The role of environmental and genetic factors in cardiovascular diseases and deaths

Ph.D Theses

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Introduction

The weather conditions basically influence human mental and physical conditions. The consequences of climate change will not only become a determining factor in today's socio-economic level, but will also have a huge impact in human life, health and disorders.

Cardiovascular diseases are the leading causes of death across in Europe, with nearly 4 million deaths in every year. Almost half of the total mortality (47%; 1.9 million) due to cardiovascular origin (52% female, 42% male), the most frequently diseases are the coronary disease and stroke. Coronary diseases are the most common causes of death in itself with 1.8 million deaths per year. In both gender every fifth people die due to coronary heart disease. Cardiovascular diseases are responsible for more than 50% of the mortality among women, which is especially typical in Central and Eastern European countries. However under 65 years of age across in Europe cardiovascular diseases are the leading causes of death, geographical and economic differences can be observed. In Western European countries, coronary heart disease death rates have decreased over the last 30 years.

The impacts of environmental and meteorological factors in cardiovascular deaths and their relationship can be examined in various ways, such as epidemiological studies or analysis of pathomorphological changes. The relationship between climatic factors and cardiovascular mortality is now generally accepted and genetic basis of cardiovascular disease is becoming even more known. However, there are no conclusive studies which could prove that there is correlation between the changing weather induces physiological responses and the genetic background of cardiovascular diseases. Having regard to the importance of the impact of climate changes it is an important objective for the future researchers on medical meteorology to verify the link between climate adaptation and genetic factors.

Distribution of environmental-related death cases across Europe - Environmental accidents

The frequency, intensity, and duration of meteorological disasters have increased since the 1920s. More recently the climate disasters, along with extreme meteorological events, earthquakes, tsunami, heat waves, infectious diseases, water- and food-borne illnesses and air pollution are important components of climate change that impact mortality.

Objectives

The purposes of this study were to demonstrate the proportion of death cases in connection with environmental-meteorological factors across the European countries; to find differences between cold and hot climates areas, and to attrack the attention for the importance of environmental-related accidenst to improve more effective prevention strategies.

Material and methods

Mortality data were collected from the WHO European Detailed Mortality Database from 32 European countries - where data were available - in the time period between 2000 and 2011. The 10th revision of the International Classification of Diseases (ICD) was used for the determination of cause of death. Mortality data were collected in all age groups and both genders summarized. The study material was subdivided into two main groups: I.) natural death, and II.) non-natural death, such as accident, suicide, and homicide. We started to use the *environmental accident* term to those accidents where the environmental- meteorological conditions due to death. In connection with the environmental factors we selected the following ICD codes for the determination of environmental-releated death cathegory: accidental drowning in natural water, exposure to extreme hot or cold weather, hypothermia, lightning, earthquake, storm, landslide and other natural forces. The prevalence of environmental-releated death cases was compared to all death cases (total/ TOT mortality), to all non-natural death cases, and to all accidents. From the all accidents group we devided the other accidents (traffic accidents, unintentional hit/struck/bitten by person or animal, unintentional poisoning, etc.) and compared to the environmental accident group. We investigated the most frequent causes of environmental death cumulatively in all countries and looked for differences between cold and hot climates regions.

Results

All together 51,578,325 (49,004,334 /95.01% natural, 2,573,991 /4.99% non-natural) death cases were collected from the selected 32 European countries between the year of 2000 and 2011. In most of countries the rate of environmental accidents in TOT mortality was higher than homicide rate, except in Denmark, Ireland where it was equal; and in Belgium, Germany, Italy, Luxembourg, Netherlands, Portugal, Serbia, Spain and TFYR Macedonia where it was less.

During the examined period in non-natural death cases there were 80,195 (3.12%) environmental accidents, 1,767,841 (68.68%) other accidents, 664,093 (25.8%) suicides and 61,862 (2.4%) homicide death cases. In the WHO database all together 1,848,036 death cases were defined as accidental deaths, and this was 71.80% of non-natural death cases. In non-natural death categories the environmental-related death cases were highest in Estonia (10.87%), Latvia (11.08%) and Lithuania (12.23%). The least rate was in Netherland (0.68%). The other types of accidents rate in non-natural death cases was the most frequent in Cyprus (82.89%), in Italy (78.2%) and in the United Kingdom (75.49%).

