

Sudden cardiac death in young Danes

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PREFACE

This PhD thesis is the result of the work carried out in the Laboratory of Molecular Cardiology, Department of Cardiology, The Heart Centre, University Hospital Copenhagen, Rigshospitalet in the period 2007-2010. The work was carried out with financial support from The Danish Heart Foundation, The Danish National Research Foundation Centre for Cardiac Arrhythmia (DARC), The Heart Centre Research Foundation at Rigshospitalet, Villadsen Family Foundation, Direktør Jacob Madsen og hustru Olga Madsens Fond, Bønnelykkefonden, Fonden 17-12-1981, Lægernes Forsikringsforening af 1891, and Kontorchef Gerhard Brøndsteds Rejsselegat, for which I am deeply grateful.

The thesis is based on 5 scientific papers:

Paper I: Winkel BG, Holst AG, Theilade J, Kristensen IB, Thomsen JL, Hansen SH, Svendsen JH, Haunsø S, Tfelt-Hansen J. Sudden unexpected death in infancy in Denmark. *Scand Cardiovasc J.* 2011 Feb;45(1):14-20.¹

Paper II: Winkel BG, Holst AG, Theilade J, Kristensen IB, Thomsen JL, Ottesen GL, Bundgaard H, Svendsen JH, Haunsø S, Tfelt-Hansen J. Nationwide study of Sudden Cardiac Death in persons aged 1-35 years. *Eur Heart J.* 2011 Apr;32(8):983-90.²

Paper III: Holst AG, Winkel BG, Theilade J, Kristensen IB, Thomsen JL, Ottesen GL, Svendsen JH, Haunsø S, Prescott E, Tfelt-Hansen J. Incidence and etiology of Sports Related Sudden Cardiac Death in Denmark – Implications for preparticipation screening. *Heart Rhythm* 2010 Oct;7(10):1365-71.³

Paper IV: Winkel BG, Holst AG, Theilade J, Kristensen IB, Thomsen JL, Hougen HP, Bundgaard H, Svendsen JH, Haunsø S, Tfelt-

Hansen J. Differences in investigations of sudden unexpected deaths in young people in a nationwide setting. *Int J Legal Med* 2011 Jul 21 [Epub ahead of print].⁴

Paper V: Winkel BG, Hollegaard MV, Olesen MS, Svendsen JH, Haunsø S, Hougaard DM, Tfelt-Hansen J. Whole-genome amplified DNA from stored dried blood spots is reliable in high resolution melting curve and sequencing analysis. *BMC Med Genet.* 2011 Feb 9;12:22.⁵

The papers are referred to as Paper I, Paper II, Paper III, Paper IV and Paper V in the thesis.

ABSTRACT

Sudden Cardiac Death (SCD) in the young (aged 1-35 years), although presumably rare, is always a tragic and devastating event often occurring in apparently healthy persons. Through the last decades, research have been undertaken to estimate the incidence rate and underlying causes of these deaths. However, because autopsy is not always conducted, the true incidence of SCD might be underestimated.

The incidence of sudden infant death syndrome (SIDS) has previously been thoroughly investigated, also in Denmark. However, data has not been precise in sudden unexpected death in infancy (SUDI) estimates. SIDS is a diagnosis of exclusion and an ICD-10 diagnosis (R95.9), but to what extent this diagnosis is being accurately applied has not been investigated in Denmark.

A genetic screening for mutations in an otherwise unexplained death, might identify a likely cause of (inherited) death. It would be of great clinical interest if DNA derived from the Danish Neonatal Screening Biobank, containing DNA from all Danes born after 1981, could be used in this respect.

In this thesis we provide nationwide data on SCD, SUDI and SIDS in Denmark for the period 2000-2006 by reading death certificates, autopsy reports, and registry data. We report the highest possible incidence rate of SCD in the young. We elaborate on regional differences in post-mortem investigations of sudden death cases in Denmark and validate a method for whole-genome amplification of DNA from Guthrie cards to be used in genetic screening for disease causing mutations.

We found 7% of all deaths in the young could be attributed to SCD. A total of 25% of sudden unexpected death in the 1-35 years old were not autopsied. The incidence of SCD of 2.8 per 100 000 person-years - when including non-autopsied cases - was higher than previously reported. Unexplained deaths were abundant and accounted for 22% of all sudden unexpected deaths. Sudden deaths occurring during competitive sports, however, were only

seen in few cases. We found that regional differences exist in the investigation of sudden unexpected deaths. Fewer deaths were medico-legally investigated by external examinations (retslægeligt ligsyn) in some parts of Denmark compared to other parts. The same was the case in autopsy ratios.

In infant deaths we found that almost 1 in 2000 live-borns died suddenly and unexpectedly during their first year of life. The R95.9 diagnosis did not reflect the SIDS cases we identified.

We were able to get DNA from the Danish Neonatal Screening Biobank on 93 cases of unexplained deaths (including SIDS). Due to the limited amount of DNA available from the dried blood spots, we performed whole-genome amplification on the DNA (wgaDNA). We investigated the use of wgaDNA for genetic screening and it completely resembled genomic DNA (gDNA). Future research will focus on the genetics substrate of sudden unexplained death. In addition, we will investigate the causes of death in the 36-49 years old, as these may also suffer from cardiac disease that can be predisposed in the family.

1. INTRODUCTION

1.1 EPIDEMIOLOGY OF SUDDEN UNEXPECTED DEATH IN INFANCY

Sudden unexpected death during infancy (SUDI) is defined as the sudden unexpected death (of natural causes) in an infant below the age of 1 year and often occurs during sleep. It is a tragic and devastating event, often occurring in an infant that was seemingly healthy prior to death. If an autopsy is performed, established causes of death in many cases will be of either infectious or cardiac origin.^{6,7} However, in the majority of deaths, no cause of death can be established and are hence termed Sudden Infant Death Syndrome (SIDS) or cot death.

SIDS has received a great deal of attention through the last decades. Especially in the 1970's SIDS was a feared and somewhat abundant cause of death with incidences peaking in the area of 1.9 deaths per 1000 births in Denmark, in other countries higher.⁸⁻¹⁰ Many studies investigated possible risk factors of these deaths. Some of the most compelling evidence emerged from different epidemiologic studies in the late 1970's all pointing towards sleeping on the stomach (prone sleeping) being one of the greatest risk factors of SIDS. This led to the "back to sleep" campaigns launched throughout the world in the late 1980's and early 1990's. The effect was enormous. In Avon, UK, prone sleeping decreased from 59% in 1988 to 2% in 1992. This was paralleled by a dramatic fall in SIDS rates from 3.8/1000 births to 0.3/1000 - in just 4 years.⁹ In Denmark, the same pattern was observed with rates decreasing from 1.8/1000 births to 0.2/1000 births following the risk reduction campaign launched December 1991.⁸ Following these risk reduction campaigns, the incidence of SIDS stabilized in most countries.

Thus, there is no doubt that sleeping on the stomach is a great risk factor of SIDS. However, the underlying mechanism(s) involved in SIDS was not explained by this epidemiologic finding. Many mechanisms have been proposed over the years including serotonergic brainstem abnormalities¹¹, fatty acid oxidation disorders¹², and infections.¹³ In the 1970's Schwartz et al proposed a link between the inherited cardiac disease Long QT Syndrome and SIDS.^{14,15} But it took another two decades before SIDS cases were directly correlated with a longer QT interval than non-SIDS deaths and healthy controls.¹⁶ These data initiated the search for underlying genetic causes to SIDS related to malignant arrhythmias.

