Systems-level perspective of sudden infant death syndrome

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Sudden infant death syndrome (SIDS) remains one of the primary causes of infant mortality in developed countries. Although the causes of SIDS remain largely inconclusive, some of the most informative associations implicate molecular, genetic, anatomical, physiological, and environmental (i.e., infant sleep) factors. Thus, a comprehensive and evolving systems-level model is required to understand SIDS susceptibility. Such models, by being powerful enough to uncover indirect associations, could be used to expand our list of candidate targets for in-depth analysis. We present an integrated WikiPathways model for SIDS susceptibility that includes associated cell systems, signaling pathways, genetics, and animal phenotypes. Experimental and literature-based gene-regulatory data have been integrated into this model to identify intersecting upstream control elements and associated interactions. To expand this pathway model, we performed a comprehensive analysis of existing proteomics data from brainstem samples of infants with SIDS. From this analysis, we discovered changes in the expression of several proteins linked to known SIDS pathologies, including factors involved in glial cell production, hypoxia regulation, and synaptic vesicle release, in addition to interactions with annotated SIDS markers. Our results highlight new targets for further consideration that further enrich this pathway model, which, over time, can improve as a wiki-based, community curation project.

Sudden infant death syndrome (SIDS) is an idiopathic syndrome that results in the sudden and unexpected death of an infant (less than 1 year of age), almost always during deep sleep. It is diagnosed when no cause of death can be found by autopsy. A number of common biological risk factors have been linked to SIDS susceptibility, including age of the infant, sex, ethnicity, gestational prematurity, and prenatal maternal exposure to tobacco or alcohol (1). In addition, a number of environmental factors have been demonstrated to increase the likelihood of SIDS, including a prone sleeping position, the sleeping environment, infection, and stress (2). However, the lack of consistent diagnosis, difficulty in distinguishing SIDS from asphyxia, suffocation or shock, and (in most cases) the presence of multiple external risk factors make it challenging to identify the underlying cause of death.

Although no clear diagnostic markers currently exist, several polymorphisms have been identified that are significantly over-represented in distinct SIDS ethnic populations (3–14). The large majority of these polymorphisms exist in genes associated with neuronal signaling, cardiac contraction, and inflammatory responses. This diversity of potential innate risk factors, associated signaling pathways, and genetics makes it difficult to obtain an integrated view of SIDS susceptibility. Indeed, SIDS may be the result of multiple distinct disorders with a common endpoint—spontaneous death during sleep—rather than a single disease entity. Although a large number of studies have been performed, there are several significant limitations for research in the area of SIDS molecular pathology. These limitations include: (i) impact of simple behavior modification on infant mortality, (ii) critical time window for susceptibility, (iii) low overall incidence in the population, and (iv) limited access to postmortem tissues. These factors, along with differences in gender, ethnicity, and infection, present significant challenges for identifying statistically significant molecular differences that may impact survival. Hence, to better understand the wealth of existing data and focus research efforts, we require a comprehensive biological model of SIDS that accounts for candidate factors, phenotypes, and interactions. Such models will be critical for prioritizing the genes, interactions, and pathways that could lead us to the underlying genetic determinants of SIDS.

