OVERLAPPING GENETIC RISK IN THE SPECTRUM OF SUDDEN DEATH

by

Christine Troskie

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Abstract

Sudden Infant Death Syndrome (SIDS) is a sudden death occurring during sleep in infants below 1 year of old, which devastates the impacted families. By nature, SIDS deaths are those where all alternative causes of death, like suffocation or strangulation are eliminated, leaving families with few answers. While SIDS impacts infants, a spectrum of Sudden Death disorders exists across all age ranges and with comorbid syndromes, many of which occur sudden and unexpectedly during sleep. There is genetic overlap in risk genes in these disorders, and most notably between SIDS, Sudden Unexpected Death in Epilepsy (SUDEP), and Sudden Unexpected Death (SUD). Known and suspected Sudden Death (SD) genes are alternatively spliced in the developing brain in age and region dependent patterns, which may explain the differential timing of sudden death disorders despite their shared molecular risk factors. The objective of my project was to identify genes associated with sudden death and determine the timing of their alternative splicing. A literature search followed by an integrated pathway analysis generated a Sudden Death (SD) candidate genes (N=248). Analysis of whole exome sequences for eight (8) sudden death probands (SIDS (N=4), SUDEP (N=3) and SUD (N=1) samples was performed and Variant Effector Predictor was used to annotate the variants. The overall number of variants in the exomes for the SIDS individuals ranged from 40869-69978, SUDEP ranged from 19162-63954, and SUD contained 63217. Within the SD candidate genes, the number of variants in SIDS (707-1160), SUDEP (379-1078), and SUD (1026) did not allow for distinguishing between cohorts. All probands regardless of age carried multiple pathogenic variants in genes associated with disorders with high incidences of sudden death such as Long QT Syndrome, Dilated Cardiomyopathy,

Dravet Syndrome, and Infantile Epileptic Encephalopathy many of which impacted one or more gene isoforms. The expression patterns of these genes of interest were evaluated using the Allen Brain Atlas for the Developing Mouse Brain to identify when, and what form each gene product is expressed. These patterns of personal genetic risk can be used to identify potential targets for molecular diagnostic screening and prevention.

Preface

This thesis is based on the work done by me in the lab of Dr. Tara L. Klassen. The sudden death blood samples came from Othon Mena at the San Diego Medical Examiner's Office, Whole Exome Sequencing was performed by the lab of Dr. Corey Nislow, and the bioinformatic data processing was done by Patrick Boutet.

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List of Abbreviations

5-HT 5-Hydroxytryptamine

ARVC5 Arrhythmogenic Right Ventricular Cardiomyopathy Type 5

BQSR Base Quality Score Recalibration

CPVT Catecholaminergic Polymorphic Ventricular Tachycardia

DCM Dilated Cardiomyopathy

DNA Deoxyribonucleic acid

EIEE Early Infantile Epileptic Encephalopathy

HCM Hypertrophic Cardiomyopathy

LQTS Long QT Syndrome

NCBI National Center for Biotechnology Information

PhastCons Phylogenetic Analysis with Space/Time models Conservation

SCD Sudden Cardiac Death

SD Sudden Death

SIDS Sudden Infant Death Syndrome

SUD Sudden Unexpected Death

SUDC Sudden Unexpected Death in Childhood

SUDEP Sudden Unexpected Death in Epilepsy

SUDI Sudden Unexpected Death in Infancy

SUDY Sudden Unexpected Death in the Young

SUND Sudden Unexpected Nocturnal Death

VEP Variant Effect Predictor GATK – Genome Analysis Toolkit

VQSR Variant Quality Score Recalibration

WES Whole Exome Sequencing

WGA Whole Genome Amplification

WGS Whole Genome sample

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Chapter 1: Background Knowledge and Scope of Thesis

1.1. Sudden Infant Death Syndrome

In the Western world, the leading cause of death for infants before one year of age is Sudden Infant Death Syndrome (SIDS) (1). Epidemiological reveals that the peak age for a SIDS death occurs at roughly the 3rd month of life but can occur up to 12 months (2). These infants are predominantly found during sleep, face down in the prone position in an otherwise healthy infant. Like the spectrum of sudden death disorders, the overarching criteria for a SIDS diagnosis is that it eliminates all alternative causes of death, such as suffocation and infection and requires a full autopsy including neuropathological assessment (3). It has been postulated that cardiac regulation via vagal tone was found to be involved in the parasympathetic control of respiration and variations in heart rate influenced by sleeping position. The function of the cardiac pacemaker was also found to be influenced by the intrauterine environment, hormones, and postnatal maternal care (4). In 1992, the American Academy of Pediatrics' recommendation to not place infants to sleep in the prone position (5), as well as the 1994 Back to Sleep campaign (6), resulted in a notable decrease in the number of infants that unexpectedly died from SIDS presumably by mitigating the environmental and physiological risk. While post-neonatal mortality has not decreased since this time, the way in which infant deaths are classified has evolved. Specifically, there has been an increase in the number of accidental deaths caused by strangulation and suffocation (7). It is now believed that many infant deaths were wrongly diagnosed with SIDS.

There is still a prevalent belief that a subpopulation of infants harbors an intrinsic risk of unexpected death. These include infants born prematurely who have an

increased risk of unexpected death, due to a compromised and underdeveloped nervous and respiratory system (8). Additional factors for SIDS in this subpopulation include African American race, tobacco or alcohol use using pregnancy, and male gender (8). In recent post-mortem studies a number of pathogenic gene variants in inflammatory and hypoxia-response genes, as well as genes involved in neural myelin sheath developmental pathways have been identified. However, voltage and ligand-gated ion channels continue to represent the largest category of causative risk genes due to their inherent role in establishing and regulating cellular excitability in heart, lung, brain and vagal nerve (3).

The brainstem houses a number of homeostatic regulatory nuclei, like the Dorsal Motor Nucleus of the Vagus, Raphe Nuclei (9), and Trigeminal Nerve, which act to communicate and control both cardiac and respiratory systems, perturbation of which can lead to death. Cellular apoptosis in the brainstem occurs more frequently in SIDS infants compared to children that survive infancy while dysfunction of the vagus nerve and aberrant neurocardiac signaling are known to be a cause of sudden death disorders (10). Intriguingly, the signal transduction properties of sympathetic and parasympathetic tracts originating in the brainstem, continue to mature *exutero* with changing excitability properties up to 3 months of age (11).

1.1.1 Overlap in the Spectrum of Sudden Death

Like SIDS (1), there are a number of clinical disorders where the cause of death is unexpected and unexplained even upon autopsy. These are classified by either known comorbid disorders, like Sudden Cardiac Death (SCD) (12) or Sudden Unexpected Death in Epilepsy (SUDEP) (13), the age of death as in SIDS, Sudden Unexpected Death in Children (SUDC) or Sudden Unexpected Death in the Young

(SUDY) (14) or by apparent cause of death, Sudden Unexpected Nocturnal Death (SUND) (15) or complete lack of other defining features as in Sudden Unexpected Death (SUD). The unifying definitions for the spectrum include autopsy-negative deaths in a range of population. (TABLE 1.1, FIGURE 1.1). SCD occurs most frequently in individuals aged 1-35 but can occur across the entire age spectrum because of structural defects or electrical heart arrhythmias underlying mortality. The latter are frequently referred to as Sudden Arrhythmia Death Syndromes (SADS) where the most prevalent disorders are Long QT Syndrome (LQTS), Brugada Syndrome, Hypertrophic Cardiomyopathy (HCM), Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT), and Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) due to underlying genetic risk factors (12,16,17). Intriguingly, the risk factors across the sudden death spectrum appear to share features, including ventricular tachycardia and fibrillation, where young males have highest risk of SUND in males from Thailand, Cambodia, Japan and the Philippines (15).

Unlike SUD which accounts for 15-20% of all natural deaths (18), SUDEP is the single most common cause of death in those with epilepsy, accounting for 40-50% of deaths in epilepsy patients (13). These unexpected deaths are 40 times more likely in people suffering from epilepsy, when compared to the general population (13). The risk factors of SUDEP include being between 19-45 years of age, male, poor medication compliance, lack of seizure control even on multiple antiepileptic drugs, a history of tonic-clonic seizures, and prolonged seizure duration (19). Similar to SIDS, the majority of SUDEP cases occur during sleep. These SUDEP deaths occur with or without evidence of preceding seizure, where it is believed an environmental or physiological insult aggravates the underlying genetic predisposition resulting in the

fatality (20). While relatively infrequent in pediatric epilepsies, SUDEP accounts for 30-50% of the deaths in severe early onset infantile epileptic encephalopathies, affecting between 1 in 500 and 1 in 1000 epilepsy patients yearly (21),where the seizure onset of occurs within the first 6 months of life where neuro-cardiac and/or neuro-respiratory dysfunction have both been shown as causative (19,20,22).

With the extensive genetic testing involved in two of the most prevalent sudden death disorders in the world, SUD and SUDEP, it has been revealed that they have overlapping etiology and risk patterns, which are shared with SIDS. In the US, prevalence of SUDEP is roughly 1.16 cases for every 1000 people with epilepsy per year (23). In comparison, a study in Denmark found that among 1-35 year old individuals, the incidence of SCD was 1.9 cases per 100 000 person-years (24), while 1 in 2000 infants in the Western world will die from SIDS within the first year of life (8). Indeed, the identification of shared genetic cause in a known Sudden Cardiac Death gene; *KCNQ1* encoding KvLQT1 (Kv7.1), historically known as the cardiac delayed rectifier potassium channel (25) is considered a cause of death in all three disorders; SIDS (26), and SUDEP (25) as well as SUD (27) and SUDY (28). Mutations in the *KCNQ1* gene result in dysfunctional signaling in the protein product Kv7.1 (KvLQT1) which results in cardiac arrhythmia (Long QT Syndrome) and/or epilepsy and/or sudden death in both mouse and man (29–32). Retrospective and prospective analyses revealed a phenotypic overlap and prevalent misdiagnoses where 30% of patients with a cardiac arrhythmia also report a presumptive epilepsy phenotype which further confounds personal genetic risk prediction (33).

It has been proposed that due to the range of seizure manifestation in children and requirement for a diagnostic EEG, many children with undiagnosed epilepsy may die of SUDEP, which may be confused with SIDS, SCD and SUDC resulting from

cardiac arrhythmia, hypoxemia or apnea with or without a seizure (34,35). Intriguingly, hippocampal malformations and morphologies are believed to play a role in sudden death in children even in the absence of epilepsy (36). Hallmarks of excitability defects in SIDS infants have been detected postmortem where granule cell dispersion and bilamination in the hippocampus were observed in 42% of 112 SIDS cases. This granule cell dispersion and bilamination within the hippocampus is also known to be a characteristic of temporal lobe epilepsy which is highly intractable and where patients are prone to cardiac dysfunction resulting in high rates of mortality (36). Volume loss within the autonomic region of the brainstem has also been seen in patients with temporal lobe epilepsy, in addition to SUDEP cases (37) Pediatric patients with *SCN1A* mutations causing Dravet syndrome also present with malformations in cortical brain development which persist as patients age (38). Thus, the potential that many SIDS cases are in fact misdiagnosed SUDEP, SUDC or even SCD cases is possible given the limited diagnostics performed in otherwise healthy infants prior to death demanding further improvements in preemptive genetic testing.

1.1.2 SIDS Triple Risk Hypothesis

Current understanding of SIDS risk and causation involves epidemiological and physiological risk factors, coined the Triple Risk Hypothesis (38). Simply, it is postulated that SIDS is caused by the summative effect of overlapping risk factors, working in concert, resulting in the sudden catastrophic and unpredictable death of the infant. The Triple Risk factors are; 1) environmental factors (such as sleeping position or infection), 2) genetic predisposition (e.g. cardiac ion channel gene mutation) and 3) a vulnerable developmental age (~2-4 months of age).

1.1.3 Environmental Risk Factors and Sudden Death

According to the Triple Risk Hypothesis for SIDS (39), the environment can play an important role in risk of death (40). Along the SD spectrum, environmental factors are also key players in controlling the risk of unexpected death. It has been long held that the principle risk of SIDS has been associated with an infant being placed in the prone sleeping position, where it appears infants are less able to regulate arousal and breathing (41). In 1994, the "Back to Sleep" campaign (42) in the United States promoted supine sleeping for infants and a 50% drop in the SIDS rate in the US was observed by 1999.

Following the reduction of SIDS deaths after the implementation of the "Back to Sleep" campaign, other risk predisposing factors became apparent as infants continued to die suddenly and unexpectedly despite this environmental intervention. These risk factors include being of African American descent, exposure to alcohol or tobacco during the prenatal period, premature birth and male gender (8). Additional environmental considerations such as temperature and control and regulation of respiratory drive were also investigated. Detailed analyses on key environmental and physiological factors (43) using birth certificates and infant death registries analyzed between 1990 and 2012, in Colorado, revealed that altitude is independently associated with SIDS risk. Infants born at higher altitudes have an increased risk of dying from SIDS potentially due to decreased cerebral oxygenation and hypoxia (44).

1.1.4 Physiological Risk Factors and Sudden Death

More recently, the risk of SIDS has expanded to include challenge by a viral or bacterial infection, where it is believed that underlying physiological risk can be further exacerbated by a prone sleeping position during sleep. The brainstem,

particularly the medulla oblongata, regulates blood pressure, breathing, and heart rate during physiological arousal and awakening and can be influenced by local and systemic cytokine concentrations (45). Inflammatory cytokine release caused by infection, in combination with abnormal serotonin neurotransmitters in the brainstem, has been implicated as a cause of SIDS (45). More than one half of SIDS infants have abnormalities in the medullary serotonin (5-hydroxytryptamine (5-HT)) system, with decreased 5-HT receptor binding, while simultaneously showing elevated interleukin (IL)-6 cerebrospinal fluid levels in SIDS infants. In these infants, the IL-6 levels are the hallmark of infection with the medullary 5-HT system having significantly higher levels relative to controls. Here, the overexpression of IL-6 receptors on cells within the medulla, in some infants, leads to the increased risk of death (45,46).

Intriguingly, abnormal expression of 5-HT receptors in the brainstem has been observed in murine SUDEP models (47), while the use of Selective Serotonin Reuptake Inhibitors (SSRIs) in epilepsy patients has been shown to decrease SUDEP risk (48). Similarly, sleep position has been shown to impact cardiac rhythmicity, where shifting a newborn, male infant, born with a congenital heart defect, from the supine position to the prone position lengthened the QT interval significantly (49). SIDs infants have also been shown to have longer QT rhythms during sleep when recorded in the first days of life compared to normal infants which was also correlated with the observed autonomic instability observed in these same patients implicating the 5-HT innervation and regulation of the cardio-respiratory pathways (50). It has also been suggested that a similar campaign, where back sleeping is promoted, could decrease the risk of SUDEP (20).

1.1.5 Role of Genetic Variants in the Predisposition of Sudden Death Spectrum Disorders

The spectrum of sudden death has shared genetic risk factors and genetic risk in SIDS is a component of the Triple Risk hypothesis (51)(39)(50) and encode proteins that have known roles in epilepsy, cardiac arrhythmia, muscle contraction, and respiratory regulation as well as general cellular processes and immune responses. This overlap implicates the same gene as causative in more than one syndrome which confounds the utility of preventative molecular diagnostics. If the same gene can cause multiple disorders, predicting what an individual may suffer from can be difficult.

Cardiac Genes: With the high incidence of inherited cardiac arrhythmia and fatal infantile inheritance (53), the role of abnormal cardiac conduction and heart development have been implicated in SIDS. More than 50% of SIDS infants have symptoms of cardiac abnormalities, such as shortness of breath, tachycardia, apnea, and records of >1 lengthened Qtc interval on EKG during the first week of life (44). Of the known mechanisms of cardiac arrhythmia, SIDS has been principally linked with dysfunctional cardiac sodium currents regulating the depolarization of heart tissue (55). In the heart, sodium current is selectively conducted through the voltage gated sodium channel $Na_v1.5$, encoded by the *SCN5A* gene, a known monogenic cause of Brugada syndrome and Short QT syndrome as well as SCD (56). In addition to the Nav1.5 pore forming alpha subunit, the sodium channel beta subunits Na_vB-4 (*SCN1B-SCN4B* genes) interact with and consequently modify ion conductance through the channel and regulate the conduction of sodium ions across cardiac cell membranes and in themselves are cardiac arrhythmia genes (55). Importantly, non-ion channel proteins involved in cardiac function and cellular processes like glycerol-3-phosphate dehydrogenase-like protein (*GPD1L*), alpha1-syntrophin (*SNTA1*), and

caveolin 3 (*CAV3*) have also been implicated in SIDS. A retrospective study performed on archived Danish neonatal blood spot genomic DNA for infants born between 2000 and 2006 revealed that 8/66 SIDS probands harbored a mutation in one of these candidate genes (53). Out of the 66 proband individuals, 6 mutations in *SCN5A* were identified as well as 1 mutation each in *CAV3*, *GPD1L*, and *SCN3B*. Intriguingly, one infant had bioinformatically nonpathogenic population mutations in both *SCN5A* and *GPD1L* when assessed independently, but it has been recognized that synergistic effects of compound and digenic mutations can result in excitability disorders and sudden death. Two *SCN5A* missense mutations, one on each allele, were found in an individual with Brugada Syndrome (57). When family members only presented with one of the mutations, no disease phenotype was seen (57).

Most recently, a whole exome analysis revealed that >35% of Sudden Death in Infancy (SUDI) cases, which is a larger classification encompassing deaths due to SIDS, accidental asphyxia, infection, or cardiac causes (58), have a presumed pathogenic variant in a gene regulating cardiac function, and that 8/47 (17%) had an ion channel gene mutation in a known cardiac arrhythmia gene (59). These findings are consistent with retrospective molecular autopsy studies in SUDEP where ~13% of cases had a deleterious variant in *KCNH2*, *KCNQ1* or *SCN5A* (the three principle genes for cardiac arrhythmias) (60), and 18% had variants within *HCN1-4*, which regulate automaticity within the cardiac conduction system (61,62) .

Respiratory Genes: The serotonin 5-hydroxytryptamine (5-HT) system originates in the medulla of the brainstem and is responsible for baseline respiratory regulation (13,63). This acts in concert with noradrenaline, also regulated by the brainstem, to regulate respiratory rhythm. The dynamic balance between the sympathetic and parasympathetic systems have been implicated in an increased SIDS

risk (63). Monoamine oxidase A (*MAOA*) is responsible for regulating both presynaptic levels of serotonin and noradrenaline, yet despite this central role, genetic risk in *MOA* in SIDS is controversial. Two cohorts of >200 SIDS infants had differential genetic risk of common polymorphisms within the promoter of *MAOA* leading to a low and normal expression of the enzyme (63,64). In the smaller cohort (N=213), the male SIDS cases had a significantly increase in the low expression allelic form, which is hypothesized to reduce the *MAOA* enzyme in the brain stem and in turn increases the serotonin and noradrenaline levels in SIDS infants. However, this is in direct contrast to the current pathophysiological theory that SIDS risk is increased in infants with reduced brain serotonin levels, and thus continues to be debated as a genetic cause (64). Like SIDS, the 5-HT system is also associated with SUDEP risk, where low levels of serotonin have been found in patients who have died from SUDEP believed to result from reduced respiratory drive and response to resulting hypoxia (19,47). DBA mice, an animal model of SUDEP with dysfunction in 5-HT regulation, exhibit seizure-induced respiratory arrest (S-IRA), which leads to cardiac arrest following an audiogenic, sound-cause seizure (65). SSRIs have also been shown to prevent S-IRA and fatal downstream cardiac arrest in these mice, such that increasing synaptic levels of serotonin is protective effect against seizure induced neurorespiratory dysfunction (47). In mice, the 5-HT neurons have also been shown to switch their function from tonic respiratory drive during the postnatal period, needed to provide the baseline respiratory level, to chemoreceptive respiratory control, stimulating above baseline levels, as the mice mature (65). This suggests that This is similar to the recorded human SUDEP cases where cerebral silencing and respiratory depression precede fatal cardiac arrest leading to death (19).

In addition to these central regulatory pathways other genes have been shown

to have altered gene expression during respiratory challenge. These include Heme oxygenase-1 (HO-1 encoded by *HMOX1*)) which is down regulated in alpine climbers with a corresponding constitutively high heme levels in the same individuals (66). Similarly, the roles of Fanconi anemia (FA) protein *FANCD2* involved in the DNA repair pathway, have also been revealed to have modified expression and transcriptional repression in hypoxic conditions (53).

Serotonin: 5-hydroxytryptamine (HT) neurons have been shown to regulate baseline respiratory control during the postnatal period in mice and respiratory arrest caused by seizures are an established mechanism in SUDEP (47)(65). Within the brainstems of the DBA mouse model, the serotonin receptors are under expressed where administering fluoxetine, a serotonin reuptake inhibitor, to these mice was able to reduce the incidence of sudden death, presumably by increasing synaptic serotonin levels (47). DBA mice also have abnormal, both increased and decreased, expression of the *HTR2B, HTR1A, HTR1B, HTR1D, HTR3D, HTR3E, HTR2C, HTR5A, HTR2A, HTR7* genes, out of the total 14 known serotonin receptors (47,67,68). Mutations in the serotonin transporter *SLC6A4* have shown to be implicated in neurodevelopmental abnormalities in infants, including decreased wakefulness and increased irritability (69,70).

Immune Genes: Infants between 2-4 months may be more susceptible to SIDS resulting from a developing immune system with corresponding low levels of systemic antibodies and thus, are at an increased risk of infection by common bacteria and viruses (71). IL-6 and IL-10 levels during an immune response is highly regulated by genetic factors where up to 50% of a response is due to personal genotype (72). Specifically, infants with low expression levels of interleukin-10 (*IL10*) are at an increased risk of SIDS due to an impaired ability to inhibit the

proinflammatory response that occurs due to an infection leading ultimately inducing a prolonged inflammatory response (73). Analysis of cerebrospinal fluid in SIDS infants contains elevated levels of, interleukin-6 (*IL6*) compared to other infant deaths as well as Vascular Endothelial Growth Factor (*VEGF*) (46,74). *VEGF* involved in the development of the respiratory system such that abnormal levels may impair infantile breath regulation during vulnerable developmental periods. It is important to note that while limited in scope and yet to be done on a systematic scale, small cohort studies have shown that immune genes, and more specifically variants, at the population level, involved in the regulatory regions of inflammatory genes are involved in SIDS risk (75).

Neuronal Regulation: Known SIDS risk genes encode ion channels and other proteins that are involved in neuronal regulation and signaling in the infantile brain. Genes encoding for subunits of the neuronal nicotinic acetylcholine receptors, *CHRNA2* and *CHRNA4*, have been implicated in SIDS but also have a role in in autosomal dominant nocturnal frontal lobe epilepsy (76). Several gene non-ion channel genes associated with myelin sheath development and synaptic transmission, including *GAP43, MBP, TPPP, SLC1A3, SLC25A4, PHOX2B, SNAP25*, and VAMP2 have also been shown to contribute to SIDS risk (77,78).

Muscle Regulation: Other genes associated with SIDS risk , such as *MYOM1*, that express proteins involved in muscle cell regulation have a role in cardiac death(79). *MYOM1* encodes for a protein expressed in the myofibraller M band of muscle cells (80). Similarly, a *SPTAN1* gene mutation, which encodes for plasma membrane-stabilizing spectrin proteins (80), implicated in Structural Focal Epilepsy was found in an individual who died from SUDEP. This supports nominating genes involved in abnormal muscle regulation and response in heart, diaphragm or

vasculature response as potential Sudden Death (SD) risk genes (81).

Cytoskeleton & General Cell Processes: Genetic variation in the *TMEM43* gene, encoding for a protein involved in maintaining the structure of the nuclear envelope has been identified in Brugada patients (82). Like other SIDS genes, this protein has also been implicated in Arrhythmogenic Right Ventricular Cardiomyopathy Type 5 (ARVC5) a non-ion channel cause of SCD and SUD (83). *LGI1*, *SMC4*, and *COL6A3* also involved in various aspects of cellular regulation and maintenance have been shown to be significantly associated with SUDEP when compared to disease controls (84). *LGI1* stabilizes synapses and regulates voltage-gated potassium ion channels, while *SMC4* encodes for a chromosomal structural maintenance protein and *COL6A3* encodes for collagen, a cell-binding protein (80). Thus, dysfunction of these proteins are presumed to have ubiquitous regulatory effects on cell functions in all tissues in the body, including the brain, heart and lungs.