Today strong evidence point towards malfunction in cardiac ion channels to be responsible for more than 10% of SIDS cases.¹⁷

This new knowledge, while important when counselling the bereaved parents in relations to inheritability of a disease in the family, has also put challenge on how to define SIDS: By definition, a SIDS case should be unexplained. A genetic variant, however, causing an alteration in the cardiac action potential thereby possibly predisposing for a lethal cardiac arrhythmia, can explain an otherwise unexplained SIDS case. Obviously, this case should then no longer be classified as a SIDS case. The problem, though, arises because no systematic genetic screening is performed in SIDS cases. Can an unexplained death be termed SIDS if a genetic screen has not been carried out (i.e. have all other diseases been ruled out)? The current international classification system of diseases issued by the World Health Organisation (ICD version 10, or ICD-10, published in 1992) offers no sub-classification system of SUDI/SIDS cases. Only the diagnosis R95.9 (denoting SIDS) is possible in the classification system. Today, it is not clear how R95.9 is applied in the cause of death registries. This is a potential problem, because all nationwide SIDS mortality rates derive from cause of death registries. Recently, a classification system for SIDS was proposed by an international working group.¹⁸ This classification system deals with many aspects of SIDS, including non-autopsied cases (termed Unclassified Sudden Infant Death, USID) and degrees of SIDS depending on autopsy results and risk factors prior to death. This classification system, however, is difficult to apply on a retrospective analysis of SIDS, but may be helpful in prospective studies.

1.2 EPIDEMIOLOGY OF SUDDEN CARDIAC DEATH IN THE YOUNG

Sudden cardiac death (SCD) in the young (1-35 years old) has not been as thoroughly investigated as SIDS. The general accepted definition of SCD today is the sudden, natural unexpected death of unknown or cardiac cause; in unwitnessed cases as a person last seen alive and functioning normally less than 24 hours before being found and in witnessed cases as an acute change in cardiovascular status with time to death being less than 1 hour.¹⁹⁻²¹

It is a major problem in the retrospective identification of SCD cases that it is not possible to extract sudden deaths from a cause of death registry. Therefore, surrogate measures have been used throughout the years in order to describe SCD in the young, for instance only out of hospital deaths, only witnessed deaths, or only autopsied cases. All these surrogate measures, albeit it comes from best available data, has the potential bias of under-reporting the true incidence of SCD in the young. Today, the total number of studies reporting incidences of SCD in the young is low and incidence rates together with causes of death varies considerably between the studies.²²⁻³⁰ No study has systematically investigated all deaths in a nationwide setting by reviewing all death certificates, autopsy reports, and registry entries on previous known disease in order to assess a highest possible estimate of SCD in the young.

A certain subgroup of SCD, the sports-related deaths in athletes, has received a great deal of attention. An attention partly driven by the extensive media exposure these deaths sometime have. The largest prospective studies on these deaths derive from Italy where preparticipation screening of all athletes has been mandatory since 1982.³¹ Some of the major conclusions from the Italian studies were that sudden death in athletes was 2.3/100 000 athletes and in non-athletes 0.9/100 000 and that preparticipation screening lowers the incidence of SCD among athletes.³² These results were in contrast to other (retrospective) studies within the field.^{33,34} The conclusions from Italy, while accepted

and endorsed by many countries and sport societies, has been criticized by others.^{35,36} In Denmark, there has been no systematic preparticipation screening. The Italian studies have hitherto been the only ones that provide data on Sportsrelated SCD (SrSCD) in an unscreened population.

A potential bias in epidemiology of SCD is the non-autopsied cases. A small study from Ireland recently noted that not all sudden death cases were autopsied.³⁷ In Denmark, the forensic pathologists have been aware that at least some sudden unexpected death cases were not autopsied. However, little is known about which deaths are not autopsied. In Denmark it is ultimately a police decision, if a medico-legal external examination (in the following referred to as external examination) and a subsequent forensic autopsy is to be performed. An external examination is mandatory by law in all cases of sudden and unexpected death. A forensic autopsy, on the other hand, is mandatory only if mode of death is not well established (for instance natural death or accident) or if a criminal act is suspected. It is not known if regional differences exist in Denmark in relations to investigations of sudden unexpected deaths presumed to be of cardiac origin.

1.3 GENETIC ASPECTS OF UNEXPLAINED DEATHS

It is well established that unexplained sudden deaths, whether it is an unexplained death in a 25 year old adolescent or in an infant aged 3 months, can be attributable to mutations in genes coding for ion channels involved in the cardiac action potential.^{38,39}

The ion currents orchestrating the cardiac action potential mainly consists of Na⁺, K⁺ and Ca²⁺ movements across the cell membrane (see figure 1).⁴⁰

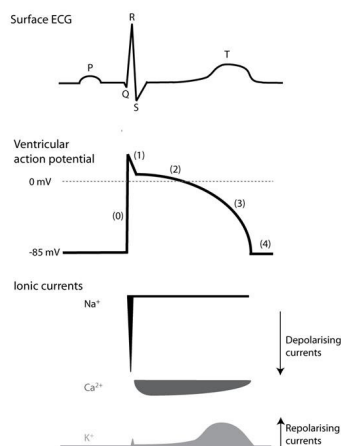


Figure 1
Ionic currents underlie the cardiac action potential and thereby the electrocardiogram. Illustration of a standard 12-lead electrocardiogram (ECG), of an intracellular recording of a ventricular action potential, and the relative time-dependent contributions of the different ion fluxes in the different phases of the action potential. With permission from Tfelt-Hansen et al, JCE 2010.

These movements of ions across the cell membrane happen through ion channels formed by proteins assembled into highly selective and complex units. The fluxes of ions results in contractions of the heart muscle cells leading to a heart beat. This delicate setup can be disturbed, for instance if a mutation in one the genes encoding these proteins alters the function of the protein thus changing the properties of the ion channel and subsequently

the ion fluxes. This may increase the risk of developing arrhythmias.

One of the most well investigated diseases in this regard is the Long QT Syndrome (LQTS). The disease is characterized by prolongation of the QT interval on the surface ECG together with a clinical history of syncope and/or sudden death and/or a family history of SCD in young age.⁴¹ The prolonged QT interval arises due to a prolonged repolarisation time of the ventricles. A prolongation of the QT interval in most cases has shown to be the result of either an increased Na⁺ current or a decreased K⁺ current.⁴² Mutations in 3 genes, KCNQ1, KCNH2 and SCN5A, can explain approximately 75% of LQTS, but another 9 genes are also associated with the disease.⁴² Furthermore, large deletions or duplications in the LQTS associated genes, not detectable with traditional sequencing techniques, might explain another 5% of LQTS.⁴³ Today, 35-40% of unexplained deaths in the young can be explained by genetic alterations in genes involved in the cardiac action potential.^{38,43,44} As already mentioned, 10% of SIDS cases can be attributed to genetic alterations causing cardiac arrhythmias.¹⁷

Thus, genetic screening for mutations in an otherwise unexplained death might identify a likely cause of death. This, however, requires DNA from the deceased – something that is not always readily accessible. The quality of DNA from formalin-fixed paraffin embedded tissue from autopsies is typically poor. In contrast, DNA from fresh frozen tissue or drawn blood samples is reliable and thus is preferred. Unfortunately, these samples have not been collected routinely at all autopsies in Denmark. The use of DNA derived from the Danish Neonatal Screening Biobank could potentially solve this problem. This biobank stores (at -20 degrees Celsius) Guthrie Cards on all newborns in Denmark since 1982.⁴⁵ Each Guthrie Card contains 2 blood spots of approximately 1 cm in diameter. It would be of great clinical significance if DNA from these blood spots could be amplified and subsequently used for genetic testing.