Because disease research and discovery rapidly accelerate, it is essential to maintain a model that can be augmented and corrected over time. WikiPathways is an online repository of community-curated biological pathways that includes online and offline tools for editing and annotation. As opposed to gene lists or ontology categories, a WikiPathway model includes qualitative interactions and annotations that can be more intuitively understood by the typical laboratory biologist. Unlike static-pathway images, such as those presented in a typical review article, a WikiPathway contains structured relationships and embedded gene, metabolite, and reference information that can be updated by any researcher. Any WikiPathway can be reverted to a previous version or compared between distinct versions using built-in visualization tools. Because a WikiPathway can be exported to multiple formats and read by pathway- and network-analysis programs such as GenMAPP (15), PathVisio (16), and Cytoscape (17), it can be merged
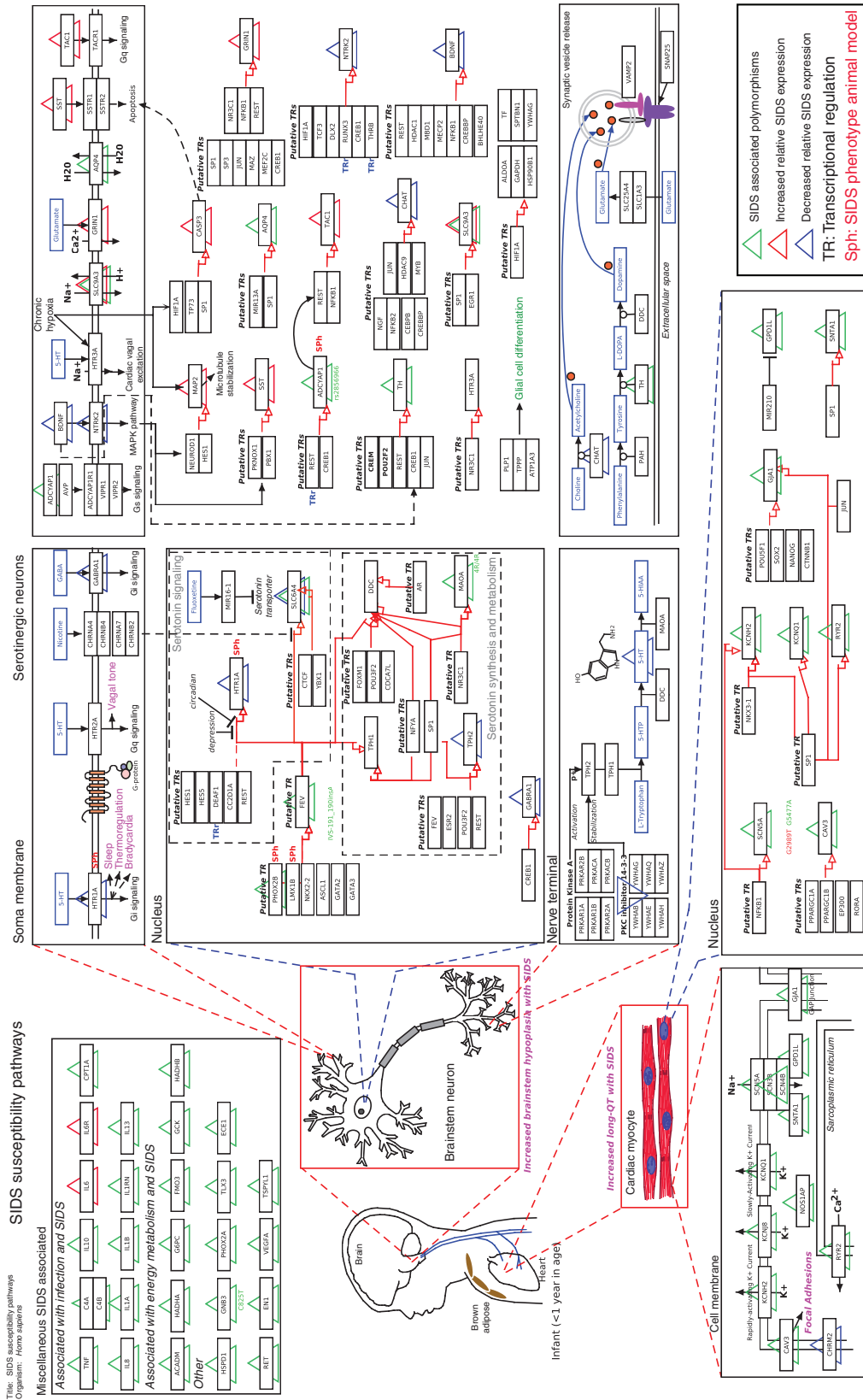
Figure 1. Integrated sudden infant death syndrome (SIDS) susceptibility pathway. Graphic export of the WikiPathway model of SIDS susceptibility (http://www.wikipathways.org/index.php/Pathway:WP706 revision 70114). A comprehensive list of DOI citations, associated gene identifiers, interactions, and metabolites are present in the online version (76). Red arrows denote transcriptional regulation. Genes are indicated by black nodes and other molecules by blue. Experimentally defined SIDS-associated molecules are indicated by an overlapping triangle denoting the type of evidence (genetic = green, increased abundance = red, decreased abundance = blue). Reprinted through Creative Commons Attribution 3.0 Unported license. Available at http://creativecommons.org/licenses/by/3.0.

with quantitative whole-genome expression, epigenetic, polymorphism, protein or metabolic data, thus allowing for highly integrated and advanced data interpretation.

