Non-Cardiac Genetic Predisposition in Sudden Infant Death Syndrome

Short Title: Gray –Non-Cardiac SIDS-Susceptibility Genes

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Abstract

Purpose:

Sudden infant death syndrome (SIDS) is the commonest cause of sudden death of an infant however the genetic basis remains poorly understood. We aimed to identify non-cardiac genes underpinning SIDS and determine their prevalence compared to ethnically matched controls.

Methods:

Using exome sequencing we assessed the yield of ultra-rare non-synonymous variants (MAF ≤ 0.00005, dominant model; MAF ≤ 0.01, recessive model) in 278 European SIDS cases (62% male; average age = 2.7±2 months) versus 973 European controls across 61 non-cardiac SIDS-susceptibility genes. The variants were classified according to ACMG criteria. Case-control, gene-collapsing analysis was performed in 8 candidate biological pathways previously implicated in SIDS pathogenesis.

Results:

Overall 43/278 SIDS cases harbored an ultra-rare SNV compared to 114/973 controls (15.5% vs 11.7%, p=0.10). Only 2/61 non-cardiac genes were significantly over-represented in cases compared to controls (ECE1, 3/278[1%] vs 1/973[0.1%] p=0.036; SLC6A4[2/278 [0.7%] vs 1/973[0.1%] p=0.049])]. There was no difference in yield of pathogenic or likely pathogenic variants between cases and controls (1/278 [0.36%] vs 4/973 [0.41%]; p=1.0). Gene-collapsing analysis did not identify any specific biological pathways to be significantly associated with SIDS.

Conclusion:

A monogenic basis for SIDS amongst the previously implicated non-cardiac genes and their encoded biological pathways is negligible.

Keywords
Genetics; molecular autopsy; sudden infant death syndrome; exome sequencing

Introduction

Sudden infant death syndrome (SIDS) is defined as “the sudden death of an infant under 1 year of age which remains unexplained after thorough investigation including detailed clinical and pathological review”.[1,2] The peak incidence occurs between 2-4 months of age and has been often associated with environmental risk factors such as prone sleep position and maternal smoking.[3] Despite successful targeted risk reduction campaigns such as the “back-to-sleep” campaigns in the 1990s, SIDS remains a leading cause of sudden infant death, occurring at a rate of 27/100,000 live births in the UK and the USA respectively.[3-5]

Research in SIDS has proposed that unexplained infant deaths result from “abnormalities at birth that make them vulnerable to potential life-threatening challenges in infancy”[6]. The “triple-risk hypothesis” proposed the convergence of three over-lapping factors: 1) a “vulnerable” infant, 2) a critical development period, and 3) an exogenous stressor.[7] Accordingly, SIDS does not typically occur in normal infants, but rather, in vulnerable infants with an underlying abnormality.[3]

Rather than a single etiology underlying the majority of infant vulnerability, SIDS may be due to multiple distinct genetic disorders with a common final endpoint of sudden death occurring during sleep.[8] Several studies have implicated both common and rare genetic variants within genes involved in several biological pathways including neurological conditions, neuronal signaling, inborn errors of metabolism, respiratory control, musculoskeletal conditions, immune response, and genetic heart disease (GHD) as a basis for underlying infant vulnerability. [2,9-14]

Using exome sequencing and a targeted analysis of 90 GHD-susceptibility genes in over 400 SIDS cases, we determined recently a 5% prevalence of GHD-associated “pathogenic” or “likely pathogenic” variants as a potential monogenic basis for SIDS.[15] There was only an excess burden of rare variants in the major channelopathy genes when

nearly 300 Caucasian cases were compared to approximately 1000 Caucasian controls.

Here, we conducted a SIDS-susceptibility variant analysis in the same cohort examining the previously published, non-cardiac SIDS-susceptibility genes followed by a gene-collapsing rare variant burden analysis involving multiple non-cardiac, biological pathways implicated previously in SIDS pathogenesis.