The overall aims of this thesis were to

- (1) Describe the incidence of sudden cardiac death, sudden unexpected death in infancy and sudden infant death syndrome in Denmark by reading death certificates, autopsy reports, and registry data
- (2) Establish autopsy ratios in sudden unexpected death, thereby providing a highest possible estimate of the magnitude of sudden cardiac death and sudden unexpected death in infancy in a nationwide setting
- (3) Describe the incidence rate of sportsrelated sudden cardiac death in Denmark where systematic preparticipation screening is currently not in place.
- (4) Describe regional differences in the post-mortem investigations of sudden death cases in Denmark
- (5) Validate a method for whole-genome amplification of DNA from Guthrie cards to be used in genetic screening for disease causing mutations

2. RESULTS

2.1 EPIDEMIOLOGY OF SUDDEN UNEXPECTED DEATH IN INFANCY

2.1.1 Paper I

During the 7 year study period (2000-2006) there were at total of 455 091 births in Denmark. A total of 1 962 infant deaths (below 1 year) occurred, of which we classified 192 (10%) as sudden and unexpected (SUDI). A previous medical history was present in 17 (9%) of the SUDI cases; autopsy was performed in 87% of cases. In

Cause of death, autopsied SUDI cases (n = 167)	n	Cardiac disease diagnosed prior to death, n
SIDS	82	
Congenital heart malformations	29	
Coarctation of the aorta	6	
Transposition of the great arteries	5	1
Malformation of cardiac chambers, unclassified	4	1
Truncus arteriosus communis	3	
Hypoplastic left ventricle	3	2
Single ventricle	3	
Ebsteins anomaly	1	
Valvular disease	2	1
Malformation of coronary artery	1	
Aneurysm of pulmonal artery	1	
Other heart diseases	11	
HCM	2	
Endocardial Fibroelastosis	2	
Left ventricular hypertrophy	2	
Endocarditis	1	
Myocarditis	1	
Benign tumor, heart	1	1
ARVC	1	
Other cardiomyopathy	1	
Infectious disease	28	
Pneumonia	13	
Sepsis	9	
Meningitis	4	
Epiglottitis	1	
Encephalitis	1	
Cerebral disease	4	
Intestinal volvulus	2	
Asphyxia	2	
Metabolic disease	1	
Other causes	8	
Conclusion after autopsy		
SUDI, cardiac cause	40 (24%)	
SUDI, non cardiac cause	45 (27%)	
SIDS	82 (49%)	

Abbreviations: HCM = Hypertrophic Cardiomyopathy, ARVC = Arrhythmogenic Right Ventricular Cardiomyopathy, SUDI = sudden unexpected death in infancy, SIDS = sudden infant death syndrome.

Table 1
Causes of death after autopsy in the 167 autopsied SUDI cases in Denmark 2000–2006, categorized in major groups.

the 167 autopsied SUDI cases 24% (n=40) died of congenital heart malformations or other heart disease, while 27% (n=45) died suddenly and unexpectedly of non-cardiac causes. Knowledge of congenital heart disease would less frequently elicit an autopsy. In 49% (n=82) of autopsied SUDI cases no cause of death was found and were therefore classified as sudden infant death syndrome, or SIDS (cot death). A total of 16 deaths – in which the infant had no known disease prior to death – were not autopsied (unclassified sudden infant death, USID cases). Thus the highest possible number of SIDS cases was 98 in the study period (average 14 SIDS cases/year). Incidence rate of SUDI was 0.42 pr. 1 000 births. Incidence rate of SIDS was 0.18 pr. 1 000 births. Highest

possible incidence rate of SIDS – including USID cases – was 0.22 pr 1 000 births. Causes of sudden death during infancy are shown in table 1.

We validated the Cause of Death Registry by comparing the cause of death after reading the autopsy report and the official ICD-10 diagnosis denoting SIDS (R95) in the registry. The Cause of Death Registry correctly categorized 81 of the 98 (83%) SIDS and USID cases. In 17 cases (17%) the ICD-10 SIDS classification was not used. In addition, 10 cases (10%) were incorrectly classified as SIDS in the Cause of Death registry. Thus, of the 91 SIDS cases identified by the Cause of Death Registry, 27 (30%) were misclassified.

2.2 EPIDEMIOLOGY OF SUDDEN CARDIAC DEATH IN THE 1-35 YEARS OLD

2.2.1 Paper II

In the 1-35 years old there were a total of 6 396 Danes dying in 2000-2006. From the review of the death certificates we identified 625 sudden unexpected death cases, of which 156 (25%) were not autopsied. The majority of autopsies (89%) were performed in one of the three Forensic Pathology Departments.

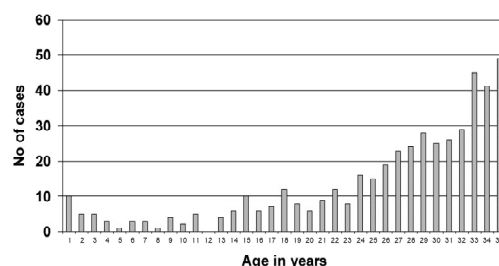


Figure 2
Age-related distribution of 470 sudden cardiac deaths in persons aged 1–35 years in Denmark 2000–06. Male deaths constituted 67% of all sudden cardiac deaths. There were no differences in the age distribution between males and females (data not shown).

The rest (11%) was performed in hospital pathology departments. In the 469 autopsied cases, 155 (33%) died a sudden non-cardiac death, while 314 deaths were SCD or unexplained. Median age was 29 years. Age distribution is shown in figure 2 and illustrates that increasing age was associated with increased risk of dying of SCD – the risk of dying of SCD was more than 10 times higher for the 30-35 years old than for the 1-10 years old. In addition, males died twice as often of SCD than females.

The most common cardiac cause of death was ischemic heart disease (IHD). In 22% of all sudden unexpected deaths (28% of autopsied cases), cause of death remained unknown after autopsy, denoted sudden unexplained death (SUD). Cardiac causes of sudden death together with the SUD cases are shown in figure 3.

A previous medical history would less frequently elicit an autopsy. Interestingly, explained SCD cases would significantly more often

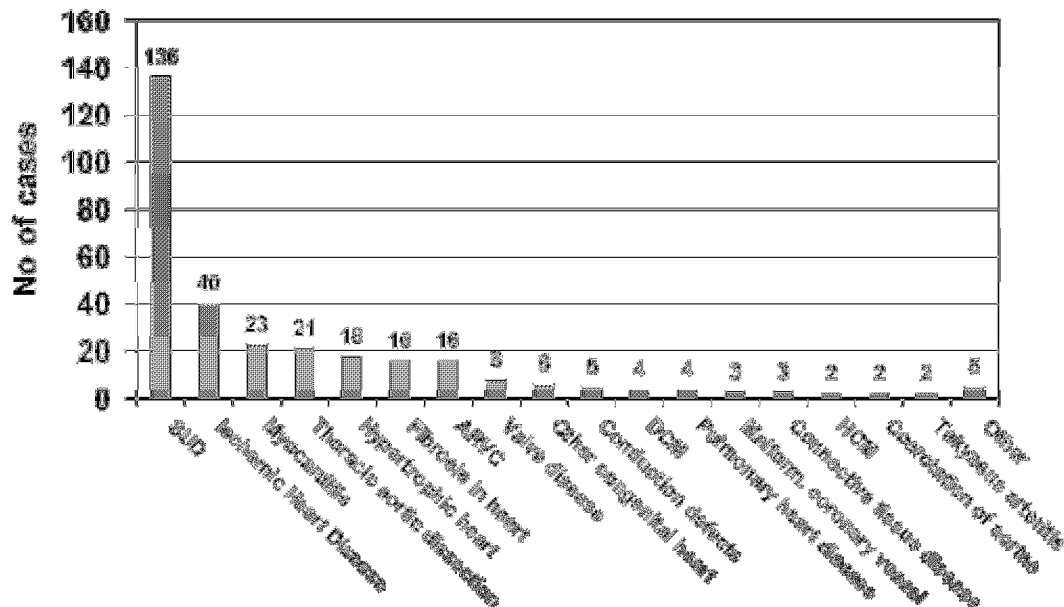


Figure 3
 Distribution of the causes of death in the 314 autopsied cases of sudden cardiac death in persons aged 1–35 years in Denmark in 2000–06. SUD, sudden unexplained death; ARVC, arrhythmogenic right ventricular cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy.

be witnessed (62% vs. 34%, $p < 0.0005$) and died less often during sleep (24% vs. 46%, $p < 0.0005$) than SUD cases. Highest possible annual incidence rate of SCD in the 1-35 years old in Denmark was 2.8 per 100 000 person-years with a higher male than female incidence rate (3.7 vs. 1.9). Excluding non-autopsied sudden unexpected death cases the incidence rate declined to 1.9 per 100 000 person-years. Of these, 1.1 pr 100 000 were explained SCD upon autopsy. By validating the cause of death registry in SCD cases, we found that 76% of deaths were correctly grouped within cardiovascular or ill-defined causes. It was not possible from the cause of death registry to extract which deaths were sudden and unexpected.