In this study, we developed an integrated view of SIDS as a WikiPathway (**Figure 1**). The purpose of this model is threefold: (i) to serve as an overview of the research in this field, (ii) to identify novel crosstalk between implicated molecular components, and (iii) to recommend new candidates for focused experimental analyses. For genome-wide analyses, such candidates would further allow for prioritization of regulated genes that directly or indirectly interact with molecular components relevant to SIDS biology. Through computational analysis of transcriptional targets, we identified previously implicated and novel upstream regulators of SIDS-associated factors. Integration of this pathway model with a recently published proteomics analysis of SIDS and control brainstem samples allowed us to identify novel biological markers linked to known SIDS pathology that may eventually provide novel mechanistic insights into SIDS susceptibility.

METHODS

SIDS WikiPathway Creation
A comprehensive survey of the literature was performed from the PubMed database using the search terms "Sudden Infant Death", along with "protein", "gene", "RNA", "DNA", "variant", or "genetic", yielding 249 associated genes from 1,141 articles. Results from all species were considered. After a careful examination of the literature for each gene, only 67 were considered to have sufficient evidence for involvement in SIDS based on the reported conclusions of the manuscript authors and supplied statistical evidence (see **Supplementary File S1** online). Associated genes, proteins, and metabolites were further segregated based on associated cell-type or organ system, i.e., neuronal, cardiac, immune, or other system.

Additional biological interactions were included based on the literature (protein or DNA interactions) or using the computational tool GO-Elite to identify common candidate upstream transcriptional regulators [PAZAR and Amadeus databases (18–20)]. Genes were then further organized based on cellular compartment (e.g., membrane, cytoplasm, postsynaptic terminal, and nucleus). All relevant literature references were included.

Proteomics Analysis
Raw proteomic data were generously provided by Hannah Kinney (Harvard University) in the form of an MS-Excel sheet. This dataset is also available as supplemental information in the work by Broadbelt et al. (21). In this study, brain samples from the gigantocellularis nucleus of the medial reticular formation of six confirmed infants with SIDS and four control ("acute") infants were isolated by micropunch. Infants with SIDS were specifically selected that possessed previously identified decreases in both TPH2 protein and 5-HT levels. Acute controls were defined as infants who died within 48 h of illness but not as a result of SIDS. A significant difference in the postmortem interval existed between the SIDS cases and acute controls (SIDS, 19.0 ± 1.3 h; acute controls, 14.0 ± 3.9 h). Although the authors were able to eliminate postmortem interval as a driving factor in the differential expression of 14-3-3 family of protein kinase inhibitors using a larger validation cohort, such an analysis was not performed for the remaining proteomics predictions evaluated in this small cohort. Hence, postmortem interval should be considered a potential confounding variable for these analyses. To compare and integrate these findings into our *in silico* SIDS model, we reanalyzed the published dataset of assigned protein spectral-count values output from the software Scaffold (version 3.1.2, Proteome Software, Portland, OR). One sample, SIDS:82, was excluded from this analysis because of the lower overall spectral

counts relative to all other samples (878 counts vs. mean counts of 2,500 in the remaining set). Quantile normalization was applied to the remaining sample protein expression values (total spectral counts per protein) and all subsequent values incremented by 1 spectral count to obtain conservative fold changes. Gene annotations were obtained by translating the International Protein Index identifiers to Ensembl gene IDs, or where no Ensembl translation existed (in the Ensembl 62 and 65 databases), from the provided gene symbol. Moderated *t*-test *P* values (Limma equivalent) were calculated from these normalized protein-expression values in AltAnalyze (22) (see **Supplementary File S2** online). In total, 63 proteins were obtained from this analysis with a $P < 0.1$, the same filter used by Broadbelt et al.; however, the original list of targets identified was unavailable for comparison. Enriched pathways, ontology terms, and other gene sets were identified for differentially regulated proteins using the GO-Elite analysis module of software AltAnalyze version 2.0.8.