In the comparison between the rate of environmental accidents and other accidents in all accidental mortality we found that the 80,195 environmental-related death cases represented 4.34% of all accidents in the overall results during this eleven years period. In Netherlands the environmental accidents means less than 1% among in all accidents. An increasing tendency can be observed between the years of 2000 and 2004, and decrease was started in the year of 2005. The lowest rate was in 2008. In the year of 2009 and 2010 the tendency started to increase again.

With regard to the examined causes of death during this period hypothermia was the most frequent event (43.02% in environmental accidents, 1.34% in non-natural death cases), followed by the drowning in natural water (33.97% in environmental accidents and 1.06% in non-natural death cases). The lowest number of cases in environmental accidents connected to volcanic eruption (less than 0.01%), exposure of sunlight (0.08%), flood (0.13%) and cataclysmic storm (0.14%). It is a remarkable statement that hypothermia was more frequent in the Central and Eastern European countries (for example: Romania, Hungary, the Baltics countries), than in the northernmost areas, such as in Norway, Sweden or Denmark. The most frequent hypothermia cases were in the Baltics countries, principally in Estonia (0.81% in all death causes). In the southern places (for example Spain, Portugal, Italia) the number of

hypothermia was nearly the same as in the North. In the Central and Eastern European countries the rate of hypothermia in all death causes by countries was 0.09-0.81%, in the northernmost areas the ratio was 0.02-0.05% and the southern places it was 0.01-0.02%.

Conclusion

From the datas we calculated that the most frequent fatal environmental death cases in the investigated countries were hypothermia and drowning in natural water. We found that that hypothermia was more frequent in the Central and Eastern European counties, than in the northernmost areas, and in the southern places the number of hypothermia was nearly the same as in the North. Characterization of environmental risk factors is important to determine preventative strategies against the global environmental changes. We concluded that greater attention needs to be placed on the social context of climate change impact, the vulnerability and adaptation. After evaluating the large mortality database from 32 European countries we are able to demonstrate the existence of a separate subgroup for which we propose to use the term *environmental accident.* Regarding to the global climate change and the effects of meteorological conditions on fatal outcomes, the environmental factor-related death cases have an increasing importance, but we can not ignore the aggravating socio-economic factors connected to them.

Characteristics of cardiovascular deaths in forensic medical cases in Budapest, Vilnius and Tallinn

Cardiovascular diseases are one of the leading cause of mortality in Europe. Several studies have demonstrated that cardiovascular mortality has a characteristic seasonal distribution, and the inverse relationship between average daily temperatures and ischaemic heart disease mortality has also been shown.

Objectives

The main purpose of this study was to investigate sudden unexpected cardiovascular death and to study how different geographical climatic influences may affect cardiac mortality in three capitals: Budapest, Vilnius and Tallinn.

Material and methods

In our study we collected cardiovascular death cases autopsied at the Forensic Departments of National Forensic Institutes in three capitals (Budapest, Vilnius and Tallinn) from the period of 2005-2009. The rate of cardiovascular mortality was 36.50% (Budapest: 4 765 deaths), 18.30% (Vilnius: 2 716 deaths) and 19.20% (Tallinn: 1 001 deaths) among medico-legal autopsy cases. The 10th version of the International Classification of Diseases (ICD) was used. We applied the following codes: ICD 120.0 (unstable angina), ICD 125 (chronic ischaemic heart disease), ICD 105-109 (chronic rheumatic heart diseases), ICD 151.7 (cardiomegaly), ICD 142 (cardiomyopathy), ICD 150 (heart failure), ICD 170 (atherosclerosis), ICD 171 (aortic aneurysm and dissection), ICD 180 (phlebitis and thrombophlebitis), ICD 195-199 (other vascular disease). We used the definition of sudden death defined by the World Health Organization (WHO), i.e., sudden death happens in 24 h after the first symptoms, in cases with no sign of previous diseases and no external cause of death or any sign of violence.