Materials and Methods

Study Population

As previously described, the SIDS cohort (N=427) consisted of 95 coroners' cases from the United Kingdom (UK; London, Sheffield, Edinburgh and Bristol) and 332 coroner/medical examiner/forensic pathologist-referred cases collected from six ethnically and geographically diverse United States (US) populations.[15] Enrolment criteria included 1) sudden unexplained death of an infant < 1 year of age, 2) reported European descent, and 3) a comprehensive negative medico-legal autopsy including a negative toxicology screen and death scene investigation. Infants with asphyxia or specific disease causing death were excluded. Ethnicity was self-reported by the referring coroner/medical examiner. This study complies with the Declaration of Helsinki; locally appointed ethics committees including Mayo Clinic's Institutional Review Board have approved the research protocol.

Control Population

A total of 973 control exomes (509 females, 464 males) from the ICR1000 UK exome series and the 1958 Birth Cohort study were included for analysis.[16] As previously reported, exome sequencing was performed using the Illumina TruSeq and Illumina instruments.[16]

Exome Sequencing

As previously described, genomic DNA isolated from each SIDS case underwent exome sequencing at the KCL-GSTT Biomedical Research Centre Genomics Platform, London, UK or Mayo Clinic's Medical Genome Facility, Rochester, Minnesota. To avoid potential confounding due to population stratification resulting from genetic admixture, a principal component analysis (PCA) was performed as previously described.[15] Furthermore, quality control metrics excluded 7 cases due to insufficient exome coverage and one individual from a half-sibling pair. A case-control dataset was established for 278 SIDS cases (confirmed as Caucasian by PCA) and 973 European controls. Detailed methodology can be found in the **Online Supplement**.

Case-Control Non-Cardiac SIDS Susceptibility-Gene Specific Variant Analysis

A list of 55 SIDS-susceptibility genes involving multiple, non-cardiac biological pathways implicated previously in SIDS pathogenesis was derived from Salomonis' Integrated Mechanism Review article, "*Systems-level perspective of Sudden Infant Death Syndrome"* published in 2014.[8] This literature review based list included genes with sufficient evidence for involvement of SIDS based on the reported conclusions of manuscript authors.[8] Based on our own literature search of articles from 2014 to 2018, 6 additional SIDS-susceptibility genes were included for a total list of 61 non-cardiac, candidate genes (see **Online Supplement eTable 1**).[17-21]

Following exome sequencing, single nucleotide variants (SNVs) and insertion/deletions (INDELs) were filtered to identify variants which followed either a dominant or recessive inheritance pattern using Ingenuity Variant Software (Qiagen, Redwood City, CA). All variants within the 61 non-cardiac SIDS-susceptibility genes were first filtered for a call quality score ≥ 20 and a read depth ≥ 10. Only non-synonymous (NSV, i.e. amino acid altering: missense, nonsense, splice-error, frame-shift insertion/deletion [INDEL], or in-frame INDEL) were considered potentially pathogenic.

For the dominant model, only ultra-rare variants (minor allele frequency [MAF] ≤ 0.00005 (1: 20,000 alleles) in Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org) were considered. Variants with a MAF > 0.00005 in any ethnic group of gnomAD were excluded, unless observed only once in that ethnic group. For the recessive inheritance model, only rare (MAF ≤ 0.01 in gnomAD) variants present as either homozygotes or compound heterozygotes (two unique pathogenic variants in the same gene) were included. Importantly, for compound heterozygotes, it was assumed that the variants were present in trans; however, parental DNA was unavailable to confirm this. Variants with a homozygous frequency > 0.0001 in gnomAD were excluded from analysis. A comparison of yield of NSVs for both the dominant and recessive model was performed for all 61 non-cardiac SIDS-susceptibility genes.

The American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) standards and guidelines for the interpretation of sequence variants was used to further assist in the interpretation and annotation of our genetic findings.[22] Automatic variant classification was performed using InterVar; a freely available web-based bioinformatics software tool for clinical interpretation of genetic variants by the ACMG/AMP 2015 guideline.[23]

SIDS Candidate Biological Pathway Gene-Collapsing Analysis

We identified previously a list of 90 genetic heart disease (GHD)-associated genes.[15] Using PubMed as our search engine, with the key phrase of ‘sudden infant death’ and ‘gene’, ‘polymorphism’ or ‘mutation’ and OMIM, with the key words of ‘sudden infant death’, ‘epilepsy’, ‘inborn errors of metabolism’, an additional list of 241 “non-cardiac” genes was identified for a gene-collapsing rare variant burden analysis. Only population-based SIDS cohorts, case reports and literature reviews between 1990 and 2016 were used. Studies based on definitions of SIDS contrary to current practices were excluded.