SIDS Pathway Extension
A database of gene interactions and gene-to-pathway associations were obtained from curated biological pathways (WikiPathways, KEGG). Databases were downloaded on 8/1/2012. All annotated WikiPathways (5,621) and KEGG-pathway (20,259) interactions were obtained from PathVisio-formatted GPML files distributed at http://www.wikipathways.org and at http://www.pathvisio.org, respectively. Novel interactions between the SIDS WikiPathway (core) and the SIDS regulated protein dataset (regulated) were obtained by identifying nodes 1–2 degrees away from the core and regulated nodes, based on the combined interaction database. Associations between core and regulated nodes were inferred by identifying the pathways shared by genes in these two groups. Statistical enrichment for the number of associated interactions obtained between the 63 regulated proteins and the 67 SIDS core markers was determined by comparing the observed number to that obtained for 10,0000 permuted randomized gene sets of the same size regulated set ($n = 63$).

RESULTS

WikiPathway Creation
Pathway design. Given the heterogeneity of SIDS-associated polymorphisms, animal genetics, and postmortem findings, our model focuses separately on relevant neurological, cardiac, and immunological interactions. For each of these cell/organ systems, genes and genotypes with the strongest reported SIDS associations were compiled from the literature and annotated in the pathway. Annotated protein-protein, protein-metabolite, microRNA-mRNA (i.e., translational repression) and protein-DNA (i.e., transcriptional regulation) interactions were extended out for each of the SIDS-associated genes. In total, 67 implicated SIDS factors and 57 interacting genes were assembled into the resulting WikiPathway (revision 49768). Although the majority of these observations were not re-evaluated in independent studies, these are included for the purpose of identifying broader candidate interactions. Custom graphics were used for each implicated cell or organ system as a means of intuitively organizing data from these different systems (**Figure 1**). Biological pathway-level enrichment analysis indicated that a large subset of these genes are involved in response to hypoxia, neuron projection, nervous system development, spinal cord injury, apoptosis, and localization to mitochondrion among others (see **Supplementary File S1** online). Examination of disease ontologies indicated an enrichment in basal ganglia diseases, depressive/anxiety disorder, respiratory signs and symptoms, tauopathies, hypertension, and cerebrovascular disorders among others.

SIDS and the central nervous system. Several lines of evidence indicate that SIDS has a strong autonomic nervous system component, in particular within neurons of the brainstem (23). The raphe nuclei of the medulla oblongata are strongly implicated in SIDS, where ultrastructural, cellular, and molecular changes have been reported in infants with SIDS (24). The raphe nuclei synthesize a large proportion of the serotonin that is produced in the human body and function in the regulation of normal cardiopulmonary activity (25), sleep (26), and thermoregulation (27).

In the proposed model, genes controlling serotonin synthesis and signal transduction were observed to have the strongest genetic and protein-expression associations with SIDS. Principal among these genes were the serotonin biosynthetic enzyme TPH2, the serotonin transporter SLC6A4, and the serotonin receptor HTR1A (3,4,24,28). Using the software GO-Elite, we discovered that several of these genes were regulated by a common transcription factor, FEV (the human orthologue of PET-1(5)). In mice, PET-1 knockout results in the loss of up to 90% of serotonin neurons (29), whereas polymorphisms in FEV are over-represented in African–American infants with SIDS (5).

Vasopressin signaling molecules were also included in our model as putative regulators of cardiopulmonary regulation (30). A protein that is associated with vasopressin signaling, named pituitary adenylate cyclase–activating polypeptide (gene name ADCYAP1), results in a SIDS-like phenotype— characterized by a high increase in spontaneous neonatal death, exacerbated by hypothermia and hypoxia—when disrupted in mice (31). Pituitary adenylate cyclase–activating polypeptide is widely expressed throughout the central nervous system, including autonomic control centers (32), and seems to be polymorphic in African–American SIDS cases (6). Although it is not clear whether ADCYAP1 and HTR1A are expressed in the same cells, these proteins regulate opposing G protein–coupled receptor signaling pathways.

