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Editorial note:

The article in this issue of Topical Update is contributed by the specialty of Forensic Pathology. Investigation of sudden death with "negative autopsy" is always a challenge to forensic pathologists. Dr. WM POON brings up for discussion the potential and practical implications of molecular autopsy of unexplained sudden death. We welcome any feedback or suggestions. Please direct them to Dr. Bobby Shum (e-mail: bsfshum@graduate.hku.hk) of Education Committee, the Hong Kong College of Pathologists. Opinions expressed are those of the author or named individuals, and are not necessarily those of the Hong Kong College of Pathologists.

Molecular Autopsy of Unexplained Sudden Death

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Introduction

Investigation of sudden death is the commonest challenge encountered by Forensic Pathologists. Most cases of sudden death are due to cardiovascular abnormalities evident at macroscopic and/or microscopic examination, such as coronary heart disease, myocarditis, cardiomyopathies, aortic dissection, etc. Unfortunately, a significant number of sudden death, estimated to be 1-5%[(1)], remains unexplained despite a thorough autopsy including toxicology, histology and other laboratory tests. This article attempts to look into some recent advances in the understanding of these "negative autopsies". Issues related to "negative autopsies" in infancy, which in itself merits another separate article, will not be covered in this article.

Most of these cases of "negative autopsies" are believed to be caused by cardiac arrhythmias in "morphologically normal hearts" [(2)]. Many of these "morphologically normal hearts", however, are genetically abnormal with gene defects in ion channels (i.e. channelopathies) in the myocytes leading to rhythm disturbances, ECG abnormalities and increased risk of sudden death. There is a growing list of inherited and congenital arrhythmia disorders caused by mutations in genes encoding defective ionic channels proteins governing the cell membrane transit of sodium, potassium and calcium ions including long QT syndrome (LQTS), short QT syndrome (SQTS),

Brugada syndrome and catecholaminergic polymorphic ventricular tachycardia (CPVT).

Long QT syndrome (LQTS)

Long QT syndrome (LQTS) is characterized by delayed repolarization of myocardium, prolonged QT interval in ECG, and recurrent syncope from a specific form of polymorphic ventricular arrhythmia called 'Torsades des Pointes'. It is a genetically heterogeneous disease affecting 1 in 5000 persons and is an important cause of unexplained sudden cardiac death in young people. It can be inherited as an autosomal dominant or recessive trait although it can also be caused by de novo mutations.

More than 500 mutations distributed in 10 genes have been described in this condition. Approximately 75% of LQTS is caused by mutations in 5 cardiac channels encoding genes: KCNQ1 (LQT1), KCNH2 (LQT2), SCN5A (LQT3), KCNE1 (LQT5) and KCNE2 (LQT6), encoding for critical ion-channel subunits that are responsible for the orchestration of the cardiac potential [(3)]. KCNQ1 and KCNE1 interact to form the cardiac IKs (inward slow potassium) current; while KCNE2 integrate with KCNH2 to form Ikr (inward rapid potassium) current and SCN5A encodes the sodium channel. Loss in function of delayed rectifier potassium channels which allow the efflux of K+ from the cell, or gain in function of the sodium or calcium channels resulting in excessive ions entering the cell, result in delayed repolarization and causes electrical heterogeneity leading to early after depolarization. Early after depolarization can facilitate the occurrence of 'Torsades des Pointes', which can progress to ventricular fibrillation and cardiac arrest.

Molecular genetic studies have yielded important genotype-phenotype correlations. In patients with LQT3 mutations, cardiac events occur predominantly during sleep or rest, whereas auditory triggers and events occurring during the period after childbirth tend be associated with LQT2 mutation. LQT1 mutation, on the other hand, is shown to be heavily associated with swimming and exertion-induced cardiac events [(4)].

The role of LQTS in apparent drowning cases was explored in a study of molecular screening for long QT mutations in 165 consecutive bodies recovered from water (i.e. putative drowning cases), a mutation in KCNH2 was found in a death originally classified as suicidal drowning [(5)]. In another study, 2 out of 10 cases of juvenile sudden and unexplained death were silent carriers of a mutation in KCHQ1 gene [(6)].

Brugada syndrome

Brugada syndrome was first described in 1992 [(7)]. The syndrome is identified by a distinct electrocardiographic pattern of right bundle branch block and persistent ST segment elevation in precordial leads (V1 through V3), a high incidence of ventricular fibrillation and sudden cardiac death. It is claimed to be responsible for up to 12% of all sudden deaths and approximately 20% of deaths with structurally normal hearts. Population studies searching for the distinct electrocardiographic pattern of Brugada syndrome in healthy adults showed a prevalence of 0.05% to 0.4% [(3)]. Recent evidence suggested that sudden unexplained nocturnal death (SUNDS), a disorder found in Southeast Asia, in fact represents Brugada syndrome [(8)].

