GENE AND GENE-ENVIRONMENT RISK FACTORS IN SUDDEN UNEXPECTED DEATH IN INFANTS

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ABSTRACT

Sequencing of the human genome has expanded our understanding of the molecular basis of many diseases and the complexity of genotype-phenotype relationships. Knowing the genotype does not define the clinical characteristics or phenotype, however, since phenotype is also influenced by gene-gene and gene-environment interactions. Studies in SIDS infants have now identified polymorphisms in 25 genes that are present in increased frequency compared to controls. These include polymorphisms in 8 cardiac channelopathy genes, 3 genes related to serotonin (5-HT), 7 genes related to autonomic nervous system development, 6 genes related to inflammation, and 1 gene related to energy production. The polymorphisms related to cardiac channelopathies and 5-HT have been confirmed in several reports. Confirmation is less robust, however, for the polymorphisms in other genes, in particular as related to energy production. We still know very little about the associated clinical phenotypes and the environmental perturbations required to unmask antemortem phenotypes having increased risk for sudden unexpected death in infants (SUDI). The recent identification of multiple genetic risk factors for SIDS and enhanced understanding of gene-environment interactions are contributing to our knowledge related to SUDI. The challenge now is to capitalize on these hypothesis-generating studies to identify opportunities for effective assessment and intervention in infants who will otherwise die suddenly and unexpectedly. This review summarizes current knowledge regarding gene and gene-environment risk factors that interact to yield phenotypes susceptible to SUDI.

KEY WORDS: sudden unexpected death in infants (SUDI), sudden infant death syndrome (SIDS), gene polymorphisms, serotonin (5-HT), cardiac channelopathy, autonomic nervous system, inflammation
“All illnesses have some hereditary contribution. Genetics loads the gun and environment pulls the trigger”

Francis Collins, M.D., Ph.D.
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INTRODUCTION

Sequencing of the human genome has resulted in a rapidly expanding understanding of the molecular basis of many human diseases and the complexity of genotype-phenotype relationships (1-4). Some genes are expressed only in healthy individuals or in disease conditions, only at specified ages, or in response to specific perturbations or states (e.g., sleep). Some genes contribute to susceptibility to disease, but other genes and their polymorphisms contribute to protection against illness.

Knowing the genotype even in single gene disorders does not necessarily identify the clinical characteristics or phenotype. Phenotype is also influenced by gene-gene and gene-environment interactions. Multiple genes interact with multiple environments to both increase and decrease the risk for clinical disease, and epigenetic processes resulting from environmental factors can lead to altered gene expression (5). Common examples of major disorders with polygenic inheritance, genetic heterogeneity, and multiple environmental exposures determining phenotypic expression include asthma, diabetes, and cancer (3). For such complex disorders, the whole is not only greater but may be different than the sum of its parts. This review summarizes the major gene and gene-environment risk factors that interact to yield phenotypes susceptible to
sudden unexpected death in infancy (SUDI). For the purpose of our review, SUDI are deaths that remain unexplained after a complete postmortem evaluation.

GENETIC RISK FACTORS

There are presently 25 genes for which SIDS victims have been reported to differ significantly from control groups of infants (3, 6) (Table).

Sodium and Potassium Cardiac Channelopathies
Sodium and potassium channelopathies resulting in prolonged or shortened QT intervals can result in arrhythmias or sudden cardiac death in children and adults and can be the cause of SUDI and SIDS (6-10). These channelopathies are reviewed in detail in a separate article in this issue and are not further discussed in this article.

No antemortem analyses of QT intervals are available in SIDS infants in whom a postmortem cardiac channel gene polymorphism has been identified. However, one infant with an ALTE was found to have LQTS in association with a spontaneous mutation on the SCN5A gene (10). Based on the aggregate of all studies, it is presently estimated that 10-15% of SUDI are associated with a primary cardiac channelopathy and resulting lethal arrhythmia (3, 7).

