida *et al.*[41]	2022
SUDY	Animal experiment, NGS	Pathogenic variant	*RBM20*	Yamamoto *et al.*[88]	2022
Pulmonary embolism	Case report, NGS	Pathogenic variant	*PROS1*	Miura *et al.*[81]	2022
SUDY	Case report, NGS	Pathogenic variant	Comprehensive	Hata *et al.*[66]	2022
Barlow's disease	Case report, NGS	Rare variant	Comprehensive	Hata *et al.*[67]	2022
SUDI		Risk variant	*PHOX2B*	Ueda *et al.*[47]	2022
Idiopathic Bradyarrhythmia	NGS	Pathogenic variant	Comprehensive	Hata *et al.*[68]	2023
Noonan syndrome	NGS	Pathogenic variant	*LZTR1*	Unuma *et al.*[69]	2023
SUDY	NGS	Pathogenic variant	Comprehensive	Takahashi *et al.*[70]	2023

NGS: Next generation sequencing; SUDI: Sudden unexpected death in infancy; SUDY: Sudden unexpected death in the young.

of medical practice, whereas postmortem genetic investigation is now performed as research. Therefore, the establishment of a suitable system is necessary in the future.

The relationship between genetic variants and sudden death has been studied extensively. As described above, variation in the serotonin transporter gene is famously considered a cause of sudden infant death[45, 71, 72]; *ACADM*, *PHOX2B*, *eNOS*, *SCN5A*, *NOS1AP*, *TNNI3*, *CPT2*, and

LQTS-related gene variants have also been analyzed in this context[31, 46, 47, 49, 50, 73–76]. In the following section, representative cases that we experienced are described. All cases have been reported elsewhere.

Case Presentations

Case 1: Metabolic autopsy for sudden unexpected death in infancy[33]

A 6-month-old boy with a fever for several days suddenly lost consciousness and died. External examination revealed no injury, and macroscopic examination revealed no distinctive abnormalities. Drug screening results were negative. Microscopic examination revealed liver steatosis, suggestive of Reye-like syndrome.

Reye-like syndrome sometimes occurs because of inherited metabolic disorders[77, 78]. Thus, postmortem biochemical analysis was performed to analyze the metabolic products, and the results revealed an increase in long-chain acylcarnitines, suggesting that the deceased had long-chain fatty acid oxidation defects, such as CPT II deficiency or carnitine-acylcarnitine translocase deficiency. Genetic analysis of these two metabolic diseases was performed using Sanger sequencing, and two substitutions in the *CPT2* gene were detected. Finally, CPT II deficiency was diagnosed by metabolic autopsy; however, this was time consuming and costly because these two genes have many exons.

Case 2: Metabolic autopsy with NGS for the remaining relatives and a new life[37]

An 11-month-old girl with a fever and vomiting for several days suddenly lost consciousness and died. No distinctive abnormalities were detected on postmortem CT imaging and macroscopic examination; furthermore, drug screening was negative. Microscopic examination revealed fatty liver. However, morphological examination did not reveal a background pathophysiology. Unlike in Case 1, NGS was available at the time. A comprehensive postmortem genetic analysis was performed using a fatty acid oxidation-related gene panel. The results revealed two variants of *CPT2* in only a single run within a few days.

In this case, pedigree analysis confirmed that each variant was inherited from the parents. Thus, we contacted the family doctor to prevent potential metabolic crises in any other children from future pregnancies, and newborn babies could then be subjected to medical follow-up during their asymptomatic period. Thus, forensic autopsies can help save new lives.

Case 3: Sudden arrhythmogenic death in sudden infant death[51]

A 3-month-old female infant died while sleeping. External examination revealed no injury and macroscopic and microscopic examinations revealed no abnormalities. SIDS was suspected to be the cause of death.

Postmortem genetic analysis revealed a D85N-*KCNE1* variant, which was reported to be associated with LQTS[79]; it was thus suggested as a possible cause of sudden infant death at first glance. However, family screening was necessary to increase the diagnostic yield[80]. Trio analysis revealed that the infant's healthy living mother who had no electrocardiogram abnormalities had the same variant. This suggested that this disease-associated genetic variant alone did not cause sudden infant death, as no symptoms were found in relatives, including parents. Thus, the genetic investigation of a single case may have misled the diagnosis.

Case 4: Molecular autopsy in a morphological abnormality case[81]

Molecular autopsy is not performed to diagnose the direct cause of death, but to reveal the pathophysiology of dying.

In this case, macroscopic autopsy revealed pulmonary embolism from an organizing thrombus in the inferior vena cava as the cause of death. If the aim of a forensic autopsy was only to determine the cause of death, this attempt might have been meaningless. However, the aim was not only to diagnose the cause of death but also to uncover its pathophysiology. The presence of an organizing thrombus with fibrosis and calcification and the absence of a history of trauma or chronic disease suggested that the deceased had subclinical congenital thrombophilia. Therefore, postmortem genetic analysis revealed that the deceased had the A139V-*PROS1* variant. This variant has been reported to result in low Protein S activity and total Protein S antigen levels[82]. Therefore, we considered that subclinical congenital thrombophilia was related to the pathophysiology of pulmonary embolism in the deceased. It is an inherited disease, thus necessitating familial screening. To this end, information regarding the cause of death triggered an examination of family members.

