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SHORT COMMUNICATION

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Molecular screening of selected long QT syndrome (LQTS) mutations in 165 consecutive bodies found in water

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Abstract The association of the long QT-syndrome (LQTS) with single accidental drowning or near-drowning cases has been recently emphasised, but no data on the prevalence of LQTS among drowning victims are currently available. In this study, we have retrospectively screened specific founder mutations in KCNQ1 (KVLQT1) and KCNH2 (HERG) genes in 165 consecutive bodies found in water in Finland. We found a KCNH2-Fin mutation in a 44-year-old woman whose death was classified as suicidal drowning, whereas no other carriers of the two LQTS founder mutations were identified among the remaining 164 victims. This study provides the first estimate of the minimum prevalence of LQTS (0.61%, CI_{95}: 0.02–3.33) in such a setting and demonstrates the value of genetic analysis of LQTS in putative drownings. The detection of a LQTS founder mutation in a body found in water is a relatively rare event based on our study sample. This finding is, however, of utmost medico-legal importance, since it broadens the spectrum of potential causes and manners of death.

Keywords Long QT syndrome · LQTS · Mutation · Drowning · Molecular screening

Introduction

The long QT syndrome (LQTS) consists of a group of acquired or genetic disorders which are clinically characterised by a prolonged QT interval, tachyarrhythmia, syncope, and at times sudden death, in any age group [1, 2, 3, 4]. In its inherited form, the LQTS (OMIM*192500) predisposes to cardiac arrhythmia due to hundreds of known mutations in at least five genes (i.e. KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2), all encoding cardiac ion channels [1, 2, 5]. These mutations have been shown to cause disturbances in the potassium or sodium ion channel function, leading to delayed ventricular repolarisation. The most common mutations occur in the KCNQ1 (KVLQT1) and KCNH2 (HERG) genes and reduce the potassium outward current during the plateau phase and phase 3 of repolarisation.

Preliminary studies suggest that gene-specific triggers, such as exercise, emotion, rest/sleep, and neurosensorial stimulation may initiate life-threatening arrhythmia in LQTS patients [6]. The association of LQTS with accidental drowning or near-drowning and the arrhythmogenic role of water immersion and swimming in patients with the LQTS, has also been recently emphasised, particularly in individuals carrying a KCNQ1 gene mutation [7, 8]. However, no data are currently available on the exact prevalence of LQTS among victims of drowning.

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Materials and methods

Autopsy material

We collected autopsy data from 165 consecutive bodies found in water (males/females ratio 3.0, mean age 49.1 years, age range 1–89 years) in southern Finland (population 1.4 million) and autopsied at the Department of Forensic Medicine, University of Helsinki, during the period June 1998 to March 2001. In our department, a blood stain dried on dedicated paper (FTA GeneCard, Life Technologies, Carlsbad, Calif., cat no. 10786–036) is collected from each autopsy, preserved in the archives as a part of the documentation in the quality assurance process and serves as a source of biological specimens for further studies when appropriate. As determined by the forensic pathologists in the autopsy reports and death certificates, the study material consisted of 107 (64.8%) accidents, 37 (22.4%) suicides, 15 (9.1%) deaths of undetermined intent, and 6 (3.6%) natural deaths.

DNA analysis

In order to obtain data on the prevalence of LQTS among drowning victims, we retrospectively screened specific Finnish founder mutations in the KCNQ1 and KCNH2 genes (KCNQ1-Fin:

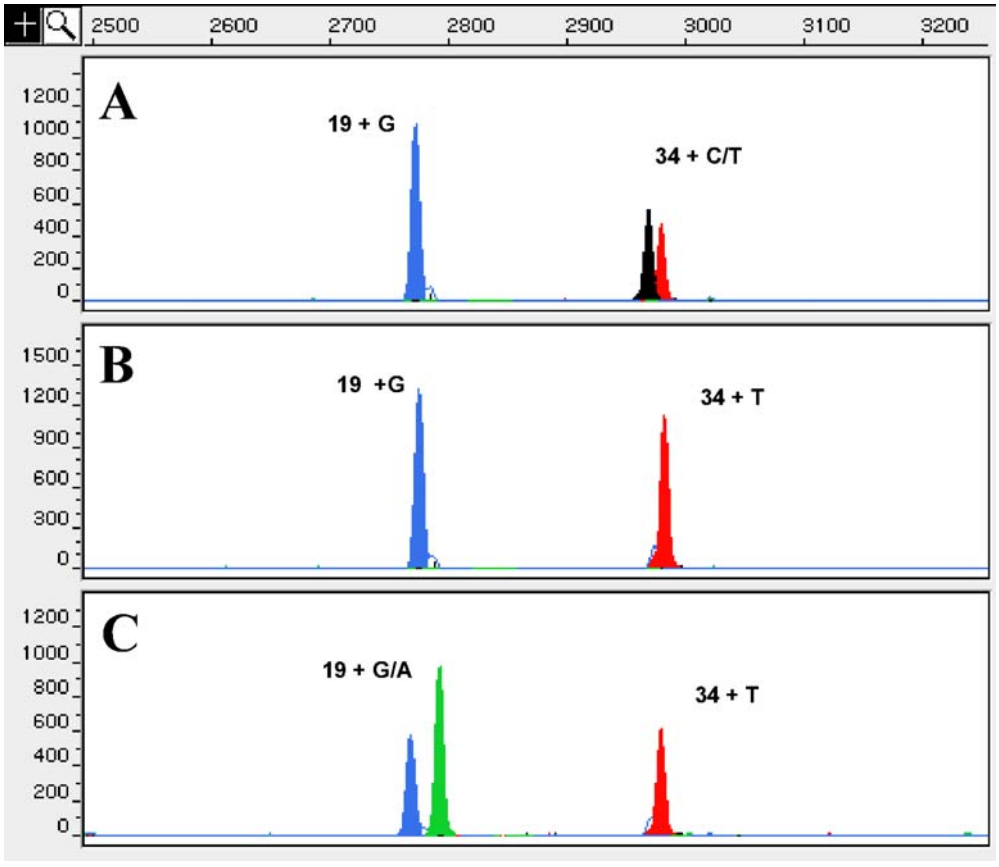

Fig. 1A–C Detection of specific founder mutations in the KCNQ1 and KCNH2 genes (KCNQ1-Fin:Gly589Asp and KCNH2-Fin:Leu552Ser) using the primer extention method. **A** In our case mutation analysis revealed a heterozygote KCNH2-Fin mutation (34+C/T). **B** A control showing wild-type alleles (19+G and 34+T) at both loci. **C** A KCNQ1-Fin mutation control sample (19+G/A). Electrophoretic images were edited to remove unspecific minor background noise <20% of specific peak heights

Gly589Asp, KCNH2-Fin: Leu552Ser) in all these 165 samples. The post-mortem (PM) mutation screening from dried bloodstains was accomplished using duplex PCR amplification of 136 bp (KCNQ1-Fin; primers 5'-GGC CCT GAT TTG GGT GTT TTA-3' and 5'-AGG ACG CTA ACC AGA ACC AC-3') and 237 bp (KCNH2-Fin; primers 5'-TGC CCC ATC AAC GGA ATG TGC-3' and 5'-CCA GCC GAT GCG TGA GTC CA-3') fragments. In order to verify successful amplification of PM samples, the PCR products were run on a 1.5% agarose gel, and visualised using ethidium bromide staining. Thereafter, a primer extension reaction with the aid of 19 nt (5'-CGC GGC AGC AAC ACG ATC G-3') and 34 nt (5'-T_{16}-GCG CGG CCG TGC TGT TCT-3') forward detection primers for KCNQ1-Fin and KCNH2-Fin mutations, respectively, was employed in a single tube, and genotypes were visualised using fluorescence detection in capillary electrophoresis (CE 310, Applied Biosystems, Foster City, Calif.) (Fig. 1).

Results and discussion

The role of LQTS-associated life-threatening arrhythmia in sudden unexpected death (SUD) has been recently emphasised and demonstrated in individual cases [2, 3, 5]. However, no data on the exact prevalence of LQTS in large series are currently available. A range of common activities (e.g., exercise, emotion, rest/sleep, swimming) and neurosensorial stimulation may trigger life-threatening arrhythmias in LQTS patients in a gene-specific manner [6]. The potential role of swimming is of great clinical importance for drowning prevention and may explain some fatalities occurring without apparent cause in experienced and sober swimmers. Furthermore, it has been recently hypothesised that a consistent proportion of sudden natural deaths in water, e.g., related to LQTS arrhythmias, can be actually misdiagnosed as dry drowning, which is traditionally attributed to laryngospasm [9].