Serotonin Transporter (5-HTT)
Several polymorphisms have been identified in the promoter region of the serotonin (5-HT) transporter protein (5-HTT) gene which is located on chromosome 17 (3, 6). Variations in the promoter region of 5-HTT affect 5-HT membrane uptake and regulation.
The long "L" allele increases effectiveness of the promoter and hence would lead to reduced extracellular 5-HT concentrations at nerve endings compared to the short “S” allele (11, 12). The L/L genotype is associated with increased 5-HTT binding on postmortem neuroimaging and binding studies (11).

Caucasian, African American, and Japanese SIDS victims are more likely than matched controls to have the “L” allele (11, 12). Among 27 Japanese SIDS victims and 115 controls, for example, frequency of the L allele is higher in SIDS victims versus controls (22.2% versus 13.5%, \( p=0.003 \)). Among 44 Caucasian and 43 African American SIDS victims and matched controls, there is an association between SIDS and the 5-HTT genotype distribution (\( p=0.022 \)), specifically with the L/L genotype (\( p=0.048 \)), and between SIDS and the 5-HTT L allele (\( p=0.005 \)). There is also a negative association between SIDS and the S/S genotype (\( p=0.011 \)).

An association has also been observed between SIDS and a 5-HTT intron 2 polymorphism which differentially regulates 5-HTT expression (13). There are positive associations between SIDS and the intron 2 genotype distributions (\( p=0.041 \)) in African American (AA) SIDS, specifically with the 12/12 genotype (\( p=0.03 \)) and with the 12 repeat allele (\( p=0.018 \)).

The fifth Ewing variant (FEV) gene encodes a transcription factor critical for serotonergic (5-HT) neuronal development. An apparently rare intronic mutation has been associated with SIDS in African-American cases of SIDS (14). This finding supports the hypothesis that altered genes interfering with development of the brainstem 5-HT system may increase the known risk for African-Americans. However, in a replication study in different infant populations, this FEV polymorphism was found to occur in both SIDS and
control infants from all ethnic groups, not just SIDS cases of African-American descent (15). Since neurochemical studies indicate an association with medullary 5-HT abnormalities in about 70% of SIDS cases, however, these authors conclude that it still will likely be important to elucidate potential abnormalities in the genetic programming of 5-HT neuronal development in infants dying suddenly and unexpectedly (11, 15). They recommended, therefore, that a full genetic screen of the FEV gene is needed to better understand the molecular pathogenesis of a potential association between FEV polymorphisms, medullary 5-HT abnormalities, and sudden unexpected infant death.

**Autonomic Nervous System (ANS)**

Molecular genetic studies in SIDS victims have identified mutations pertinent to early embryologic development of the ANS (3, 6). The relevant genes include MASH 1, BMP 2, PHOX2a and PHOX 2b, RET, ECE 1, EDN 1, TLX3, and EN 1 (Table). Eleven protein-changing rare mutations have been identified in 14/92 SIDS cases among the PHOX 2a, RET, ECE 1, TLX3, and EN 1 genes. Only one of these mutations (TLX 3) was found in 2/92 controls. Each of these mutations occurred in a single SIDS case, except for the TLX3 base change that occurred in 4 SIDS and the 2 control infants. African American infants accounted for 10/11 mutations in SIDS cases and the protein-changing mutations in 2 controls.

Eight polymorphisms in the third exon of the PHOX2B gene occur significantly more frequently in SIDS compared to control infants (6). Two of the 8 polymorphisms identified were protein-altering missense mutations occurring in 9 SIDS (10%) and 4 controls (4%).
Infection and Inflammation

Loss-of-function polymorphisms have been identified in 3 anti-inflammatory genes in SUDI infants, including 2 complement C4 genes (3, 4). In a study of 104 SIDS victims, 19 infection-related infant deaths, and 84 healthy infant controls, SIDS victims with mild upper respiratory infection prior to death were more likely to have deletion of either the C4A or the C4B gene compared to SIDS victims without infection, to deaths caused by infection or to living controls (p=0.039) (16). These data suggest that partial deletions of C4 in combination with a mild upper respiratory infection place these relatively hypo-immune infants at increased risk for SUDI. Loss-of-function polymorphisms in the gene promoter region for IL-10, an anti-inflammatory cytokine, have also been observed in 46 SIDS victims compared to 660 living controls (3, 17). These IL-10 polymorphisms result in decreased IL-10 levels and hence could contribute to SIDS by delaying initiation of protective antibody production or reducing capacity to inhibit inflammatory cytokine production.