2.2.2 Paper III

We investigated all the deaths in persons aged 12-35 years with a particular focus on deaths occurring during sport. In Denmark there is no formalized preparticipation screening. Therefore, our study was well suited to establish the incidence of Sportsrelated SCD (SrSCD) in a nationwide unscreened population. During the 7-year study period we identified 15 cases of SrSCD, corresponding to a median of 2 cases per year (range 0-5 deaths/year). See Table 2 for details regarding the deaths. The incidence rate of SrSCD in the age group (athletes and non-athletes) was 0.13 (95% CI 0.07 - 0.22) per 100 000 person-years whereas the incidence rate was 1.21 (95% CI 0.68 - 2.00) per 100

000 athlete person-years. Predominant sports were running (n=5) and soccer (n=5). Autopsy was performed in all but one case. Arrhythmogenic Right Ventricle Cardiomyopathy (ARVC) (n=4) was the most frequent cardiomyopathy.

2.2.3 Paper IV

From the review of death certificates, we noted that 25% of sudden unexpected deaths suspicious of SCD were not autopsied. We hypothesized that there would be regional differences in the investigations of these sudden unexpected deaths. In the study period (2000-2006) Denmark was divided into 14 counties. For each county we investigated the ratios of conducted external examinations and autopsies in the sudden unexpected deaths. We found that of the 625 sudden unexpected deaths, 526 (84%) had a medico-legal external examination. Significant differences existed between the counties with ratios varying between 63% and 93% ($p = 0.004$). External examinations were conducted more frequently in urban counties than in rural counties (88% vs. 81%, $p = 0.014$) (figure 4). There was a significant difference in autopsy rates between the Danish counties (varying between 60% and 88%, $p = 0.001$). Autopsy was more often conducted in urban counties (80% and 71% respectively, $p = 0.009$) as well as in Eastern Denmark (82% vs. 66%, $p < 0.0005$).

Age at death	Sport	Sport arena	Witnessed	Autopsy results	Prodromal symptoms	Antecedent symptoms	Body weight (kg)	Heart weight (g)
15	Running	Yes	Yes	CCAA	None	Chest pain, no relation to exercise	72	454
17	Soccer	Yes	Yes	No autopsy	None	Dyspnea, treated as asthma	N/A	N/A
18	Running	No	No	ARVC	None	None	70	360
18	Soccer	Yes	Yes	ARVC	None	None	77	500
26	Soccer	Yes	Yes	Sarcoidosis	None	None	83	500
27	Running	Yes	Yes	SUD	None	None	82	395
27	Soccer	Yes	Yes	SUD	None	Dyspnea, treated as asthma	76	332
28	Cycling	No	Yes	ARVC	None	Exercise-related syncope	81	535
29	Aerobics	Yes	Yes	SUD	Syncope minutes before	None	78	460
30	Running	No	No	Pos-HCM	None	Exercise-related syncope diagnosed with athletes heart	106	680
30	Handball	Yes	Yes	SUD	None	Presyncope 1 week before during training	104	530
31	Boxing	Yes	Yes	CAD	None	None	92	510
32	Running	Yes	Yes	CAD	None	Exercise-related syncope	70	400
33	Handball	Yes	Yes	ARVC	Dizziness	2 admissions because of chest pain in preceding year	86	615
35	Soccer	No	No	Myocarditis	Dizziness	None	83	359

Table 2
Cases of sports-related sudden death in Denmark, 2000-06.

All decedents were male. Prodromal symptoms was defined as symptoms in the minutes leading up to the death, and antecedent symptoms was defined as symptoms in the year to months leading up to the death. ARVC = arrhythmogenic right ventricular cardiomyopathy; CAD = coronary artery disease; CCAA = congenital coronary artery anomaly; LV = left ventricular; N/A = not available; Pos-HCM = possible hypertrophic cardiomyopathy; SUD = sudden unexplained death.

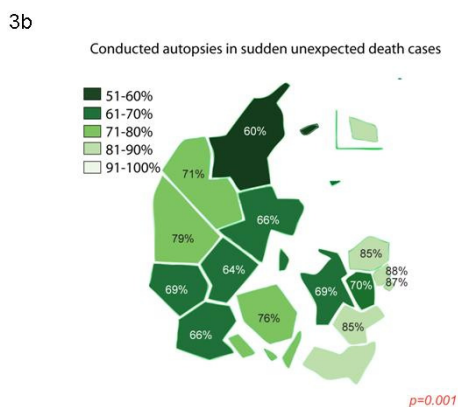
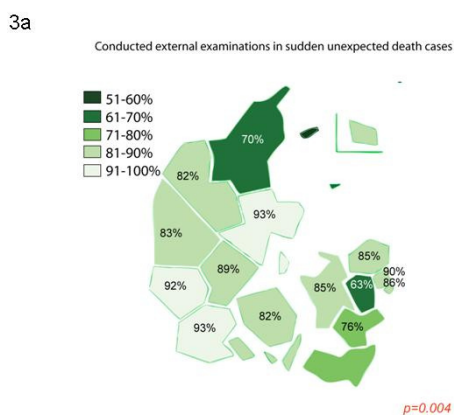


Figure 4

For the counties in Denmark are shown in scaled colours a) Conduction of external examinations in sudden unexpected death, i.e. in how many deaths were the police requesting a death scene investigation thereby involving the medical officer of public health ensuring a thorough examination of the death and b) Total autopsy rate (forensic and hospital autopsies) of sudden unexpected death, i.e. how many of the sudden unexpected death cases were autopsied in each county.

2.3 THE USE OF BLOOD SPOTS FROM THE DANISH NEONATAL SCREENING BIOBANK IN CANDIDATE GENE SCREENING

2.3.1 Paper V

We performed a validation study to investigate if whole-genome amplified DNA (wgaDNA) from dried blood spots could reliably be used in genetic screenings. We analyzed blood derived genomic DNA (gDNA) from 10 lone atrial fibrillation patients and compared it with the same patients wgaDNA obtained from their Guthrie Cards. The Guthrie Cards had been stored in the Danish Neonatal Screening Biobank at Statens Serum Institut for 19 to 28 years. We compared high resolution melting curve analysis (HRMCA) and sequencing data from the patient's gDNA and wgaDNA. We analyzed the genes SCN5A and KCNA5, which constitutes 27 exons (35 amplicons) and 1 exon (5 amplicons) respectively. Number of basepairs investigated was above 10.500 per patient. In the wgaDNA group a total of 85 of the 400 amplicons had altered curves. In the gDNA it was 81 amplicons. When we analyzed the sequencing results of the 10 wgaDNA samples a total of 31 variants were discovered. Of the 31 variants, six led to a change in the amino acid sequence, three of which were the previously reported common variant H558R in SCN5A (rs1805124).⁴⁶ The last amino acid change in SCN5A was R340Q. The 2 variants identified in KCNA5 were T155C and R578K. When