The following parameters were evaluated: age, gender, cause of death, months of death, week days, BAC levels, place of death (private home, public institute, road, work place, hospital, ambulance, others). The BAC test was performed in most cases in Vilnius and Tallinn and about 10% of the cases of death in Budapest. We analysed blood alcohol concentrations (BAC) in a range of categories: slight (BAC: 51e80 mg/100 ml), mild (BAC: 81e150 mg/ 100 ml), moderate (BAC: 151-250 mg/100 ml), severe (BAC: 251-350 mg/100 ml), and very

severe (BAC: above 351 mg/100 ml). The tissue samples removed from the body were fixed in a 4% formaldehyde solution, and after embedding in paraffin, 3-4 mm sections were made. The haematoxylin and eosin stained slides were examined under a light microscope. Microscopical examination of heart and other tissue samples was performed in all cases in Vilnius and Tallinn, but not in every case in Budapest. Statistics were amassed using the STATA 10 statistical package and included a Pearson Chi-square test for dichotomous variables, 2-ways summary table method, and the Spearman rank order correlations. A p-value of 0.05 was considered as statistical differences.

Results

In our material there were 8 482 (5 753 male and 2 729 female) cardiovascular deaths between the years 2005 and 2009 in Budapest, Vilnius and Tallinn. The mean age was 64.07 ±14.33 year. Male predominance was observed in all the three capitals, and 59.83% (5 075) of cases involved males over the age of 60. The highest rate was observed in the age group of 71-80 years (35.17%) and 51-60 years (24.45%). Our data suggests that under the age of 40 years the rate of cardiovascular death is rare (4.33%). The most common cause of death in each capital was chronic ischaemic heart disease: Budapest: 40.23%; Vilnius: 77.10%; Tallinn: 37.36%. In the test group, chronic rheumatic heart diseases were detected in the fewest number (No. 71), besides, almost the same proportion pulmonary embolism (No. 223), aneurysms (No. 230) and chronic heart failure (No. 241) occurred least often.

The highest number of cardiovascular deaths occurred in January (805/9.49%) and December (770/9.07%). In Tallinn statistically significant seasonal distribution was observed with winter prevalence (280/ 3.30%). In Vilnius there was spring prevalence (760/8.90%) and in Budapest mortality data did not show seasonal distribution, however the most death cases were detected in the winter period, there were no significant differences between the seasons. There was a correlation between the winter period and old age (more than 56 years). During the winter period the mean age of victims was 66 year in Budapest, 63.5 year in Vilnius, and 61 year in Tallinn. Correlation with other factors (e.g. age, gender, BAC) was not statistically significant.

In addition, most cases were found dead on Mondays (1373/16.19%), in every capital. Most of the victims died at home (Budapest 45.50%, Vilnius 74.08%, Tallinn 45.60%), in hospital (Budapest 20.80%, Vilnius 3.17%, Tallinn 3.80%), or in a public place (Budapest 14.20%,

Vilnius 8.65%, Tallinn 21.00%). A BAC test was done in 513 (10.77%) cases in Budapest, in 2 704 (99.56%) cases in Vilnius and in 975 (97.40%) cases in Tallinn. In 2 954 (70.47%) cases the BAC tests were negative. The BAC test was generally positive with victims with chronic ischaemic heart disease.

Conclusion

Based on our results we can conclude that there are several factors that may influence cardiovascular mortality. For example, environmental-geographical parameters may affect natural cardiovascular death. This study results support the hypothesis that a colder climate might represent a risk factor for elderly persons with chronic ischaemic heart diseases. The more frequent winter occurrence of cardiovascular death has long been known, but it is not detectable in all countries. Our study points out the occurrence of cold-induced mortality should be analysed in more details, with comparing several different climate areas and exploring other influencing factors for the better understanding of the underlying processes.

Evaluation of meteorological and epidemiological characteristics of fatal pulmonary embolism

Pulmonary embolism represents a main public health problem worldwide with a great mortality, in about 25% of victims, and it could be the first manifestation of sudden death.

Objectives

The purposes of this study are to examine epidemiological and pathological patterns detected during medico-legal autopsies in PE cases, to identify the meteorological risk factors for the occurrence of PE death, to predict the expected mortality of PE as a function of meteorological elements and to provide a descriptive temporal analysis of cumulated number of fatal PE cases.

Material and methods

The survey target groups included cases of PE in the capital Budapest. Based on the database of the Department of Forensic and Insurance Medicine, Semmelweis University, there were 23 892 cases autopsied between 1 January 2001 and 31 December 2010 in our institute. Among these cases, there were 467 (1.95 %) PE defined as cause of death in this time period. We collected PE cases when death happened as sudden unexpected death even if it was after suffering traumatic injuries.