Gain-of-function polymorphisms in pro-inflammatory cytokines, resulting in increased inflammatory response to infectious or inflammatory stimuli, would also contribute to an imbalance between pro-inflammatory and anti-inflammatory cytokines. Significant associations with SIDS have been observed for a vascular endothelial growth factor (VEGF) polymorphism, two IL-6 polymorphisms, and for TNF-alpha (3, 18, 19). As apparent proof-of-principle, elevated levels of IL-6 and VEGF have been reported from cerebrospinal fluid (CSF) in SIDS infants (3, 4).

Energy Production

Mitochondria are cytoplasmic organelles that provide most of cell energy. Mitochondria contain their own DNA, mitochondrial DNA (mtDNA), which has a high rate of disease-
causing mutations (20). Several studies of mtDNA have been performed in SIDS and control infants, some of which have demonstrated significant differences in mutations in SIDS compared to control infants, including a high substitution rate in the HVR-1 region of the D-loop and an association between a high number of these substitutions and mutations in coding areas of mtDNA. Of additional potential interest, cardiac arrhythmias, including LQTS, have been observed in families with mitochondrial disease (21). It is thus possible that some mtDNA mutations may be genetic variants that when combined with environmental factors not present in controls could predispose to SUDI (4).

Studies in SIDS infants have thus identified an increasing number of polymorphisms in genes with disparate regulatory functions having increased frequency compared to control infants. However, there are several limitations to these studies. All of the genetic studies to date have been based on relatively small sample sizes, which limit their generalizability. Also, these studies are limited to SNPs, making it difficult to evaluate gene-gene interactions. Although candidate gene studies are founded on physiological hypotheses for SIDS, studies of complex diseases have demonstrated the limitations of this approach in identifying major risk loci. Resources from the International HapMap Project (22, 23) and affordable, accurate high-throughput genotyping technologies now permit unbiased analysis of the entire human genome using association methods. This approach has produced a revolution in human genetics over the past 3 years, with genome wide association studies (GWAS) successfully identifying several novel loci for complex diseases (24). Thus far, however, there are no published GWAS for SIDS in any population.
CLINICAL AND NEUROPATHOLOGIC PHENOTYPES ASSOCIATED WITH SUDDEN UNEXPECTED INFANT DEATH

The genetic polymorphisms documented in SIDS infants (Table) cannot yet be directly linked with any defined phenotype that has been ascertained antemortem. The ANS-related polymorphisms are consistent with postmortem data in SIDS infants and with the physiologic data in at-risk infants and infants later dying of SIDS. Overall, these pathophysiologic data are indicative of impaired cardiorespiratory control and arousal regulation (3, 4, 6). Brainstem muscarinic cholinergic pathways develop from the neural crest and are important in ventilatory responsiveness to CO₂. The muscarinic system develops from the neural crest, and the RET proto-oncogene is important for this development. RET knockout mice have a depressed ventilatory response to hypercarbia. Neurotransmitter studies of the arcuate nucleus in SIDS infants have identified multiple receptor abnormalities relevant to autonomic control overall and to ventilatory and arousal responsiveness in particular.