We performed case-control, gene-collapsing analyses of ultra-rare (MAF <

0.00005), NSV's with a Combined Annotation Dependent Depletion (CADD) score ≥ 20 in candidate biological pathways previously implicated in SIDS pathogenesis. A CADD score ≥ 20 is equivalent to a 0.99 probability that the variant has a functional impact.[24] The unit of analysis was a collection of genes from each pathway (See **Online Supplement eTable 2**): GHD (90 genes), epilepsy (72 genes), inborn errors of metabolism (69 genes), other neurological (33 genes), respiratory system (37 genes), autonomic nervous system (13 genes), immune system (12 genes), and nicotine response (3 genes).

Statistics

Categorical variables were expressed as absolute numbers and percentage, and compared with Fisher's exact or Chi-square tests. Probability values were based on two-sided tests considered significant at $P < 0.05$. For this exploratory analysis, a Bonferonni correction of $p < 0.0008$ (0.05 divided by 61) was not applied. Analysis was conducted with SPSS version 18.0 software (SPSS Chicago III).

Results

Demographics

The case cohort consisted of 278 European SIDS cases (173 males, 105 females; average age = 2.7 ± 1.98 months), described previously by our group.[15] The epidemiologically high risk age group of 2–4 months (55.4%) and male gender (62.2%) accounted for the majority of the cases. Sleep characteristics were known in 60% of the cohort, of whom 66/172 (38%) were co-sleeping at the time of the SIDS death (**Table 1**).

SIDS-Susceptibility Gene-Specific Analysis of the Non-Cardiac Genes Previously Implicated in SIDS

Considering a dominant inheritance model, a total of 44 unique (42 novel), ultra-rare, NSVs (20 missense, 2 in-frame deletions, 1 frame-shift deletion, and 1 stop-loss) were identified in 43/278 (15.5%) SIDS cases overall (**Figure 1**). Further, 2/278 (0.72%) SIDS cases hosted > 1, ultra-rare NSV. In comparison, a total of 115 unique (104 novel), ultra-

rare, NSVs (109 missense, 1 in-frame deletions, 2 frame-shift deletions, 1 start-loss, and 1 stop-loss) were identified in 114/973 (11.7%, p =0.10) European controls (**Figure 1**). Further, 6/973 (0.62%) European controls hosted > 1, ultra-rare NSV.

The gene-specific yields for the SIDS cohort and the European controls are shown in **Table 2**. For 59 of the 61 genes, there was no over-representation of ultra-rare NSVs in SIDS cases versus controls at even the low stringent $p < 0.05$ threshold (**Table 2**). Two genes hosted more ultra-rare NSVs in SIDS cases than controls at this threshold: *ECE1* (endothelin converting enzyme, [3/278 (1.1%) cases vs 1/973 (0.1%) controls; p=0.036]) and *SLC6A4* (solute carrier family 6 member 4, also known as the serotonin transporter 1 [2/278 (0.7%) cases vs 0/973 (0%) controls; p=0.049]) (**Table 2**).

Following variant classification using the strict ACMG guidelines, 1 of the 44 (2.2%) SIDS case variants and 3 of the 115 (3.5%) European control variants, achieved either a "pathogenic" or "likely pathogenic" designation. All other variants were classified as variants of uncertain significance (VUS). There was no difference in overall yield of "pathogenic" or "likely pathogenic" variants between the European SIDS and control cohorts (1/278 [0.36%] vs 3/973[0.31%]; p=1.0 see **Online Supplement eTable 3 and 4**).