Nicotinic acetylcholine receptors, which are expressed in serotoninergic raphe neurons throughout development (33), were also included in this model because of the potential implication in SIDS. The application of nicotine or exposure to cigarette smoke is sufficient to inhibit electrical activity of raphe serotoninergic neurons (34), and chronic nicotine infusion in rats decreases expression of the SLC6A4 gene (35). Furthermore, nicotine exposure reduces immunoreactivity of the serotonin receptors 5-HT1A (HTR1A) and 5-HT2A (HTR2A) in several nuclei of the brainstem (36).

Many neuronal-implicated SIDS genes (ADCYAP1 (37), HTR1A (38), TPH2 (39), GRIN1 (40), CHRNB2 (40), TH (41), and TAC1 (42)) can all be transcriptionally regulated by REST (repressor element-1 silencing transcription factor). REST, along with three other genes (HNF4A, SP1, and CREB1), was independently enriched in our transcriptional regulatory analysis, suggesting that these factors may be novel targets of interest in SIDS susceptibility.

SIDS and the heart. Several studies have identified a critical link between cardiac arrhythmia (i.e., long-QT syndrome) and

SIDS (9). Indeed, several research groups hypothesize that a subset of diagnosed SIDS deaths are identical in etiology to adult forms of cardiac sudden death (43). A number of genetic association studies identified function-modifying mutations in critical cardiac channels in as many as 15% of all SIDS cases (9). These mutations have been predicted to predispose infants to lethal cardiac arrhythmias and cardiomyopathies associated with sudden death. The highest proportion of SIDS-associated mutations (both inherited and sporadic) is found in the sodium channel gene SCN5A. SCN5A mutations cause long-QT3 syndrome, which is responsible for the highest incidence of spontaneous death during sleep; therefore, this disorder may account for a significant proportion of SIDS deaths (44). Mutations in other crucial cardiac-signaling genes, including connexin-43 (GJA1), ryanodine receptor 2 (RYR), and several others, have also been implicated (9). In addition, further examination highlighted a diverse set of putative transcriptional regulators for these genes, as well as the relatively common transcription factor, SP1 (45–47).

SIDS and the immune system. Infection is considered a significant risk factor for SIDS (48), and several mutations have been identified in genes associated with inflammatory responses and thermoregulation. For mutations in inflammation-associated genes, such as TNF-alpha (10), interleukin (IL-10 (11)), and complement component-4 (12), many are only significant in the presence of infection and SIDS. In SIDS cases, cerebrospinal fluid levels of IL-6 are also increased as are IL-6 receptor levels in the arcuate nucleus of the brain, another major site of serotonin synthesis (49). Genes such as IL-6 and ADCYAP1 have also been linked with inflammatory processes (12,50), suggesting that pathways associated with inflammation in the brain can promote or predispose SIDS (49).

Other implicated SIDS genes. Regulation of thermogenesis by brown adipose tissue has been proposed to be an important component of SIDS, given that SIDS incidence is highest in the winter months and that animal models of SIDS demonstrate variations in body temperature compared with controls (51–53). Interestingly, the activation of raphe HTR1A decreases both shivering and peripheral vasoconstriction in piglets (27). Other miscellaneous genes with genetic associations for SIDS cases are also shown.

Replication of genetic findings. Although many of the indicated genes in our molecular model were implicated from small cohort analyses and often for rare variants, a number of these genetic observations have been re-evaluated in larger independent cohorts. Chief among these are the neuronal signaling components MAOA, PHOX2B, and SLC6A4, where multiple studies have evaluated specific variants in SIDS (54–62). Only in the case of MAOA are there independent studies, which demonstrate the significant enrichment of a genetic variant in SIDS cases (promoter length polymorphisms) relative to controls (54,55); however, there are an equal number of studies that dispute this claim (56,57). MAOA is an attractive

candidate molecular regulator because it is encoded on the X-chromosome, and hence, may influence the greater propensity of male infants to succumb to SIDS. Many other variants have been evaluated in replication studies; however, nearly all of these do not reach statistical significance on *post hoc* analyses or are borderline significant (58,62–64). In the case of both IL-1 (65) and IL-1 receptor antagonist (66) gene variants, significant replication in a single study has been reported in SIDS.