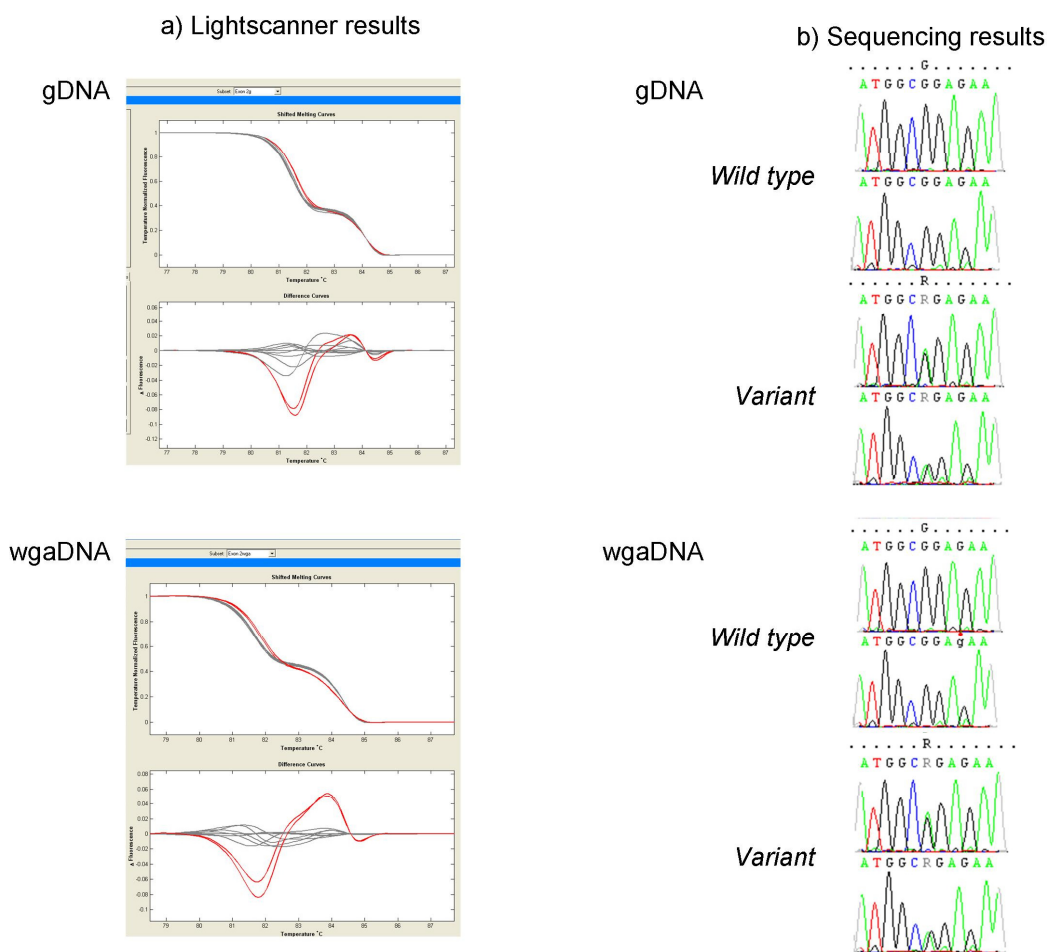


Figure 5
Comparison of high resolution melting curve analysis (panel A) and sequence analysis (panel B) of wgaDNA and gDNA. In panel A is shown melting curves for amplicon 2. In panel B is shown an example of one of the two corresponding variants which both was a heterozygous G->A substitution in position 87 that did not result in any aminoacid change. It is noteworthy that the spike alteration due to the aminoacid change in wgaDNA completely resembles that of gDNA. This was the case for all variants in this study.

we analyzed the wgaDNA we found the same 31 variants, in the exact same pattern. Examples of melting curves and sequencing results are provided in figure 5. All variants were found to have altered melting curves in both gDNA and wgaDNA samples.

3. DISCUSSION AND FUTURE PERSPECTIVES

3.1 METHODOLOGICAL CONSIDERATIONS

3.1.1 Death certificates review

The incidence rate of sudden cardiac death (SCD) in the young has been difficult to establish. Rates have varied greatly possibly due to geographical, ethnic, socioeconomic, and cultural distinct patterns influencing disease and death in different parts of the world in combination with different designs of the studies.^{22-31,33,37,47-50}

In theory, to assess the incidence of SCD in the general population, there are three possible approaches to identify sudden unexpected death cases: (1) Identification through autopsied cases, (2) identification from cause of death registries, or (3) identification from death certificates. Identification through autopsied cases

provides the best data on causes of death.⁵¹ One of the potential problems, though, is that not all sudden unexpected deaths might be autopsied. Subsequently, a report from an autopsy series might be biased in incidence rates and the distribution of causes of sudden death. Identification from cause of death registries has the benefit that all deaths are registered (at least in Denmark). There are, however, some major drawbacks using the cause of death registry. First of all, whether or not a death is sudden is not registered. Hence, the registry can not identify sudden death cases alone. Therefore, it is necessary to look at the causes of death to distinguish those who died of cardiac causes from those who died of other causes. This, however, overestimates the incidence of SCD, since not all cardiac deaths are sudden and unexpected. The next filter to apply, then, would be to include only those who died out-of-hospital. While this, in theory, are getting closer to the sudden and unexpected deaths, one major problem remains: Is the diagnosis in the cause of death registry correct, i.e. are some death misclassified? The cause of death registry is primarily an extract of what is written on the death certificate decoded by non-medical staff. Thus, the use of cause of death registry in the primary identification of sudden unexpected death cases can be flawed by many factors.

This leaves the death certificates as the last potential alternative to address the magnitude of SCD in the young. Previously, however, it has been shown that the use of death certificate derived data for identifying SCD in the United States yielded a sensitivity of 59% and a positive predictive value of a mere 19%.⁵² In other words, only 59% of all SCD cases were identified through death certificates, and only 19% of the deaths initially thought to be SCD by the death certificate review, actually were SCD. Even though the study had a different study population (mean age 81 years vs. 29 years for ours 1-35 year old study population), this would naturally lead to concern whether our initial use of death certificates would be a reliable method. However, unlike the US death certificates,⁵³ the Danish death certificates allows for an extensive additional information, thereby making them suitable for a primary screening tool for identification of sudden unexpected death in the young (supplementary 1 in Paper II). This extensive information is written in the supplemental information field in all cases of external examinations (conducted by the medical officers of public health or the forensic pathologists themselves) and usually to some extent also in the absence of an external examination. The information, when written by the medical officers of public health, always at a minimum includes (1) a summary of existing diseases and the physical condition prior to death as described by relatives, (2) a description of the events immediately preceding the death as described by witnesses, (3) a summary of the actions taken by the EMS on the scene, (4) what actions was taken at hospital (if any), and (5) a thorough outer inspection of the body. In this regard, it is noteworthy that an external examination was carried out in 84% of all sudden unexpected death cases.

The positive predictive value of identifying SCD from death certificates (that is, how many death initially thought to be SCD actually turned out to be so after including data from autopsies) was 50% (Paper II). This, however, was calculated using only autopsied cases as numerator and as such could never exceed the autopsy ratio of 75%. This suggests that death certificates in Denmark contain a high level of information. While this is sufficient information in regards to identifying sudden unexpected death cases, it is insufficient information to identify SCD cases, especially SUD cases, as one-third of autopsied sudden unexpected death cases turned out not to be SCD but rather sudden death of other causes. With the method we used, we therefore were able to go beyond the inherent weaknesses of using only autopsy reports, death certificates or the Cause of Death registry as means of describing SCD.

When interpreting on 8358 death certificates, mistakes as to the initial categorization are inevitable. Therefore we made an inter-observational approach in which all death certificates were categorized by two MD's separately (the author of this thesis being the principal investigator reading all 8358 death certificates and 3 other each reading +2500 death certificates). In the event of disagreement, the death certificate was reviewed again, and a consensus was made. Therefore all death certificates were reviewed twice, and all disagreements were reviewed and discussed a third time in plenum. Thus, most likely only a few cases have been misclassified during this initial death certificate review.

3.1.2 Autopsy review

As noted, autopsy is essential both in establishing the incidence rate of SCD and the underlying causes i.e. what cardiac diseases are involved.⁵¹ Autopsies, however, can be more or less focused on particular organs, especially when performed in hospital pa-

thologist departments. Standardization of the procedure is important in a thorough protocol. For instance, it is important always to go through all organs in all cases. It is also important always to perform histology and to perform a toxicology screen if a death is unexplained. Recently, a review was published which addressed the state of the art forensic investigation of SCD.⁵⁴ One of the key conclusions was that the quality of the autopsy is strictly linked to the use of a rigorous protocol in dissection, collection of samples, and the use of histology, toxicology and molecular biology.

Each of the 3 forensic departments in Denmark has a standardization of the autopsy in place: All organs are examined systematically in all cases and histology and toxicology are used routinely. We found that histology was performed in 96% of all autopsied SCD cases and toxicology was performed in 82% of all sudden unexpected death (SUD) cases (Paper II). Of note, 11% of all autopsies were conducted at hospital pathology departments, where toxicology is never performed (probably due to the high cost). Thus, we believe the quality of the majority of autopsies in young sudden death cases in Denmark is high. Because of the retrospective design, though, the pathologists have not had particular focus on the heart in all cases – thus some descriptions of the heart were less detailed than others, especially in cases where a non-cardiac cause of death was found.