The syndrome exhibits an autosomal dominant inheritance with variable and probably age-dependent expression. Currently over 100 mutations in the SCN5A gene, primarily missense mutations, have been linked to Brugada syndrome [(9)]; all of these mutations create a decreased sodium current either by influencing the trafficking or gating function of the sodium channel. In addition, mutations of genes that modulate sodium channel function such as the glycerol-3-phosphate dehydrogenase 1-like (GPD1-L) gene are also associated with Brugada syndrome.

Abnormalities of the sodium current are not the only genetic defects in Brugada syndrome. Mutations in the gene encoding the L-type calcium channel (CACNA1C) or its β 2b subunit (CACNB2b) were found in Brugada patients with unusually short QT intervals, indicating that

reduced calcium current can also contribute to the development of Brugada syndrome.

Brugada syndrome is a disease that manifest in adulthood with incomplete penetrance, and a high proportion of carriers remain asymptomatic, the value of genetic analysis for reproductive screening and reproductive counselling is less obvious than other conditions associated with sudden death in childhood and adolescence. Genetic analysis is useful, however, in non-penetrant mutation carriers and in family members of genotyped probands to detect early manifestation of the disease.

Catecholaminergic polymorphic ventricular tachycardia (CPVT)

CPTV is an inherited arrhythmia syndrome characterized by polymorphic ventricular tachycardia triggered by vigorous physical exertion or acute emotion usually in childhood and adolescence with normal resting ECG and the absence of structural heart disease. The most characteristic arrhythmia of CPVT is bidirectional ventricular tachycardia presenting with an alternating QRS axis. Clinically it may present as syncope or sudden death. About 30% of cases have a family history of sudden cardiac death [(3)].

Two genetic variants of CPVT have been identified, one is caused by mutations in the RyR2 gene transmitted in autosomal dominant form, while the other is caused by autosomal recessive mutations in the cardiac-specific isoform of the calsequestrin gene (CASQ2). Mutations in the RyR2 gene, which encodes the calcium release channel in the sacroplasmic reticulum that is essential for regulation of excitation-contraction coupling and intracellular calcium level, can be identified in approximately 70% of patients. More than 60 RyR2 mutations have been identified so far. Mutations in the RyR2 gene have also been found in victims of sudden infant death syndrome (SIDS), suggesting that it may also cause lethal arrhythmias in infant.

Autosomal recessive transmitted mutations in CASQ2 gene, which codes for calsequestrin, a calcium binding protein in the terminal cisternae of sacroplasmic reticulum that is bound to the ryanodine receptor and participates in the control of excitation-contraction coupling, have been found in only 7% of CPVT cases. Other CPTV genes are likely to exist as many cases showed no mutation in either RyR2 or CASQ2 genes.

Genetic analysis is very helpful in the diagnosis of the disease as affected individuals show an unremarkable ECG and no structural heart abnormalities, and CPVT is a malignant disease if left untreated. Genetic evaluation for family members of CPTV cases is highly indicated.

Short QT syndrome

The short QT syndrome is a recently recognized entity characterized by the ECG findings of persistently short QT interval (QTc < 300ms) with tall, symmetric and peaked T waves. Its clinical picture consisted of sudden death, syncope, palpitations and malignant arrhythmia. Most cases have a positive family history of sudden death. The syndrome has been linked to gain-of-function mutations in some of the genes implicated in LQTS including KCNH2, KCNQ1 and KCNJ2, causing in an increase in intensities of rectifier potassium currents in the repolarization phase of the cardiac cycle. Recently, a new clinical entity combining Brugada and short QT phenotypes caused by loss-of-function mutations in the α 1- and β -subunits of the L-type cardiac calcium channels has been described.

Practical Implications

The identification of mutations capable of causing sudden cardiac death with “structurally normal heart”, and the availability of molecular testing for these conditions opens up new avenues for the correct diagnosis and classification of sudden death cases that would otherwise be labelled as “unknown” or “undetermined”. A recent series of molecular autopsy on sudden unexplained death identified mutations in LQTS and CPTV associated genes in over one third of cases [(10)]. Enabled by molecular testing, the pathologist is able to assist the responsible legal authority to

arrive at the correct conclusions regarding the cause and manner of death, in cases with natural as well as unnatural causes of death e.g. drowning precipitated by an arrhythmia caused by an inherited channelopathy. On top of his duty to the legal authority, the pathologist also has an obligation to explain the autopsy results and cause of death to family members, who are confused and unable to make sense of the sudden demise of a loved one. With the advent of preventive measures such as implantable defibrillators, genetic screening of surviving family members to identify at risk individuals and to institute preventive measures need to be considered. Consultation with and referral to appropriate clinical specialists should be arranged.