Case 5: Molecular autopsy for death investigation without autopsy[83]

An autopsy is the most reliable method for detecting the cause of death[11, 84]. However, the autopsy rate in Japan is low (approximately 1.6%)[1]; therefore, all unnatural deaths are not subject to autopsy examinations. However, this does not imply that a death investigation is not conducted. Although postmortem radiological, needle pathological, toxicological, and biochemical examinations cannot be considered alternatives to autopsy, they may be used to suggest the cause of death.

An eight-month-old Japanese girl with polycystic kidney disease (PKD) died of acute cardiac failure after acute-onset dyspnea at the hospital. Autopsies are not performed in Japan for such non-criminal cases. The pediatrician proposed a postmortem molecular autopsy of the family and consulted our laboratory. Performing autopsies is the main task of forensic science; however, our laboratory also receives requests for postmortem molecular analysis. In this case, postmortem genetic analysis revealed *JPH2* (p.T237A/p. I414L) as the causative variant of DCM and *PKD1* (p.Q4193*) as the causative factor of PKD. PKD is re-

portedly associated with DCM (PKD cardiomyopathy)[85, 86]. Therefore, in this case, the *PKD1* variant may have accelerated the onset and progression of DCM through impaired calcium cycling in cardiomyocytes.

Case 6: RNA sequencing and model animal experiments[63]

The deceased was a man in his 20's with a diagnosis of sudden natural death. Postmortem genetic analysis revealed the I536T *RBM20* variant. The *RBM20* gene is known to be one of the causative genes for DCM via splicing abnormalities; however, the significance of the I536T variant is unknown[87]. To reveal the significance of this variant, RNA sequencing of cardiac samples from the deceased, splicing reporter assays, and knock-in mouse experiments were performed[88]. These investigations revealed that the I536T variant affects the splicing of cardiac structural proteins, resulting in the development of DCM.

As comprehensive genetic analyses have progressed, several variants of uncertain significance have been detected in sudden death cases. This underscores the importance of performing molecular autopsy and interpreting the significance of detected variants in diagnosing the cause of death.

Investigation of Drug-related Death Using Molecular Autopsy

There are two types of drug-related deaths. The first is death in which a fatal dose is detected and intoxication is the cause of death, as described above. The second involves a drug-induced secondary pathogenesis. Matsusue *et al.* focused on the genetic variants in methamphetamine users[89]. They examined the *RYR1*, *CPT2*, *ACADVL*, and *CYP2D6* genes and found no obvious relationship between these genetic variants and rhabdomyolysis. Drug-induced arrhythmias are known to occur. LQTS is a life-threatening arrhythmic disease often caused by an inherited genetic variant, as described above; however, it is sometimes induced by drugs[90]. Kamei *et al.* performed a genetic analysis of *KCNQ1* and *KCNH2* in 10 cases of sudden death involving patients administered psychotropic medication, and concluded that administering psychotropic drug therapy to individuals carrying the G643S variant of *KCNQ1* may increase the risk of prolonged QT intervals and life-threatening arrhythmias[91]. Nagasawa *et al.* also analyzed the *KCNQ1* and *KCNH2* genes in 20 cases (of which 10 died after taking methamphetamine and 10 died after using new psychoactive substances) and concluded that the use of new psychoactive substances, particularly synthetic cathinones, is associated with an elevated risk of serious cardiac arrhythmia and sudden death in individuals carrying G643S *KCNQ1*[92]. Overall, genetic predisposition can provide important information for future mortality investigations.

Important Notes Regarding Genetic Variants

The importance of molecular autopsy has increased in both medical and legal fields. Of note are the incidents of scientific sleuths solving murder mysteries[93]. For instance, a mother whose child died after labored breathing, uncontrolled vomiting, and gastric distress was sent to prison for murder because the child was diagnosed with ethylene glycol poisoning. However, the diagnosis was later corrected to methylmalonic acidemia using metabolic autopsy[28]. Furthermore, in a case where a mother was convicted of the murder of three of her children and manslaughter of the fourth, Brohus *et al.* reported a genetic predisposition in the family, highlighting the importance of identifying genetic variants[94, 95].

As described above, many genetic variants have been detected since the development of molecular autopsy using NGS, most of which would have remained unknown if it was not for molecular autopsy. Most of these variants show incomplete penetrance, and because the medical information of patients is lacking in cases of sudden death, it remains unclear whether a disorder is associated with a detected variant[13]. Further accumulation of knowledge and interpretation of such genetic analyses is thus necessary to prevent sudden death among bereaved family members[96].

Some guidelines recommend saving samples in sudden death cases[97]. This facilitates genetic analyses during autopsy and later reinvestigation because toxicological examination and molecular autopsy, as described above, are not mandatory procedures.

Conclusion

In this review, we introduced a method for investigating the cause of death, especially in cases with non-morphological abnormalities, and described representative cases. Although toxicological examinations and molecular autopsy are not always performed, except during forensic autopsies, they can help detect potential crimes and accidents, as well as subclinical genetic abnormalities, providing important information to the surviving relatives. Thus, although classical autopsy (macroscopic and microscopic examinations) is the most important part of death investigations, other examinations, including molecular and metabolic autopsy, are also essential.

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