An intriguing strategy to evaluate the penetrance of reported SIDS polymorphisms is in reference to the large and growing DNA sequence and single-nucleotide polymorphism databases. To evaluate the incidence of channelopathy-associated genetic variants for CAV3, KCNH2, and SCN5A in the general population, Andreasen et al. (67) recently evaluated these variants from Exome Sequencing Project analyzed subjects. The incidence of these variants was found to be high (1 in 29) relative to the known incidence of SIDS. However, these analyses do not consider the distribution of these variants in SIDS cases or the presence of extrinsic risk factors, such as sleep position, which are considered necessary for triggering SIDS in a large subset of cases.

Integration With SIDS Proteomics Data

All of the molecular associations discussed thus far were derived using a classical candidate gene or molecular approach. However, to identify novel regulators that could open new avenues for the mechanistic evaluation of SIDS, we require unbiased discovery using high-throughput experimental strategies such as genomics and proteomics. Recently, Broadbelt et al. (21) performed a proteomic survey of the brainstem of a small cohort of infants who died of SIDS. The infants with SIDS evaluated in this study all had prior detected decreases in the amount of TPH2 and 5-HT. Although their analysis focused on alterations in the 14-3-3 family of protein kinase inhibitors occurring in SIDS compared with control infants, several dozen additional proteins were predicted to be regulated (list unpublished).

Although only a small cohort of samples were analyzed by Broadbelt et al. using proteomics, we reanalyzed this data to determine whether any regulated peptides can be bioinformatically implicated in known SIDS pathways or with our presented integrated model. Such an analysis further provides an important workflow for augmenting the presented WikiPathway model. Similar to the study by Broadbelt et al., we identified 63 proteins that achieved a $P < 0.1$ moderated t-test cutoff when comparing SIDS vs. acute controls. Similar to the original analysis, 14-3-3 protein family members were among the most significant proteins identified (see **Supplementary File S2** online). To discover functionally informative gene sets, we performed GO-Elite analysis on upregulated and downregulated genes for pathways, ontologies, transcription targets, and cellular biomarkers. This analysis revealed a number of gene sets associated with SIDS pathology, including those that regulate synaptic transmission (SNAP25, VAMP2, SLC1A3, and SLC25A4), myelin sheath development and glial cell differentiation (GAP43, MBP, PLP1, TPPP, ATP1A3, and TUBB4A),

and response to hypoxia (ALDOA, GAPDH, HSP90B1, TF, SPTBN1, and YWHAG). Novel categories discovered include gene sets involved in glycolysis and gluconeogenesis (ALDOA, GAPDH, GOT1, and GOT2), the tricarboxylic acid cycle (ACO2 and IDH2), prolactin signaling (PPIA, SIRPA, YWHAG, and YWHAZ), cytoskeletal organization (KRT17, KRT2, KRT9, MAPT, MSN, SPTBN1, and TUBB), and transcriptional regulation by the transcription factors NFIA (ALDOA, MBP, and PLP1), SP1 (ALDOA, ATP1A3, and MBP), HSF1 (ATP1B1, CRYM, and HSPA1A), FOS (GOT1, MBP, STIP1, and TUBB4B) and HNF4A (ANXA6, GAPDH, GNB1, GOT1, HSP90B1, and TF) (see **Supplementary File S1** online).

Synaptic transmission–associated proteins collectively had the largest observed fold changes in this dataset. SNAP25 and VAMP2 protein expression was downregulated 7- and 3.5-fold, respectively, in SIDS vs. acute controls (**P < 0.01). In addition, the neurotransmitter reuptake transporters SLC25A4 and SLC1A3 were downregulated 4.8- and 3.9-fold, respectively (*P < 0.05) (**Figure 2a**).

Connecting Proteomics Results With Validated SIDS Markers

To evaluate how proteins identified through the proteomics approach can interact with more established SIDS markers, we identified genes in both sets that participate in the same biological pathways or share annotated interactions. All known SIDS markers were considered for these two queries.

For the analysis of shared pathways, 29 of 67 established SIDS markers were found to overlap with 30 of the 63 targets identified from the brainstem proteomics analysis in one or more pathways (23 common pathways in all) (**Table 1**). An example is the co-occurrence of MBP (myelin basic protein) and the transcription factor PHOX2B in the neural crest differentiation pathway. Both MBP and PHOX2B are regulated by the transcription factor SOX10 in this pathway. This pathway co-occurrence analysis identified several additional factors that would not have been connected to SIDS biology using other methodologies.