We reviewed autopsy reports from deceased persons in 2000 through 2006 (Paper I-IV). All autopsy reports were read by the author of this thesis, extracting all relevant information from the report and transferring it into our database. In a number of cases, there was a discrepancy between the conclusion in the autopsy report, and our impression from the findings. In the majority of these cases the discrepancy was whether a cardiac finding could explain the death or not. In cases like this, a senior forensic pathologist from the respective department was consulted and the entire case in all its contents was reviewed again. In a number of cases, additional samples for histology were prepared in order to further elucidate the death. Expert cardiac pathologists have also been consulted in selected cases. After this second review of the case, the forensic pathologist decided the classification of the death. Our approach is very similar to the hitherto largest study on SCD in the young published in 2005 and probably reflects the best alternative in the retrospective review of autopsy reports.²⁷ An alternative strategy, although not feasible in the present study design, could have been to extend the inter-observational design from the review of the death certificates to encompass the review of the autopsy reports as well. It is unknown if this approach would have yielded a higher accuracy.

3.1.3 Whole genome amplification of DNA

Drawn blood samples are the best and most readily available source of DNA for genetic analysis and are thus the golden standard for which all other tissues are compared against.⁵⁵ However, in some cases the available amount of DNA is low, and thus a comprehensive genetic analysis might not be possible. This, for instance, is the case for blood spots stored in the Danish Neonatal Screening Biobank. It would therefore be of great clinical value if an amplification of the whole genome (whole genome amplification, WGA) could safely be applied to these samples thereby producing great quantities of wgaDNA from a small amount of gDNA. However, a potential major drawback to these WGA samples may be random errors incorporated into the DNA product i.e. false positives. To overcome this, Statens Serum Institut has previously validated and optimized different WGA protocols and

has shown that wgaDNA is reliable and performs as well as gDNA in Genome Wide Association Studies (GWAS).^{56,57} One of the key factors making the wgaDNA reliable, is to perform the amplification 3 times in separate wells, because this step in theory, when combining the products from the wells in the end, would dilute a possible random error to a non-detectable size. While this strategy has proven to be feasible in GWAS and SNP studies, it has not been shown before that it could be applied to sequencing analysis.

We therefore set up the validation study (Paper V), in which we compared gDNA from drawn blood samples with wgaDNA from dried blood spots (DBS) collected through the Danish Neonatal Screening programme and stored at Statens Serum Institut for approximately 20 years. Ten patients with lone atrial fibrillation were selected for high resolution melting curve analysis (HRMCA) and sequencing analysis in the genes SCN5A and KCNA5. Comparison of wgaDNA with golden standard gDNA is a solid way to validate the use of wgaDNA for HRMCA and sequence analysis. HRMCA is used as a primary screening tool for detection of possible alterations in the examined amplicon and can thus be used to restrict the number of amplicons necessary to sequence – a cost and time consuming step in genetic analysis. It is crucial, however, that false negatives do not appear, i.e. that if a variant is present in the amplicon of the gene examined, an altered curve will appear at HRMCA. We found a high false positive rate when applying the HRMCA to the samples. This might be due to the conservative approach when analyzing melting curves in combination with our software setup: Melting curves were selected as altered if they were not completely identical to the others. At the same time, our software setup for sequencing analysis examined only 50 basepairs upstream and downstream of every exon, even though the primers covered a larger part of the intronic sequences. Thus, it is possible that altered melting curves reflect a true variant in the intronic sequence that we can not see in the software setup. Previously it has been shown that wgaDNA might produce slightly greater numbers of false positives on HRMCA compared to gDNA.⁵⁸ We found no significant difference between wgaDNA and gDNA (n=85 and n=81, respectively, p=0.74). Neither in the gDNA nor in the wga samples did we observe false negatives.

We included 10 patients in the study. In our lab we did not have many patients young enough to have been born after 1981 – a criterion that was needed in order to obtain the wgaDNA from the DBS sample. However, prior results have been very convincing in regards to call rate and concordance rate.⁵⁶ From the WGA's performed on the 10 DBS samples, our yield of DNA was very different (ranging between 52.4 and 150 ng/ul) despite the same method applied. DBS samples, therefore, are different and as such one must suspect that a sample will not perform once in a while. However, our results indicate that when the WGA step is successful, the resulting wgaDNA is of excellent quality. Hence, the number of patients is of less importance. It is more important to investigate several amplicons. In Paper V, we in total compared more than 100 000 basepairs from 40 different amplicons (400 in total) and found no difference between gDNA and wgaDNA.

3.2 DISCUSSION OF RESULTS

3.2.1 Epidemiology of sudden unexpected death in infancy

3.2.1a Paper I

The decline in SIDS incidence rates in Denmark has previously been well described especially by the Nordic Epidemiologic SIDS

Studies in the 1990's.^{8,59–61} SIDS a diagnosis of exclusion and subsequently is difficult to apply on non-autopsied deaths. Furthermore, it is not clear, how the diagnosis is used in the Danish cause of death registry. These questions, together with the lack of a general overview of SUDI in Denmark, initiated our analysis of SUDI and SIDS in Denmark.

The summaries provided on the death certificates provided detailed information on the circumstances of the deaths. To supplement data from the death certificates we also used data from the registries in Denmark on all prior in- and outpatient hospital contacts, as well as all autopsy reports on deaths suspicious of SUDI. Using this approach, we circumvented the inaccuracies in the official statistics from The National Board of Health regarding SIDS incidences (derived from The Cause of Death registry). Furthermore the data were not affected by the lack of registration of SUDI cases in the official registries.

In our study, 24% (n=40) of autopsied SUDI cases were found to have structural cardiac disease, of which 29 of them were congenital malformations. The most abundant cardiac cause of death was coarctation of the aorta (n=6) followed by transposition of the great arteries (n=5). Interestingly, of the 40 cases of structural heart disease only 6 of them were diagnosed prior to death. The 34 undiagnosed fatal cases equal 5 deaths per year. It is speculative that at least some of these deaths could have been avoided. In addition, the proportion of SUDI cases with congenital heart malformations is many times bigger than what has previously been reported.⁷ Whether it is because we are more encompassing in our study, or other countries are better at diagnosing and treating congenital heart malformations, remains unknown.

We report differentiated incidence rates of SIDS in the study. One of the reasons was that we wanted to provide the highest possible incidence rate of SIDS in Denmark. The definition of SIDS has been difficult to assess, but the newest and most widely accepted definition has been proposed by Krous et al. They define SIDS as the sudden unexpected death of an infant <1 year of age, with onset of the fatal episode apparently occurring during sleep, that remains unexplained after a thorough investigation, including performance of a complete autopsy and review of the circumstances of death and the clinical history.¹⁸ Previously, deaths before the age of 7 days was not considered SIDS, but it is now accepted that some otherwise unexplained deaths occur in the first 7 days of life, and thus should be termed SIDS. The definition also states that the fatal episode apparently should occur during sleep. This is primarily based upon previous epidemiologic observations, together with a paper from 2002 that reported on the causes of death in infants that were awake at time of death.⁶² One of the major problems in this regard, however, is this study describes only deaths with structural heart disease. Hence, no data from the study was available on unexplained deaths in infants dying while awake. However, there has been a previous report on these deaths.⁶ In that study they conclude that these deaths might be secondary to arrhythmias caused by e.g. LQTS. As 10% of our unexplained cases died while awake, we did not exclude them even though they were not included in the definition by Krous et al. We therefore reported both the incidence rate of SIDS in infants who died during sleep (0.16 deaths per 1 000 births) and the incidence rate when including deaths while awake (0.18 deaths per 1 000 births). Based on our findings, we believe that deaths while awake, if otherwise unexplained, should be included in a future revised definition of SIDS. It is possible that a larger proportion of these deaths can be explained by channelopathies such as LQTS and Brugada Syndrome, but as no evidence

exists comparing the two groups genetically, it remains speculative.