Ion Channels & Associated Proteins: Ion channels are believed to play a major role in SUDEP, SIDS, and SUD. Inherited channelopathies play a role in cardiac diseases, such as Long QT Syndrome and Brugada Syndrome, and sudden cardiac death (85). In addition, *SCN1A* and *KNCA1* have been shown to be implicated in both epilepsy and SUDEP (3). *KCNQ1* loss-of-function mutations lead to Long QT Syndrome 1 (LQT1), LQT2 is caused by mutations in potassium channel gene *KCNH2,* LQT3 is caused by sodium channel gene *SCN5A* mutations, LQT7 is caused by potassium channel gene *KCNJ2* mutations, and LQT8 is caused by calcium channel gene *CACNA1C* mutations (85).

1.1.6 Genetic Overlap in Disorders Predisposing to Sudden Death Nominates Novel SIDS Genes

Despite the previously identified candidate genes in SIDS, the overlap in the predisposing comorbid or pathophysiological-related conditions nominates a range of other known genetic causes, of which the majority are related to excitability disorders in brain (epilepsy) and heart (cardiac arrhythmias). These genes include the most common causes of SCD in young adults; Hypertrophic Cardiomyopathy (HCM), Dilated Cardiomyopathy (DCM), and Familial Atrial Fibrillation (AF) where impaired cardiac function is the principle cause of mortality (86). Even within these disorders there is considerable overlap in causative genes which underscores the complexity of risk prediction in the spectrum of SD. Importantly, while most disease genes encode ion channels, there are others that encode protein kinases, scaffolding proteins, as well as myosin and actin cardiac muscle filaments. Similarly, SUDEP risk is not equal across all patients and in addition to the idiopathic generalized genetic epilepsies (IE/GGE) it occurs most frequently in Early Infantile Epileptic Encephalopathies (EIEE), and patients with Dravet Syndrome (DS), carrying between 5.7-10% and being responsible for approximately 60% of fatalities (87). Cardiac arrhythmias have been seen during focal or tonic-clonic seizures in both mouse and man which has been established as a cause of SUDEP (13,34,88).

Hypertrophic Cardiomyopathy (HCM) - HCM can lead to sudden cardiac death due to thickening of heart septum and ventricle walls, thus restricting blood flow through the heart (89). Prevalence is roughly 1/500 and mortality is 0.7-1% per year for patients (90). Additionally, it has been shown that the 15 year cumulative incidence of SCD was 6% (86). Genes associated with HCM include *CAV3*, *OBSCN, LAMP2, MYL2, MYL3, MYOZ2, PRKAG2, TNNI3, CSRP3, ACTN2, TPM1, PLN,*

MYBPC3, TNNC1, TNNT2, ACTC1, and MYH7 (79,81,89,91,92,61).

Dilated Cardiomyopathy (DCM) –DCM leads to reduced blood flow due to thinning of the cardiac walls and enlargement of the cardiac chambers (89). Similar to HCM, the 15 year cumulative incidence of SCD was 5% (86). Genes associated include *MYH6, MYH7, TNNT1, TNNT2, TNNC1, BAG3, RBM20, CTF1, DES*, and *EMB, TAZ, CSRP3, TPM1, ACTC1, MYBPC3* (89,93–95).

Familial Atrial Fibrillation (AF) –AF is an inherited condition that leads to abnormal heart rhythms due to improper electrical activity in the atria of the heart. A meta-analysis of 7 studies looking at the incidence of SDC in 6061 atrial fibrillation patients found a pooled relative risk of 1.88 and an absolute risk increase of 0.6/1000 participant years (96). No studies have been conducted to look at the risk of SCD for patients newly diagnosed with atrial fibrillation (97). Overall, atrial fibrillation is diagnosed in roughly 5% of adults over the age of 65, with risk factors including age, high blood pressure, diabetes, and heart disease (98). However, there is syndromic overlap and comorbidities. In inherited atrial arrhythmias, a risk factor for SADS, both atrial fibrillation (AF) and atrial flutter, are commonly seen in individuals with Brugada Syndrome and Long QT Syndrome. In these individuals the initial occurrence of AF is during nighttime (99,100). The prevalence of AF increases with age, where among adults younger than 55 years old was 0.1%, and the prevalence for adults older than 80 was 9% (101). Genes with causative variants for AF include *LMNA*, *GJA5, KCNA5*, and *KCNE1* the latter being a known regulatory gene for the epilepsy and cardiac channelopathy gene *KCNQ1* which causes SCD, SUD and SUDEP (81,102–104).

Early Infantile Epileptic Encephalopathy (EIEE) – Commonly known as Ohtahara Syndrome, this severe infantile epilepsy syndrome is diagnosed prior to 3

months of age, with the first seizures typically occurring in utero or during the first 10 days of life (76). Up to 75% of the infants have onset of symptoms within a month of birth with high rates of morbidity and mortality with ~ 25% of patients dying before the age of 2 (105). Surviving children have severe physical and developmental disabilities. These may progress to further diagnoses of West Syndrome and Lenox-Gastaut Syndrome, both seizure disorders found in older children (106). West Syndrome (commonly referred to as Infantile Spasms) and Lenox-Gastaut Syndrome are both currently diagnosed etiolgically based on seizure type and disease progression, with the former characterized by full body stiffness-type seizures compared to those with Lenox-Gastaut Syndrome suffering from a variety of different types of seizures (107). Similar to EIEE, the incidence of Ohtahara Syndrome is roughly 1/50 000 in the UK and 1/100 000 in Japan (104). Genes associated with Ohtahara Syndrome include *CDKL5, SLC12A5, STXBP1, SPTAN1, PCDH19, SCN2A, SCN8A*, and *TBC1D24* , where *PDCH19*, *SCN2A* and *SCN8A*, overlap with other infantile epilepsies like Dravet Syndrome (104,108,109).

Early Myoclonic Encephalopathy (EME) – Similar to EIEE, EME also has an early, infantile onset. Seizures are observed within 3 months of life, with most beginning within the first few weeks. The principal differential diagnostic is that EME involves partial, myoclonic seizures, whereas EIEE involves tonic spasms (110). Similar to EIEE, the prognosis for children with EME is poor, with roughly half of children passing away before the age of 2 (110). Many of the genes implicated in EIEE overlap with EME due to their role in neuronal excitability and neuronal development (105).

Severe Myoclonic Epilepsy of Infancy (SMEI) and Dravet Syndrome (DS) – While the syndromic overlap between DS and SMEI remains contentious the unifying

features of these syndromes is the onset prior to the age of 1, with the incidence ranging from 1/20 000 and 1/40 000 live births (105,111). Febrile seizures are a common instigator; however afebrile patients are also observed due to the underlying genetics. Over 80% of patients with a SMEI/DS clinical phenotypes have a molecular diagnosis with a gene mutation in *SCN1A* while other causative genes include *SCN2A, SCN1B, GABRAG2* and *PCDH19* (112). The rate of SUDEP within Dravet Syndrome is the highest in the pediatric population, with an incidence ranging from 5.7-10% and accounting for roughly 60% of fatal epilepsy deaths (13). Cardiac arrhythmias have been seen during focal or tonic-clonic seizures in Dravet patients, implicating neuro-cardiac dysfunction as a cause of SUDEP (13,34).

1.2 Project Rationale and Scope of Thesis

Personalized medicine has marked a new milestone in clinical practice as the demand for genetic diagnosis is rising steeply, including pre-emptive screening, familial and population genetic profiling, and molecular autopsy for medico-legal purposes. Although pathogenic mutations have been identified in the Spectrum of Sudden Death, they lack the ability to accurately predict clinical phenotype. Risk assessment is further complicated by the overlap of causative genes and shared mechanisms of neuro-cardiac and neuro-respiratory dysfunction observed in these patients. There is an urgent priority to identify and translate genetic predictors of Sudden Death into clinical practice for risk stratification and prevention.

1.2.1 Hypothesis

I hypothesize that life-stage dependent expression patterns of alternatively spliced isoforms of key candidate genes in the brainstem cause differential timing of sudden death syndromes across age groups. Here, personal patterns of genetic

variation within my Sudden Death Candidate genes will be analyzed in the context of transcript variability and pathogenicity to determine if there is a role in an individual's person genetic risk.

1.2.2 Specific Aims

Specific Aim 1: Identification of Sudden Death Candidate Genes: A list of candidate genes that play a known or potential role in the spectrum of sudden death disorders with a focus on SIDS, SUDEP, and SUD was compiled from an in-depth analysis of the published literature. This produced a tiered list of known and suspected "Sudden Death Candidate Genes" (SD genes). Using the preliminary seed terms related to Sudden Death and pathophysiological mechanisms such as 1) respiratory distress; 2) immune dysfunction; 3) hypoxia; 4) cellular apoptosis 5) inflammation, the literature nominated additional genes implicated as causative or contributory in other diseases. This initial gene list was then analyzed for pathway-network analysis using the webserver GeneMANIA (113) to expand the list to biologically related genes using functional co-expression or co-localization as criteria.

Specific Aim 2: Genetic Variation in Sudden Death Exomes: Due to the relative rarity of Sudden Death disorders and the inability to predict who will die when, both retrospective DNA and at time of death blood samples were collected on Guthrie cards (Neonatal) from individuals who have died from SIDS, SUDEP, and SUD. Whole genomic DNA was extracted and subjected to Whole Genome Amplification (WGA) Library preparation was performed using the Nextera Rapid Capture Exome Kit (Illumina, San Diego) followed by data collection on the Illumina HiSeq2500. WES for a total of eight deidentified and anonymized probands was obtained. This includes four SIDS cases (two male; two female), three SUDEP (three male) and one

SCD (one male) cases for variant analysis. An integrated bioinformatics pipeline including transcript annotation, and multiple pathogenicity algorithms was employed. The number, nature and functional consequences of variants within the SD genes were compared across SD disorders and by individual to identify the potential cause of death.

Specific Aim 3: Spatio-temporal expression patterns of SD Candidate genes : In this aim, the central hypothesis is tested in that the expression of key SD genes that were predicted to be deleterious and pathogenic in SD exomes from Aim 2 , will be further analyzed using the resources available as part of the Allen Brain Atlas (114). The Allen Brain Atlas for the Developing Mouse Brain was used to identify relative expression levels of the SD genes within the brain and specifically the brainstem through development. A total of 8 stages are available; Embryological day (E)11.5, E13.5, E15.5, E18.5, Postnatal day (P)4, P14, P28, P56. According to Translating Time, P15.5 corresponds to a 4 month old infant and P28 is roughly a 1 year old infant. According to Translating Time (115), 35 day post-conception mice (PC) is equivalent to 379 days PC in humans and 45 day PC mice is equivalent to 621 days PC in humans. Building in this biological context, the pathogenic variants in SD genes were mapped to their transcription patterns in the developing and mature brain to evaluate if their dysfunction influences the brainstem in an age dependent manner. This provides further stratification of risk patterns in the overlapping risk genes observed in the eight Sudden Death samples.

Table 1.1: Spectrum of Sudden Death by age and diagnosis			
	SIDS	**SUDEP**	**SUD/SCD**
Age	0-1 year old	Lifelong	>18
Diagnosis	Autopsy-negative death. Usually found lying face down in bed.	Autopsy-negative death. Usually found lying face down in bed.	Autopsy-negative death. SCD when cardiac cause is believed.

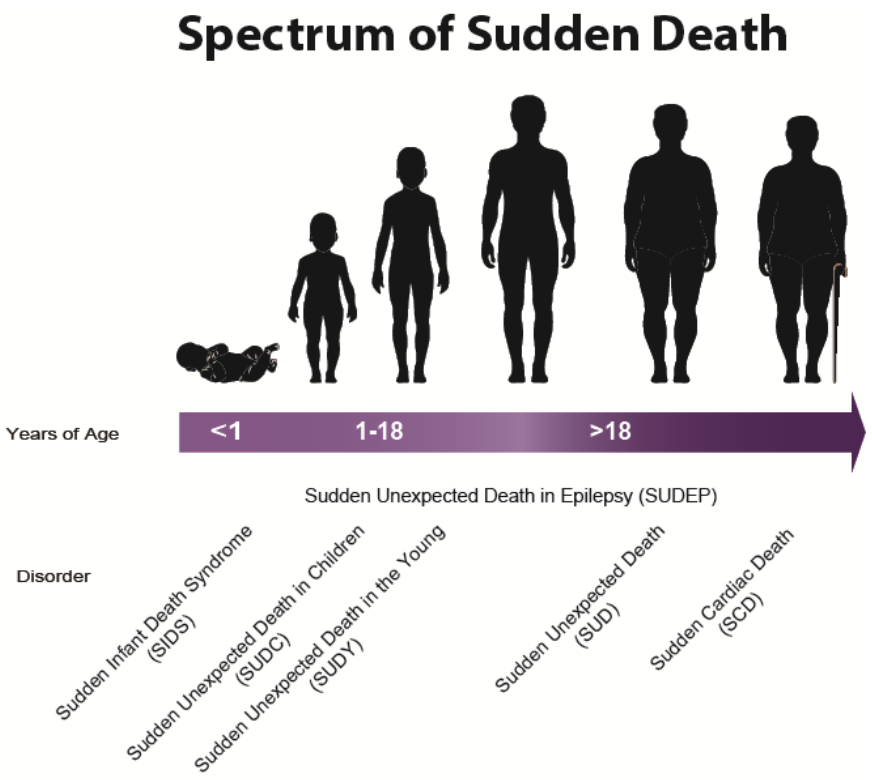


Figure 1.1. The Spectrum of Sudden Death. Sudden Infant Death Syndrome (SIDS) occurs between the age of 0-1 year old, where all other possible causes of death are eliminated during autopsy. Children who die from Sudden Unexpected Death in Children (SUDC) are usually between the ages of 1 and 18 years old, whereas Sudden Unexpected Death in the Young affects young adults (18 to age 40) and is similar to both SIDS and SUDC because individuals are also generally found lying face down. SUDY by definition is a sudden unexpected death, however historically there has been an implication of cardiac involvement as an underlying mechanism. This is similar to Sudden Unexpected Death (SUD) which impacts individuals over the age of 18 and can be specified as Sudden Cardiac Death (SCD) if cardiac regulation is thought to play a role in the death. Sudden Unexpected Death (SUDEP) can occur at any age in an individual with epilepsy however pediatric SUDEP deaths are rare with the exception of the severe Infantile Epileptic Encephalopathy disorders like Dravet syndrome where up to 30% of patients die of SUDEP.

Chapter 2: Materials and Methods

2.1 Compiling the Sudden Death Candidate Gene List

A detailed literature review of SIDS, SUDC, SUDEP, and SCD on PubMed was done to establish a primary list of the genes known to be causative or contributory in multiple patients via pathophysiological mechanism in these diseases. All literature up to and including January 31, 2016 was reviewed using a systematic key word search to first identify the syndrome (e.g. SIDS) and then a review of the results for genes or key molecules was performed for the top 200 papers returned. The results from the top 50 genes were used to populate a candidate gene table for each disorder. A subsequent search of syndrome and the terms; i) genes; 2) genomics; 3) genetics; 4) exome; 5) sequencing; 6) inheritance; 7) autopsy; 8) diagnostics; 9) polymorphism; 10) testing (e.g. SIDS genes; SIDS exome; SIDS genetics); 11) mutations; 12) variants was performed to further expand the candidate list. This formed the basis of gene based searches for diseases and disorders associated with the SD genes and their known or presumed pathophysiological mechanisms.

To better understand the mechanistic and functional overlap across the SD genes, a bioinformatics pathways analysis was performed to stratify and categorize the candidate genes. The original genes acquired from the PubMed literature search (N=169) were entered into GeneMANIA Webserver (113). Here, the underlying database and association algorithm of GeneMANIA uses available biological and disease datasets to identify additional genes involved in the same pathways, DNA-protein interactions, or protein-protein interactions based on the input gene list. In addition, GeneMANIA provides filtering functions enabling a tiered pathway and network analysis of *H. sapiens* associated datasets specifically. This increases the

likelihood that risk genes identified within specific pathways relate to the function, coexpression or colocalization of different combinations of SD genes. GeneMANIA allowed for the inclusion of an additional 79 genes, bringing the total number of SD candidate genes to 248.

2.2 DNA Samples

In collaboration with Dr. Alica Goldman (BCM Center for SUDEP Research Director NIH funded S.T.O.P SUDEP Biorepository), Dr. Torbjorn Tomson (Karolinska) and Dr. Othon Mena (San Diego Coroner) we have performed the first in depth review of SUDY cohort (ages 0-35 years old). This review of 13,050 deaths registered between 2008 and 2013 in the San Diego Medical Examiner's Office identified 122 definite SUDY cases of which 48%, 28%, and 23% fulfilled clinical diagnosis of SUDEP, SIDS, and SCD cases, respectively (unpublished). Of these samples, we used 4 SIDS (2 male, 2 female), 3 SUDEP (3 male), and 1 SUD (1 male) for this project, where we were blinded for cause of death until all variant annotation was complete. Using the novel innovations supported by the CURE Grant (PI-Goldman, Co-Is Klassen, Tomson) we have piloted WGS from trace amounts of blood card derived genomic DNA (gDNA). gDNA was extracted with the QiaAmp Mini Spin Kit (Qiagen, Hilda, Germany) amplified with the Repli-g Ultrafast Whole Genome Amplification (WGA) mini kit (Qiagen) and the Genomeplex WGA kit (Sigma-Aldrich, St. Louis, MO) was used to fragment the template DNA (116).

2.3 Whole Exome Sequencing and Processing

Whole genome amplification (WGA) was performed on the DNA samples from the blood cards to bring up the total amount of DNA to the minimum of 50

ng/uL. For the 8 samples, the concentration of DNA measured by the 2200 TapeStation Instrument (Agilent Technologies, Santa Clara, USA) ranged from 53 ng/μL to 408 ng/μL. Library preparation was performed by the UBC Pharmaceutical Sciences Sequencing Center for 8 Sudden Death individuals using a single sequencing library from the 8 samples, using Nextera Rapid Capture Exome Kit (Illumina, San Diego, USA). Per sample, 50 ng of DNA was required for the library preparation step. The initial part of the library preparation fragmented the DNA and tagged each sample with a unique index pair. The samples were then pooled based on DNA concentrations, assessed by fluorimetry, and hybridized to exome-specific capture probes. The final library went through minor PCR amplification and cleanup, prior to quality control and sequencing. Importantly, this exome dataset will only reveal variants in the transcript encoding portion of the genome, generally referred to as the protein coding region of the genome. Whole exome sequencing was chosen to keep costs down for the pilot project, in addition to allowing us to only look at variants found within the exons of genes. While this limits the amount of variation available for analysis compared to Whole Genome Sequencing, this approach provides the high-quality reads required to address the principle hypothesis of a singular variant having pathogenic effects on multiple transcripts within the same gene of interest.

Following library preparation, the samples were read using the Illumina Hi-Seq 2500 (Illumina, San Diego, USA) and the paired end FASTQ files containing the raw sequencing data were produced for each individual. The FASTQ files were run through FastQC as a quality assurance step. Trim_galore (117) was then used to remove Nextera adapter sequences. Following this step, SolexaQA++ (118,119) was used for more read trimming and selection.

Following the current standard in personalized epilepsy genomics, the Broad

Institute's GATK 3.4 Best Practices workflow (120) was implemented to process and annotate the WES variants. Here, data collection was obtained by runs across 2 lanes resulting in 2 distinct paired end files that were merged prior to downstream processing. As described above, the FASTQ files were pre-processed into paired end files with library linkers were removed, the resulting WES FASTQ file was subsequently aligned to the Hg19 reference genome and the base quality score is generated using the Burrows-Wheeler Aligner (121), resulting in the output of a SAM file. Picard tools (122) was used to convert the SAM file to a BAM file, in addition to being used to mark duplicates. Removal of duplicate artifacts where the same DNA fragments may be sequenced several times, limits the evidence for calling a putative variant, enabling the GATK pipeline to ignore these duplicates using an internal read filter algorithm.

The GATK pipeline performed and refined local sequence alignments around insertion/deletion (indel) events. Principally, this step refines the initial mapping step which produces artifacts, such as reads flank indel events which are, in the absence of refinement, mapped with mismatching bases, that are called as variants but are in fact mapping artifacts. The realignment process employed here identifies the most consistent placement of the reads relative to indels, to minimize abnormal calls which improves the accuracy of the base recalibration. Base Quality Score Recalibration (BQSR) was then used to correct sequencing errors and other experimental artifacts through the employment of a quality score to improve variant calls within each sequencing read. These quality scores were per-base estimates of error from the sequencing runs as generated by the Illumina HiSeq 2500. BQSR was a process in which machine learning is used to model these errors empirically from the input data and adjust the quality scores per the algorithm, improving the accuracy of the variant

calls. Here, base recalibration used a model of covariation, based on the data and a set of known variants, and adjusted the base quality scores in the data based on the model (120). This was followed by a second round of marking duplicates and realignment in the absence of low quality base calling, as recommended by the GATK Best Practices.

The second phase of analysis was coined Variant Discovery, which identifies those bases or regions of the individual exome data that are different from the reference genome. These variant regions are identified and given a quality score where both false positives, also known as specificity, and false negatives, also known as sensitivity, based on high stringency filtering were minimized to capture the amount and extent of variation. The whole genome amplification (WGA) required to bring the DNA concentration up to 50 ng/μL prior to library preparation can introduce more errors as compared to normal, whole DNA preparations. This step converts the BAM file into a Variant Call Format file (VCF). During this conversion, a Variant Quality Score Recalibration (VQSR) is generated, which allows variants filtering and prioritization based on the probability that they are true genetic variants instead of sequencing or data processing artifacts. In addition, this step includes the layering of functional annotations (such as gene structure) onto the individual genetic variants, allowing for predictions of the protein level implication effects of the variants. The final resulting VCF file is a text file that captures nucleotide insertions/deletions (indels), single nucleotide polymorphisms (SNPs) also known as single nucleotide variants (SNVs), and structural variation calls which include multiple nucleotide, duplication, deletion and repeat expansions. Importantly, as per GATK Best Practices for small (N=8) datasets, we included the analysis of 22 whole exome raw BAM files retrieved from phase 1 of the 1000 Genome Project in our data analysis pipeline to

bring the total number of samples up to the recommended 30 (123,124). To minimize variant call errors, ethnic and gender matched controls were selected where 6 samples were Europeans of which 4 were female and 2 were male, while, 16 samples were retrieved from the North American region, of which 9 were male and 7 were female. This reflects the white Caucasian/white Hispanic nature of the SD samples collected by the San Diego Coroner's office. The resulting VCF file was then used for Bioinformatics analysis and functional annotation.

2.4 Bioinformatic Analysis

The WES VCF file for the 8 probands was subjected to variant annotation. This annotation was done employing both Variant Effector Predictor (VEP) (125,126), providing Ensembl (127) transcript annotation, and SnpEff variant annotation software (version 4.11, build 2015-10-03 by Pablo Cingolani) (128,129), providing the National Centre for Biotechnology Information (NCBI) (130) transcript annotation. From the literature, it appeared that the Ensembl set of transcripts was larger than the NCBI set (131). In addition, VEP was able to retrieve more transcript information for our probands (FIGURE 2.2) compared to SnpEff. Thus, we chose to only focus on the transcript data from VEP. Data for analysis included the 1) chromosome and 2) nucleotide position, 3) SNP ID (dbSNP rs accession) if known, 4) reference allele at the position of interest as reported in Hg19, and 4) the alternative allele encoded by the variant in that individual. In addition to being mapped to all known transcripts in the Ensmbl (VEP) and NCBI (SnpEff) databases, the gene variants were also annotated as to transcript (e.g. intronic, missense, nonsense, splice site) and functional consequence (e.g. potentially or probably pathogenic, benign, deleterious) or consequence score (e.g. amino acid substitution matrix) using

bioinformatics tools. These include 1) CADD (Combined Annotation Dependent Depletion) Phred, 2) PolyPhen 2 HDIV Pred, 3) SIFT Pred, and 4) PhastCons 100Way Vertebrate (132,133). These annotations used the most current Ensembl VEP and the dbNSFP (version 2.9) (134) annotation databases to determine the effects of the variants (TABLE 2.1). The physiochemical consequences of amino acid substitution were calculated as a Grantham score using a Perl script graciously provided by Stephane Flibotte, in the Department of Zoology at the University of British Columbia (135,136).