When possible annotated interactions between established SIDS markers and proteomics findings were examined, four genes (VAMP2, RAP1B, MAPT, and MBP) were found to connect indirectly to the gap junction protein connexin-43 (GJA1) through protein kinase or MAP kinase intermediates (Figure 3C). In addition, the genes MAPT and SLC directly and indirectly interact through the SIDS associated protein CASP3, respectively. These interactions were statistically enriched when compared with a permuted set of random proteins relative to SIDS annotated markers ($P = 0.04$).

DISCUSSION

Although the incidence of SIDS has decreased substantially through educational campaigns that promote safe sleep, the number of SIDS deaths remains high. Improvements in educational efforts and legislation around safe sleep are still needed, as are diagnostic methods to identify at-risk infants in need of stringent monitoring or possible prophylactic intervention
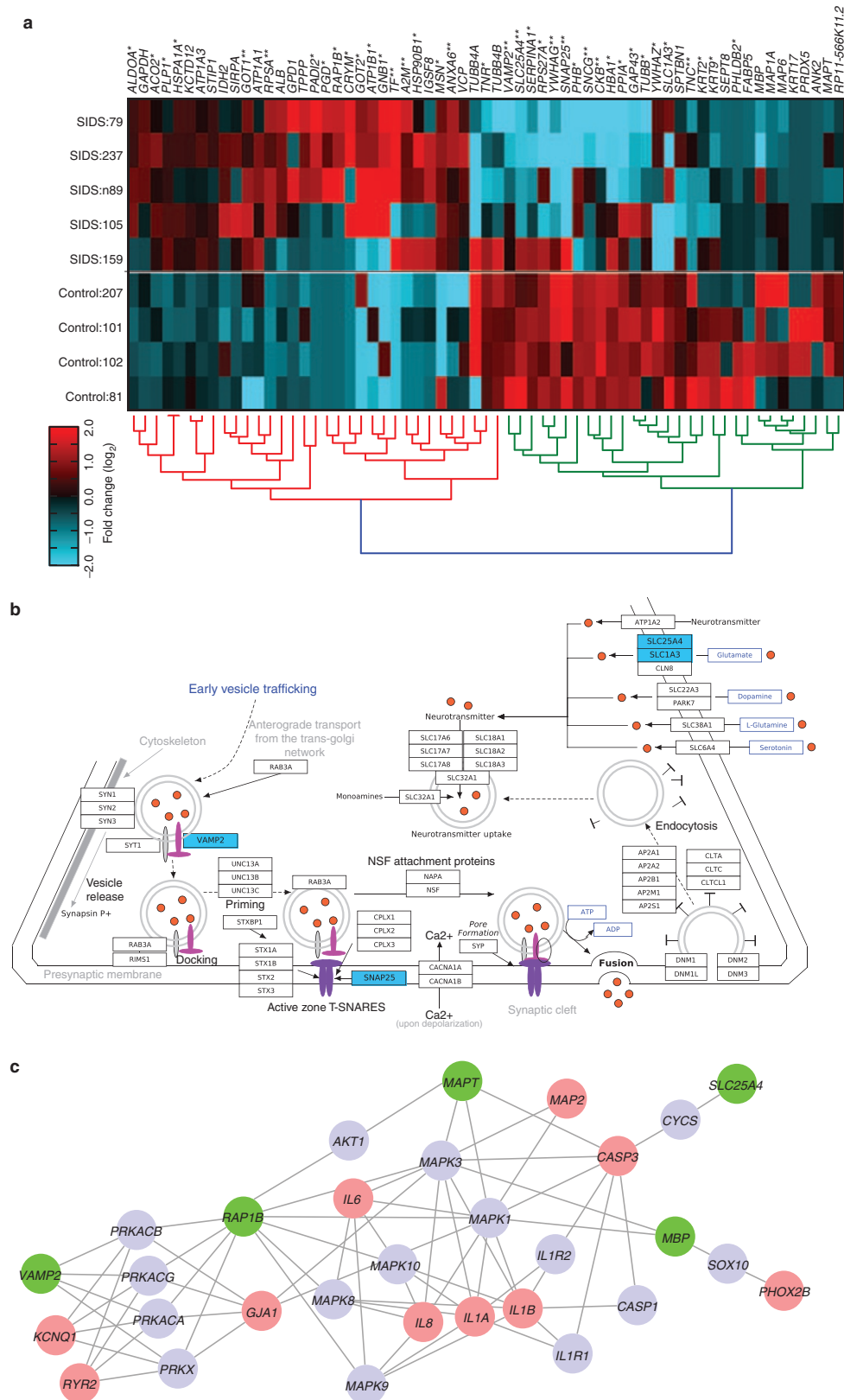
Figure 2. Novel proteomic sudden infant death syndrome (SIDS) associations. Differentially regulated proteins identified through re-analysis of SIDS brainstem proteomic profiles. (**a**) AltAnalyze produced hierarchically clustered SIDS and acute control protein-expression profiles relative to the mean of all samples; blue and red indicate up- and downregulation, respectively (*$P < 0.05$, **$P < 0.01$). (**b**) AltAnalyze visualization of the WikiPathways Synaptic Vesicle Pathway (WP2267), with proteins downregulated in the SIDS proteomic analysis in blue. (**c**) Annotated interactions between differentially expressed proteins in SIDS cases (green), previously implicated SIDS genes (light red), and interacting proteins (light blue) in Cytoscape.