PE was determined based on the autopsy pathomorphological findings as large thrombi lodged at the bifurcation of the main pulmonary artery or the lobar branches, and the thrombi originated in the deep venous system of the lower extremities. Histological assessment of the embolus samples was also performed in conjunction with the venous sites of thrombosis. Histological investigation of fat emboli (Sudan III) has been performed in every case when long bone fractures or massive blunt injury soft tissue was found during the medico-legal autopsy. Cases with fat emboli were excluded from our study. Based on the macroscopic and histological findings, the post-mortem changes were excluded.

In PE cases, we reviewed the autopsy reports that contained the information of age, gender, manner of death, place of death, cause of death, clinical anamnestic data and previous diseases, traumatic injuries, survival period, immobilisation, surgery, and source and severity

of emboli. The following post-mortem data were also obtained: scene investigation, macroscopic and histological findings, post-mortem BAC tests and toxicology.

Regarding to the manner of death, we investigated cases in four different groups, as natural death, accidents, suicides and homicides. In our material, we categorised the studded cases in two groups based on the manner of death. We sorted into the natural death group those cases where no previous violent event happened. The violent death group contains the cases where accident, suicide, or homicide resulted in injury and led to pulmonary embolism. Based on the existence of the well-known risk factors in the anamnestic data, we separated the idiopathic VTE cases (in the absence of any known risk factors) from the associated VTE cases (cases with risk factors). The 10th revision of the International Classification of Diseases (ICD) was used for the determination of cause of death. Based on the post-mortem retrospective data collecting study, ethics approval is not required.

Meteorological data were obtained from the gridded E-OBS data sets. Meteorological data were analysed from the region of capital Budapest. The following variables were used: daily maximum, minimum and mean temperature; temperature change between days with PE death and the previous days; daily atmospheric air pressure; and atmospheric air pressure change between days with PE death and the previous days.

In the first phase, we performed a Bayesian estimation for weather; detecting the relationship between PE deaths and then, we studied the growth of registered PE deaths depending on the time. Cox processes are adequate models for the number of registered PE deaths in time. The number of registered PE deaths up to the nth day can be interpreted as a Cox process which intensity parameter is modulated by the weather. The conditional expectation extrapolated to 300 days was introduced as a new quantity to measure the effect of weather on the incidence of fatal PE because the monthly number of fatal PE during 10 years was considered and E300 is comparable to the magnitude of this quantity. Local or seasonal changes in incidence of fatal PE can be accurately modelled, but global trends (over 10 years) are not in general because it is possible that there are some unknown effects which are not taken into consideration in the model. For that reason, in the second phase of the statistical analysis, we performed a crude power law regression to detect global trends. Computations were done by Wolfram® Mathematica 9.0.1.0.

Results

There were 467 (211 males, 256 females) fatal PE cases investigated in the study period. VTE caused fatal PE in 2.51% (No 320/12 749) of natural death cases, in 2.18% (No 136/6 231) after accidents, in 0.22 % (No 10/4 453) after suicides and in 0.21% (No 1/459) after homicidal injuries. We compared the risks and pathological characteristics of PE between the natural and the violent death groups. In most cases, the source of embolism could be found; the most frequent locations were the lower limb (No. 276) and the pelvic venous plexus (No. 34). In 151 cases, source of emboli was not found as the thrombus probably completely moved to pulmonary arteries. The average age was higher in the violent death group (74 years) than in natural death group (68 years). In natural sudden unexpected death cases, the death occurred mostly at home (No 182); second, in an ambulance (No 52), or in hospital (No 46), or at public place (No 39), at work place, only one case was registered. All the trauma patients died in hospital, and as expected, immobilisation, surgery and antithrombotic prophylaxis were more frequent in this group.

We did not find any relevant differences in the source and in severity of embolism, or in the incidence of obesity or malignancy between the two groups. In our study, there were more long bone fractures among trauma patients (81/147 in lower extremities, 21/147 in upper extremities); however, in these cases, fat emboli investigation showed a negative result. Fatal fat embolism cases were excluded from this material as previously mentioned. In the violent death group, 7 (4.76 %) of the patients died in the first 24 hours, 25 (17.01%) died between 1 and 7 days, 82 (55.78 %) deaths occurred in 1 to 4 weeks after the traumatic injury, and 33 (22.45 %) died after 1 month. In our material, 228 idiopathic VTE and 239 associated VTE cases have been found. In violent death cases with high rate of immobilisation and surgical treatment, combined aetiology of PE has been frequently detected (No.131).