Initially, transcript information was collected from both the NCBI and Ensembl databases, using SnpEff and VEP respectively. As an example, for proband 2069 who died from SUDEP, FIGURE 2.1 represents the variants found in the candidate gene list, along arbitrary positions on the chromosomes. Generally, Ensembl provided information on more transcripts when compared to NCBI, thus we chose to focus on the data from VEP annotations.

Because of the diversity of transcripts to which the resulting WES data were mapped, the functional annotations were difficult to categorize using the annotation categories. Thus, variants were binned into 3' Untranslated Region (UTR), 5' UTR, Synonymous, Nonsynonymous, Splice Site, Splice Region, Nonsense, Intronic, Regulatory, and Insertion/Deletion. These classes were arbitrary but related to the presumed functional and/or biological consequence resulting from the variant effect on one or more transcripts.

UTR Variant- A mutation located in the flanking Untranslated Regions (UTR) regions of a gene 3' variants are located downstream of the gene, whereas 5' UTR variants are located upstream of a gene (137) based on which is the encoding strand. Thus, a 5' UGT variant on the sense strand of DNA would be complimented by the 3'

location on the antisense strand. Variants in these regions are known to XYZ on functional impact.

*Splice Site Varian*t bin included variants from both splice acceptor and splice donor variants. Splice acceptor variants are mutations that changes the two bases of 3' end of an intron, whereas a splice donor changes the two bases of the 5' end of an intron.

Splice Region Variant – Single nucleotide substitutions that will change either between the 3rd and 8th base from the start or end of an intron, or within 3 bases of an exon. This bin included variants that were also contained within the splice region but also overlapped with intron variants, synonymous variants, and nonsense-mediated decay (NMD) variants depending on the isoform sequence they were annotated with. An NMD variant is a sequence of DNA that is the target of nonsense-mediated decay resulting in no translation of the mRNA into protein equating to a complete loss of function at the protein level.

Intronic Variant - intron variants, noncoding exon variants, and noncoding transcript variants. Variants that were labelled as intron variants by SnpEff (128,129) and VEP (125) were located within the introns of a gene but not within 8 bases of its ends. Non-coding exon transcript variants were variants that changed non-coding exons in non-coding transcripts. Ensembl defines the impact of these variants as "modifiers" due to the difficulties in predicting the impact of non-coding variants. Since this is similar to the effect than an intron variant would cause, due to not being present in the final protein, these were grouped them together. A noncoding transcript variant is a variant located within the noncoding RNA gene.

Synonymous variant bin included variants that resulted in no change to the encoded amino acid but do occur within the amino acid coding portion of the exon.

Due to the redundancy of the genetic code, multiple codons can code for the same amino acids, this is frequently referred to as the "wobble hypothesis" (138). Variants result in a different triplet codon however it continues to code for the same amino acid and thus are presumed to be less pathogenic than other types of variants. Within the synonymous variant bin, we included synonymous variants that were also labelled as targets for nonsense-mediate decay (NMD).

*Nonsynonymous varian*t bin included missense variants and stop lost variants. This category is presumed to be the most pathogenic under molecular diagnostic testing as missense variants involve changing the codon such that a different amino acid is expressed. Stop lost variants involve the changing of one of the bases in the stop codon of a gene, resulting in the loss of the termination signal at the end of translation.

Premature Stop Codon/Lost Start Codon variant bin traditionally considered to be a nonsynonomous variant because it involves the coding of a premature stop codon or the loss of a start codon, here these are binned separately due to their known pathogenic role in excitability disorders. These mutations result in premature termination of translation during protein synthesis. The resulting truncated protein formed leads to a complete loss of function.

Insertion/Deletion bin contained any variants where the alleles for an insertion or deletion of 1 or more bases were included. This includes expansions, repeats and duplications or deletions impacting the structural nature of the underlying gene in the chromosome. Many of these variants were annotated based on their presumed impact on protein coding however they were binned separately due to the change in underlying chromosomal organization rather than impact on mRNA or protein.

Regulatory variant bin includes upstream, downstream and intergenic

variants. Upstream variants are located at the 5' end of a gene, within the regulatory region of whereas a downstream variant is located at the 3' end of a gene, also within the regulatory region. Intergenic variants are variants located in the regions between genes and may also overlap with regulatory regions of DNA. Variants within these regions are of importance due to the possibility of affecting gene promotor regions or other areas that may play a role in regulating gene expression.

Spanning Deletion bin was used variants where the allele was missing in that sample, due to the presence of an upstream deletion resulting in a frameshift mutation (139). These are overlapping deletions that are so large that they cover multiple variant sites and are captured in the VCF v4.3 specifications using a "*" (140).

Three decision trees were generated to provide a focus for analyzing the exome data from the SD cohorts (FIGURE 2.1). The first decision tree follows the analysis of the complete whole exome data for each proband, comparing the total number of variants between cohorts, as well as between probands. Following filtering the exome data to only include the SD candidate genes, the second decision tree follows the logic of determining the differences in number of variants within the SD candidate genes. If the resulting data demonstrated that the number of SD candidate genes impacted by variants differed among probands and did not allow for prediction of cause of death. The third decision tree looks specifically at the number of transcripts that are impacted by variants within the SD candidate genes. Once the variability in the number of impacted transcripts is determined, the spatio-temporal expression of genes containing pathogenic variants can be determined.

2.5 Allen Brain Atlas and Alternative isoforms

Using our compiled gene list, we have begun to query the NCBI, Ensembl and

UCSC gene repositories to identify and compare the alternatively spliced isoforms reported for our candidate genes. Importantly, there are a number of key risk genes with a large number of splice forms, such as CACNA1C encoding the Cav2.1 voltage-gated calcium channel, which has 32 alternative protein forms. These results were also used during the variation annotation phase of Aim #2.

We then evaluated the expression patterns of each SD gene using the Allen Brain Atlas. Based on ISH analysis, the molecular probes in Allen Brain are limited to the principle (i.e. longest) transcript and/or the isoform that was successfully hybridized in subsequent rounds of optimization. While many of the candidate genes were present within the adult mouse brain atlas, there were fewer represented across the developing murine brain. Where possible, the location and relative abundance of the SD gene expression patterns were noted. Genes with high levels of expression within the brainstem were noted and will be compared against what is known about tissue expression patterns in cardiac and pulmonary tissues as well as the developing and mature vagal and phrenic nerves.

The Allen Brain Atlas (114) for the Mouse Brain and the Developing Mouse Brain was used to determine the presence of the longest form of each candidate gene within areas of the brain, with key interest in the brainstem. Thus, if evidence exists for the expression of a gene with a potentially deleterious variant in one of our sudden death samples, this could be used as supporting evidence for cause of death.

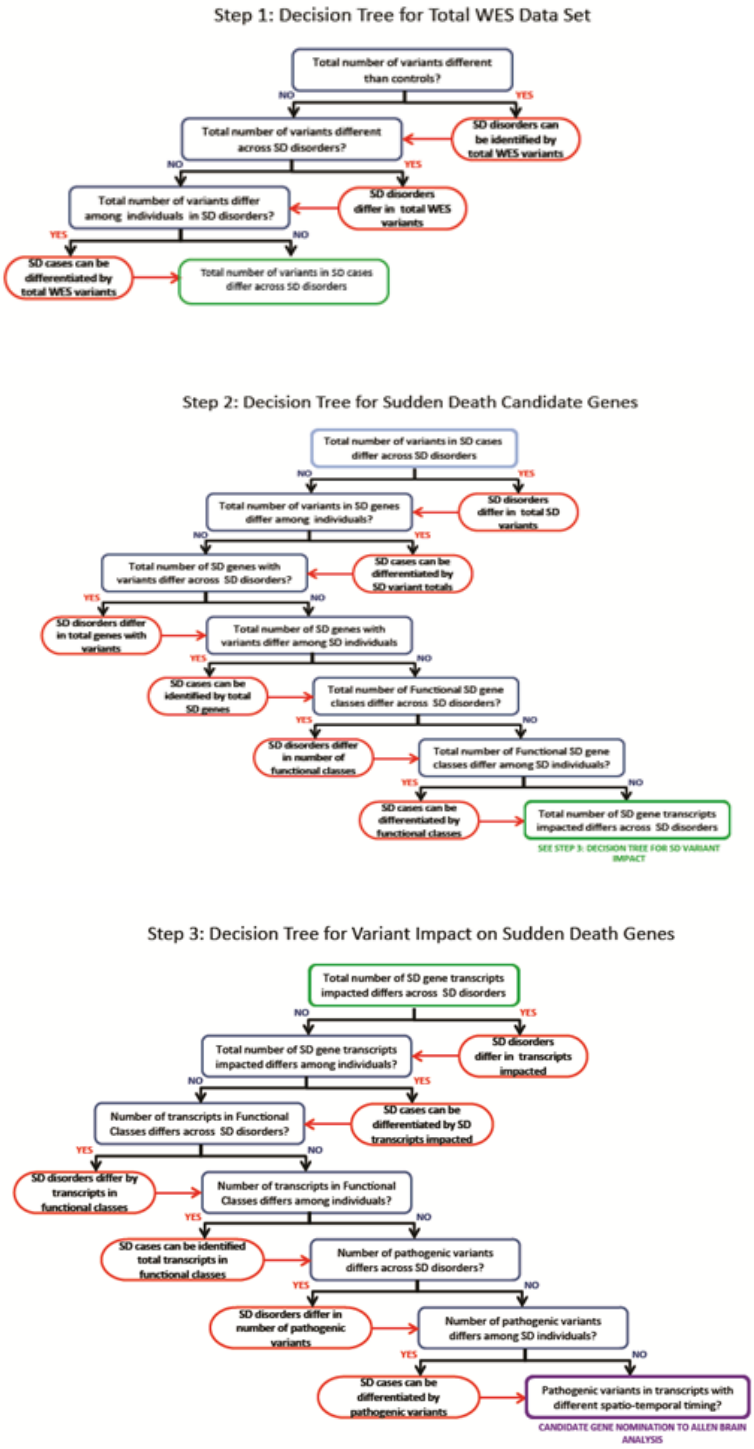


Figure 2.1: Three Step Decision Pathway for analysis of the 8 whole exome Sudden Death samples (4 SIDS, 3 SUDEP, 1 SUD). Initially beginning with the raw data files for our 8 exomes, Step 1 was to evaluate total amounts of variation in the cohort and individual cases at the level of variant and gene. Step 2 was to evaluate the amount of variation in the Sudden Death genes as a candidate gene set and further subdivided into functional classes while Step 3 placed an emphasis on variant pathogenicity and transcript involvement.

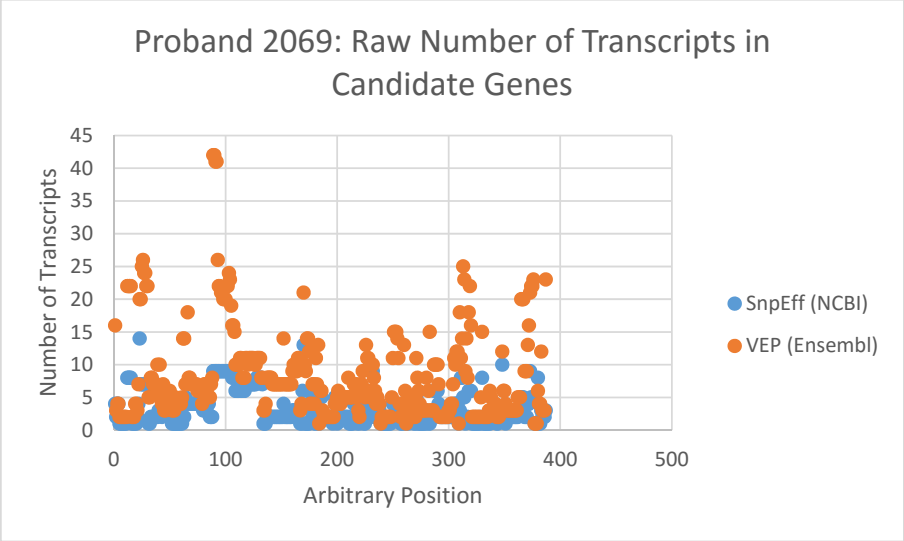


Figure 2.2. Scatter plot of the number of transcripts shown to be impacted by variants, as annotated by SnpEff and VEP. SnpEff retrieves transcript information from NCBI, whereas VEP retrieves transcript information from Ensembl.

Table 2.1: Variant Annotation Classifications and Definitions	
Variant Annotation	**Scoring/Function**
CADD Phred	CADD was acquired from dbNSFP (version 2.9 for hg19) (134). Scores are determined using a matrix to create a score between 0 and 99. A score of >10-20 means potentially deleterious, meaning that they likely reduced the overall fitness of the organism (141,142).
PolyPhen2 HDIV Phred	Acquired from dbNSFP (version 2.9 for hg19) (134). Determines if a mutation will be benign (B), possibly damaging (P), or damaging (D) by looking at the amino acid substitution that occurs (143).
SIFT Pred	Acquired from dbNSFP (version 2.9 for hg19) (134). Determines if a mutation will lead to a change in the overall charge of the protein. If a change occurs, then the variant is deemed deleterious (D). If a change in the net charge does not occur, the variant is deemed tolerated (T) (143).
phastCons 100 Way Vertebrate	Acquired from dbNSFP (version 2.9 for hg19) (134). Using multiple alignments of 100 vertebrate genomes, including the human genome to determine the probability (between 0 and 1.0) that the site belongs to a highly evolutionarily conserved sequence. If the site is highly conserved, it likely has an important function. The larger the score, the more conserved (143). A cutoff of 0.84 for conservation has previously been used (144).
Grantham	Score is used to determine the physicochemical nature of an amino acid substitution. A higher score means a greater difference in chemical properties between two amino acids, in terms of polarity and molecule volume. Scores range from 0 to 215. A score of 0-50 is Conservative, 51-100 is Moderately Conservative, 101-150 is Moderately Radical, and >151 is Radical. (135,136,145).
dbSNP	Acquired from SnpSift (132,133) and provides the rsID, showing if the variant is present in the dbSNP database.

Chapter 3: Results

3.1. Identification of Sudden Death Candidate Genes

3.1.1 Genes Identified in the Spectrum of Sudden Death

The compilation of SD candidate gene list using previously published literature on SIDS, SUDEP and SUD resulted in 248 genes (TABLE 3.1-3.9). While it is recognized that a pathophysiological and mechanistic overlap exists across the sudden death spectrum, which includes key risk genes central to cardiorespiratory function, the full extent of risk across the entire age and syndrome spectrum has yet to be evaluated in a concerted way. It is generally assumed that sudden death is induced by neurological dysfunction, as in epilepsy, heart dysfunction, and cardiology. However, SIDS research also implicates environmental and immune triggers as potential causes. Importantly, respiratory infections and the subsequent inflammatory response may play a role in triggering sudden death in infants during a vulnerable age. Here, known causative and contributory genes were entered into GeneMANIA (113) and those genes implicated through co-expression and co-localization pathway and interaction analysis were included in the SD candidate gene list (TABLE 3.1-3.9). The original sudden death genes collected from the literature (N=169), prior to expansion using GeneMANIA, encode proteins involved in various cellular response roles, including ion channels, sarcomeric regulation in muscle cells, and cytoskeletal regulation. As expected by pathway analysis, the additional GeneMANIA acquired genes are involved in the same regulatory roles within cells and are found in key tissues implicated in the presumed disease mechanism.

In addition to genes implicated in SIDS, SUD and SUDEP, the full 248 candidate genes evaluated for involvement in other diseases including those

considered high risk for sudden death, such as epilepsy and cardiac arrhythmias (TABLE 3.10). These genes may or may not be genes undergoing molecular diagnostic or laboratory hematological diagnostic testing in these conditions. 70% of SD genes (N=174) are implicated in disease, the majority lead to cardiac arrhythmias including Long QT Syndrome and Brugada Syndrome where the causative genes have a high rate of mutations identified in post-mortem analysis of SUDY, SUD or SCD. Notably, there are a number of genes which are involved in more than one disease further complicating risk prediction (TABLE 3.10). In addition to excitability disorders, genes found in the development of cancer were also found to be implicated in SIDS and nominated via pathway analysis (N=10). Specifically, these were genes involved in the inflammatory and or immune response, such as *TBX21*, or cell death, such as *APPBP2* which would support the environmental component of the Triple Risk Hypothesis for SIDS.

3.1.2 Sudden Death Candidate Genes as Disease Genes

The genetic overlap between genes implicated in SIDS, SUDEP, SUD, cardiac arrhythmias, and epilepsy was observed within a subset (N=174) candidate genes (FIGURE 3.1). Of these a total of 19 genes were only implicated in SUDEP, 26 genes were only implicated in SIDS, 2 genes were only implicated in SUD. Similarly, 26 genes were only implicated in epilepsy, and 31 genes were only implicated in cardiac arrhythmias, while 17 genes were involved in both SUD and cardiac arrhythmias, and 4 genes were involved in both epilepsy and cardiac arrhythmias. Importantly, analysis of the SD genes revealed that *KCNQ1* (N=1) was identified in all five (5) categories of sudden death (SIDS, SUD, SUDEP) and causative excitability disorders (cardiac arrhythmia and epilepsy).

3.1.3 Sudden Death Candidate Genes Have Different Biological Functions

The SD candidate genes were categorized based function (80). The nine functional categories were: muscle regulation, cardiac regulation, neuronal regulation, cytoskeletal role, hypoxia, inflammation, serotonin receptors and transporters, general cellular processes, and ion channels and associated proteins. Genes that encoded proteins associated with the proper function of muscle cells within the body were binned into the muscle regulation category (N=16) (TABLE 3.1). Genes that were specifically involved in homeostasis within the heart were binned into the cardiac regulation category (N=45) (TABLE 3.2). However, ion channels, regardless of their role in different tissues, were binned into the ion channel category (N=47) (TABLE 3.3). Genes, excluding ion channels, that play a role in the brain were binned into the neuronal regulation category (N=30) (TABLE 3.4). Any of the SD candidate genes with a role in the cellular cytoskeleton were binned into the cytoskeletal role category (N=14) (TABLE 3.5), whereas genes that had an overall role in cellular homeostasis were placed in the general cellular processes category (N=65) (TABLE 3.6). Serotonin receptors and transporters were categorized separately (N=11) (TABLE 3.7). The genes that have been shown to specifically play a role in hypoxia within the body were binned together (N=10) (TABLE 3.8), and genes with a role in the immune system and the inflammatory response were categorized together (N=10) (TABLE 3.9).

3.2. Genetic Variation in Sudden Death Exomes

3.2.1. Variation Observed in SD Whole Exomes

Using the established decision tree, the whole exomes for the SD probands were analyzed. Initially, the total number of variants in the whole exome were counted and compared to a literature value for the average number of variants in a healthy individual who on average had a range of 25 000-55 000 variants observed in whole exome data depending on capture kit, coverage and platform used. Whole exome data from SIDS proband blood spot gDNA following WGA had a range of 40869-69978 variants (N=4) (TABLE 3.11) where the average number of variants is 55257 (SEM ± 6098) which is similar to results obtained from whole blood analysis of those with Amyotrophic Lateral Sclerosis (ALS) (146). The range of variants observed for the SUDEP probands, was 19162 to 63954, variants were present in the whole exomes while the single SUD proband, a total of 63217 variants were present in the whole exome data (TABLE 3.11). These variants were distributed across the entire genome and included both known and novel variants and polymorphisms, similar to results observed previously in epilepsy, cardiac arrhythmia and other sudden death cases. There were no discernable differences between SD categories, such that the total number of variants in the whole exome did not distinguish SIDS from those in SUDEP or SUD cohorts, despite the large range observed in SUDEP cases.

3.3. Personal Patterns of Variation SD Candidate Genes

3.3.1. Variation within SD Candidate Genes

The total amount of whole exome variation was subsequently filtered to consider only the nominated SD genes (N=248) to evaluate the overlap or

independent patterns of variation within the probands. As observed in the whole exome, the amount of variation was extensive and encompassed many different candidate genes, including those with established overlap. There were no distinguishing features across the amount of variation by cohort, where SIDS probands (N=4), had an average of 1014.5 (SEM ±125) variants present within the exomes compared to the SUDEP probands (N=3), an average of 787 (SEM ±209) variants were present within the exomes. For the single SUD proband, a total of 1026 variants were present within the exomes.

When the SD groups are compared, the personal variation observed in the SD candidate genes is extensive when only the totals are considered, even among the same cause of death (TABLE 3.12). For the SIDS group, the amount of variation ranged from 774 to 1160 variants while the SUDEP group ranged from 379 to 1078 variants. The single SUD proband had 1026 variants in the SD candidate genes. Thus, even when only the presumed mechanistic genes are considered, the amount of personal variation is incapable of distinguishing the cause of death and cannot discriminate between the individuals within each category suggesting patterns rather than total gene variation is important for diagnostic purposes.

SD candidate gene variants were further evaluated by considering the total number of genes containing variants by functional category (TABLE 3.13). Out of the 4 SIDS probands, 94% (15/16) of the SD candidate genes involved in muscle regulation (FIGURE 3.2), all of the 43 genes involved in cardiac regulation, 79% (23/29) of the genes involved in neuronal regulation, 93% (13/14) of the genes involved in the cellular cytoskeleton, 50% (9/18) of the

genes with roles in hypoxia, 83% (5/6) of the genes involved in the immune response, 82% (9/11) of the serotonin receptor and transporter genes, 77% (49/64) of the genes involved in general cellular processes, 98% (46/47) of the genes encoding ion channels and their associated proteins contained variants.

The SUDEP probands (N=3) had the majority of genes located in the muscle regulation (88% (14/16)) and cardiac regulation categories (88% (38/43) (FIGURE 3.3). Variation in cellular cytoskeleton (71% (6/18)), hypoxia (56% (11/18)), serotonin receptor (55% (6/11)) and ion channel genes (89% (42/47) were similar to SIDS probands however there were slight differences in which genes contained variants. Interestingly, each of the immune response genes (N=6) had one or more variants in at least one proband. The numbers and patterns of variants observed in the single SUD proband did not differ significantly from those in the SIDS or SUDEP patients, including having a variant in 4 out of the 6 immune response genes (FIGURE 3.4). These personal patterns of variation by category are indistinguishable across the SD cohorts further suggest the genetic and mechanistic overlap underlying risk in the spectrum of sudden death.

To further evaluate personal risk in SD disorders, the individual probands were compared within each cohort to further stratify risk prediction. First, the total variants by individual within the SD candidate genes were compared. For SIDS probands, the total genes containing a variant ranged from 109 to 213 while SUDEP probands had 93 to 159 genes impacted while the SUD proband had 168 genes impacted. The second analyses further subdivided the number of SD genes containing variants into their functional categories, which regardless of

proband showed the same distributions as observed at the cohort level. The SIDS probands had equal distribution across General Cellular Processes (29:46:32:40 genes), Cardiac Regulation (24:49:37:38), and Ion Channel (25:51:29:37) categories (TABLE 3.13). This equitable distribution, and in fact the number of genes impacted were similar in the SUDEP probands where 24, 33 and 34 ion channel genes contained variants.

3.3.2. Genetic Variation in SD Candidate Gene Transcripts

The WES SD gene variants were annotated to all possible isoform transcripts reported in Ensembl using the VEP algorithm. This revealed that individual variants can impact multiple alternate splice variants depending upon which coding exon the variant is contained in, and how frequently that exon is used in a transcript. In SIDS probands (N=4), the genetic variation in the SD gene impacted 3226-9388 transcripts with an average of 5836 (SEM ±1333) transcripts (TABLE 3.14). This range was similar to the 1671-7046 SUDEP probands (mean=4415 SEM ±1552; N=3) and the SUD proband who had 7269 variant-affected transcripts within the SD candidate genes. The large variation in affected transcripts within SD cohorts and similar ranges between cohorts means that it is not possible to use the number of affected transcripts to determine cause of death.