Consistent with the evidence for 5-HT polymorphisms in some SIDS infants (Table), the neuropathologic data provide compelling evidence for 5-HT dysregulation in up to 70% of infants so evaluated. 5-HT is an important neurotransmitter and the 5-HT neurons in the medulla project extensively to neurons in the brainstem and spinal cord that influence respiratory drive and arousal, cardiovascular control including blood pressure, circadian regulation and non-REM sleep, thermoregulation, and upper airway reflexes (3, 6, 11). Medullary 5-HT neurons may be respiratory chemosensors and may be involved with respiratory responses to intermittent hypoxia and respiratory rhythm generation. A recent study of multiple serotonergic brainstem abnormalities in SIDS infants further confirms a critical role for medullary 5-HT neuropathology (11, 15). These extensive abnormalities include increased 5-HT cell density and morphologic immaturity,
reduced 5-HT<sub>1A</sub> receptor binding sites in regions of the medulla involved in homeostatic function, and reduced 5-HT transporter (5-HTT) binding relative to the number of 5-HT cell bodies in the medulla. Of interest, male SIDS infants have lower receptor binding density than female SIDS infants and have a higher risk of SIDS than females. These findings suggest that the synthesis and availability of 5-HT is altered within 5-HT pathways and hence alters neuronal firing. There is a link between neuropathological raphe defects and 5-HTT promoter region polymorphisms in SIDS, including a significant correlation between raphe defects and the 5-HTT Long (L) allele (25). Of additional note, maternal smoking during pregnancy, another well-known risk factor for SIDS, was also associated with these neuropathological findings and the L allele. Although the neuropathologic data do not clarify whether medullary 5-HT levels are increased or decreased in SIDS infants, the 5-HTT polymorphism data are consistent with decreased extracellular or synaptic 5-HT concentrations.

There are thus an unknown number of antenatal phenotypes that could be associated with the observed 5-HT polymorphisms and neuropathologic findings, but no overt antenatal phenotype has yet been identified. Further, since many genes are involved in the control of serotonin synthesis, storage, membrane uptake, and metabolism, causal polymorphisms may not be limited to the 5-HTT gene. A recent study suggests a possible link between the medullary 5-HT system and the expression of IL-6 receptors (26). The arcuate nucleus is the site for several neurotransmitter abnormalities in SIDS, including 5-HT, muscarinic, and kainate receptor binding, and increased expression of IL-6 receptors (IL-6 R) is evident in the arcuate nucleus in SIDS infants, 44% of whom had signs of mild infection immediately prior to death. There was also a marginally significant increase in IL-6 R in the arcuate nucleus in sudden infant deaths attributed to infection (26). The arcuate nucleus contains 5-HT and glutamatergic neurons that at
least in animals are critical to chemosensitivity and involved with protective responses to hypercapnia, potentially induced by the combined effects of prone position and mild infection (27). CO₂ levels are reported to be elevated during severe neonatal infections and even mild respiratory infection (present in about 50% of SIDS infants) may increase CO₂ levels in infants >3 months of age (28, 29). These neuropathologic observations are consistent with the documented polymorphisms associated with upregulation of proinflammatory cytokines including IL-6 (Table) and underscore the need for continued genetic and neuropathologic research related to the brainstem, cytokines, infection, and chemosensitivity in sudden unexpected infant deaths.

A recent SIDS case report provides proof-of-concept for a link between altered antemortem physiology (phenotype) data and abnormal 5-HT receptor binding abnormalities at autopsy (30). Although no 5-HT polymorphism was identified, physiologic studies shortly after birth demonstrated altered autonomic and respiratory function not associated with an overt and clinically recognizable phenotype.

Other categories of sudden unexpected death appear to have some neuropathologic and genetic similarities with SIDS, and new data also suggest that our gaps in knowledge may be diminished by incorporating lessons learned from these other categories. Similar to observations in SIDS, medullary arcuate nucleus hypoplasia and hypoplasia of one or more nuclei of both the rostral and caudal raphe groups occur in a high frequency of sudden intrauterine unexplained death (SIUD) (25, 31). Also of note, neuropathological raphe defects, 5-HTT promoter region polymorphisms and maternal smoking during pregnancy are associated in SIUD just as in SIDS (25). Emerging evidence suggests that both sudden unexpected death in epilepsy and sudden
unexpected death in childhood may have some causal neuropathological and genetic similarities to SIDS (32, 33).