A host of questions need to be answered before molecular testing can be applied to the investigation of unexplained sudden death and the genetic counselling of surviving family members. The rapid advance of molecular genetic would inevitably lead to an ever-expanding list of candidate genes and mutations for sudden cardiac death. The pathologist, without the benefit of antemortem clinical information or ECG, would find it difficult to decide which tests to order. The cost of an exhaustive screening for all potentially relevant mutations would be prohibitive, and funding for these tests, outside of a research setting, is hard to come by. Moreover, the interpretation of the results is not a simple task, and one of the major questions that remain for the pathologist is: What does it mean to find nonsynonymous polymorphisms instead of mutations when autopsy is negative for anatomic and histopathological findings? [(11)]. How much weight to attribute to these polymorphisms in deciding on the cause of death? In addition, non-coding regions of candidate genes are conceivably responsible for many of the arrhythmia syndromes mentioned above. Should we extend screening to non-coding regions of candidate genes for all the arrhythmia syndromes when coding region screening of these genes have not yielded any results. And what are the implications for family members? Given the incomplete penetrance of some candidate genes and the uncertain significance of genetic polymorphisms, the identification of genetic abnormalities/variations does not necessarily equate to a predisposition to sudden cardiac death. What to tell family members? When is genetic screening on family members warranted? The questions go on and on.

Conclusion

Recent advances in DNA technology enables the identification of mutations in cardiac ion channel genes capable of causing sudden cardiac death with structurally normal hearts, thus allowing deaths previously labelled as "unknown"/"undetermined" to be correctly classified as to their cause and manner of death. It also opens the door to genetic screening of surviving family members with a view to identifying at risk individuals and the institution of preventive measures. However, there are still problems concerning cost, the choice of appropriate candidate genes/mutations to screen for and the interpretation of test results that need to be addressed, before molecular tests can be applied widely in the investigation of sudden death and the genetic counselling of family members.

References:

(1) S.D. Cohle, B.A. Simpson, The negative autopsy: sudden cardiac death or other? Cardiovascular Pathology, 10 (2001) 219-222.

(2) Steven B. Karch, Changing times: DNA resequencing and the "nearly normal autopsy", Journal of Forensic and Legal Medicine, 14 (2007) 389-397.

(3) M.S. Rodriguez-Calvo et al., Molecular genetics of sudden cardiac death, Forensic Science International, 182 (2008) 1-12.

(4) P.J. Schwartz, S.G. Priori, C. Spazzolini, Genotype-phenotype correlation in the long Q-T syndrome. Gene-specific triggers for life-threatening arrhythmia, Circulation 103 (2001) 89-95.

(5) Philippe Lunetta et al., Molecular screening of selected long QT syndrome (LQTS) mutations in 165 consecutive bodies found in water, International Journal of Legal Medicine, 117 (2003) 115-117.

(6) Marco Di Paolo et al., Postmortem molecular analysis in victims of sudden

unexplained death, The American Journal of Forensic Medicine and Pathology, 25 (2004) 182-184.
(7) P. Brugada, J. Brugada, Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report, J. Am. Coll Cardiol. 20 (1992) 1391-1396.
(8) M. Vatta et al., Genetic and biophysical basis of sudden unexplained nocturnal death syndrome (SUNDS), a disease allelic to Brugada syndrome, Hum. Mol. Genet. 11 (3) (2002) 337-345.
(9) C. Antzelevitch, Genetic basis of Brugada syndrome, Heart Rhythm 4 (2007) 756-757.
(10) DJ Tester et al., Targeted mutational analysis of the RyR2-encoded cardiac ryanodine receptor in sudden unexplained death: a molecular autopsy of 49 medical examiner/coroner's cases, Mayo Clin. Proc. 79 (2004) 1380-1384.
(11) Antonio Oliva, Vincenzo L Pascali, Kui Hong, Ramon Brugada, Molecular autopsy of sudden cardiac death – the challenge of Forensic Pathologists to the complexity of genomics, The American Journal of Forensic Medicine and Pathology, 26 (2005) 369.