For the three SD groups, number of SD candidate genes containing variants were all within similar ranges where there was no substantial or distinguishing differences in the number of affected genes between the three groups. For the SIDS probands, a large proportion were in the Cardiac Regulation, General Cell Processes, and Ion Channel categories (TABLE 3.15).

There was some variation between the SIDS probands, where proband 2098 had more impacted SD candidate genes in all categories when compared to the other three probands. Variation between the three SUDEP probands was also seen, with several impacted genes also belonging to the Cardiac Regulation, General Cell Processes, and Ion Channel categories. Lastly, the single SUD probands also has numerous variant-containing candidate genes within the Cardiac Regulation, General Cell Processes, and Ion Channel categories. Since the same three categories contained the majority of impacted SD candidate genes for all three SD groups, it is not possible to predict the cause of death simply by the SD categories that contain the most variants.

For both the SIDS and SUDEP groups, most impacted transcripts were located within SD candidate genes in the Cardiac Regulation and Muscle Regulation categories. For the single SUD proband, most transcripts located were in SD candidate genes in the Cardiac Regulation and Ion Channel categories.

3.4. Sudden Death Gene Variants Are Pathogenic in Multiple Isoforms

Because variant containing exons can be spliced in alternate patterns, including their absence in the protein coding isoform, individual variants may have different functional effects. When this variant impact is considered as part of the analysis, it is observed that the SIDS probands (N=4) have multiple variants impacting untranslated regions with both 5' and 3' Untranslated Regions (UTR) having between 36-102 and 35-103 transcripts respectively. Potentially pathogenic variants impacting the splice region (53-153 transcripts) and splice sites (0-8 transcripts) of transcripts were also observed. Unexpectedly, the SIDS probands had a number of potentially pathogenic variants impacting multiple splice variants including

nonsynonomous variants encoding amino acid substitutions (259-692 transcripts) as well as those encoding premature stop or lost start codons (0-783 transcripts) and large insertions and deletions (301-699 transcripts) (TABLE 3.16).

Within the SUDEP probands (N=3), multiple variants impact transcripts in the 3' and 5' UTR regions, where 18-136 and 7-75 transcript are impacted, respectfully. The splice region (43-100) and splice site (0-3) are also impacted, in addition to the nonsynonymous variants (163-528) and variants leading to a premature stop codon or lost start codon (0-1). When looking specifically at large insertions or deletions, 199-693 transcripts were affected (TABLE 3.16).

The single SUD proband had 130 and 77 transcripts affected in the 3' UTR and 5' UTR regions, respectfully, by variants in the SD candidate genes. Within he transcripts, the splice regions (134 transcripts) and splice sites (3 transcripts) were also impacted by the variants. Additionally, 853 transcripts were affected by synonymous variants and 504 were affected by nonsynonymous. Premature stop codons and lost start codons affected no transcripts, whereas large insertions and deletions affected 631 transcripts (TABLE 3.16). The SD cohorts cannot be distinguished using the number of transcripts similarly impacted by variants due to the high similarity in ranges.

3.5 Personal Pathogenic Variants in Sudden Death Probands

Overall, the majority of variants deemed pathogenic using bioinformatics variant annotation cutoffs impacted an array of SD candidate genes in the SIDS proband. The principle impacted functional categories impact were the Ion Channel, General Cellular processes, Cardiac Regulation, and Muscle Regulation categories (FIGURE 3.5). Most of the pathogenic variants within the transcripts were found to

be nonsynonymous in nature, or located within intronic or regulatory regions (FIGURE 3.6, 3.7, 3.8). These patterns of personal variation were also observed in the SUDEP and SUD probands (FIGURE 3.9, FIGURE 3.10).

For SIDS proband 2095, key pathogenic variants were identified in *SCN2A*, *RYR3*, *DAG1*, *IL10RA*, *TTN*, and *TRPM6 (*TABLE 3.17). All of these genes have been previously associated with excitability and sudden death disorders, including SIDS, SUDEP, Early Infantile Epileptic Encephalopathy, Epilepsy, and HCM. Variants within three of these genes; RYR3, *DAG1*, and *IL10RA* have previously been reported in dbNSP, however variants in *SCN2A, TTN* and *TRPM6* are novel to this proband. Depending on the specific gene the number of transcripts containing these six key variants is from 2 to 21.

DSP, *KCNJ5, KCNH2*, *SCN1B*, *OBSCN* and *ABCC8* were observed to contain pathogenic variants in SIDS proband 2098 (TABLE 3.18). Not only have these genes been previously associated with disease states such as HCM, Long QT Syndrome, Dravet Syndrome, SUD, as well as Rapid Onset Dystonia-Parkinsonism, and Hyperinsulinemic Hypoglycemia of Infancy. Like the other SIDS proband, variants in *DSP*, *KCNJ5*, *KCNH2*, and *OBSCN* are known. These variants impact between 2 to 7 transcripts depending on gene isoform.

The final SIDS probands had pathogenic variants in some of the same genes (TABLE 3.19) as the above where proband 2475 had a variant in *SCN2A* while proband 2477 had a variant in *SCN1B* (TABLE 3.20). Interestingly, not only did these probands have overlap with the other SIDS probands, but also each other where S*CN8A* also contained a variant in each proband. The variants in these probands were also a combination of novel and previously reported variants. These SD candidate genes are associated with SIDS, Neonatal Bartter Syndrome, Early Infantile Epileptic

Encephalopathy, HCM and Cancer. The range of affected transcripts for these variants is from 2 to 21 and 3-110 transcripts in proband 2475 and 2477 respectively.

As predicted, the personal variation in the SUDEP probands shared similar pattersn of risk genes impacting multiple functional categories. SUDEP proband 2231 has key pathogenic variants located in *HTR3E*, *KCNH2*, *RYR3*, *OBSCN, ERBB2*, and *TTN* (TABLE 3.21). These genes have been previously associated with include SUDEP, SIDS, Epilepsy, Cardiac Arrhythmias, Long QT Syndrome, HCM, and Cancer. All 6 variants have previously been reported in dbSNP. There is overlap between SUDEP proband 2429 and both the SUDEP and SIDS probands where pathogenic variants are observed in *SCN1B, KCNJ5, KCNH2* and *OBSCN* genes, all of which have been identified to cause excitability defects in heart or brain and are associated with the spectrum of sudden death disorders (TABLE 3.22). Intriguingly, SUDEP proband 2069 had a different risk gene profile with compound variants in *ATP1A3,* as well as variants in *TMEM214, LMNA, TRDN* and *TTN* (TABLE 3.23).

The single SUD proband, 2460, contained key pathogenic variants in *GOT2*, *OBSCN*, *RBM20*, *SCN5A*, *TTN*, and *DAG1* (TABLE 3.24). All 6 variants have been seen previously in the and dbSNP and impact 1 to 21 transcripts. The genes that contain the variants are associated with SUD, SUDEP, SIDS, DCM, HCM, Epilepsy, Long QT Syndrome, and Brugada Syndrome.

Collectively, several genes, including *OBSCN* (TABLE 3.25), was observed to contain pathogenic variants in all 8 probands. However, the number of transcripts impacted by the personal variants was not the same ranging from5-12 transcripts. Each proband's unique set of pathogenic variants, as well as the number of transcripts impacted by the variant, can be used to distinguish between the SD cohorts. Specifically, the SIDS probands all had variants that impacted genes associated

seizure disorders with an infantile onset epilepsy. As expected SUDEP proband 2429 also contained a pathogenic variant variant in *SCN1B* causative for Severe Myoclonic Epilepsy of Infancy however it cannot be determined if this is a cause of epilepsy, sudden death or represents the previously reported overlap in risk in this patient population. Thus, looking at the specific timing of expression of different transcripts within the brainstem is an integral consideration in predicting the timing of sudden death and the potential for undiagnosed seizure disorders as a potential contributing cause.

3.6 Expression of SD Risk Genes in the Developing Brain

The In Situ Hybridization (ISH) data from the Allen Brain Atlas for the Developing Mouse Brain was used to detect expression of the SD candidate genes containing pathogenic variants within the brainstem of the mouse at different stages within development. Through the exome data, high priority genes were identified with pathogenic variants. However, due to limited data available from the Allen Brain Atlas for the Developing Mouse Brain, there was limited ability to identify and compare the expression of the genes of interest within the brain across developmental stages. It is also important to recognize that an RNA probe for only one mRNA isoform per gene was used to detect the presence of the protein. The Allen Brain for the Developing Mouse Brain using ISH showed low expression of *Mbp* in the brainstem region of the mouse at embryonic day 15.5 (E15.5), which is roughly equivalent to a 4 month old infant (FIGURE 3.11). The human equivalent of this gene, *MBP,* was found to contain pathogenic variants in some of the SUDEP probands but none of the SIDS probands. Expression of *Mbp* was shown to increase at postnatal day 4 (P4), roughly equivalent to a 1 year old child in humans (FIGURE

3.12) In comparison, *Hrc2c* had undetectable to low expression in the brainstem at E15.5 (FIGURE 3.13), which increased slightly at P4. The human version of this gene, *HRC2C,* contained pathogenic variants in the SIDS probands but none in the SUDEP or SUD probands (FIGURE 3.14). Variability in expression within the brainstem of genes containing pathogenic variants was seen across mouse brain developmental stages but did not allow identification or comparison of specific isoforms.

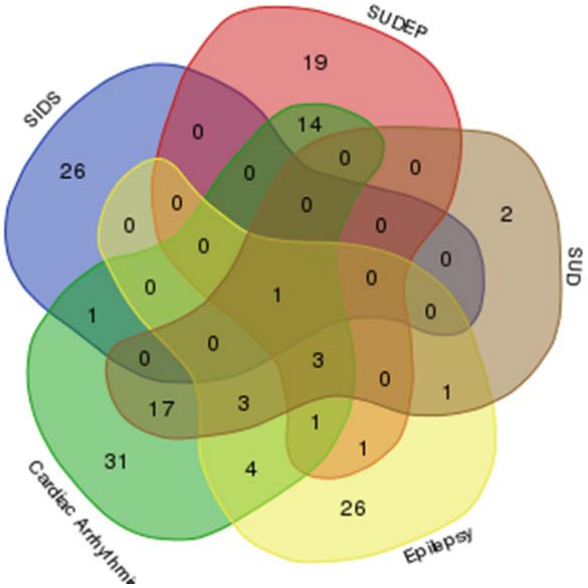


Figure 3.1. Venn Diagram showing the overlap in a subcategory of the candidate genes implicated in SIDS, SUDEP, SUD, epilepsy and cardiac arrhythmias. No genes were implicated in all five disorders (N=0), 26 were only implicated in SIDS, 19 were only implicated in SUDEP, 2 were only implicated in SUD, 26 were only implicated in Epilepsy, and 31 were only implicated in cardiac arrhythmias. Venn diagram was generated using Bioinformatics Evolutionary Genomics tool (147).

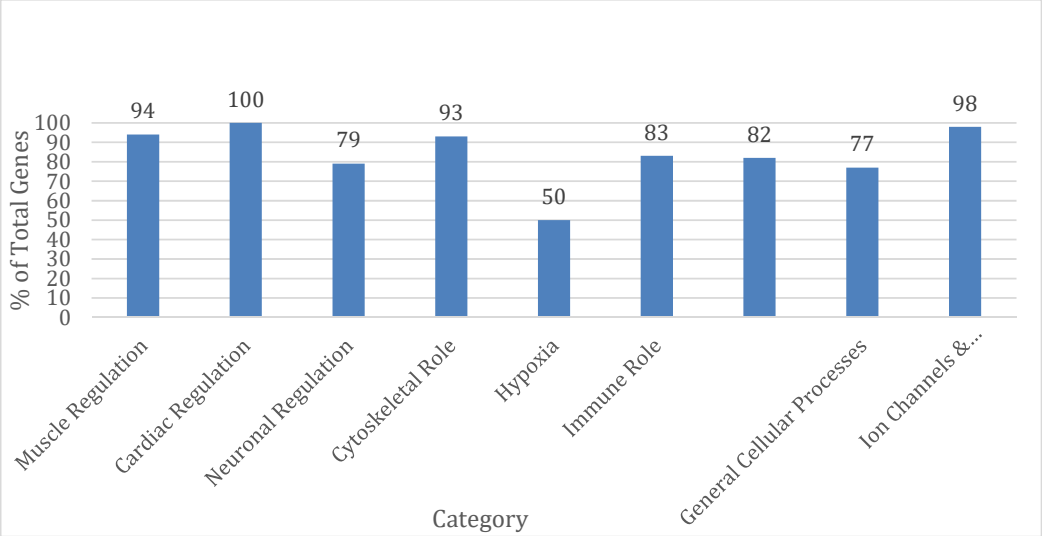


Figure 3.2. The percentage of the SD candidate genes, by functional category, that had variants present in Sudden Infant Death Syndrome (SIDS) probands.

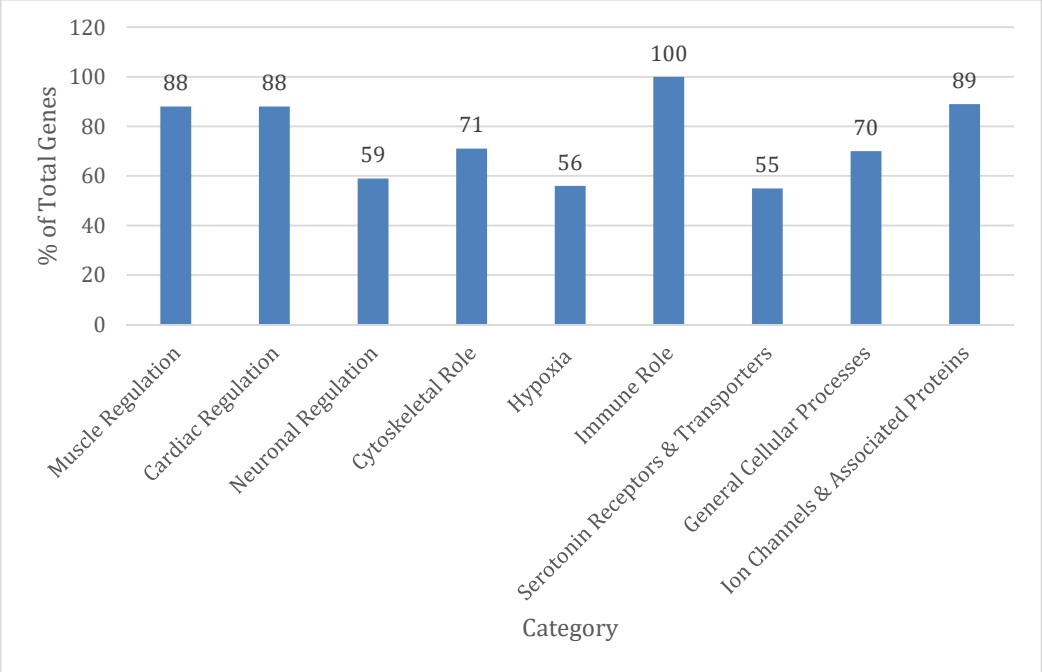


Figure 3.3. The percentage of the SD candidate genes, by functional category, that had variants in Sudden Unexpected Death in Epilepsy (SUDEP) probands (N=3).

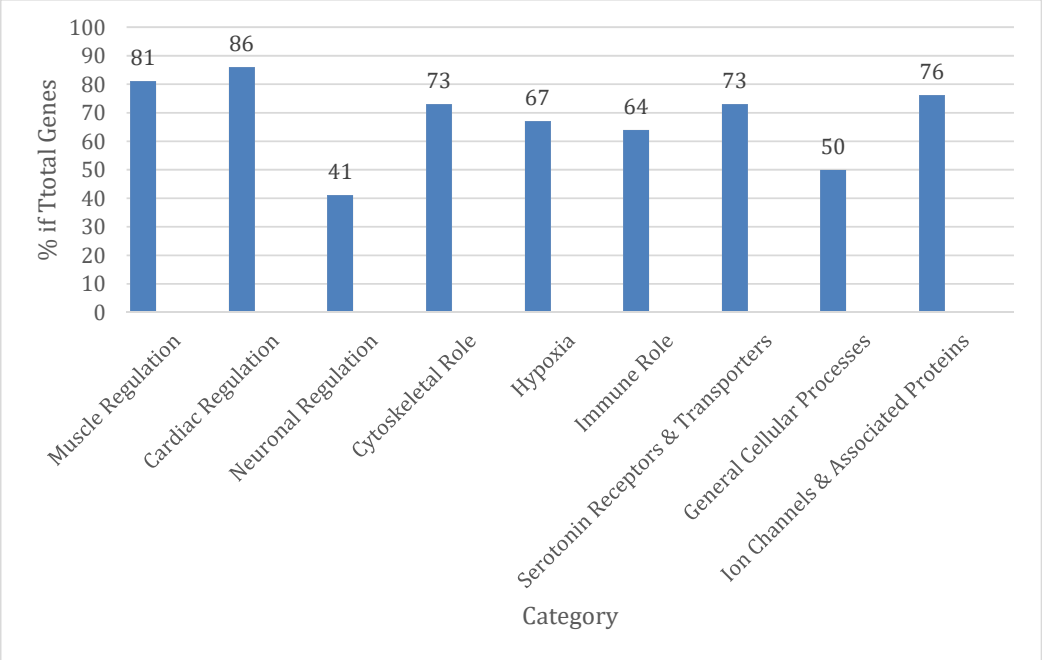


Figure 3.4. The percentage of the SD candidate genes, by functional category, that contained variants present in the single, Sudden Unexpected Death (SUD) proband.

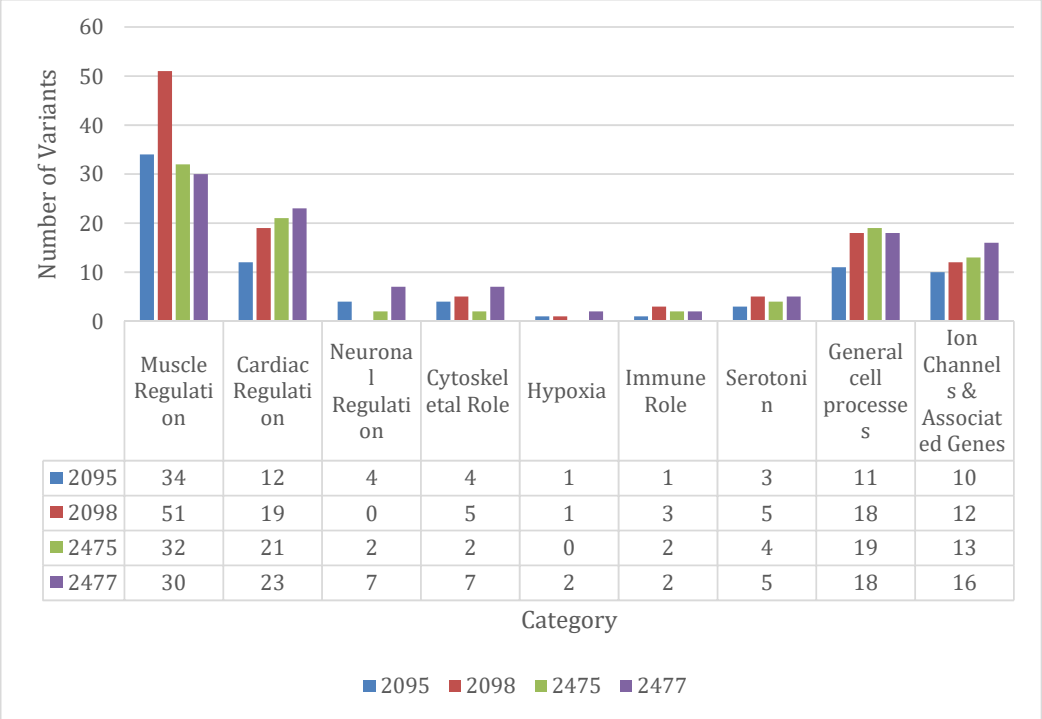
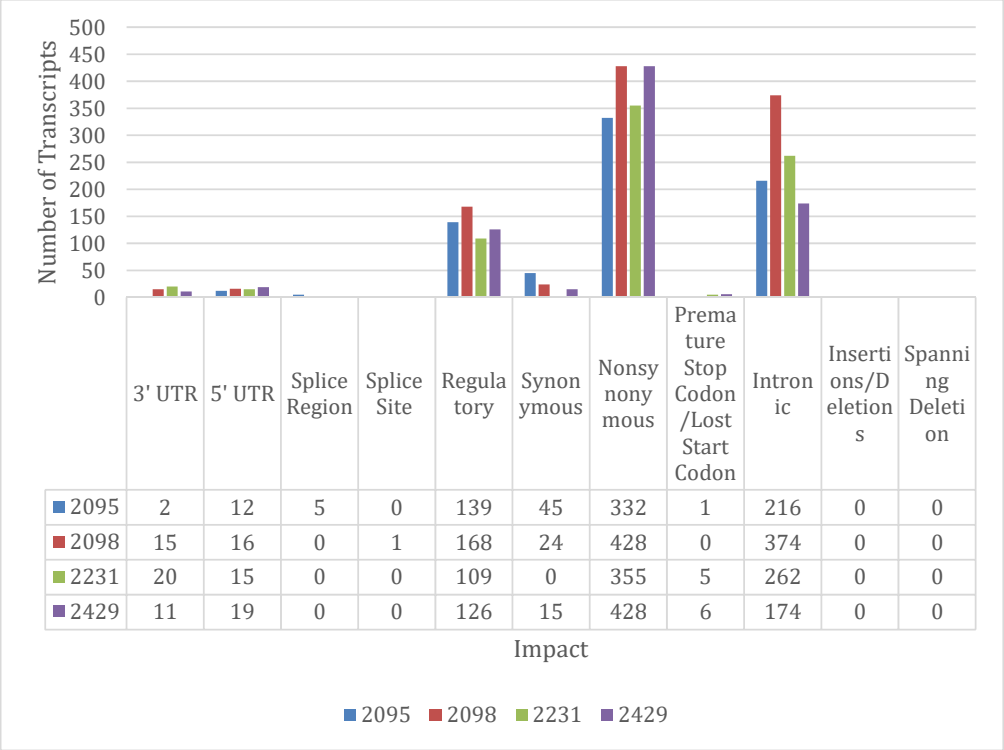


Figure 3.5. The number of pathogenic variants in Sudden Death (SD) candidate genes in the Sudden Infant Death Syndrome (SIDS) probands (N=4). Pathogenicity was defined as scoring pathogenic for at least one of the five variant annotation methods; CADD Phred (>10), PolyPhen2 HDIV Phred (Possibly damaging or Damaging), SIFT Pred (Deleterious), PhastCons 100 Way Vertebrate (>0.84), and Grantham (>100).



	3' UTR	5' UTR	Splice Region	Splice Site	Regulatory	Synonymous	Nonsynonymous	Premature Stop Codon /Lost Start Codon	Intronic	Insertions/Deletions	Spanning Deletion
2095	2	12	5	0	139	45	332	1	216	0	0
2098	15	16	0	1	168	24	428	0	374	0	0
2231	20	15	0	0	109	0	355	5	262	0	0
2429	11	19	0	0	126	15	428	6	174	0	0

Figure 3.6. The number of transcripts impacted by pathogenic variants in Sudden Death (SD) candidate genes in Sudden Infant Death Syndrome (SIDS) probands (N=4). Pathogenicity was defined as scoring pathogenic for at least one of the five variant annotation methods; CADD Phred (>10), PolyPhen2 HDIV Phred (Possibly damaging or Damaging), SIFT Pred (Deleterious), PhastCons 100 Way Vertebrate (>0.84), and Grantham (>100).