**GENE-ENVIRONMENT INTERACTIONS**

Clinical phenotypes as unmasked by the physiological studies in infants at increased risk for SIDS (a few later dying of SIDS) and neuropathological studies in SIDS infants do not clarify the extent to which causation can be attributed to at-risk genotypes, environmentally–induced DNA alterations (epigenetic changes), acute lethal environmental perturbations, or a combination thereof (5). Despite these critical knowledge gaps, however, it is evident that the risk for SIDS in individual infants is determined by complex interactions between genetic and environmental risk factors (Figure). These environmental influences or exposures may be prenatal or postnatal, and may be either cumulative (persistent or intermittent) or only present as a sudden or acute perturbation triggering a lethal sequence of events (2-4). There appears, for example, to be an interaction between prone sleep position and impaired ventilatory and arousal responsiveness. Face-down or nearly face-down sleeping does occasionally occur in prone-sleeping infants and can result in episodes of airway obstruction, but healthy infants will arouse before such episodes become life-threatening. Infants with genotypes associated with insufficient arousal responsiveness to asphyxia, however, would be at risk for sudden death. There may also be interactions between modifiable risk factors such as soft bedding, prone sleep position and thermal stress, and links between genetic risk factors such as ventilatory and arousal abnormalities and temperature or metabolic regulation deficits. Polymorphisms resulting in cardio-respiratory control deficits could be related to 5-HTT, for example, or to genes pertinent
to development of the autonomic nervous system (Table). Infants with any of these genotypes could be at increased risk for sleep-related intermittent hypoxemia and hence more susceptible to adverse effects associated with unsafe sleep position or soft bedding. Infants at increased risk for sleep-related hypoxemia and secondary acidosis could also be at greater risk for fatal arrhythmias in the presence of a cardiac channel polymorphism (34, 35).

Recent febrile illness, often related to upper respiratory infection, has been observed in 50% or more of SIDS victims (3). Although not considered to be causal per se, such otherwise benign infections could increase risk for SIDS in combination with genetically determined impaired immune responses or cytokine imbalance (Table). The reported infection and inflammatory-related polymorphisms would alter the balance between anti- and pro-inflammatory mediators, resulting in a pro-inflammatory state. The mast cell degranulation which has been reported in SIDS infants would be consistent with an anaphylactic reaction to a bacterial toxin, but has not yet been associated with a specific genotype (3).

SIDS infants have increased CSF levels of 2 pro-inflammatory cytokines, IL-6 and VEGF (3, 4). These elevations could be related to polymorphisms in these genes (Table), but there are no genotype studies in the same infants having postmortem CSF measurements. The higher VEGF levels could also be evidence of intermittent hypoxemic events since VEGF is up-regulated by hypoxia.

The increased risk for SIDS associated with fetal and postnatal exposure to cigarette smoke may be related at least in part to genetic or epigenetic factors, including those affecting brainstem autonomic control (3, 4). To date, however, no genetic studies in
SIDS infants have identified an increased frequency of any polymorphisms affecting tobacco metabolism (6). Additional genetic studies are needed, however, since both animal and infant studies indicate decreased ventilatory and arousal responsiveness to hypoxia following fetal nicotine exposure, and impaired autoresuscitation after apnea has been associated with postnatal nicotine exposure. Decreased brain stem immunoreactivity to selected protein kinase C and neuronal nitric oxide synthase isoforms occurs in rats exposed to cigarette smoke prenatally, another potential cause of impaired hypoxic responsiveness (36). Smoking exposure also increases susceptibility to viral and bacterial infections and increases bacterial binding after passive coating of mucosal surfaces with smoke components, implicating interactions between smoking, cardiorespiratory control, and immune status.

In infants with a sodium or potassium cardiac channelopathy may be at significantly increased risk for a fatal arrhythmia during sleep by predisposing perturbations that increase cardiac electrical instability, including REM sleep with bursts of vagal and sympathetic activation. Other relevant perturbations could include minor respiratory infections, and any other cause of sleep-related hypoxemia/hypercarbia, especially if resulting in acidosis (9, 10, 35, 37).