In addition to the inclusion of deaths while awake, we also included non-autopsied unclassified sudden infant death (USID) cases to provide a highest possible estimate of SIDS in Denmark. The incidence rate, however, only rose to 0.22 per 1 000 births when including these deaths. The incidence rate of SIDS cases in the present study (0.16-0.22/1000 births) is in the lower range compared to previous studies.⁶³ This could in part be explained by the higher rate of explained SUDI cases we found in our study compared with a previous study.⁶⁴

We tested the use of the SIDS diagnosis in the cause of death registry. Following the SIDS incidence rate over time, is most readily done by extracting the number of deaths from the registry. This, however, should only be applied if the diagnosis is used correctly, i.e. that all SIDS cases are actually coded as SIDS in the registry, and that the registry does not have many cases wrongly coded as SIDS. Our findings in this regard were not uplifting. Taken together, 30% of all deaths in the cause of death registry, coded as SIDS, were misclassified. If we had only collected death certificates and autopsy reports from those with the R95 code, we would have missed more than 1 in 6 SIDS cases in the subsequent analysis. Because unexplained infant deaths in the ICD-10 registry can only be coded as SIDS (R95) probably explains why the registry fails to be accurate as has also been addressed before.⁵³ More options to sub-classify SIDS would probably increase the validity. Another problem might be the two-step recording of cause of death into the Danish registry. The two-step recording is due to the delay of the results of i.e. histology and toxicology reported to the Danish National Board of Health. Because these additional analyses are neither mandatory by law, nor necessary for the death to be recorded into the registry, inaccuracies can arise if the reporting of these subsequent analyses fails to be registered.

In the future, we recommend that the successor of ICD-10 (ICD-11) would include some sub-classifications of SIDS. In addition, we think that SIDS guidelines should be revised regarding deaths while awake. The guidelines should preferentially be based on good evidence and not empiric assumptions.

3.2.2 Epidemiology of sudden cardiac death in the 1-35 years old

3.2.2a Paper II

The diagnosis of SCD can be elusive and the incidence in the young has been difficult to establish. We reported the highest possible incidence rate of SCD to be 2.8 per 100 000 person-years. Although low this is higher than previously reported with incidence rates between 1.0 and 1.8 per 100 000 person-years. Lowest incidence rate (1.0) was reported from the Veneto region,³¹ in the Netherlands it was 1.6,²² and in the UK 1.8 per 100 000 person-years.⁴⁸ The discrepancy between our incidence rate and previous reports might not be so surprising, since we have included non-autopsied cases of sudden unexpected death. Excluding those would lower the incidence rate to 1.9 per 100 000 person-years in our study. The lower incidence reported from the Veneto region might be caused by some non-athlete deaths being overlooked in the Italian study as pointed out by the authors themselves.⁴⁷ A contributing factor might also be the difference in time limits in the definition of SCD (ours <24 hour vs. the Italian study's < 1 hour). The difference in age ranges studied (1-35 years vs. 12-35 years), though, should have produced higher incidence rates in the Italian study. It would be interesting to know autopsy ratios in other countries. Unfortunately, we are only aware of one

small study which report autopsy ratio quite similar to ours.³⁷ It is conceivable - although speculative - that not all sudden death cases are autopsied in other countries as well.

We found a significant proportion of deaths (11%) occurring in-hospital and more than one-third (34%) died during sleep. Taken together this warrants for caution in the interpretation of figures that rely solely on out-of-hospital deaths and/or witnessed deaths as surrogate measures of SCD as they will likely underestimate the incidence. Sudden unexplained deaths (SUD) were frequent in our study (29% of autopsied sudden unexpected death cases). This is in line with previous findings.^{25-27,37} This high frequency might in part reflect the young median age, as older populations have fewer unexplained cases.⁶⁵ Because 37% of the SUD cases had a positive toxicology, either prescribed drugs in therapeutic concentrations or illegal drugs in trace amounts, it could be speculated that some drugs, even in therapeutic concentrations, could have caused a fatal arrhythmia through i.e. prolongation of the QT interval.^{66,67} A larger proportion of SUD died during sleep compared to explained SCD (46% vs. 24%). A similar observation has been reported previously.²⁶ Some of these persons may harbour disease causing mutations in for instance the genes *KCNQ1* and *SCN5A* known to be associated with death during sleep due to primary arrhythmogenic diseases like the Long QT Syndrome and the Brugada syndrome.^{40,44} In line with this, previous studies have demonstrated that in case of a SUD, a close family evaluation and/or a genetic testing might determine the aetiology of the death.^{44,68-70} Consistent with previous data is also IHD being the most frequent cause of SCD in our study (11%).²² The low incidence of HCM (<1%, n=2), in our study compared to previous studies (between 6% and 13%)^{27,29,31,49} is probably because we in our study report idiopathic fibrosis (IF) and left ventricular hypertrophy (LVH) as separate disease entities. If we include LVH and IF in the definition of HCM, our incidence would increase to 9% (n=18 and n=16, respectively), thereby being in concert with the other studies. It has previously been shown that some of these LVH and IF may clinically have been diagnosed as HCM.^{71,72} ARVC appears to be a significant cause of sudden cardiac death in the young in Denmark as they accounted for 5% of autopsied SCD in our study. Although a significant cause of death, it is less frequent when compared to the Italian Veneto region (13% of sudden cardiovascular deaths).³¹

Some studies rely on cause of death registry data.^{22,73} A prerequisite for this is that all SCD and SUD cases are correctly categorized in the Cause of death registry. In Denmark, we found that 24% of all autopsied cases of SCD or SUD were not categorized in the Cause of Death Registry with an ICD-code denoting cardiac or ill-defined cause of death. Thus the cause of death registry in Denmark can not be used for the identification of SCD cases. This might not be a unique problem for Denmark. Careful measures should therefore be taken when information is retrieved only from selected deaths derived from cause of death registries. In addition, most cause of death registries suffers from the inherent weakness of not registering whether a death was sudden and unexpected. In Denmark, we believe that we can not rely solely on the information on cause of deaths in the registry when it comes to identifying SCD cases.

In perspective, we would like in a future study to apply data from prescribed drugs as many drugs are known to be associated with for instance an increased risk of prolongation of the QT interval and potentially malignant arrhythmias.⁶⁷

We conclude that SCD in the young accounts for 7% of all deaths. It has become increasingly interestingly, however, to broaden the perspective and investigate deaths until the age of 50. These

intermediate aged SCD cases (36-49 years) are most likely numerous compared to the already studied group, and probably the main cause of death will be premature IHD. It is conceivable that many of these deaths are caused by familiar hypercholesterolaemia (FHC) and therefore a family evaluation of these deaths is also very important.

3.2.2b Paper III

Previous reports on SrSCD have been limited to either media searches or from autopsy series from defined regions and/or subpopulations.^{30,32} In our study, by examining all death certificates and autopsy reports in a nationwide setting, we believe that only a few if any SrSCD cases were missed. To be certain that we had not overlooked any SrSCD cases, we also performed a media search in The Danish media surveillance database Infomedia (www.infomedia.dk), a database of approximately 400 printed, 2200 web based, and the major radio and television Danish media. All national and most regional media are included in the database. We identified only 3 deaths through this approach. All 3 had also been identified through the death certificate review. According to the National Danish Health and Morbidity Study 10.9% of the Danish population in the age group 16-35 year reported participating in competition level sports.⁷⁴ We extrapolated this figure and consequently estimated the athlete population to be 10.9 % corresponding to 177 070 athletes aged 12-35 years old.

The distribution of cases according to sports was proportional to the popularity of the sports in Denmark. The data thus does not give any further information on risk of SrSCD in relation to type of sport. The incidence rate of SrSCD was 1.21 per 100 000 athlete person-years. This is in close alignment to what has been found in other studies (range 0.61-0.93).^{31,32,50,75} The studies, however, are not entirely matched on gender, age and inclusion criteria (US studies, for instance, included aborted SCD). The study that comes closest to our population is the Italian. They found a post screening incidence rate of SrSCD to be 0.87 per 100 000 athlete person-years. Interestingly, the reported decline to this incidence in Italy (from 4.19 per 100 000 athlete person-years) has primarily been explained by the screening initiative. In Denmark, however, we do not have any formalized screening program preceding our incidence rate, and thus must be compared to the 4.19 per 100 000 athlete person-years. The value of preparticipation screening, therefore, may be limited in Denmark as we already have an incidence rate of SrSCD comparable to the post-screening era rate in Veneto. A comparison between Minnesota and Veneto by Maron et al recently led to similar conclusions regarding the USA, although the two populations differed in respect to gender and age distribution.⁷⁵ As 73% of SrSCD occurred in a sports arena it is conceivable that the placement of an Automated External Defibrillator (AED) might have saved some of these lives.⁷⁶ We do not know how many lives have been saved by AED, as we did not include aborted sudden cardiac arrest in our study.