A heterozygous "pathogenic" p.V153fs*41-SLC22A5 variant was identified in a 4-month-old female SIDS case. The p.V153fs*41-SLC22A5 variant has been observed previously in patients with primary carnitine deficiency, an autosomal recessive disorder of the carnitine cycle resulting in defective fatty acid oxidation.[25] However, because a second *SLC22A5* variant was not identified in this SIDS case, it is unlikely this infant had undiagnosed primary carnitine deficiency. Two controls also hosted ultra-rare "likely pathogenic" heterozygous variants (p.Y447C-SLC22A5, and p.G827R-GRIN1). Interestingly, the p.G827R-GRIN1 variant has also been identified previously as a de novo heterozygous variant in three unrelated individuals with severe intellectual disability, movement disorder and seizures.[26,27]

Considering a recessive inheritance model, homozygous or compound heterozygous variants were observed in 2/278 (0.72%) SIDS cases compared to 3/973 (0.31%) controls (p=0.31). A homozygous p.R78Q-SULT1A1 variant was identified in a 2-month-old male SIDS case and a hemizygous p.V37I-MAOA variant was identified in a 3.8-month-old male SIDS case. In European controls, there was a homozygous p.R297Q-MAOA variant in one control and a homozygous p.V231I-CHRM2 variant in a second control; a third control hosted compound heterozygous *HADHA* variants (p.K249N and p.E510Q; see **Online Supplement Table 5 and 6**). All of the variants were classified as a VUS except for the p.E510Q-HADHA variant which was classified as "pathogenic". The p.E510-HADHA variant has been reported previously in both homozygous and compound heterozygous cases in a large number of individuals and families with long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency and demonstrated to result in significant loss of enzyme activity with this variant.[28]

There were no significant differences in the yield of dominant/recessive NSVs among all 61 SIDS-susceptibility genes when comparing sex, sleep position (supine vs prone), or co-sleeping (yes vs no) (**Table 3**).

SIDS Biological Pathway Gene-Collapsing Analysis

A rare variant, gene-collapsing burden analysis performed on gene sets involving 8 different biological pathways (genetic heart disease, epilepsy, inborn errors of metabolism, respiratory control, other neurological conditions, autonomic nervous system, immune system, and nicotine metabolizing) previously implicated in SIDS pathogenesis also failed to yield any significant associations (**Table 4**).

Discussion

Since the proposal of the "triple-risk hypothesis" over twenty years ago, investigators have been searching for monogenetic explanations as a substrate for infant vulnerability to SIDS. Some of the suspected sources of an infant's "underlying vulnerability" include

genetic determinants leading to dysfunction of the central and autonomic nervous system, inborn errors of metabolism, and cardiac channelopathies (**Supplemental Reference list**). While there have been no clear diagnostic markers identified, several common polymorphism have been identified to be significantly over-represented in distinct SIDS ethnic populations.[8]

Recently, we completed exome sequencing-based molecular autopsy with a genetic heart disease (GHD) gene-specific analysis for 278 unrelated European ancestry SIDS cases in order to determine the contribution of monogenic heart disease to SIDS pathology. Less than 12% of the European SIDS cases hosted an ultra-rare (MAF < 0.005%) "potentially informative" variant in one of the 90 GHD-susceptibility genes analyzed. However, according to the American College of Medical Genetics (ACMG) guidelines only 4.3% of the cases possessed immediately clinically actionable GHD-associated variants (i.e. "pathogenic" or "likely pathogenic").

Our current study now examines the potential contribution of "non-cardiac" genes in the pathogenesis of SIDS using a similar approach to examine 61 published non-cardiac genes previously implicated in SIDS [8,17-21]. The majority had been identified as potential "SIDS-susceptibility" genes following both common and rare variant association studies, typically involving promoter region variants. However, only approximately 55% had been associated previously with either a dominant or recessive rare monogenic disease. Although 28 (46%) have never been associated with any monogenic disorder (dominant or recessive) and 18 (29.5%) have only been associated with recessive disease, we chose to interrogate all 61 genes under both dominant and recessive inheritance models to examine the potential role of each gene for its involvement in the monogenic basis for SIDS.