In our material, the average monthly temperature was between 0.2 and 22.9°C. We detected seasonal distribution with fewer number of PE death cases during summer period than in other seasons. About the relative growth of conditional probability of PE against average daily temperature we can tell that a 5°C decrease in the daily mean temperature increases the odds of PE death by at least 10% in natural death group and by 20 % in violent non-natural death group (p= 0.95). Daily atmospheric air pressure and changes in air pressure between days with PE death and the previous days were detected.

About the relative growth of conditional probability of fatal PE as a function of the change of air pressure it could be said that the change of air pressure has a weak effect on the incidence of PE death (p=0.34); however, we found that in natural death group, the air pressure increases the risk of PE death. These results clearly suggest that in case of lower daily mean temperature, more fatal PEs can be expected. Moreover, the number of fatal PE cases as natural death is larger when day-to-day air pressure change is increasing than when it is decreasing. For violent death cases, tendency in air pressure is not such a clear determining factor.

Conclusion

We concluded that in case of lower temperature (i.e. winter time), more fatal PE cases are predicted to occur. In addition, the increasing tendency of air pressure is also likely to result in more PE deaths. This latter effect is much less pronounced in the violent death group and then in natural death cases. Thanks to the Bayesian statistical model, the relative growth of the probability of fatal PE can be expressed as a function of the considered weather parameters. The conditional probability function acts like a so-called forecast function. The number of fatal PE follows a power law. From this fact, we deduced that the population of Budapest City is exposed to an unknown thrombotic effect. We successfully quantified the main meteorological risk factors of PE death using Cox process based on Bayesian statistical model. The math model constructed in this study has a great potential to other epidemiological investigations aiming to detect significant connection between different time series.

A common polymorphism of the human cardiac sodium channel alpha subunit (SCN5A) gene is associated with sudden cardiac death in chronic ischemic heart disease

The information stored in DNA is called the genome, human genome consists of 23 pairs of chromosomes and nearly three billion DNA base pairs. The single nucleotide polymorphisms is a DNA sequence variation, represent only one base pair difference. The most common method used to clarify the genetic background of polygenic hereditary diseases is genetic association study aimed to establish whether the suspected risk allele's incidence is higher in the study group compared to controls.

Objectives

The aim of the present case-control study was to find genetic polymorphisms that might be independent and universal predictors of sudden cardiac death. To this end, selected single nucleotide polymorphisms occurring in the above mentioned candidate genes have been genotyped in a cohort of Hungarian patients who succumbed to different cardiovascular ailments.

Material and methods

Buccal swabs from 360 victims of natural, cardiovascular death (66.7% male, mean age: 68.02 ± 14.45 years) were collected post mortem during autopsies at the Department of Forensic and Insurance Medicine (N = 262) and the 1st Department of Pathology (N = 98) of the Semmelweis University between Sept 2011 and Nov 2013. Regarding the circumstances of death, 165 cases (45.8%) were witnessed, unexpected, 'established SCDs' occurring within an hour of symptom onset: 28 in hospital, 17 in public institutions, 37 in ambulance and 83 in urban public places. 195 unwitnessed cases (54.2%) occurred at home without obvious extracardiac cause, within 24 hours of last being observed in good, symptom-free condition, therefore fulfilling the internationally acknowledged criteria of 'probable SCD'.

Buccal sampling was carried out within four days after death on the average. Morphological inclusion criteria were valvular heart disease; myocardial hypertrophy, fibrosis, fatty degeneration or atrophy; calcification or thrombus in the coronary arteries; moderate or severe atherosclerosis in other arteries; and embolism in the pulmonary arteries. The 10th revision of

the International Classification of Diseases (ICD) was used for categorizing the cause of death. Violent death cases and cases where cardiovascular complications were consequences of underlying primary diseases (such as malignancies and pneumonias) were excluded from the study. No clinical records or data on medication, smoking, lifestyle factors etc. were considered.

The age-matched control group comprised 300 non-related volunteers (39.3% male, mean age: 65.75 ± 14.83 years) without any known cardiovascular disease. Buccal samples of the participants were collected at the Institute of Psychology, Eötvös Loránd University, Budapest, Hungary. All case and control subjects were Caucasian whites from the administrative area of Budapest. Of a multitude of polymorphisms known to associate with SCD, five single nucleotide polymorphisms (SNPs) were selected from the candidate genes.