In half of the cases (53%) the decedents had experienced probable cardiac symptoms such as dyspnea and chest pain in the weeks, months, or years leading up to their death, and in 4 of these cases (27%) even exercise related syncope and pre-syncope was reported. This underlines the importance of a thorough investigation by specialists in cardiology in all cases of athletes experiencing probable cardiac symptoms, especially if symptoms present during exercise. Thus, strategies other than preparticipation screening might be more beneficial in Denmark, namely (correct) placement of AED's and a thorough investigation of

athletes presenting with possible cardiac symptoms during exercise.

It is important to emphasize, that our study was only looking at SrSCD in athletes that might have undergone a screening if a formalized screening program had been in place in Denmark. The actual number of deaths in relation to physical activity was higher (Paper II), but many of these deaths were happening in non-athletes and the majority were not engaged in high-endurance sport at the time of death. For instance, death occurring while running after a bus, or lifting heavy items in relations to gardening was not considered as being SrSCD in an athlete even though the death happened during activity. Furthermore, as opposed to the data from Italy, in our study we did not include SCD that occurred in an athlete outside of training or competition, but according to data from USA and Veneto 80-90% of all SCD in athletes occur during sports activity, thus minimizing the problem.^{32,50} Preparticipation screening in athletes has been and still is a matter of passionate debate. This was highlighted once again when our study was published, as it gave rise to several comments.⁷⁷⁻⁷⁹ A large pan-continental prospective study addressing these issues might be the next step. However, the question is if the study will gain enough power given the suspected low number of cases.

3.2.2c Paper IV

We found 25% of sudden unexpected deaths possible due to a cardiac cause were not autopsied. The analysis of how these unexpected death cases are investigated in Denmark revealed that regional differences exist. From a cardiologist/geneticist point of view, the thorough examination of these deaths is crucial, because the cause of death – even when unexplained – is essential in the subsequent family work-up in relations to possible inherited diseases.^{69,80,81} Family members may be eligible for life-saving therapy. A number of efficient therapies exist, such as life style modifications and medicine for early ischemic heart disease, antiarrhythmic drugs for channelopathies, and implantable cardiac defibrillators for a number of the SCD causing conditions. A sudden unexpected death with no previous medical disease and no autopsy, makes the clinical work-up difficult – both because the cause of death may not be of cardiac or unknown origin, and because the genetic workup can not be targeted. In fact, a genetic work-up in the relatives of a sudden death case, is usually only targeted at the mutation thought to cause the death. Hence, if no genetic work-up has been done in the proband (the index patient), as a general rule, no genetic work-up will be performed in the family of the decedent unless some family members are showing phenotypic traits of disease.

It is of great concern that we document significant differences in the handling of sudden unexpected deaths both in the mandatory external examinations as well as in the autopsy rates. These differences could, at least in part, well be explained by population density and geographical issues. The ratio of external examinations conducted can be seen as a marker for how many deaths had involvement of the police and the medical officer of public health with the subsequent potential of being autopsied in a forensic setting. Uncertainty in regards to cause of death does not automatically convey a forensic autopsy - the police have to solicit it. This decision might be under the influence of several parties such as the medical officer of public health, the relatives and/or the head of police: The medical officer of public health will provide the police with a medical point of view; the relatives might be reluctant to have an autopsy performed and perhaps oppose it (forcing the police to present the case for a judge if they

still want a forensic autopsy). Finally, the local head of police might have a general opinion as to how the law is interpreted and what is traditionally done in a case like this. One county is interesting in this regard. Aarhus County produced the overall highest ratio of external examinations (93%), but at the same time produced one of the lowest overall autopsy ratios (66%). We have no definite explanation to this finding, but it could demonstrate the problem in having the police to decide which deaths should be autopsied.

Although significant differences appear to exist in Denmark, there are some concerns that need to be addressed. One concern is that the police districts do not match the Danish counties. Therefore, the counties acts as a surrogate measure of the police districts. This was necessary because police district data, as oppose to county data, was not always provided on death certificates, but only in cases where an external examination was carried out. Hence, the study was not feasible reporting from police districts. Another concern would be why we have not adjusted for socio economic status. We could have reported overall socio economic status for each county, but we felt that it would be to crude measures to interpret on.

In perspective, we would like to develop an algorithm for applying socio economic status.

It is the hope, that this thesis will promote focus on the problem regarding non-autopsied cases. It is the opinion of the Danish Society of Cardiology, that all sudden unexpected deaths under the age of 50, should receive a thorough investigation of the death, including an autopsy.⁸²

3.2.3 The use of blood spots from the Danish Neonatal Screening Biobank in candidate gene screening

3.2.3a Paper V

We found no difference between wgaDNA and gDNA. False positives would probably have rendered the wgaDNA useless for genetic screening. However, we fully trusted the quality of the wgaDNA.^{56,57}

A previous study has compared wgaDNA with gDNA in HRMCA and sequence analysis. This study, however, were using fresh blood and not DBS as a source material for the WGA and they did not sequence all amplicons as we did. They found that wgaDNA performs well on HRMCA, although producing more failures and discordant patterns suggesting a mutation.⁵⁸ We did not find melting curves fail more often in wgaDNA than gDNA, but we did notice a higher rate of discordant patterns, even though it did not reach statistic significance.

Demonstrating a 100% concordance with genotyping data obtained by the gDNA samples, we suggest that WGA in triplicates can safely and reliably be used on DBS samples. In the clinical setting wgaDNA from DBS samples can become very important for instance in cases of otherwise unexplained deaths where material suitable for DNA testing is otherwise not retrievable. A genetic workup in these cases might confirm or reveal inherited cardiac diseases such as Long QT syndrome and Brugada syndrome where sudden cardiac death may be the initial symptom and thereby help to identify patients at risk in the family.^{40,83} Based on the results from our validation study, we now have commenced the screening of the 93 unexplained deaths in which we have retrieved wgaDNA.

4. CONCLUSION

We have given very valid figures for the incidence rates of sudden unexpected death in infancy, sudden infant death syndrome, and sudden cardiac death in a nationwide setting. This was possible because of the design of the study in combination with the extensive registries in Denmark.

We found that SUDI constituted 10% all infant deaths and 49% of these deaths were categorized as SIDS corresponding to an incidence rate of up to 0.22 per 1000 live births. In persons aged 1-35 years, 25% of all sudden unexpected deaths were not autopsied. Incidence rate of SCD was higher than previously reported (up to 2.8 per 100 000 person-years) when including these non-autopsied cases of presumed SCD. This corresponds to 7% of all deaths in the young. Sportsrelated SCD was seldom occurring (0-5 deaths per year) and questions the benefit of preparticipation screening. Most frequent cardiac cause of death in the young was IHD. Cause of death, however, remained unknown in 29% of autopsied sudden unexpected death cases suggesting a primary arrhythmogenic cause of death.

Ratios of medico-legal external examinations as well as autopsies differed significantly between the counties in Denmark despite operating under the same set of laws. This calls for increased awareness in the handling of these deaths. Because a high proportion of these deaths are potentially inherited, autopsy is essential, and for this reason we recommend a mandatory autopsy in all sudden unexpected deaths in the young.

DNA from dried blood spots, collected routinely from all infants in Denmark for the past 30 years, can be used in genetic analysis. This has a great potential, both in the clinical setting and for research purposes.

Future work will expand the deaths studied to also encompass the 36-49 years old. In addition, we will look into the possible genetic substrate of unexplained deaths.

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