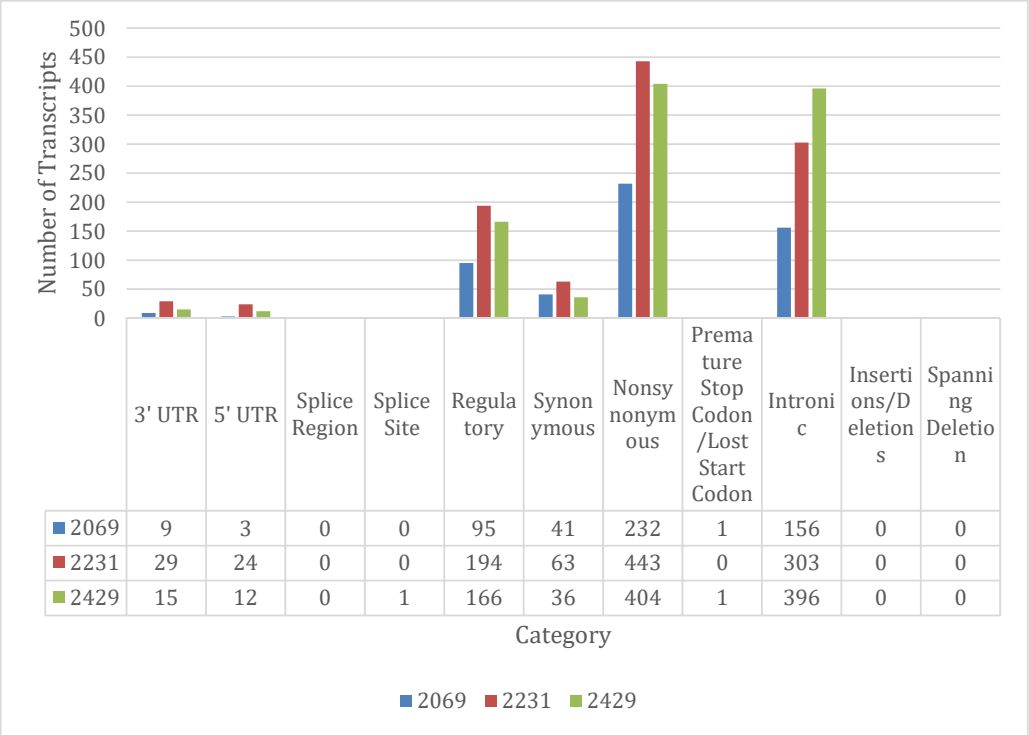


Figure 3.7. The number of transcripts impacted by pathogenic variants in the Sudden Death (SD) candidate genes in Sudden Unexpected Death in Epilepsy (SUDEP) probands (N=3). Pathogenicity was defined as scoring pathogenic for at least one of the five variant annotation methods; CADD Phred (>10), PolyPhen2 HDIV Phred (Possibly damaging or Damaging), SIFT Pred (Deleterious), PhastCons 100 Way Vertebrate (>0.84), and Grantham (>100).

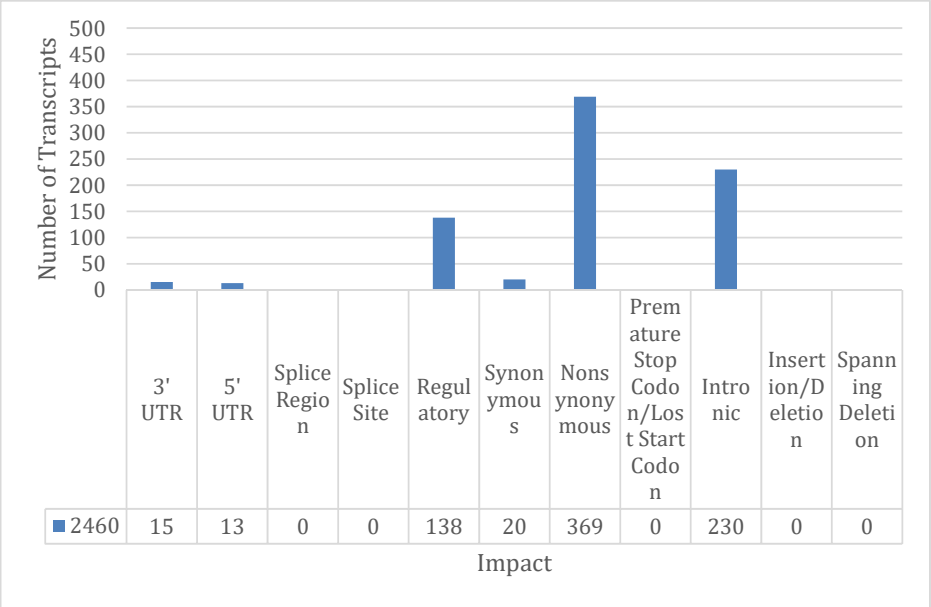


Figure 3.8. The number of transcripts impacted by pathogenic variants in the Sudden Death (SD) candidate genes in Sudden Unexpected Death (SUD) proband (N=1). Pathogenicity was defined as scoring pathogenic for at least one of the five variant annotation methods; CADD Phred (>10), PolyPhen2 HDIV Phred (Possibly damaging or Damaging), SIFT Pred (Deleterious), PhastCons 100 Way Vertebrate (>0.84), and Grantham (>100).

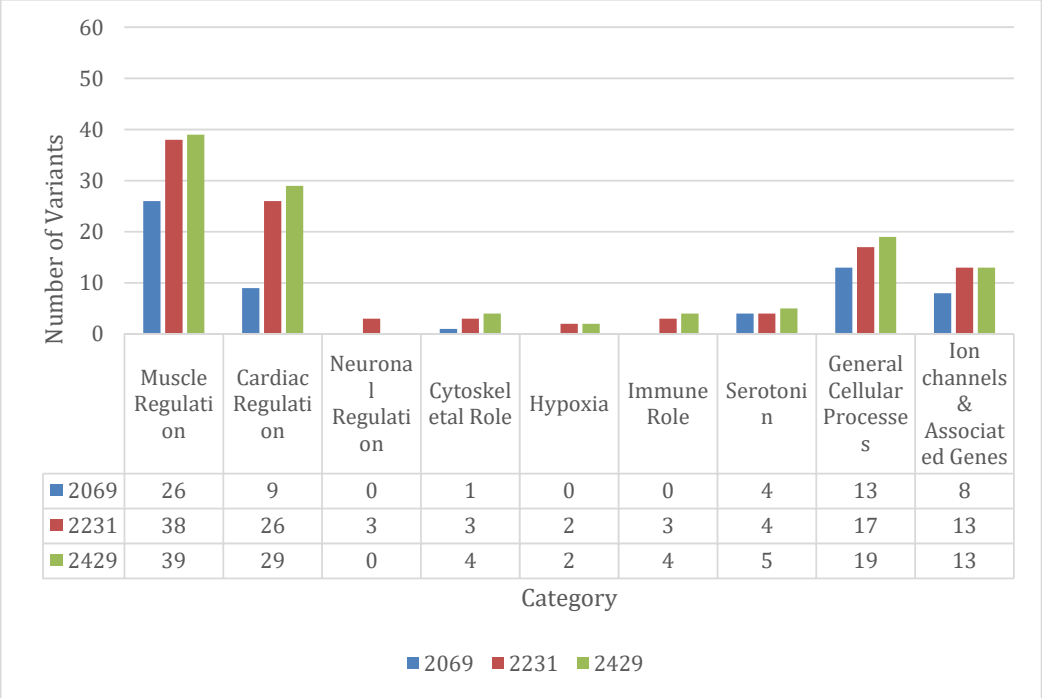


Figure 3.9. The number of pathogenic variants in Sudden Death (SD) candidate genes in the Sudden Unexpected Death in Epilepsy (SUDEP) probands (N=3). Pathogenicity was defined as scoring pathogenic for at least one of the five variant annotation methods; CADD Phred (>10), PolyPhen2 HDIV Phred (Possibly damaging or Damaging), SIFT Pred (Deleterious), PhastCons 100 Way Vertebrate (>0.84), and Grantham (>100).

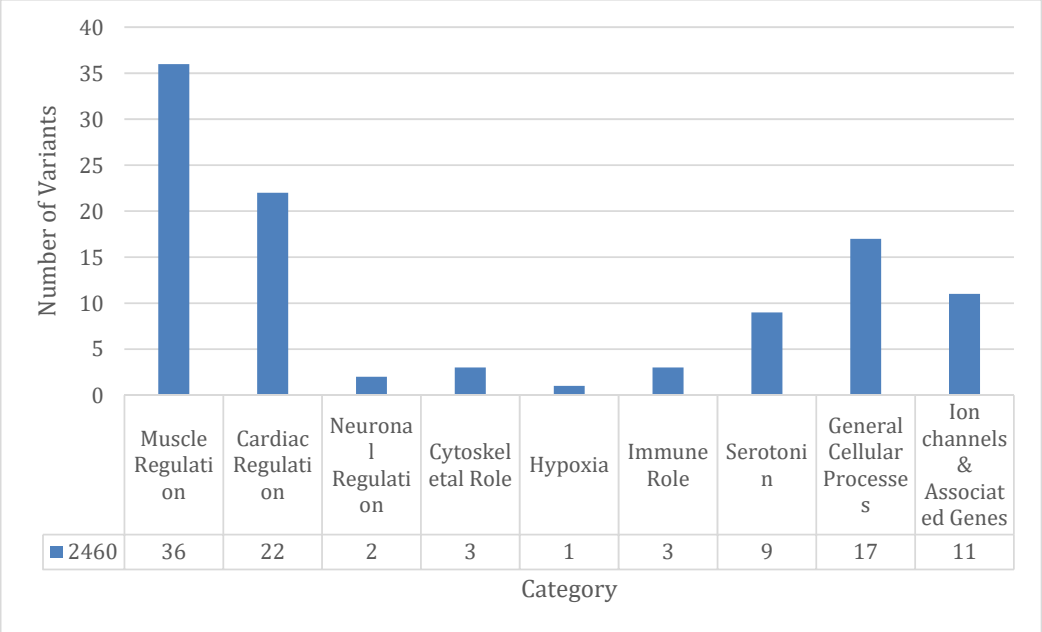


Figure 3.10. The number of pathogenic variants in Sudden Death (SD) candidate genes in the Sudden Unexpected Death (SUD) proband (N=1). Pathogenicity was defined as scoring pathogenic for at least one of the five variant annotation methods; CADD Phred (>10), PolyPhen2 HDIV Phred (Possibly damaging or Damaging), SIFT Pred (Deleterious), PhastCons 100 Way Vertebrate (>0.84), and Grantham (>100).

Figure 3.11. Allen Brain Atlas for the Developing Mouse Brain In Situ Hybridization image for *Mbp* expression at embryonic day 15.5 (E15.5), equivalent to a roughly 4 month old infant. Pathogenic variants found in *MBP* within the SUDEP probands and lacking from the SIDS probands.

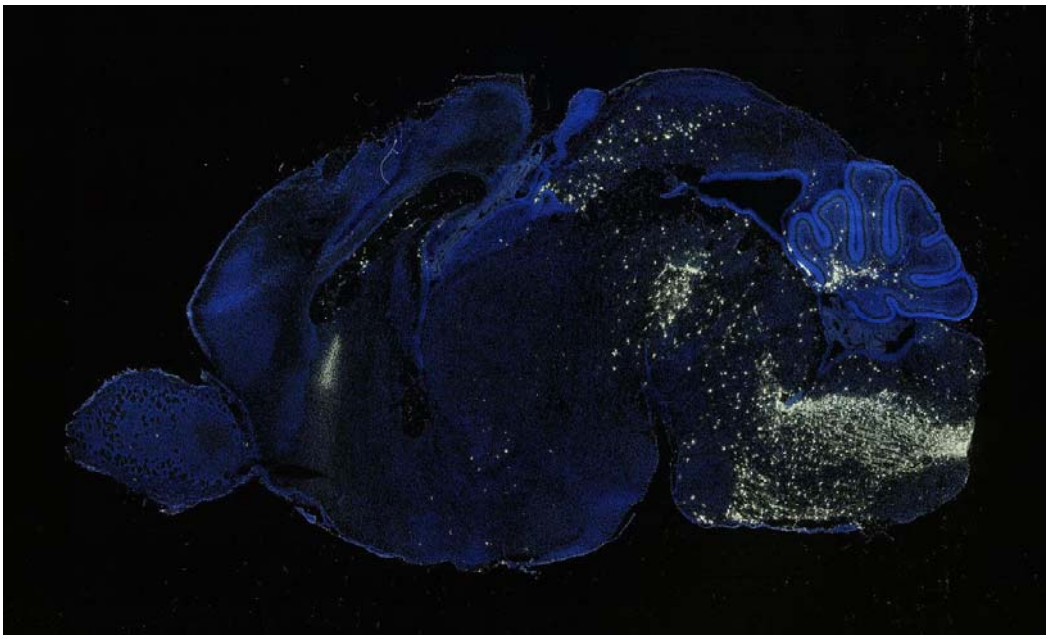


Figure 3.12. Allen Brain Atlas for the Developing Mouse Brain In Situ Hybridization image of *Mbp* expression at postnatal day 4 (P4), equivalent to roughly a 1 year old child. Pathogenic variants found in *MBP* within the SUDEP probands and lacking from the SIDS probands. An RNA antisense probe was used to detect expression levels.

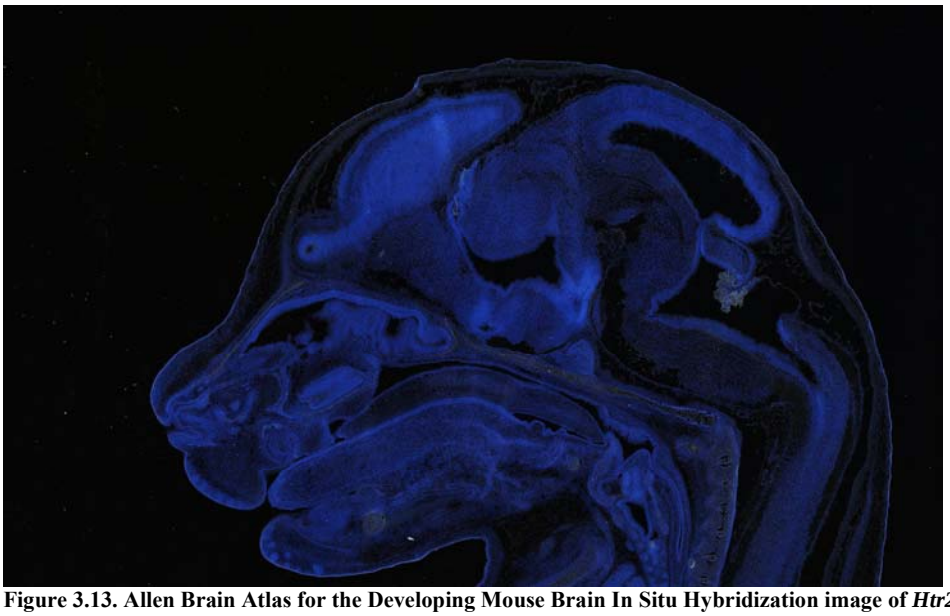


Figure 3.13. Allen Brain Atlas for the Developing Mouse Brain In Situ Hybridization image of *Htr2c* expression at embryonic day 15.5 (E15.5), equivalent to roughly a 4 month old infant. Pathogenic variants in *HTR2C* found within the SIDS probands and lacking from the SUDEP and SUD. An RNA antisense probe was used to detect expression levels.

Figure 3.14. Allen Brain Atlas for the Developing Mouse Brain In Situ Hybridization image for *Htr2c* expression at postnatal day 4 (P4), equivalent to roughly a 1 year old child. Pathogenic variants in *HTR2C* were found within the SIDS probands and lacking from the SUDEP and SUD probands. An RNA antisense probe was used to detect expression levels.

Table 3.1. Sudden death candidate genes with roles in muscle			
GENE	**LOCATION**	**Function**	**Reference**
TCAP	chr17:37,821,599-37,822,807	Mediates the antiparallel assembly of titin on the sarcomeric Z disk. Implicated in cancer.	GeneMANIA (113)
MYOM1	chr18:3,066,805-3,220,106	Component of the myofibrallar M band in muscle cells. Implicated in DCM.	Marston 2015 (79)
NEB	chr2:152,341,853-152,591,001	Encodes for Nebulin, part of the cytoskeletal matrix in the sarcomeres of skeletal muscle.	GeneMANIA (113)
SGCG	chr13:23,755,060-23,899,304	Encodes for Gamma-sarcogylcan, a sarcolemmal transmembrane glycoprotein, that interacts with dystrophin.	GeneMANIA (113)
MYL7	chr7:44,178,463-44,180,916	Encodes for Myosin Light Chain 7, a motor protein found in all eukaryotic cells.	GeneMANIA (113)
TNNI2	chr11:1861432-1862910	Encodes for a fast-twitch skeletal muscle protein, which is responsible for calcium-dependent regulation of striated muscle contraction.	GeneMANIA (113)
SPTAN1	chr9:131,314,837-131,395,941	Encodes for Spectrins, which are filamentous cytoskeletal proteins that function as scaffolds to stabilize the cell membrane and organelles.	Bagnall 2016 (81)
SLMAP	chr3:57,743,174-57,914,894	Component of conserved striatin-interacting phosphatase and kinase complex.	Ishikawa 2012 (148)
TTN	chr2:179,390,717-179,672,150	Structural protein found in striated muscle.	Campuzano 2014 (73)
LMNA	chr1:156,104,904-156,107,058	A component of the nucleoplasmic side of the inner nuclear membrane.	Guo 2015 (94)
PKP2	chr12:32,943,680-33,049,780	Links cadherins to intermediate filaments in the cytoskeleton.	Campuzano 2014 (73)
HRC	chr19:49,654,456-49,658,681	Sarcoplasmic reticulum protein that binds LDL.	GeneMANIA (113)
TRDN	chr6:123,537,484-123,958,238	Regulates Ca^{2+} release via RYR1 and RYR2, the calcium release channels in the sarcoplasmic reticulum.	Wilde and Behr 2013 (85)
SMCHD1	chr18:2,655,886-2,805,015	A component of the SMC (Structural Maintanence of Chromosomes) protein. Helps maintain X inactivation in females.	GeneMANIA (113)

Table 3.1. Sudden death candidate genes with roles in muscle			
GENE	**LOCATION**	**Function**	**Reference**
CKMT2	chr5:80,529,139-80,562,217	Encodes for mitochondrial creatine kinase, which transports high energy phosphate from the mitochondria to creatine.	GeneMANIA (113)
CHRNA1	chr2:175,612,320-175,629,200	Alpha subunit of the muscle acetylcholine receptor.	Hantai 2004 (149)

Table 3.2. Sudden death candidate genes with roles in cardiac regulation			
GENE	**LOCATION**	**Function**	**Reference**
MYL2	chr12:111,348,624-111,358,404	Encodes for the regulatory light chain that is associated with the cardiac myosin beta (or slow) heavy chain. Plays a role in HCM.	Noseworthy 2008 (92)
MYL3	chr3:46,899,357-46,904,973	Encodes for the myosin light chain 3, an alkali light chain. Plays a role in HCM.	Noseworthy 2008 (92)
GLA	chrX:100,652,779-100,663,001	Encodes for a homodimeric glycoprotein that hydrolyses the terminal alpha-galactosyl moieties in glycolipids and glycoproteins. Plays a role in HCM.	Marston 2015 (79)
LAMP2	chrX:119,560,003-119,603,204	Encodes for membrane glycoproteins. Plays a role in HCM.	Miani 2012 (91)
MYOZ2	chr4:120,056,939-120,108,944	Encodes for a sarcomeric protein that binds to calcineurin, a phosphatase involved in calcium-dependent signal transduction. Plays a role in HCM.	Noseworthy 2008 (92)
PRKAG2	chr7:151,253,201-151,574,316	Encodes for a component of AMP-activated protein kinase. Plays a role in HCM.	Bagnall 2014 (150)
TNNI3	chr19:55,663,136-55,668,957	Encodes for a subunit of the troponin complex of the thin filaments in striated muscle. Plays a role in HCM.	Bagnall 2014 (150)
CSRP3	chr11:19,203,577-19,232,118	Encodes for a protein involved in regulatory processes, as part of cellular differentiation and development. Plays a role in HCM & DCM.	Bagnall 2014 (150)
ACTN2	chr1:236,849,770-236,927,558	Encodes for a protein that ancors the myofibrillar actin filaments in skeletal	Bagnall 2014 (150)

Table 3.2. Sudden death candidate genes with roles in cardiac regulation			
GENE	**LOCATION**	**Function**	**Reference**
		muscle cells. Plays a role in HCM.	
TPM1	chr15:63,334,838-63,364,113	Encodes for an actin-binding protein that plays a role in contraction within smooth and striated muscle cells. Plays a role in HCM & DCM.	Bagnall 2014 (150)
PLN	chr6:118,869,442-118,881,587	Encodes a substrate for cAMP-dependent protein kinase in cardiac muscle cells. Plays a role in HCM.	Raghow 2016 (89)
MYBPC3	chr11:47,353,396-47,374,253	Encodes for the cardiac myosin-binding protein C. It is only expressed in heart muscle. Plays a role in HCM & DCM.	Bagnall 2014 (150)
TNNC1	chr3:52,485,107-52,488,057	Encodes for a troponin subunit, found on the actin filament in muscle cells. Plays a role in HCM & DCM.	Bagnall 2014 (150)
ACTC1	chr15:35,080,297-35,087,927	Encodes for actin, expressed in cardiac muscle cells. Plays a role in HCM, DCM & LVNC.	Campuzano 2015 (61)
MYH7	chr14:23,881,947-23,904,870	Encodes for the heavy chain of cardiac muscle myosin. Plays a role in HCM, DCM & LVNC.	Bagnall 2014 (150)
SNTA1	chr20:31,995,763-32,031,698	Encodes for a cytoplasmic peripheral membrane scaffold protein. Plays a role in Long QT syndrome.	Adler 2015 (53)
MYH6	chr14:23,851,199-23,877,486	Encodes for the heavy chain of cardiac muscle myosin.	Bagnall 2014 (150)
TNNT1	chr19:55,644,161-55,660,606	Encodes for a subunit of troponin, located on the thin filament of the sarcomere. Plays a role in DCM.	Bagnall 2014 (150)
TNNT2	chr1:201,328,136-201,346,890	Encodes for a tropomyosin-binding subunit of the troponin complex, located on the	Bagnall 2014 (150)

Table 3.2. Sudden death candidate genes with roles in cardiac regulation			
GENE	**LOCATION**	**Function**	**Reference**
		thin filament of striated muscle. Plays a role in HCM and DCM.	
BAG3	chr10:121,410,882-121,437,329	Bind to the Hsc70/Hsp70 ATPase domain. Plays a role in DCM.	Knezevic 2015 (93)
RBM20	chr10:112,404,155-112,599,227	Encodes a protein that is able to regulate splicing and binds to RNA. Plays a role in DCM.	Guo 2012 (94)
CTF1	chr16:30,907,928-30,914,881	Encodes for Cardiotropin 1, which binds to ILST/gp130 receptors. Plays a role in DCM.	Walsh 2016 (95)
DES	chr2:220,283,099-220,291,461	Encodes for a muscle specific intermediate filament protein. Plays a role in DCM.	Bagnall 2014 (150)
EMB	chr5:49,692,031-49,737,234	Encodes for a transmembrane glycoprotein involved in cell growth. Plays a role in DCM.	GeneMANIA (113)
TAZ	chrX:153,639,877-153,650,063	Encodes for a protein expressed in cardiac and skeletal muscle. Plays a role in DCM & VCN.	Bagnall 2014 (150)
NPPA	chr1:11,905,767-11,907,840	Encodes for Cardiodilatin-related peptide, involved in regulating extracellular fluid volume. Plays a role in DCM.	GeneMANIA (113)
GJA5	chr1:147,228,332-147,232,714	Encodes for connexin, which is a part of gap functions. Plays a role in Familial AF.	Bagnall 2014 (150)
CAV3	chr3:8,775,486-8,788,451	Encodes for caveolin, which are scaffolding proteins. Plays a role in HCM.	Adler 2015 (53)
MIA3	chr1:222,791,444-222,841,351	Encodes a protein required for collagen release. Implicated in heart disease.	GeneMANIA (113)