Screening of LQTS gene mutations in defined case series should be particularly fruitful in Finland, where specific founder mutations have been identified in the KCNQ1 and KCNH2 genes, and are responsible for about one third (approximately 30% and 5%, respectively) of clinically diagnosed LQTS cases [10]. In addition, accidental drowning rates in Finland are the highest among the Nordic countries with comparable geographic, climatic, and socio-cultural background, and are markedly higher than the EU average [11].

Mutation analysis revealed the KCNH2-Fin mutation (Fig. 1) in a 44-year-old woman with a mental disorder and previous suicide attempts, who was found in a bathtub and died a few minutes later in spite of resuscitation attempts [12]. After police investigations and on the basis of autopsy findings, the death was classified as suicidal drowning. Toxicology using gas chromatography screening (GC) for drugs in blood was positive for mirtazapine (0.6 mg/l), temazepam (0.2 mg/l), desmethyldiazepam (0.1 mg/l), and diazepam (0.1 mg/l). The blood alcohol concentration was 0.26‰. Retrospective examination of clinical data revealed a prolongation of the rate-corrected QT-interval (QTc) in the ECGs, which were taken during the medical care following two suicide attempts by psychotropic drugs (temazepam, chlorpromazine) 14 and 3 months be-

fore death (QTc=492 ms and 468 ms, respectively). No ECG without medication was available in the medical files of the victim. Abnormalities of QTc intervals associated with various psychotropic drug treatments in psychiatric patients have recently been emphasised [13]. However, in this case, the psychotropic drugs involved in the suicide attempts and those found post-mortem are not among those currently known to trigger life-threatening arrhythmias in acquired LQTS or in patients with non-symptomatic LQTS mutations. No other carriers of the two LQTS founder mutations were identified in the remaining 164 cases.

The results of our screening furnish the first estimate of the minimum prevalence of LQTS mutations among bodies found in water (0.61%; CI_{95}: 0.02–3.33). Considering that the two mutations investigated encompass approximately 35% of familial LQTS cases in Finland, the prevalence of LQTS among bodies found in water may be estimated to be in the range of at least 1–2% (overall prevalence in the population <0.1/100,000 persons per year).

Although the finding of an LQTS mutation is rare in our sample, the present study demonstrates the importance of molecular testing for LQTS mutations among victims of putative drowning. Moreover, it emphasises the value of an analysis of different arrhythmogenic genes in suspected drownings, since our case is the first fatal drowning associated with a KCNQ2 gene mutation. Earlier only KCNQ1 gene mutations have been reported in fatal drownings [6, 7, 8]. The LQTS-associated fatality detected in our study material also represents the first reported case where a mutation for the LQTS was found in a victim of allegedly "intentional" drowning. The medico-legal implications are of utmost importance, since detection of an LQTS mutation in a body found in water enlarges the spectrum of potential causes and manners of death. In cases with unclear circumstances, this finding may significantly influence the judicial approach and outcome of suspected crime or litigation involving payment of insurance benefits. However, it must be emphasised that even if an LQTS mutation is detected, the differential diagnosis between a sudden cardiac death in water, and a drowning death may be challenging, especially after the onset of putrefactive changes. The differences between morphological changes of lung (and possibly quantitative diatom data analysis) observed in drowning eventually associated with laryngospasm and/or rapid cardio-circulatory arrest, and those found in sudden cardiac deaths with terminal aspiration of water can be subtle and difficult to interpret. Furthermore, if based on circumstances and autopsy findings, a sudden cardiac death in water would seem more probable, it can be problematic to differentiate between a LQTS-triggered sudden death and a sudden cardiac death of another origin. In addition to LQTS, other genetically determined arrhythmias or cardiac conduction abnormalities (e.g. in epileptics), tumours of the cardiac conduction system (e.g. A-V node benign tumours), spasm of normal coronary or commotio cordis, may cause sudden death with negative or minimal autopsy findings [14, 15, 16].

In conclusion, PM molecular screening for gene mutations responsible for SUD is an emerging diagnostic tool that can change the forensic evaluation of unexplained sudden deaths [3, 4]. However, all bodies of those carrying a LQTS mutation do not necessarily represent sudden arrhythmic death or unnatural death triggered by LQTS. The development of PM molecular methods makes it necessary to critically evaluate the cause-effect relationship between genetically determined predisposition, external triggers and terminal events prior to death.

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