**SUMMARY AND FUTURE DIRECTIONS**

The list of gene polymorphisms associated with SIDS continues to expand, but no genome wide association studies (GWAS) have been reported in relevant populations, and we still know very little about the associated clinical phenotypes and the
environmental perturbations that may be required to unmask antemortem phenotypes having increased risk for sudden unexpected infant death. No definable antemortem phenotypes for genotypes affecting infection/inflammation have been established. The functional consequences of altered ANS developmental genotypes can be inferred from pathophysiologic data in SIDS infants, but no antemortem clinical phenotypes have been established and no antemortem assessments have sufficient sensitivity and specificity to identify apparently healthy infants destined to die of SIDS. Even less is known regarding antemortem clinical phenotypes in early infancy in any 5-HT-related polymorphisms in infants destined to die suddenly and unexpectedly.

The recent reports of genetic risk factors associated with SIDS and enhanced understanding of gene-environment and gene-gene interactions have led to important new hypotheses and new directions in research related to sudden unexpected death in infants (SUDI and SIDS). The challenge now is to capitalize on these hypothesis-generating opportunities and identify methods for effective assessment and intervention in infants who will otherwise die suddenly and unexpectedly. Regardless of what is known regarding genetic polymorphisms with causal links to SUDI, effective prevention will depend on defining the relevant environmental perturbations and the resulting clinical phenotypes that can be identified antemortem.
LEGENDS

TABLE  Twenty-five genes have been identified for which the distribution of polymorphisms differs in SIDS compared to control infants. Phenotypes can be inferred for the cardiac channelopathies and the infection/inflammation-related genotypes, but the resulting phenotypes from polymorphisms in ANS, 5-HT, or energy-production-related genes have not been established. See text for individual references.


Figure  Schematic illustration of potential interactions between representative environmental and genetic risk factors for SUDI and SIDS.

### TABLE

**CARDIAC CHANNELOPATHIES** (8 gene polymorphisms)

*Long and Short QT Syndromes (LQTS, SQTS)*

- Sodium channel (SCN5A)
- Sodium channel-interacting proteins
  - CAV3-encoded caveolin-3
  - Glycerol-3-phosphate dehydrogenase 1-like gene (GPD1-L)
- Potassium channel (KCNQ1, KCNH2, KCNE2)
- RyR2-encoded cardiac ryanodine receptor

**SEROTONIN** (5-Hydroxytryptamine, 5-HT) (3 gene polymorphisms)

- 5-HT transporter protein (5-HTT)
- Intron 2 of SLC6A4
  - Functional variable number tandem repeat (VNTR) polymorphism
- FEV

**AUTONOMIC NERVOUS SYSTEM DEVELOPMENT** (7 gene polymorphisms)

- Paired-like homeobox 2A (PHOX2A)
- PHOX2B
- Rearranged during transfection factor (RET)
- Endothelin converting enzyme-1 (ECE 1)
- T-cell leukemia homeobox (TLX 3)
- Engrailed-1 (EN 1)
- Tyrosine hydroxylase (THO1)

**INFECTION & INFLAMMATION** (6 gene polymorphisms)
- Complement C4A
- Complement C4B
- Interleukin (IL)-10
- IL-6
- Vascular endothelial growth factor (VEGF)
- TNF-alpha

**ENERGY PRODUCTION** (1 gene polymorphism)
- Mitochondrial DNA (mtDNA) polymorphisms
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exposure selectively alters protein kinase C and nitric oxide synthase expression
Environmental risk factors

- Smoking
- Soft bedding
- Prone or side sleeping
- Prematurity

Genetic risk factors

- 5-HTT polymorphism
- ANS polymorphism
- Cardiac ion channel polymorphism
- Complement or Interleukin polymorphism

Impaired autonomic regulation and arousal

SIDS

FIGURE