Table 3.2. Sudden death candidate genes with roles in cardiac regulation			
GENE	**LOCATION**	**Function**	**Reference**
ANKRD1	chr10:92,671,857-92,681,032	Found in the endothelium and is induced by IL1 and TNF-alpha. Plays a role in DCM.	Bogomolovas 2014 (151)
PDE3A	chr12:20,522,179-20,837,041	Encodes a protein involved in cardiovascular function, by regulating smooth muscle relaxation and contraction. Implicated in cancer.	GeneMANIA (113)
POPDC2	chr3:119,360,908-119,379,404	Transmembrane protein in skeletal and cardiac muscle.	GeneMANIA (113)
TNNI3K	chr1:74,663,947-75,010,112	MAP Kinase involved in cardiac physiology.	Theis 2014 (152)
RYR2	chr1:237205702-237997288	Calcium channel found in cardiac muscle cells. Causes the release of calcium ions that triggers cardiac muscle contraction.	Wilde and Behr 2013 (85)
TIE1	chr1:43,776,664-43,788,779	Tyrosine kinase that plays a role in angiogenesis and blood vessel stability.	Leu 2015 (84)
GPD1L	chr3:32,148,003-32,210,207	Encode a protein that binds to sodium ion channel SCN5A. Implicated in Brugada syndrome.	Adler 2016 (53)
OBSCN	chr1:228,395,831-228,566,575	Sarcomeric signaling protein implicated in HCM.	Marston 2015 (79)
DAG1	chr3:49,507,565-49,573,051	Encodes for the Dystroglycan protein. Plays a role in HCM.	GeneMANIA (113)
CASQ2	chr1:116,242,626-116,311,426	Calsequestrin, located in the sarcoplasmic reticulum of cardiac cells	Wilde and Behr 2013 (85)
VCL	chr10:75,757,872-75,879,914	Encodes for a cytoskeletal protein associated with cell-matrix and cell-cell junctions. Plays a role in HCM & DCM.	Campuzano 2014 (73)
DSP	chr6:7,541,870-7,586,946	Encodes a protein that	Bagnall 2014

Table 3.2. Sudden death candidate genes with roles in cardiac regulation			
GENE	**LOCATION**	**Function**	**Reference**
		connects intermediate filaments to desmosomal plaques forms a component of functional desmosomes. Plays a role in HCM.	(150)
RYR3	chr15:33,603,177-34,158,303	Encodes for a ryanodine receptor, functions to release calcium from intracellular storage. Implicated in arrhythmias.	Klassen 2014 (3)
AKAP9	chr7:91,570,189-91,739,987	Encodes a protein that binds to the regulatory subunit of protein kinase A. Plays a role in arrhythmias, Brugada syndrome, and Long QT syndrome.	Allegue 2015 (82)
NPPB	chr1:11917521-11918992	Encodes for Natiuretic Peptide B. High levels of this protein indicate heart failure.	GeneMANIA (113)
LDB3	chr10:88,428,426-88,495,824	Encodes a PDZ domain-containing protein, which interacts with other proteins in cytoskeleton assembly. Plays a role in LVNC.	Hata 2016 (153)

Table 3.3. Sudden death candidate genes that encode for ion channels and associated proteins			
GENE	**LOCATION**	**Function**	**Reference**
HCN4	chr15:73,612,200-73,661,605	Encodes for a voltage-gated potassium channel. Implicated in Brugada Syndrome and SUDEP.	Campuzano 2014 (62,73)
CACNA1C	chr12:2,080,229-2,080,366	Encodes for a voltage-gated calcium channel. Implicated in cardiac arrhythmias, epilepsy, and Sudden Cardiac Death.	Wilde and Behr 2013 (85)
CACNA2D1	chr7:81,579,418-82,073,031	Encodes for a voltage-gated calcium channel. Implicated in short QT syndrome, HCM, and Brugada syndrome.	Wilde and Behr 2013 (85)
CACNB2	chr10:18,429,606-18,830,688	Encodes for a voltage-gated calcium channel. Implicated in Brugada syndrome, cardiac arrhythmias, Sudden Cardiac Death and HCM.	Wilde and Behr 2013 (85)
KCND3	chr1:112,318,454-112,531,777	Encodes for a voltage-gated potassium channel. Implicated in Brugada syndrome, cardiac arrhythmias, and Sudden Cardiac Death.	Wilde and Behr 2013 (85)
KCNIP1	chr5:169,780,881-170,163,636	Encodes for a voltage-gated potassium channel. Implicated in DCM.	Horn 2006 (154)
KCNIP3	chr2:95,963,072-96,051,825	Encodes for a voltage-gated potassium channel.	GeneMANIA (113)
KCNE3	chr11:74,165,886-74,178,600	Encodes for a voltage-gated potassium channel. Implicated in Brugada syndrome,	Wilde and Behr 2013 (85)

Table 3.3. Sudden death candidate genes that encode for ion channels and associated proteins			
GENE	**LOCATION**	**Function**	**Reference**
		cardiac arrhythmias, and sudden cardiac death.	
KCNE4	chr2:223,916,648-223,920,357	Encodes for a voltage-gated potassium channel.	GeneMANIA (113)
KCNE1	chr21:35,818,986-35,828,107	Encodes for a voltage-gated potassium channel. Implicated in Long QT syndrome, Epilepsy, and Sudden Cardiac Death.	Wilde and Behr 2013 (85)
KCNE2	chr21:35,736,323-35,743,440	Encodes for a voltage-gated potassium channel. Implicated in Long QT syndrome, epilepsy, Sudden Cardiac Death and cardiac arrhythmias.	Wilde and Behr 2013 (85), Andreason 2013 (155)
KCNH2	chr7:150,642,044-150,675,402	Encodes for a voltage-gated potassium channel. Implicated in Long QT syndrome, epilepsy, Sudden Cardiac Death and cardiac arrhythmias.	Tu 2011 (60)
KCNH7	chr2:163,227,917-163,695,257	Encodes for a voltage-gated potassium channel.	GeneMANIA (113)
KCNJ2	chr17:68,165,676-68,176,183	Encodes for a voltage-gated potassium channel. Implicated in Short QT syndrome, cardiac arrhythmias, and Sudden Cardiac Death.	Wilde and Behr 2013 (85)
KCNJ3	chr2:155,555,093-155,714,864	Encodes for a voltage-gated potassium channel. Also involved in hypoxia (check ref).	Neary 2013 (1)
KCNJ5	chr11:128,761,313-128,787,951	Encodes for a voltage-gated potassium channel.	Wilde and Behr 2013 (85)

Table 3.3. Sudden death candidate genes that encode for ion channels and associated proteins			
GENE	**LOCATION**	**Function**	**Reference**
		Involved in Long QT Syndrome, Sudden Cardiac Death, and cardiac arrhythmias.	
KCNJ8	chr12:21,917,889-21,927,755	Encodes for a voltage-gated potassium channel.	Wilde and Behr 2013 (85)
KCNQ1	chr11:2,466,221-2,870,340	Encodes for a voltage-gated potassium channel. Implicated in Long QT, SIDS, Sudden Cardiac Death and SUDEP.	Tu 2011 (60)
SCN1B	chr19:35,521,592-35,525,174	Encodes for one of the beta subunit of the voltage-gated sodium channel.	Wilde and Behr 2013 (85)
SCN1A	chr2:166,845,670-166,930,180	Encodes for one of the alpha subunit of the voltage-gated sodium channel.	Wilde and Behr 2013 (85), Klassen 2013 (116), Kalume 2013 (13), Ferrari 2015 (156)
SCN2A	chr2:166,152,283-166,248,820	Encodes for one of the alpha subunit of the voltage-gated sodium channel. Implicated in early infantile epileptic encephalopathy.	Lemke 2012 (157), Howell 2015 (158)
SCN2B	chr11:118,033,519-118,047,337	Encodes for one of the beta subunits of the voltage-gated sodium channel. Implicated in atrial fibrillation.	Winkel 2015 (55)
SCN3B	chr11:123,499,895-123,525,315	Encodes for one of the beta subunits of the voltage-gated sodium channels. Implicated in Brugada syndrome, cardiac arrhythmias, and Sudden Cardiac Death.	Adler 2016 (53), Wilde and Behr 2013 (85)
SCN4B	chr11:118,004,092-118,023,630	Encodes for one of the beta subunits of the voltage-gated sodium channels.	Adler 2016 (53), Wilde and Behr 2013 (85)

Table 3.3. Sudden death candidate genes that encode for ion channels and associated proteins			
GENE	**LOCATION**	**Function**	**Reference**
		Implicated in Long QT Syndrome, cardiac arrhythmias, and Sudden Cardiac Death.	
SCN5A	chr3:38,589,553-38,674,850	Encodes for one of the alpha subunits of the voltage-gated sodium channels. Implicated in Long QT Syndrome, Brugada Syndrome, Epilepsy, Sudden Unexpected Death in Epilepsy, cardiac arrhythmias, and Sudden Cardiac Death.	Tu 2011 (60), Wilde and Behr 2013 (85)
SCN7A	chr2:167,260,083-167,343,481	Encodes for an alpha subunit of the voltage-gated sodium channels. Involved in hypoxia (ref) and neonatal epilepsy.	Okumura 2011 (159)
SCN8A	chr12:51,985,020-52,206,648	Encodes for an alpha subunit of the voltage-gated sodium channels.	Veeramah 2012 (160) , Wagnon 2014
SCN9A	chr2:167,051,697-167,232,497	Encodes for an alpha subunit of the voltage-gated sodium channels. Implicated in neuropathic pain.	Li 2015 (161)
TRPM4	chr19:49,661,016-49,715,098	Encodes for a calcium-activated nonselective ion channel, able to move monovalent cations across the membrane. Implicated in Brugada Syndrome.	Wilde and Behr 2013 (85)
KCNJ11	chr11:17,406,796-17,410,206	Encodes for a voltage gated, inward rectifying potassium channel. Implicated in Permanent Neonatal Diabetes Mellitus.	Martins 2015 (162)

Table 3.3. Sudden death candidate genes that encode for ion channels and associated proteins			
GENE	**LOCATION**	**Function**	**Reference**
KCNJ1	chr11:128,707,909-128,712,429	Encodes for a voltage-gated, inward rectifying potassium channel. Implicated in Neonatal Bartter Syndrome.	GeneMANIA (113)
HCN1	chr5:45,254,950-45,696,498	Encodes for a hyperpolarized-activated cyclic nucleotide potassium channel. Implicated in Early Infantile Epileptic Encephalopathy and cardiac arrhythmias.	Wilde and Behr 2013 (85)
HCN2	chr19:589,893-617,159	Encodes for a hyperpolarized-activated cyclic nucleotide potassium channel. Implicated in Sinoatrial Node Disease, epilepsy and cardiac arrhythmias.	Wilde and Behr 2013 (85)
KCNA1	chr12:5,019,071-5,040,527	Encodes for a voltage-gated potassium channel. Implicated in Epilepsy, SUDEP, and cardiac arrhythmias.	Glasscock 2010 (88), Klassen 2013 (116)
KCNA5	chr12:5,153,085-5,155,954	Encodes for a voltage-gated potassium channel. Implicated in Familial atrial fibrillation (AF).	Suzuki 2012 (102)
KCNEIL (also known as *KCNE5*)	chrX:108,866,929-108,868,393	Encodes for a voltage-gated potassium channel. Implicated in Brugada Syndrome.	Skinner 2005 (103)
CALM1	chr14:90,863,327-90,874,619	Encodes for Calmodulin 1, which is a EF-hand calcium-binding protein.	Wilde and Behr 2013 (85)
RANGRF	chr17:8,191,969-8,193,409	Encodes for a protein that	Wilde and Behr 2013 (85)

Table 3.3. Sudden death candidate genes that encode for ion channels and associated proteins			
GENE	**LOCATION**	**Function**	**Reference**
		functions to regulation the function of Nav1.5 cardiac sodium channel.	
RYR1	chr19:38,924,340-39,078,204	Calcium release channel in sarcoplasmic reticulum of skeletal muscle.	Lanner 2012 (163)
ATP1A2	chr1:160,085,549-160,113,381	Encodes for a subunit of the Na/K transport ATPase. Implicated in Familial Hemiplegic Migraines.	Ferrari 2015 (156)
KCNT1	chr9:138,594,031-138,684,992	Encodes for a voltage-gated potassium channel. Implicated in epilepsy.	EuroEPINOMICS 2014 (164), Moller 2015 (109)
KCNIP2	chr10:103,585,731-103,603,677	Encodes for a voltage-gated potassium channel.	GeneMANIA (113)
NIPA2	chr15:23,004,684-23,034,427	Encodes for a Selective Mg2+ transporter. Implicated in Angelman syndrome.	GeneMANIA (113)
TRPM6	chr9:77,337,411-77,503,010	Encodes a protein that contains both a protein kinase and an ion channel domain. Implicated in atrial fibrillation.	Zhang 2015 (165)
SCNM1	chr1:151,129,140-157,142,773	Encodes for a zinc finger, which modifies expression of SCN8A.	GeneMANIA (113)
SLC8A1	chr2:40,339,286-40,657,444	Encodes for a solute carrier protein, that exchanges Na and Ca across the membrane.	GeneMANIA (113)
ATP1A3	chr19:42,470,734-42,498,428	Encodes for a subunit of the Na/K transport ATPase.	Paciorkowski 2015 (166)

Table 3.3. Sudden death candidate genes that encode for ion channels and associated proteins			
GENE	**LOCATION**	**Function**	**Reference**
		Implicated in early life epilepsy, episodic prolonged apnea, and postnatal microcephaly.	

Table 3.4. Sudden death candidate genes with roles in neuronal regulation			
GENE	**LOCATION**	**Function**	**Reference**
CALM2	chr2:47,387,221-47,403,740	Encodes calmodulin, involved in binding to calcium. Plays a role in Long QT, epilepsy, and catecholaminergic polymorphic ventricular tachycardia (CPVT).	Wilde and Behr 2013 (85)
GAP43	chr3:115,342,151-115,440,334	Growth associated protein 43, expressed at high levels during neuronal development.	Salomonis 2014 (77)
MBP	chr18:74,690,789-74,844,774	Myelin sheath development and glial cell differentiation	Salomonis 2014 (77)
PLP1	chrX:103,031,754-103,047,547	Myelin sheath development and glial cell differentiation	GeneMANIA (113)
TPPP	chr5:659,977-693,510	Myelin sheath development and glial cell differentiation	Salomonis 2014 (77)
TUBB4A	chr19:6,494,330-6,502,330	Encodes for a member of the beta tubulin family, eventually assembling to form microtubules. Associated with Spastic Paraplegia.	Kumar 2015 (167)
GJA1	chr6:121,756,745-121,770,873	Encodes for connexin and forms a component of gap junctions, allowing small molecules to pass between cells.	Andreason 2013 (155)
MAOA	chrX:43,514,155-43,606,071	Encodes mitochondrial enzymes that cause oxidative deamination of neurotransmitters, such as dopamine, serotonin, and norepinephrine.	Grob 2014 (63)
PHOX2B	chr4:41,746,099-41,750,987	Encodes a protein that acts as a transcription factor, involved in the development of neurons and	Liebrechts-Akkerman 2014 (78), Salomonis 2014 (77)

Table 3.4. Sudden death candidate genes with roles in neuronal regulation			
GENE	**LOCATION**	**Function**	**Reference**
		neurotransmitters.	
NFATC1	chr18:77,160,326-77,289,323	Encodes for a component of the nuclear factor of activated T cells.	GeneMANIA (113)
NXT2	chrX:108,779,010-108,787,927	Encodes a protein that contains a nuclear transport factor 2 (NTF2) domain. Plays an important role in transporting molecules between the cytoplasm and nucleus.	GeneMANIA (113)
SLC1A3	chr5:36,606,457-36,688,436	Encodes for a high affinity glutamate transporter.	Salomonis 2014 (77)
SLC25A4	chr4:186,064,417-186,071,538	Encodes for a gated pore that translocates ADP from the cytoplasm to the mitochondrial matrix. Also transports ATP from the mitochondrial matrix to the cytoplasm.	Salomonis 2014 (77)
SNAP25	chr20:10,199,477-10,288,066	Encodes for a presynaptic plasma membrane protein involved in regulation of neurotransmitter release.	Salomonis 2014 (77)
VAMP2	chr17:8,062,465-8,066,293	Encodes for a vesicle-associated membrane protein and regulates synaptic transmission. Implicated in familial infantile myasthenia (respiratory depression).	Salomonis 2014 (77)
NOS1AP	chr1:162,069,774-162,370,475	Cytosolic protein that binds neuronal NOS. Implicated in Long QT syndrome.	Allegue 2015 (82)
CHD2	chr15:93,426,526-	Involved in chromatin	EuroEPINOMICS

Table 3.4. Sudden death candidate genes with roles in neuronal regulation			
GENE	**LOCATION**	**Function**	**Reference**
	93,571,237	remodeling.	2014 (164)
TBC1D24	chr16:2,525,147-2,555,735	Involved in membrane trafficking in the brain. Implicated in familial infantile myoclonic epilepsy.	Falace 2010 (168)
STXBP1	chr9:130,374,544-130,457,460	Role in neurotransmitter release via regulation of syntaxin, a transmembrane attached protein receptor. Implicated in Dravet Syndrome.	EuroEPINOMICS 2014 (164), Carvill 2014 (108)
GABRB3	chr15:26,543,546-26,939,539	Subunit of a ligand-gated chloride channel in the brain. Implicated in absence epilepsy.	DeLorey 1999 (169)
CHRNA7	chr15:32,322,691-32,464,722	Encodes for a subunit of the brain nicotinic acetylcholine receptor.	Machaalani 2011 (170)
CHRNA4	chr20:61,974,575-62,009,753	Encodes for a subunit of the brain nicotinic acetylcholine receptor	Chen 2015 (76)
CHRNA2	chr8:27,317,279-27,337,400	Encodes for a subunit of the brain nicotinic acetylcholine receptor.	Chen 2015 (76)
SLC12A5	chr20:44,650,329-44,688,784	Neuronal K-Cl transporter that lowers intracellular chloride concentrations.	Stodberg 2015 (171)
PAFAH1B1	chr17:2,496,504-2,588,909	Encodes for a protein that is required during brain development and neuronal proliferation.	Bagnall 2014 (150)
EN1	chr2:119,599,747-119,605,759	Encodes a protein involved in pattern formation during development of nervous system	Campuzano 2014 (73)
APH1A	chr1:150,237,799-150,241,609	Encodes a component of the gamma secretase complex, which cleaves	GeneMANIA (113)

Table 3.4. Sudden death candidate genes with roles in neuronal regulation			
GENE	**LOCATION**	**Function**	**Reference**
		integral membrane proteins. Has been implicated in Altzeimers.	
SLC1A4	chr2:65,216,495-65,251,000	Encodes for a Glutamate/Neutral Amino Acid Transporter. Implicated in Infantile Spasms.	Conroy 2016 (172)
CDKL5	chrX:18,425,583-18,653,629	Encodes for a Ser/Thr protein kinase. Implicated in Early Infantile Epileptic Encephalopathy and X-linked Infantile Spasms Syndrome.	EuroEPINOMICS 2014 (164)
SLC2A1	chr1:43,391,052-43,424,530	Encodes a glucose transporter in the blood brain barrier.	GeneMANIA (113)

Table 3.5. Sudden death candidate genes with roles in the cytoskeleton			
GENE	LOCATION	Function	Reference
ANK2	chr4:113,970,785-114,304,896	Encodes an ankyrin protein, which links integral proteins to the cytoskeleton.	Wilde and Behr 2013 (85)
KRT2	chr12:53,038,342-53,045,959	Encodes a keratin protein, which is expressed in epithelial cells.	Salomonis 2014 (77)
KRT9	chr17:39,722,094-39,728,310	Encodes a keratin protein, which is expressed in epithelial cells.	Salomonis 2014 (77)
KRT17	chr17:39,775,692-39,780,882	Encodes a keratin protein, which is expressed in epithelial cells.	GeneMANIA (113)
LAMC3	chr9:133,884,469-133,969,860	Encodes for Laminins, extracellular glycoproteins.	GeneMANIA (113)
PDLIM2	chr8:22,436,254-22,451,810	Encodes a protein involved in cell migration and adhesion. Implicated in cancer.	GeneMANIA (113)
MAPT	chr17:43,971,748-44,105,699	Encodes for microtubule-associated protein tau, expressed in the nervous system. Alternative isoforms are expressed at different stages, depending on neuronal maturation and neuron type.	Salomonis 2014 (77)
DSG2	chr18:29,078,027-29,128,814	Encodes a desmoglein protein, which is involved in cell-cell junctions. Plays a role in arrhythmias.	GeneMANIA (113)
JUP	chr17:39,910,859-39,942,964	Encodes a protein, found in the cytoplasm, which is a component of desmosomes and intermediate junctions.	Forleo 2015 (173)
VSIG1	chrX:107,288,200-107,322,414	Encodes a junctional adhesion molecule. Found to be expressed in cancer cells.	GeneMANIA (113)
DSC1	chr18:28709214-28742819	Encodes a calcium-dependent glycoprotein, expressed in epithelial cells and involved in cell-cell adhesion.	GeneMANIA (113)
CXADR	chr21:18,884,700-18,965,897	Encodes a group B coxsackievirus and subgroup C adenovirus receptor. Implicated in cancer.	GeneMANIA (113)
PCDH19	chrX:99,546,642-99,665,271	Encodes a calcium-dependent cell adhesion protein expressed in the brain.	Redies 2012 (174), Kwong 2012 (175)
ERMAP	chr1:43,282,795-43,310,660	Encodes for a transmembrane protein.	GeneMANIA (113)

Table 3.6. Sudden death candidate genes with roles in general cell processes			
GENE	**LOCATION**	**Function**	**Reference**
PCNT	chr21:47,744,036-47,865,682	Encodes a protein involved in cell adhesion.	GeneMANIA (113)
CCAR1	chr10:70,480,971-70,551,309	Encodes a protein involved in transcription regulation.	GeneMANIA (113)
TGFB3	chr14:76,424,442-76,448,092	Encodes a protein involved in embryogenesis and cell differentiation. Implicated in arrhythmias.	Dashash 2006 (71)
TMEM43	chr3:14,166,440-14,185,180	Encodes a protein involved in maintaining the nuclear envelope structure. Implicated in familial arrhythmagenic right ventricular dysplasia type 5 (ARVD5).	Siragam 2014 (83)
FAM213A	chr10:82,167,585-82,192,753	Encodes a protein involved in redox regulation of the cell. Implicated in cancer.	GeneMANIA (85)
LGI1	chr10:95,517,566-95,557,916	Encodes a protein involved in voltage-gated potassium channel regulation. The protein may also be involved in neuronal growth regulation and cell survival.	Leu 2015 (84), Klein 2016 (176)
SMC4	chr3:160,117,062-160,152,750	Encoded protein is involved in structural maintenance of chromosomes.	Leu 2015 (84)
COL6A3	chr2:238,232,646-238,323,018	Encodes for collagen, which is a cell-binding protein. Implicated in muscular dystrophy and myopathy.	Leu 2015 (84)
ALG13	chrX:110,909,043-111,003,877	Encodes a subunit of the bipartite UDP-acetylglucosamine transferase enzyme. Implicated in X-linked intellectual	EuroEPINOMICS 2014 (164)