Soberingly, there was no significantly increased burden of ultra-rare variants in all 61 genes in SIDS cases compared to ethnically matched controls (15.5% vs 11.7%,

p=0.10) in a dominant inheritance model or rare homozygous/compound heterozygous variants in cases over controls using a recessive inheritance model (0.72% vs 0.3%, p=0.31). In addition, there was a negligible yield of immediately clinically actionable disease-associated variants (i.e. “pathogenic” or “likely pathogenic”) in SIDS cases (0.36%) and controls (0.31%) with no significant difference detected. Furthermore, there was no difference in yield of variants between cases and controls for 59 of the 61 genes when analyzed independently.

Only two genes (*ECE1* and SLC6A4) achieved the $p < 0.05$ threshold; however ultra-rare SNVs in both genes may also be irrelevant as well since it would be predicted that perhaps 3 of the 61 genes would achieve this cut-off by chance alone. The *ECE1* encoded-endothelin-converting enzyme 1(ECE1) has been associated previously with autonomic dysfunction and has been proposed to play a potential role in SIDS-susceptibility. In 1999, a heterozygous loss-of-function *ECE1* variant (p.C742R), absent in 100 controls, was identified in a single patient with Hirschsprung disease, structural cardiac defects, craniofacial abnormalities, other dysmorphic features, and autonomic dysfunction.[29] In 2004, Weese-Mayer and colleagues reported the identification of a single *ECE1* missense variant (p.T354A) in one of 46 black SIDS cases that was absent amongst 46 ethnic matched controls.[30] However, both of these variants have now been observed within the gnomAD database at a MAF that would suggest that they may be too common (p.C742R present in 48/62,405 (0.08%) European individuals; p.T354A present in 106/12,015 (0.89%) African individuals) to be responsible for the disease phenotypes observed originally.

Solute Carrier Family 6 Member 4 (*SLC6A4*) gene encodes for the serotonin transporter 1 (5-HTT). SLC6A4 missense variants have not previously been directly linked with SIDS, however there have been other associations between SIDS and *SLC6A4*. A number of studies have explored the association of SIDS with two common functional

insertion/deletion polymorphisms thought to influence *SLC6A4* gene expression; one in the promoter region (5-HTT promoter polymorphism or 5-HTTLPR) and one in the second intron. Initial results showed an increased frequency in the long (L) allele of the *SLC6A4* promoter region in SIDS victims and infants with apparent life-threatening events as well as an association with the intron 2 polymorphism and SIDS. However, several studies involving larger SIDS cohorts failed to replicate these early findings.[12,31,32] In our SIDS cohort we identified two ultra-rare missense variants, V524M-SLC6A4 and A228D-SLC6A4 in two separate female 2-month old SIDS victims, both of which were classified as VUS by ACMG criteria. This is an interesting finding in our study, though the result is only borderline for "statistical" significance (p=-.049). Therefore, further functional data would be required before attributing any potential contribution of *SLC6A4* genetic variation in the pathogenesis of SIDS.

Given the prior inconsistent and weak associations between both *ECE1* and *SLC6A4* variants in SIDS, it would be way too premature to conclude that ultra-rare non-synonymous *ECE1* and *SLC6A4* variants are contributing factors to infant vulnerability for SIDS. In fact, based on our analysis, we suggest that many of the previously established SIDS-susceptibility genes should be reconsidered and potentially reclassified to "Limited Evidence" or "Refuted Evidence" disease-gene designations. Replication of these results in other large SIDS cohorts and functional data are necessary before concluding that 1-2% of SIDS cases may stem from non-synonymous *ECE1* and *SLC6A4* variants.