Handling of buccal samples and isolation of genomic DNA was performed essentially as described elsewhere. Two parallel samples were obtained and processed from each participant. Concentration of genomic DNA solutions was measured by the AccuBlue Broad Range dsDNA Quantification kit (Biotium, Hayward, USA). Concentrations of DNA stock solutions varied between 15–200ng/mL. Indicators of DNA purity were acceptable (OD 260/280 ratios were between 1.6–2.0 and OD 260/230 ratios varied between 2.0–2.2), and agarose gel electrophoretic assays revealed no significant fragmentation of genomic DNA. 1 µL samples from tenfold diluted stock solutions were used for genotyping.

Genotyping of selected single nucleotide polymorphisms (SNPs) was performed by a 7300 Real-Time PCR System (LifeTechnologies, USA) using commercially available TaqMan Genotyping Assays (Applied BioSystem, Foster City, USA), according to the manufacturer's instructions.

Chi-square test of SPSS 17.0 for Windows was used for assessing the Hardy-Weinberg equilibrium. A genotype-based logistic regression model adjusting for age and sex was used for case control analysis. The Bonferroni correction was used for multiple testing. The Alibaba 2.1 transcription factor binding prediction software and the TRANSFAC database were employed for in silico transcription factor binding analysis.

Results

In order to find a genetic polymorphism that could be used as a universal predictor of cardiac death risk, we set up an intentionally very heterogeneous cohort of subjects who died of different cardiovascular illnesses as verified through autopsy. The majority of patients deceased of chronic myocardial ischemia, witch diagnosis were more frequent among male subjects. To dispel concerns on the quality of DNA obtained from post mortem sampling, we performed PCR-based genotyping of a well-known length polymorphism in the type 4 dopamine receptor gene that had much been studied previously in our laboratory [36]. Control PCR assays were run successfully on all post mortem samples, confirming that they were applicable for genetic testing Five candidate genes of cardiac death have been selected from the current literature and one representative single nucleotide polymorphism from each gene was genotyped by quantitative PCR using allele-specific TaqMan probes. Though the list of genotyped variants is far from being comprehensive, the selected candidate genes are known to be critically involved in the pathomechanisms of sudden cardiac death which encompass dysrhythmias (SCN5A), perturbed intracellular calcium signaling (RYR2 and NOS1AP), myocardial remodeling (TGFBR2) and sympathetic activation (ADRB2). Accordingly, four SNPs analyzed in the present study (in genes ADRB2, RYR2, SCN5A and TGFBR2) have reportedly been associated with SCD (Table 2). The rs10494366 SNP in the NOS1AP gene showed strong association with cardiovascular mortality and high hazard ratio in patients treated with dihydropyridine calcium channel blockers. Here, we wished to address the issue whether this variant is associated with cardiac death in an extended, more heterogeneous patient population. Importantly, all genotype frequencies were in Hardy-Weinberg equilibrium in both the control and case population, and call rates were higher than 95% for each SNP investigated (data not shown). Genotype distributions for each SNP in both populations as well as results of the case-control association study are presented in Table 3. Genotype frequencies were compared using a logistic regression model-based multivariate analysis accounting for age and gender differences between the case and control populations (Table 3). P values and odds ratios (OR) with confidence intervals (CI) for all five polymorphisms are shown in Table 3. Our results revealed that the CC genotype of the rs11720524 (G/C) SNP of the SCN5A gene occurred more frequently in the cardiovascular death cohort compared to the control group (47.90% vs. 36.99%, $p = 0.019$, OR = 1.351), while none of the other studied polymorphisms showed any significant differences in this respect. Due to performing multiple comparisons, the accepted level of significance was

corrected to avoid false positive results. After applying the stringent Bonferroni correction for multiple testing ($p = 0.01$ [0.05/5] as 5 SNPs were studied) the effect of the CC allele turned out to be slightly below the threshold of statistical significance. The heterogeneity of the case population prompted us to perform a post hoc sub-analysis to figure out which case subgroup was responsible for the above effect. It turned out that the CC genotype occurred significantly more frequently in patients with chronic ischemic heart disease ($p = 0.012$, OR = 1.455) but not in the other patient subgroups. As the statistical strength of the association was fairly enhanced by eliminating cases other than chronic ischemic heart disease, and the other SCD cases did not associate with the variant at all ($p = 0.277$), our data support the notion that the common rs11720524 CC variant is associated with increased SCD risk in patients with ischemic cardiomyopathy only, but not with the risk of SCD in general.