Table 3.6. Sudden death candidate genes with roles in general cell processes			
GENE	**LOCATION**	**Function**	**Reference**
		disability and Congenital Disorders of Glycosylation.	
FASN	chr17:80,036,214-80,056,208	Encodes for Fatty Acid Synthetase.	EuroEPINOMICS 2014 (164)
DNM1	chr9:130,965,658-131,017,527	Encodes for GTP-binding protein.	EuroEPINOMICS 2014 (164)
APPBP2	chr17:58,520,520-58,603,580	Encodes for a protein involved in intracellular protein transport and regulated cell death. Implicated in cancer and Alzheimers.	Coppola 2013 (177)
PTRH2	chr17:57,751,997-57,784,987	Promotes caspase-independent apoptosis. Involved in infantile-onset multisystem neurological, endocrine, and pancreatic disease.	Coppola 2013 (177)
CLTC	chr17:57.697,219-57,773,671	Encodes Clathrin, a protein that is present on the cytoplasmic side of organelles.	Coppola 2013 (177)
TUBD1	chr17:57,936,851-57,970,304	Encodes for tubulin, which make up Microtubules.	Coppola 2013 (177)
CSTB	chr21:45,192,393-45,196,326	Encodes an intracellular thiol proteinase inhibitor. Implicated in epilepsy.	Striana 2010 (178)
CHD9	chr16:53,088,945-53,361,414	Encodes for Chromodomain helicase DNA brinding protein 9, which is a transcriptional coactivator of PPARA.	GeneMANIA (113)
CSTF2T	chr10:53,455,246-53,459,355	Encodes a protein involved in cleaving the 3' end of RNA in preparation for adding on a PolyA tail.	GeneMANIA (113)
COPZ2	chr17:46,103,533-	Encodes a subunit of	GeneMANIA (113)

Table 3.6. Sudden death candidate genes with roles in general cell processes			
GENE	**LOCATION**	**Function**	**Reference**
	46,115,152	the coatomer protein complex 2, involved in cellular vesicle formation.	
AP2B1	chr17:33,914,282-34,053,436	Encodes a protein that binds clathrins to vesicles.	Pignatelli 2012 (179), GeneMANIA (113)
AKR1C3	chr10:5,090,958-5,149,878	Encodes a Aldo-keto Reductase enzyme. Involved in cell growth regulation and reduction of prostaglandins.	GeneMANIA (113)
APAF1	chr12:99,039,078-99,129,211	Encodes a protein involved in initiating apoptosis.	GeneMANIA (113)
GOT1	chr10:101,156,627-101,190,530	Encodes an enzyme involved in amino acid metabolism.	Salomonis 2014 (77)
GOT2	chr16:58,741,035-58,768,246	Encodes an enzyme involved in amino acid metabolism.	Salomonis 2014 (77)
BAX	chr19:49,458,117-49,464,519	Encodes BCL2-associated X protein, which accelerates programmed cell death.	Tehranian 2008 (180)
CHD7	chr8:61,591,324-61,780,586	Encodes a protein involved in rRNA synthesis. Implicated in CHARGE syndrome, which involves heart defects and abnormal growth and development.	Fujita 2009 (181)
CPLX1	chr4:778,745-819,945	Encodes a protein involved in synaptic vesicle exocytosis. Implicated in Wolf-Hirschhorn Syndrome, which involves seizures and abnormal growth and development.	Glynn 2007 (182)
LACTB2	chr8:71,549,501-71,581,447	Encodes for Lactamase, beta 2.	GeneMANIA (113)
MANEA	chr6:96,025,373-96,057,328	Encodes an enzyme which is able to cleave oligosaccharides.	GeneMANIA (113)
GAPDHS	chr19:36,024,314-36,036,218	Encodes an enzyme involved in	Salomonis 2014 (77)

Table 3.6. Sudden death candidate genes with roles in general cell processes			
GENE	**LOCATION**	**Function**	**Reference**
		carbohydrate metabolism.	
SGPP1	chr14:64,150,935-64,194,756	Encodes a protein involved in sphingolipid metabolism.	GeneMANIA (113)
ARID4B	chr1:235,330,210-235,491,532	Encodes a protein involved in cellular proliferation, apoptosis and differentiation.	GeneMANIA (113)
SMARCA5	chr4:144,434,616-144,478,642	Encodes a gene involved in chromatin remodeling. Implicated in cancer.	GeneMANIA (113)
TNKS2	chr10:93,558,151-93,625,232	Encodes a protein involved in telomere lengthening and apoptosis. Implicated in cancer	GeneMANIA (113)
TMEM123	chr11:102,267,056-102,323,775	Encodes a protein involved in oncosis.	GeneMANIA (113)
ERBB2	chr17:37,856,254-37,884,915	Encodes for erythoblastic leukemia viral oncogene homolog 2. Implicated in cancer.	GeneMANIA (113)
VIT	chr2:36,923,833-37,041,937	Encodes for Vitrin, involved in cell adhesion.	GeneMANIA (113)
ZNF709	chr19:12,571,998-12,595,632	Encodes for Zinc finger protein 209, involved in transcription regulation. Implicated in cancer.	GeneMANIA (113)
ZZZ3	chr1:78,030,190-78,148,343	Encodes for Zinc finger, ZZ-type containing 3, involved in transcription regulation.	GeneMANIA (113)
ZNF44	chr19:12,382,625-12,405,714	Encodes for Zinc-finger, protein 44, involved in transcription regulation.	Bassuk 2013 (183)
BAD	chr11:64,037,302-64,052,176	Encodes a protein involved in programmed cell death.	GeneMANIA (113)
NPRL3	chr16:84,271-138,860	Encodes a protein	Ricos 2015 (184)

Table 3.6. Sudden death candidate genes with roles in general cell processes			
GENE	**LOCATION**	**Function**	**Reference**
		involved in increased GTP hydrolysis in the TORC1 pathway.	
FAM71A	chr1:212,797,789-212,800,120	Encodes a protein of unknown function, known as Family with Sequence Similarity 71A.	GeneMANIA (113)
C15orf41	chr15:36871812-37102449	Implicated in Congenital Dyserythropoietic Anemia Type 1. Encodes an Open Reading Frame	GeneMANIA (113)
BAZ2B	chr2:160,175,490-160,473,203	Encodes a Zinc Finger, involved in transcription regulation.	GeneMANIA (113)
SLC30A3	chr2:27,476,552-27,498,685	Encodes a Solute carrier family 30 member 3, involved in transporting zinc to the extracellular space.	GeneMANIA (113)
DEPDC5	chr22:32,149,944-32,303,012	Encodes the DEP Domain Containing 5, involved in inhibition of the amino acid-sensing brance of the mTORC1 pathway. Implicated in familial focal epilepsy.	Ricos 2015 (184)
VPS13A	chr9:779,792,269-80,036,457	Encodes a gene involved in controlling the steps in the cycling of proteins through the trans-Golgi network to the endosomes, lysosomes, and the plasma membrane. Implicated in epilepsy.	Connolly 2014 (185)
HTRA1	chr10:124,221,041-124,274,424	Encodes a Serine Protease, which regulates insulin-like growth factors.	Feng 2015 (47)
S1PR1	chr1:101,702,305-101,707,076	Encodes a sphingosine receptor, involved in cell-cell	GeneMANIA (113)

Table 3.6. Sudden death candidate genes with roles in general cell processes			
GENE	**LOCATION**	**Function**	**Reference**
		adhesion. Implicated in cancers.	
ABCC8	chr11:17,414,432-17,498,449	Encodes for ATP-binding cassette, which transports proteins across membranes. Involved in hyperinsulinemic hypoglycemia of infancy	Takagi 2013 (186)
GPR26	chr10:125,425,871-125,456,913	Encodes G-Coupled Protein Receptor 26.	GeneMANIA (113)
TMEM214	chr2:27,255,778-27,264,563	Encodes for a protein that works with CAPS4 in ER-stress induced apoptosis.	GeneMANIA (113)
RORB	chr9:77,112,281-77,308,093	Encodes for a protein DNA-binding protein that is able to bind to hormone response elements upstream of genes to enhance their expression. Implicated in epilepsy.	Baqlietto 2014 (187)
GOLGA2	chr9: 131,018,108-131,038,274	Encodes for a protein located in the Golgi apparatus.	Shamseldin 2016 (188)
GOLGA1	chr9: 127,640,646-127,710,771	Encodes for a protein located in the Golgi apparatus.	GeneMANIA (113)
MAPKAP1	chr9: 128,199,672-128,469,513	Encodes a subunit of mTORC2, which is involved in cell growth regulation.	GeneMANIA (113)
TSC1	chr9: 135,766,735-135,820,020	Encodes a protein that negatively regulates the mTORC1 pathway.	GeneMANIA (113)
BTAF1	chr10: 93,683,526-93,790,082	Encodes for a TATA box-binding protein-associated factor, involved in DNA transcription.	GeneMANIA (113)
TUBGCP5	chr15: 22,833,395-22,873,892	Encodes a protein involved in microtubule binding.	GeneMANIA (113)
ALDOA	chr16: 30,064,411-30,081,778	Encodes a protein involved in glycolysis.	Sorensen 2015 (189)
POLR3K	chr16: 96,407-103,628	Encodes a subunit of RNA Polymerase 3.	GeneMANIA (113)

Table 3.6. Sudden death candidate genes with roles in general cell processes			
GENE	**LOCATION**	**Function**	**Reference**
NACA2	chr17: 59,667,794-59,668,563	Encodes a protein that prevents polypeptides from binding to the ER in cells.	GeneMANIA (113)
METTL2A	chr17: 60,501,228-60,527,454	Encodes a methyltransferase enzyme.	GeneMANIA (113)
EIF4ENIF1	chr22: 31,832,963-31,892,094	Encodes a transport protein involved in signaling between the cytoplasm and nucleus.	GeneMANIA (113)

TABLE 3.7. Sudden death candidate genes involved in serotonin			
GENE	**LOCATION**	**Function**	**Reference**
HTR2B	chr2:231,972,950-231,989,824	Encodes a serotonin receptor.	Feng 2015 (47)
HTR1A	chr5:63,255,875-63,258,119	Encodes a serotonin receptor.	Feng 2015 (47)
HTR1B	chr6:78,171,948-78,173,120	Encodes a serotonin receptor.	Feng 2015 (47)
HTR1D	chr1:23,518,388-23,521,222	Encodes a serotonin receptor.	Feng 2015 (47)
HTR2A	chr13:47,405,677-47,471,211	Encodes a serotonin receptor.	Feng 2015 (47)
HTR2C	chrX:113,818,551-114,144,624	Encodes a serotonin receptor.	Feng 2015 (47)
HTR5A	chr7:154,862,034-154,879,102	Encodes a serotonin receptor.	Feng 2015 (47)
HTR3E	chr3:183,817,967-183,824,783	Encodes a serotonin receptor.	Feng 2015 (47)
HTR3D	chr3:183,750,619-183,757,157	Encodes a serotonin receptor.	Feng 2015 (47)
HTR7	chr10:92,500,576-92,617,671	Encodes a serotonin receptor.	Feng 2015 (47)
SLC6A4	chr17:28,521,337-28,562,986	Encodes a serotonin transporter, involved in neuronal signaling. Implicated in Obsessive Compulsive Disorder.	Blair 2016 (69)

TABLE 3.8. Sudden death candidate genes with roles in hypoxia			
GENE	**LOCATION**	**Function**	**Reference**
GAPDH	chr12:6,643,585-6,647,537	Encodes glyceraldehyde-3-phosphate dehydrogenase. Implicated in cancer.	GeneMANIA (113)
HSP90B1	chr12:104,324,112-104,341,708	Encodes a heat shock protein. Implicated in tumor formation.	Salomonis 2014 (77)
SPTBN1	chr2:54,683,454-54,898,583	Encodes spectrin, which is an actin crosslinking and scaffolding protein.	GeneMANIA (113)
TF	chr3:133,464,800-133,497,850	Encodes a glycoprotein that is able to transport iron from the intestines, liver and immune system to all other cells in the body.	GeneMANIA (113)
YWHAG	chr7:75,956,108-75,988,342	Encodes a 14-3-3 protein that binds to phosphoserine-containing proteins, in order to signal transduction.	Salomonis 2014 (77)
HMOX1	chr22:35,777,060-35,790,207	Encodes for Heme Oxygenase, which involved in converting heme to bilirubin.	Miura 2012 (65)
ALDOC	chr17:26,900,133-26,903,951	Encodes a gene involved in glycolysis, Expressed in the hippocampus and Purkinje cells of the brain.	Wang 2007 (190)
HBA1	chr16:226,679-227,520	Encodes a subunit of hemoglobin.	GeneMANIA (113)
MB	chr22:36,002,811-36,013,384	Encodes myoglobin, which is expressed in skeletal and cardiac muscle cells and is involved in oxygen storage and diffusion.	GeneMANIA (113)
FANCD2	chr3:10,068,113-10,141,344	Encodes a protein involved in chromosomal stability.	Adler 2016 (53)

TABLE 3.9. Sudden death candidate genes with roles in the immune system			
GENE	**LOCATION**	**Function**	**Reference**
IL6	chr7:22,766,766-22,771,621	Encodes a cytokine, which is released by cells in response to infection to signal an inflammatory response.	Rognum 2009 (45)
IL10R	chr11:117,857,106-117,872,199	Encodes a cytokine, which is released by cells in response to infection to signal an inflammatory response.	Opdal 2004 (191)
TNF	chr6:31,543,344-31,546,113	Encodes a cytokine secreted by macrophages and involved in the inflammatory response.	Bonny 2011 (192)
AKNA	chr9:117,096,433-117,156,685	Encodes a transcription factor that activates expression of the CD40 receptor, which is expressed on the surface of lymphocytes.	Ma 2011 (193)
ATG5	chr6:106,632,352-106,773,695	Encodes a gene involved in autophagy vesicle formation and inflammatory cell differentiation.	Kuma 2004 (194)
ERVW-1	chr7:92,098,079-92,099,695	Encodes a human endogenous provirus envelope protein, expressed in the placenta.	Ruebner 2013 (195)
PAQR3	chr4:79,839,094-79,860,582	Encodes a progestin and adipoQ receptor.	GeneMANIA (113)
PRKD3	chr2:37,477,646-37,544,222	Encodes for protein kinase D3.	GeneMANIA (113)
TBX21	chr17:45,810,610-45,823,485	Encodes T-box 21, which is a transcription factor involved in immune system development. Implicated in cancer.	GeneMANIA (113)

TABLE 3.9. Sudden death candidate genes with roles in the immune system			
NPRL2	chr3:50,384,761-50,388,522	Encodes a protein that has tumor suppressive actions.	Ricos 2016 (184)

Table 3.10. Overlapping SIDS, SUDEP, and SUD Risk Genes					
GENE	**Subgroup**	**Association with SIDS**	**Association with SUDEP**	**Association with SUD**	**Other Disease Association**
TCAP	Muscle				Cancer
MYOM1	Muscle				DCM
SPTAN1	Muscle		X		
SLMAP	Muscle				Brugada Syndrome
TTN	Muscle	X			
LMNA	Muscle				DCM
PKP2	Muscle	X			
TRDN	Muscle				Cardiac arrhythmias
CHRNA1	Muscle				Congenital Myasthenic Syndrome
MYL2	Cardiac regulation			X	HCM
MYL3	Cardiac regulation			X	HCM
GLA	Cardiac regulation				HCM
LAMP2	Cardiac regulation				HCM
MYOZ2	Cardiac regulation			X	HCM
PRKAG2	Cardiac regulation		X		HCM
TNNI3	Cardiac regulation		X		HCM
CSRP3	Cardiac regulation		X		HCM & DCM
ACTN2	Cardiac regulation		X		HCM
TPM1	Cardiac regulation		X		HCM & DCM
PLN	Cardiac regulation			X	HCM
MYBPC3	Cardiac regulation		X		HCM & DCM
TNNC1	Cardiac regulation		X		HCM & DCM
TNNT2	Cardiac regulation		X		HCM & DCM
ACTC1	Cardiac regulation			X	HCM, DCM, & LVNC
MYH7	Cardiac regulation		X		HCM, DCM, & LVNC

Table 3.10. Overlapping SIDS, SUDEP, and SUD Risk Genes					
GENE	**Subgroup**	**Association with SIDS**	**Association with SUDEP**	**Association with SUD**	**Other Disease Association**
SNTA1	Cardiac regulation				Long QT Syndrome, Brugada Syndrome
MYH6	Cardiac regulation		X		
TNNT1	Cardiac regulation		X		DCM
BAG3	Cardiac regulation				DCM, Heart failure
RBM20	Cardiac regulation				DCM
CTF1	Cardiac regulation				DCM
DES	Cardiac regulation		X		DCM
EMB	Cardiac regulation				DCM
TAZ	Cardiac regulation		X		DCM & VCN
NPPA	Cardiac regulation				DCM
GJA5	Cardiac regulation		X		Familial AF
CAV3	Cardiac regulation				Brugada Syndrome, HCM
MIA3	Cardiac regulation				Heart Disease
ANKRD1	Cardiac regulation				DCM, Heart failure
PDE3A	Cardiac regulation				Cancer
TNNI3K	Cardiac regulation				Familial AF, DCM
RYR2	Cardiac regulation			X	Epilepsy, Cardiac arrhythmias
TIE1	Cardiac regulation		X		
GPD1L	Cardiac regulation				Brugada Syndrome
OBSCN	Cardiac regulation			X	HCM
DAG1	Cardiac regulation				HCM

Table 3.10. Overlapping SIDS, SUDEP, and SUD Risk Genes					
GENE	**Subgroup**	**Association with SIDS**	**Association with SUDEP**	**Association with SUD**	**Other Disease Association**
CASQ2	Cardiac regulation		X		Cardiac arrythmias
VCL	Cardiac regulation				HCM & DCM
DSP	Cardiac regulation				HCM
RYR3	Cardiac regulation		X		
AKAP9	Cardiac regulation				Cardiac arrythmias, Brugada Syndrome, and Long QT Syndrome
LDB3	Cardiac regulation				LVNC
CALM2	Neuronal Regulation				Long QT, Epilepsy, Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)
GAP43	Neuronal Regulation	X			
MBP	Neuronal Regulation	X			
TPPP	Neuronal Regulation	X			
TUBB4A	Neuronal Regulation				Spastic Paraplegia
GJA1	Neuronal Regulation	X			
MAOA	Neuronal Regulation	X			
PHOX2B	Neuronal Regulation	X			
SLC1A3	Neuronal Regulation	X			
SLC25A4	Neuronal Regulation	X			
SNAP25	Neuronal Regulation	X			
VAMP2	Neuronal Regulation	X			Familial Infantile

Table 3.10. Overlapping SIDS, SUDEP, and SUD Risk Genes					
GENE	**Subgroup**	**Association with SIDS**	**Association with SUDEP**	**Association with SUD**	**Other Disease Association**
					Myasthenia (respiratory distress)
NOS1AP	Neuronal Regulation				Long QT Syndrome, Brugada Syndrome
CHD2	Neuronal Regulation				Epilepsy
TBC1D24	Neuronal Regulation				Familial Infantile Myoclonic Epilepsy
STXBP1	Neuronal Regulation				Epilepsy
GABRB3	Neuronal Regulation				Absence epilepsy
CHRNA7	Neuronal Regulation	X			
CHRNA4	Neuronal Regulation				Epilepsy
CHRNA2	Neuronal Regulation				Epilepsy
SLC12A5	Neuronal Regulation				Epilepsy of Infancy with Migrating Focal Seizures
PAFAH1B1	Neuronal Regulation		X		
EN1	Neuronal Regulation	X			
APH1A	Neuronal Regulation				Alzheimer's Disease
SLC1A4	Neuronal Regulation				Infantile Spasms
CDKL5	Neuronal Regulation				Early Infantile Epileptic Encephalopathy, X-linked Infantile Spasms Syndrome
ANK2	Cytoskeleton			X	Cardiac arrhythmias
KRT2	Cytoskeleton	X			

Table 3.10. Overlapping SIDS, SUDEP, and SUD Risk Genes					
GENE	**Subgroup**	**Association with SIDS**	**Association with SUDEP**	**Association with SUD**	**Other Disease Association**
KRT9	Cytoskeleton	X			
PDLIM2	Cytoskeleton				Cancer
MAPT	Cytoskeleton	X			
DSG2	Cytoskeleton				Cardiac arrhythmias
JUP	Cytoskeleton				Cardiac arrhythmias
VSIG1	Cytoskeleton				Cancer
CXADR	Cytoskeleton				Cancer
PCDH19	Cytoskeleton				Dravet Syndrome
GAPDH	Hypoxia				Cancer
Hsp90b1	Hypoxia	X			Tumor formation
Ywhag	Hypoxia	X			
IL6	Inflammation	X			
IL10R	Inflammation	X			
TNF	Inflammation				Brugada Syndrome
AKNA	Inflammation	X			
ATG5	Inflammation				Autophagy in neonates
ERVW-1	Inflammation				Pathological pregnancies (preeclampsia, intraeuterine growth restrictions, and high elevated liver and low platelets syndrome)
TBX21	Inflammation				Cancer
NPRL2	Inflammation				Epilepsy
HTR2B	Serotonin		X		

Table 3.10. Overlapping SIDS, SUDEP, and SUD Risk Genes					
GENE	**Subgroup**	**Association with SIDS**	**Association with SUDEP**	**Association with SUD**	**Other Disease Association**
HTR1A	Serotonin		X		
HTR1B	Serotonin		X		
HTR1D	Serotonin		X		
HTR2A	Serotonin		X		
HTR2C	Serotonin		X		
HTR5A	Serotonin		X		
HTR3E	Serotonin		X		
HTR3D	Serotonin		X		
HTR7	Serotonin		X		
SLC6A4	Serotonin	X			Obsessive Compulsive Disorder
TGFB3	General cell processes				Cardiac arhythmias
TMEM43	General cell processes				Familial Arrhythmagenic Right Ventricular Dysplasia Type 5 (ARVD5)
FANCD2	General cell processes				Brugada Syndrome
FAM213A	General cell processes				Cancer
LGI1	General cell processes		X		
SMC4	General cell processes		X		
COL6A3	General cell processes		X		Muscuar Dystrophy, Myopathy
ALG13	General cell processes				Epilepsy, X-linked Intellectual Disability, Congenital Disorders of Glycosylation
FASN	General cell processes				Epilepsy
DNM1	General cell processes				Epilepsy
APPBP2	General cell processes				Epilepsy, Cancer, Alzheimers

Table 3.10. Overlapping SIDS, SUDEP, and SUD Risk Genes					
GENE	**Subgroup**	**Association with SIDS**	**Association with SUDEP**	**Association with SUD**	**Other Disease Association**
PTRH2	General cell processes				Epilepsy, Infantile-onset Multisystem Neurological, Endocrine, and Pancreatic Disease
CLTC	General cell processes				Epilepsy
TUBD1	General cell processes				Epilepsy
CSTB	General cell processes		X		Epilepsy
GOT1	General cell processes	X			
GOT2	General cell processes	X			
BAX	General cell processes				Cognitive impairment after traumatic brain injuries
CHD7	General cell processes				CHARGE Syndrome, involving sight problems, deafness, heart defects, slow growth, and urinary tract problems.
CPLX1	General cell processes				Wolf-Hirchhorn Syndrome, involving developmental disabilities and seizures.
RORB	General cell processes				Epilepsy
GOLGA2	General cell processes				Epilepsy
ALDOA	General cell processes				Cancer
HCN4	Ion channels & related genes				Brugada Syndrome, SUDEP

Table 3.10. Overlapping SIDS, SUDEP, and SUD Risk Genes					
GENE	**Subgroup**	**Association with SIDS**	**Association with SUDEP**	**Association with SUD**	**Other Disease Association**
CACNA1C	Ion channels & related genes			X	Epilepsy, cardiac arrhythmias, Centrally Mediated Ventilation
CACNA2D1	Ion channels & related genes			X	Cardiac arrhythmias, Short QT Syndrome, HCM, and Brugada Syndrome
CACNB2	Ion channels & related genes			X	Cardiac arrhythmias, Brugada Syndrome, HCM
KCND3	Ion channels & related genes			X	Cardiac arrhythmias, Brugada Syndrome
KCNIP1	Ion channels & related genes				DCM
KCNE3	Ion channels & related genes			X	Cardiac arrhythmias, Brugada Syndrome
KCNE1	Ion channels & related genes			X	Epilepsy, cardiac arrhythmias, Long QT Syndrome
KCNE2	Ion channels & related genes			X	Epilepsy, cardiac arrhythmias, Long QT Syndrome
KCNH2	Ion channels & related genes			X	Epilepsy, cardiac arrhythmias, Long QT Syndrome
KCNJ2	Ion channels & related genes			X	Cardiac arrhythmias, Short QT Syndrome
KCNJ3	Ion channels &				Long QT Syndrome,

Table 3.10. Overlapping SIDS, SUDEP, and SUD Risk Genes					
GENE	**Subgroup**	**Association with SIDS**	**Association with SUDEP**	**Association with SUD**	**Other Disease Association**
	related genes				Epilepsy
KCNJ5	Ion channels & related genes			X	Cardiac arrhythmias, Long QT Syndrome
KCNJ8	Ion channels & related genes	X			
KCNQ1	Ion channels & related genes	X	X	X	Long QT Syndrome,
SCN1B	Ion channels & related genes		X	X	Epilepsy, Sudden Cardiac Death, cardiac arrhythmias
SCN1A	Ion channels & related genes		X	X	Epilepsy, cardiac arrhythmias, Familial Hemiplegic Migraines
SCN2A	Ion channels & related genes				Early Infantile Epileptic Encephalopathy
SCN2B	Ion channels & related genes	X			Atrial Fibrillation
SCN3B	Ion channels & related genes			X	Cardiac arrhythmias, Brugada Syndrome
SCN4B	Ion channels & related genes			X	Cardiac arrhythmias, Long QT Syndrome
SCN5A	Ion channels & related genes		X	X	Epilepsy, Long QT Syndrome, Brugada Syndrome
SCN7A	Ion channels & related				Neonatal Epilepsy

Table 3.10. Overlapping SIDS, SUDEP, and SUD Risk Genes					
GENE	**Subgroup**	**Association with SIDS**	**Association with SUDEP**	**Association with SUD**	**Other Disease Association**
	genes				
SCN8A	Ion channels & related genes			X	Epilepsy
SCN9A	Ion channels & related genes				Neuropathic pain
TRPM4	Ion channels & related genes			X	Cardiac arrhythmias, Brugada Syndrome
KCNJ11	Ion channels & related genes				Transient Neonatal Diabetes Mellitus
KCNJ1					Neonatal Bartter Syndrome
HCN1	Ion channels & related genes				Early Infantile Epileptic Encephalopathy, Cardiac arrhythmias
HCN2	Ion channels & related genes				Sinoatrial Node Disease, Epilepsy, and Cardiac arrhythmias
KCNA1	Ion channels & related genes		X		Epilepsy, and cardiac arrhythmias
KCNA5	Ion channels & related genes				Familial Atrial Fibrillation, DCM
KNCE5	Ion channels & related genes		X		Brugada Syndrome, cardiac arrhythmias
CALM1	Ion channels & related genes				Cardiac arrhythmias
RANGRF	Ion			X	Cardiac

Table 3.10. Overlapping SIDS, SUDEP, and SUD Risk Genes					
GENE	**Subgroup**	**Association with SIDS**	**Association with SUDEP**	**Association with SUD**	**Other Disease Association**
	channels & related genes				arrhythmias
RYR1	Ion channels & related genes			X	
ATP1A2	Ion channels & related genes				Familial Hemiplegic Migraine
KCNT1	Ion channels & related genes				Epilepsy
NIPA2	Ion channels & related genes				Angelman Syndrome
ATP1A3	Ion channels & related genes				Early life epilepsy, episodic prolonged apnea, and postnatal microcephaly.