Following our recent SIDS case-control gene-collapsing ultra-rare variant burden analysis involving the 4 major cardiac channelopathy genes (*KCNQ1*, *KCNH2*, *SCN5A*, and *RYR2*), we extended our case-control gene-collapsing burden analysis to include 331 genes thought to be important in 8 different candidate biological pathways previously implicated in SIDS. While the gene-collapsing analysis did not identify any specific biological pathways to be significantly associated with SIDS, the association between

"other neurological genes" was borderline for statistical significance (p=0.05), suggesting this may be an important pathway for SIDS pathology. In fact, for one of the "other neurological genes", *SCN4A*, we recently demonstrated a significant (p= 0.0057) over-representation of functionally disruptive variants in European SIDS versus ethnic-matched controls. [33] The *SCN4A* encoded skeletal muscle voltage-gated sodium channel (Nav1.4) is important in controlling skeletal respiratory muscle contraction. Interestingly, we did not find an association between specific epilepsy genes on gene-collapsing burden analysis (18% in cases vs 18.3% in controls, p=0.58) despite other groups recently demonstrating an association with epilepsy variants and SIDS, particularly *SCN1A* in those infants with hippocampal abnormalities.[34]

Conclusions

Investigation of all previously implicated non-cardiac, SIDS-susceptibility genes in a large European SIDS case-control analysis has failed to show any significant associations of ultra-rare or novel variation consistent with autosomal dominant and recessive inheritance patterns. Furthermore, there are few pathogenic or likely pathogenic variants. This demonstrates clearly that there is very little monogenic disease involving these specific genes underlying SIDS, at least within their translated open reading frames and canonical splice sites.

Whether or not an unbiased analysis of the open reading frames/canonical splice sites of all 20,000+ genes will reveal any novel monogenic substrates for infant vulnerability remains to be determined. It also remains to be seen whether more common genetic variation may associate with infant vulnerability to sudden death thereby supporting a complex polygenic inheritance model for infant vulnerability. In the interim, these previously implicated non-cardiac SIDS-susceptibility genes should be demoted to "Limited Evidence" genes at least in terms of a penetrant, monogenic basis for SIDS.

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Tables

Table 1. Summary of the Sudden Infant Death Syndrome Cohort Demographics

Demographics		European Ancestry (n=278)
Sex	**Male**	173 (62.2%)
	Female	105 (37.8%)
Age	**Average (months)**	2.7 ± 1.98
	Range (months)	0.1 -12
Age Group	**< 2 months**	81 (29.1%)
	2 – 4 months	154 (55.4%)
	> 4 months	43 (14.7%)
Sleep Position	**Supine**	85 (30.6%)
	Prone	52 (18.7%)
	Side	29 (10.4%)
	Seated	2 (0.72%)
	Unknown	110 (39.6%)
Co-Sleeping	**Yes**	66 (23.7%)
	No	106 (38.1%)
	Unknown	106 (38.1%)

Values are n (%) or mean ± SD

Table 2. Gene Specific Yield of Ultra-Rare Non-Synonymous Variants in Cases and Controls

Gene	Cases (n=278)	Percent Cases	Controls (n=973)	Percent Controls	p-value
ECE1	3	1.1	1	0.1	0.036
SLC6A4	2	0.7	0	0.0	0.049
CHRNB4	2	0.7	2	0.2	0.216
NOS1AP	2	0.7	2	0.2	0.216
SLC9A3	2	0.7	2	0.2	0.216
FEV	1	0.4	0	0.0	0.220
HSPD1	1	0.4	0	0.0	0.220
HTR1A	1	0.4	0	0.0	0.220
IL1B	1	0.4	0	0.0	0.220
MBL2	1	0.4	0	0.0	0.220
TLX3	1	0.4	0	0.0	0.220
RET	2	0.7	3	0.3	0.309
SLC22A5	2	0.7	3	0.3	0.309
HTR3A	0	0.0	6	0.6	0.348
ACADS	1	0.4	1	0.1	0.395
NTRK2	1	0.4	1	0.1	0.395
SST	1	0.4	1	0.1	0.395
AQP4	1	0.4	2	0.2	0.530
CLCNKB	1	0.4	2	0.2	0.530
GCK	1	0.4	2	0.2	0.530
IL6R	1	0.4	2	0.2	0.530
IL13	0	0.0	4	0.4	0.581
MAP2	5	1.8	14	1.4	0.589
CHAT	0	0.0	5	0.5	0.592
GRIN1	0	0.0	5	0.5	0.592
CHRNB2	2	0.7	4	0.4	0.620
OPRM1	2	0.7	4	0.4	0.620
ACADM	0	0.0	1	0.1	1.000
ADCYAP1	0	0.0	1	0.1	1.000
BDNF	0	0.0	1	0.1	1.000
C4A	0	0.0	0	0.0	1.000
C4B	0	0.0	0	0.0	1.000
CASP3	0	0.0	0	0.0	1.000
CHRM2	0	0.0	1	0.1	1.000
CHRNA4	0	0.0	3	0.3	1.000
CHRNA7	0	0.0	1	0.1	1.000
CPT1A	1	0.4	4	0.4	1.000
CPT2	2	0.7	6	0.6	1.000
EN1	0	0.0	0	0.0	1.000
FMO3	0	0.0	1	0.1	1.000
G6PC	1	0.4	3	0.3	1.000
GABRA1	0	0.0	1	0.1	1.000
GNB3	1	0.4	3	0.3	1.000
HADHA	2	0.7	9	0.9	1.000