Table 3.11. Overall number of Variants in Whole Exomes for Sudden Death Probands obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification	
Individual	**Number of Variants**
Proband 2095 (SIDS)	51,737
Proband 2098 (SIDS)	69,978
Proband 2475 (SIDS)	40,869
Proband 2477 (SIDS)	58,445
Proband 2069 (SUDEP)	19,162
Proband 2231 (SUDEP)	63,954
Proband 2429 (SUDEP)	50,226
Proband 2460 (SUD)	63,217

Table 3.12. Total personal variation in SD Candidate Genes in the Whole Exomes for Sudden Death Probands, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification.	
Sample	**Number of Variants**
Proband 2095 (SIDS)	765
Proband 2098 (SIDS)	1121
Proband 2475 (SIDS)	697
Proband 2477 (SIDS)	958
Proband 2069 (SUDEP)	379
Proband 2231 (SUDEP)	1062
Proband 2429 (SUDEP)	921
Proband 2460 (SUD)	1001

Table 3.13. Number of Sudden Death candidate genes with variants, per category, in Whole Exomes for Sudden Death Probands, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification

Category	Muscle Regulation	Cardiac Regulation	Neuronal Regulation	Cytoskeletal Role	Hypoxia	Immune Role	Serotonin Receptors & Transporters	General Cellular Processes	Ion Channels & Associated Proteins	Total
Proband 2095 (SIDS)	4	24	6	8	5	2	3	29	25	109
Proband 2098 (eererSIDS)	17	49	20	8	8	7	7	46	51	213
Proband 2475 (SIDS)	9	37	11	8	11	4	8	32	29	151
Proband 2477 (SIDS)	14	38	12	11	8	5	8	40	37	173
Proband 2069 (SUDEP)	7	16	6	7	3	3	3	24	24	93
Proband 2231 (SUDEP)	12	32	12	10	7	4	4	45	33	159
Proband 2429 (SUDEP)	9	36	13	12	10	5	3	37	34	159
Proband 2460 (SUD)	13	38	12	8	8	4	8	41	36	168

Table 3.14. Number of transcripts affected by variants in Sudden Death candidate genes in Whole Exomes for Sudden Infant Death Syndrome (SIDS), Sudden Unexpected Death in Epilepsy (SUDEP), and Sudden Unexpected Death (SUD) probands, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification.		
SIDS (N=4)	**SUDEP (N=3)**	**SUD (N=1)**
3226	1671	7269
9388	7046	
4437	4527	
6293		

Table 3.15. Number of transcripts in SD candidate genes, per category, in Whole Exomes for Sudden Infant Death Syndrome (SIDS), Sudden Unexpected Death in Epilepsy (SUDEP), and Sudden Unexpected Death (SUD) probands, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification.

	SIDS (N=4)	SUDEP (N=3)	SUD (N=1)
Muscle Regulation	990 2322 1069 1233	226 1741 14	1306
Cardiac Regulation	1086 2640 1029 2278	333 1913 1510	1757
Neuronal Regulation	70 342 180 325	96 247 196	305
Cytoskeletal Role	190 389 202 283	71 213 280	378
Hypoxia	428 547 433 721	249 591 489	369
Immune Role	64 185 85 151	30 104 141	935
Serotonin	43	26	70

Table 3.15. Number of transcripts in SD candidate genes, per category, in Whole Exomes for Sudden Infant Death Syndrome (SIDS), Sudden Unexpected Death in Epilepsy (SUDEP), and Sudden Unexpected Death (SUD) probands, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification.

	SIDS (N=4)	SUDEP (N=3)	SUD (N=1)
	79 52 72	76 42	
General Cellular Processes	631 1086 654 1071	314 997 836	925
Ion Channels & Associated Proteins	323 1798 733 1093	326 1164 1019	1224

Table 3.16. Number of transcripts affected by type of variant by impact in Whole Exomes for Sudden Infant Death Syndrome (SIDS), Sudden Unexpected Death in Epilepsy (SUDEP), and Sudden Unexpected Death (SUD) probands, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification.

	SIDS (N=4)	SUDEP (N=3)	SUD
3' Untranslated	36	18	130
Region	102	136	
	76	70	
	98		
5' Untranslated	35	7	77
Region	103	70	
	50	75	
	76		
Splice Region	53	43	134
	153	100	
	78	63	
	126		
Splice Site	0	1	3
	8	0	
	1	3	
	1		
Regulatory Region	529	304	1279
	1315	1085	
	729	814	
	1001		
Synonymous	328	152	853
	369	744	
	544	561	
	643		

Table 3.16. Number of transcripts affected by type of variant by impact in Whole Exomes for Sudden Infant Death Syndrome (SIDS), Sudden Unexpected Death in Epilepsy (SUDEP), and Sudden Unexpected Death (SUD) probands, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification.

	SIDS (N=4)	SUDEP (N=3)	SUD
Nonsynonymous	250 692 426 503	163 528 294	504
Premature Stop **Codon**	0 1 8 0	1 0 1	0
Intronic	1693 5074 2233 3146	783 3788 2149	3653
Insertion/Deletion	301 967 472 699	199 693 497	631
Spanning Deletion	1 4 0 0	0 2 0	5

Table 3.17. Key Pathogenic Variants in Whole Exome for Sudden Infant Death Syndrome (SIDS) proband 2095, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification.

Gene	***SCN2A***	***RYR3***	***DAG1***	***IL10RA***	***TTN***	***TRPM6***
CADD Phred	29.6	17.02	21.6	14.51	14.29	28
PolyPhen	-	Potentially damaging, Damaging	Benign	Benign	Benign	Damaging
SIFT Phred	-	Tolerated, Deleterious, Deleterious	Tolerated	Tolerated	Deleterious	Deleterious
PhastCons	1.0	0.491	1.0	0.013	1.0	0.99
Grantham	-	180	177	215	107	160
Amino Acid Substitution (NCBI Transcripts)	-	p.Arg1641Cys	p.Ser14Trp	p.Cys4Trp	p.Glu8144Ala	p.Asp1126Tyr
Number of Transcripts Affected	5	2	21	10	11	7
rsID from dbNSP	-	rs4780144	rs2131107	-	rs16866465	-
Disease	Early Infantile Epileptic Encephalopathy, Early Myoclonic Encephalopathy, Dravet Syndrome, Severe Myoclonic Epilepsy of Infancy	SUDEP	HCM	SIDS	SIDS	Epilepsy

Table 3.18. Key Pathogenic Variants in Whole Exome for Sudden Infant Death Syndrome (SIDS) proband 2098, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification.

Gene	***DSP***	***KCNJ5***	***KCNH2***	***SCN1B***	***OBSCN***	***ABCC8***
CADD Phred	18.35	17.19	16.87	9.129	11.49	26.6
PolyPhen	Damaging	Benign	Potentially damaging, Damaging,	Benign	Benign	Damaging
SIFT Phred	Deleterious	Tolerated	Tolerated	Deleterious	Tolerated	Deleterious, Tolerated
PhastCons	0.859	1.0	1.0	0.41	1.0	0.003
Grantham	180	29	78	110	152	125
Amino Acid Substitution (NCBI Transcripts)	p.Arg1537Cys	p.Gln282Glu	p.Lys897Thr	p.Ser248Arg	p.Val1600Asp	p.Gly111Arg
Number of Transcripts Affected	2	3	7	6	6	4
rsID from dbNSP	rs28763967	rs7102584	rs1805123		rs7532342	-
Disease	Rapid Onset Dystonia-Parkinsonism (RDP), Alternating Hemiplegia of Childhood (AHC) involving neonatal seizures	SUD, Long QT Syndrome	SUD, Epilepsy, Long QT Syndrome	Dravet Syndrome, Severe Myoclonic Epilepsy of Infancy	HCM	Hyperinsulinemic hypoglycemia of infancy

Table 3.19. Key Pathogenic Variants in Whole Exome for Sudden Infant Death Syndrome (SIDS) proband 2477, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification.						
Gene	***SCN8A***	***HSP90B1***	***SLC1A4***	***SCN1B***	***SCN1B***	***GOT2***
CADD Phred	25.9	14.19	32	9.129	2.198	21.8
PolyPhen	Damaging	Damaging	Damaging	Benign	Benign	Benign
SIFT Phred	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious	Tolerated
PhastCons	1.0	1.0	1.0	0.41	0.034	1.0
Grantham	56	98	109	110	71	109
Amino Acid Substitution (NCBI Transcripts)	p.Glu1223Lys	p.Pro321Leu	p.Gly361Val	p.Ser248Arg	p.Arg250Thr	p.Val346Gly
Number of Transcripts Affected	4	5	5	110	71	3
rsID from dbNSP	-	rs116891695	-	rs67701503	rs67486287	rs30842
Disease	SUD, Epilepsy	SIDS	Infantile Spasms	Severe Myoclonic Epilepsy of Infancy, Dravet Syndrome	Severe Myoclonic Epilepsy of Infancy, Dravet Syndrome	SIDS

Table 3.20. Key Pathogenic Variants in Whole Exome for Sudden Infant Death Syndrome (SIDS) proband 2475, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification.						
Gene	***KCNJ1***	***PKP2***	***SCN8A***	***SCN2A***	***SMACA5***	***DAG1***
CADD Phred	32	28.6	34	29.4	32	21.6
PolyPhen	Potentially damaging	Damaging	Damaging	Damaging	Damaging	Benign
SIFT Phred	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious	Tolerated
PhastCons	1.0	1.0	1.0	1.0	1.0	1.0
Grantham	56	155	64	89	125	177
Amino Acid Substitution (NCBI Transcripts)	p.Glu258Lys	p.Phe424Ser	p.Ala1575Val	p.Arg1163Cys	p.Arg620Gly	p.Ser14Trp
Number of Transcripts Affected	7	2	3	5	2	21
rsID from dbNSP	-	-	rs182326351	rs17183814	-	rs2131107
Disease	Neonatal Bartter Syndrome	SIDS	Early Infantile Epileptic Encephalopathy, Early Myoclonic Encephalopathy	Early Infantile Epileptic Encephalopathy, Ealry Myoclonic Encephalopathy, Dravet Syndrome	Cancer	HCM

Table 3.21. Key Pathogenic Variants in the Whole Exome for Sudden Unexpected Death in Epilepsy (SUDEP) proband 2069, obtained from Dried Blood Spots (DBS) gDNA and subjected to Whole Genome Amplification.						
Gene	**ATP1A3**	**ATP1A3**	**TMEM214**	**LMNA**	**TRDN**	**TTN**
CADD Phred	4.325	28.9	22.7	17.50	13.72	18.53
PolyPhen	Benign	Deleterious	Deleterious	Benign, Potentially damaging	Benign	Benign
SIFT Phred	Deleterious	Deleterious	Deleterious	Deleterious	Tolerated	Tolerated
PhastCons	1.0	1.0	1.0	1.0	1.0	1.0
Grantham	-	159	21	29	142	142
Amino Acid Substitution (NCBI Transcripts)	-	p.Gly633Cys	p.Val351Met	p.Arg401His	p.Ile438Ser	p.Ser1295Leu
Number of Transcripts Affected	5	5	13	22	2	11
rsID from dbNSP	rs919390	-	rs1124649	rs141490569	rs2873479	rs1552280
Disease	Rapid Onset Dystonia-Parkinsonism (RDP), Alternating Hemiplegia of Childhood (AHC) involving neonatal seizures	Rapid Onset Dystonia-Parkinsonism (RDP), Alternating Hemiplegia of Childhood (AHC) involving neonatal seizures	Seizures	DCM	Cardiac Arrhythmias	SIDS

Table 3.21. Key Pathogenic Variants in Sudden Unexpected Death in Epilepsy (SUDEP) proband 2231, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification.						
Gene	**HTR3E**	**KCNH2**	**RYR3**	**OBSCN**	**ERBB2**	**TTN**
CADD Phred	17.31	18.92	17.02	15.77	22.1	20.6
PolyPhen	Benign	Benign	Potentially damaging, damaging	Damaging	Potentially damaging	Damaging
SIFT Phred	Tolerated	Tolerated	Tolerated, Deleterious, Deleterious	Deleterious	Deleterious	Deleterious, Tolerated
PhastCons	0.995	0	0.491	1.0	1.0	1.0
Grantham	58	102	180	125	27	109
Amino Acid Substitution (NCBI Transcripts)	p.Ala86Thr	p.Arg1047Leu	p.Arg1641Cys	p.Gly4209Arg	p.Pro1170Ala	p.Gly34278Val
Number of Transcripts Affected	7	7	2	5	20	41
rsID from dbNSP	rs7627615	rs36210421	rs4780144	rs56218706	rs1058808	rs3731752
Disease	SUDEP	Epilepsy, Cardiac Arrhythmias, Long QT Syndrome	SUDEP	SUD, HCM	Encodes for erythroblastic leukemia viral oncogene homolog 2. Involved in cancer.	SIDS

Table 3.22. Key Pathogenic Variants in Whole Exome for Sudden Unexpected Death in Epilepsy (SUDEP) proband 2429, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification.						
Gene	**OBSCN**	**TTN**	**SCN1B**	**HTR3D**	**KCNJ5**	**TRDN**
CADD Phred	18.33	20.6	9.129	21.8	17.19	13.72
PolyPhen	Benign	Damaging	Benign	Potentially damaging, benign	Benign	Benign
SIFT Phred	Deleterious	Deleterious, Tolerated	Deleterious	Tolerated	Tolerated	Tolerated
PhastCons	0	1.0	0.41	0.024	1.0	1.0
Grantham	180	109	110	29	20	142
Amino Acid Substitution (NCBI Transcripts)	p.Arg5619Cys	p.Gly3427Val	p.Ser248Arg,.	p.Arg435His	p.Gln282Glu	p.Ile438Ser
Number of Transcripts Affected	5	41	6	4	3	2
rsID from dbNSP	rs3795800	rs3731752	rs67701503	rs6789754	rs7102584	rs2873479
Disease	HCM	SIDS	Dravet Syndrome, Severe Myoclonic Epilepsy of Infancy	SUDEP	SUD, Cardiac Arrhythmias, Long QT Syndrome	Cardiac Arrhythmias

Table 3.23. Key Pathogenic Variants in Whole Exome for Sudden Unexpected Death (SUD) proband 2460, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification.						
Gene	***GOT2***	***RBM20***	***OBSCN***	***SCN5A***	***TTN***	***DAG1***
CADD Phred	21.8	25.7	11.49	22.4	22.6	21.6
PolyPhen	Benign	Damaging	Benign	Potentially damaging, Damaging	Damaging	Benign
SIFT Phred	Tolerated	Deleterious	Tolerated	Deleterious	Deleterious	Tolerated
PhastCons	1.0	1.0	1.0	1.0	1.0	1.0
Grantham	109	94	152	144	160	177
Amino Acid Substitution (NCBI Transcripts)	p.Val346Gly	p.Gly232Asp	p.Val1600Asp	p.Ser524Tyr	p.Asp2243Tyr	p.Ser14Trp
Number of Transcripts Affected	3	1	6	10	10	21
rsID from dbNSP	rs30842	rs61735268	rs7532342	rs41313691	rs138787974	rs2131107
Disease	SIDS	DCM	SUD, HCM	SUDEP, SUD, Epilepsy, Long QT Syndrome, Brugada Syndrome	SIDS	HCM

Table 3.24. OBSCN Variants in the eight Whole Exomes for Sudden Death probands, obtained from Dried Blood Spot (DBS) gDNA and subjected to Whole Genome Amplification.

Proband	Number of Variants	Number of Transcripts Affected
2095(SIDS)	7	9, 6, 12, 12, 9, 5, 4
2098 (SIDS)	9	5, 6, 12, 12, 5, 5, 6, 6, 4
2475 (SIDS)	5	5, 6, 12, 12, 4
2477 (SIDS)	2	9, 5
2069 (SUDEP)	2	6, 5
2231 (SUDEP)	12	5, 6, 12, 12, 9, 5, 5, 6, 6, 6, 4
2429 (SUDEP)	10	12, 12, 12, 11, 5, 5, 5, 7, 4, 4
2460 (SUD)	11	9, 6, 12, 12, 11, 9, 5, 6, 6, 4, 4

Chapter 4: Discussion and Conclusions

4.1 Challenges in Personalized Risk Prediction

To achieve truly personalized risk prediction, the clear defined decision tree can provide appropriate questions to be evaluated when profiling or predicting potential risk of sudden death even in small cohorts. Here, the inability to differentiate among the SD probands regardless of population, cohort, or personal comparison underscores the challenges of molecular diagnostics where risk prediction, and potentially therapeutic intervention. The amount and nature of personal genetic variation and the biological integration of spatio-temporal expression patters during development requires a new analytical paradigm where statistical predictions are superseded by integrated risk analysis.

4.2 Genetic Variation in Sudden Death Probands is Pathogenic

Currently, the Triple Risk Hypothesis which include environmental factors, a vulnerable age, and genetic predisposition is employed to encompass all presumed contributors to risk. Here, the comparison of Sudden Death probands representing infants (SIDS), syndromes (SUDEP) and adult sudden death (SUD) allowed for the consideration of genetic variation and pathogenicity in alternatively spliced transcripts in a subset of SD candidate genes. Not only was the overall exomic variation extensive, but the distribution, relative contribution and functional classes affected by variants was similar across all probands regardless of cause of death. Even when transcript data was considered and pathogenic consequences calculated, the individual patterns

were complex and indistinguishable between individuals or cause of death. This unequal valence and contribution underscores the challenge molecular diagnostic risk prediction has clinically, and required further consideration of select candidate genes in the developing brain where spatio-temporal patterns were noted. Specifically, the development of the brainstem and regulatory cardio-respiratory nuclei had changing patterns of expression of the candidate genes across age ranges, including pre and postnatally.

Individual risk genes were observed to contain potentially pathogenic variants using one or more of the bioinformatics variant annotation methods. Thus, simply having a pathogenic mutation within this gene is not predictive of the type of death that will occur. Instead, it is highly likely, that as proposed in the Triple Risk Hypothesis for SIDS, risk factors, in this instance genetic variation combines to increase risk such that the cause of death in each of these individuals is likely a result of the compounded functional affect of multiple pathogenic variants within their exome.

4.3 Limitations and Future Directions

As seen in the decision trees (Figure 2.1), it was not possible to predict the cause of death in the probands by the number of variants in all the genes within the whole exomes or in the subsection of the SD candidate genes. The eight probands did have more variants on average than controls from the literature (146,196) where roughly 55,727 variants were found in an individual. It is possible that these additional variants contribute to a compounding affect that leads to sudden death. Large variability in the number of genes containing

variants, the number of impacted transcripts, and the specific number and type of pathogenic variants between different probands within the same cause of death group.

For all three group; SIDS, SUDEP, and SUD, numerous affected transcripts fell into the Intronic Variant bin (Table 3.16), which included non-coding exon variants, noncoding transcript exon variants, and intron variants. While these variants do not modify protein structure, it does not diminish possible biological impact as many variants of this type impact distal regulatory elements, even up to 100 kb away from the target region (197). The alteration of gene regulation frequently impact expression levels leading to an increase or decrease in protein expression. Similarly, many variants coded directly to the regulatory regions which can modify transcription factor or DNA polymerase binding also modifying gene expression patterns. Variants with established pathogenic effects like Nonsynonymous, and Indels were also present in large numbers in all individuals. Missense mutations encode amino acid substitutions that impact protein packing, alter protein function or protein biogenesis. The larger insertions and deletions would result in large frameshifts in the coding sequence, altering the codons required to attract the proper amino acids during protein translation.

For some genes, multiple variants were located within the same gene. The number of transcripts affected by the variant was counted separately and compound mutations were not taken into consideration. Therefore, the true overall number of transcripts affected in each of the probands is less than presented because some transcripts were impacted by multiple variants and thus, counted multiple times.

The conclusions that can be drawn from our results are limited, due to the small number of samples that we have. However, the exact cause of death appears to be different even within the three sudden death categories due to the large differences between individuals in terms of the genes affected and the number of variants present within these genes. However, it was intriguing that all of the SIDS probands had pathogenic variants in genes involved in epilepsy, such as Early Infantile Epileptic Encephalopathy, also known as Ohtahara Syndrome. It is possible that the infants had mild, undetectable seizures or that the seizures only occurred when the parents of these infants were not present. It also underscores the role of the brain in the regulation of cardiac and respiratory function such that abnormal signalling via the brainstem is a viable pathophysiological mechanism consistent with the Triple Risk Hypothesis and matches the etiology of death observed in both SUDEP and SIDS.

4.4 Summary and Conclusions

Small cohorts, oligogenic heterogeneity, and large number of rare private variants in Sudden Death cases require novel approaches in variant prioritization and functional impact prediction such as incorporating spatio-temporal expression patterns for genetic variant pathogenicity predictions. At the moment, due to the role of the same genes in a variety of disorders across the SD spectrum, it is difficult to predict the developmental timing of risk. By understanding the role of lifestage-dependent expression of different transcripts for the same genes, and the variability of mutations within these transcripts, we will gain a greater understanding and ability to predict the timing of death risk within the populations along the SD spectrum.

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