HADHB	0	0.0	0	0.0	1.000
IL10	0	0.0	0	0.0	1.000
IL1A	0	0.0	0	0.0	1.000
IL1RN	0	0.0	1	0.1	1.000
IL6	0	0.0	0	0.0	1.000
IL8 (CXCL8)	0	0.0	3	0.3	1.000
LMX1B	0	0.0	0	0.0	1.000
MAOA	0	0.0	3	0.3	1.000
PHOX2A	0	0.0	0	0.0	1.000
PHOX2B	0	0.0	0	0.0	1.000
SULT1A1	1	0.4	3	0.3	1.000
TAC1	0	0.0	0	0.0	1.000
TH	0	0.0	3	0.3	1.000
TNF	0	0.0	0	0.0	1.000
TPH2	0	0.0	2	0.2	1.000
TSPYL1	0	0.0	2	0.2	1.000
VEGFA	0	0.0	0	0.0	1.000

Genes are listed in order by p-value. A p-value < 0.05 was considered potentially significant.

Table 3. The Effect of Various Demographics on the Yield of Ultra-Rare Gene Variants in SIDS Cases

Demographic		Overall (n=278)	P value
Sex	**Male**	28/173 (16.2%)	1.0
	Female	17/105 (16.2%)	
Age	**2-4 months**	22/154 (14.3%)	0.33
	Other	23/124 (18.5%)	
Sleep Position	**Prone**	5/52 (9.6%)	0.40
	Supine	14/85 (16.5%)	
	Side	4/29 (13.4%)	
	Seated	0/2 (0.0%)	
	Unknown	22/110 (20.0%)	
Co-Sleeping	**Yes**	10/66 (15.2%)	0.24
	No	13/106 (12.3%)	
	Unknown	22/106 (20.8%)	

Table 4. Gene-Collapsing Rare Variant Analysis Involving All Previously Implicated SIDS-Associated Biological Pathways

Biological/Disease Pathway	Number of Genes	p-Value	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
Genetic Heart Disease	90	0.65775	0.95207	89 (32%)	322 (33%)
Epilepsy	72	0.57748	0.97947	50 (18%)	178 (18.3%)
Inborn Errors of Metabolism	69	0.97726	0.67451	30 (10.8%)	148 (15.2%)
Respiratory Control	37	0.45849	1.0665	17 (6.1%)	56 (5.8%)
Other Neurological Conditions	33	0.05391	1.4808	31 (11%)	76 (7.8%)
Autonomic Nervous System	13	0.18094	1.9606	5 (1.8%)	9 (0.9%)
Immune System	12	0.71602	0.87464	1 (0.4%)	4 (0.4%)
Nicotine Metabolizing	3	0.62886	1	2 (0.7%)	7 (0.7%)

Pathways listed by number of genes.

Figure Legends

Figure 1. Yield of Ultra-Rare Non-Synonymous Variants in Previously Published, Non-Cardiac SIDS-Susceptibility Genes– Bar graph depicting the percent yield of ultra-rare (minor allele frequency < 0.00005), non-synonymous variants identified among the 61 non-cardiac SIDS-susceptibility genes for the SIDS case and European control cohorts.