Table 1. Implicated SIDS genetic loci and observed SIDS-regulated brainstem proteins occurring within common biological pathways (KEGG or WikiPathways)

Shared pathway	SIDS implicated	SIDS regulated proteins
Protein digestion and absorption: KEGG-hsa04974	KCNQ1, SLC9A3	ATP1B1, ATP1A1, ATP1A3
Pancreatic secretion: KEGG-hsa04972	KCNQ1, RYR2	ATP1B1, RAP1B, ATP1A1, ATP1A3
Arginine and proline metabolism: KEGG-hsa00330	MAOA	CKB, GOT1, GOT2
PPAR signaling pathway: KEGG-hsa03320	ACADM, CPT1A	FABP5
Nicotine activity on dopaminergic neurons: WP1602	CHRNA4, CHRNB2, TH	GNB1
Chemokine signaling pathway: KEGG-hsa04062	GNB3, IL8	GNB1, RAP1B
Glutamatergic synapse: KEGG-hsa04724	GNB3, GRIN1	GNB1, SLC1A3
Malaria: KEGG-hsa05144	IL10, IL1B, IL6, IL8, TNF	HBA1
Selenium pathway: WP15	IL1B, IL6	HBA1, PRDX5, ALB
Pathways in cancer: KEGG-hsa05200	CASP3, IL6, IL8, RET, VEGFA	HSP90B1
Endocytosis: KEGG-hsa04144	CAV3, RET	HSPA1A
Toxoplasmosis: KEGG-hsa05145	CASP3, IL10, TNF	HSPA1A
Prion diseases: KEGG-hsa05020	IL1A, IL1B, IL6	HSPA1A, STIP1
Neural crest differentiation: WP2064	PHOX2B	MBP
Measles: KEGG-hsa05162	IL1A, IL1B, IL13, IL6	MSN, HSPA1A
GPCRs, class A rhodopsin-like: WP455	CHRM2, HTR1A, HTR2A	RP11-566K11.2
Calcium signaling pathway: KEGG-hsa04020	CHRM2, CHRNA7, GRIN1, HTR2A, RYR2	SLC25A4
Mineral absorption: KEGG-hsa04978	SLC9A3	TF, ATP1B1, ATP1A1, ATP1A3
Focal adhesion: KEGG-hsa04510	CAV3, VEGFA	TNC, TNR, RAP1B
Gap junction: KEGG-hsa04540	GJA1, HTR2A	TUBB, TUBB4B, TUBB4A, RP11-566K11.2
Calcium regulation in the cardiac cell: WP536	CHRM2, GJA1, GNB3, RYR2	YWHAG, ANXA6, ATP1B1, YWHAZ, GNB1
Myometrial relaxation and contraction pathways: WP289	GJA1, GNB3, IL1B, IL6, RYR2	YWHAG, YWHAZ, GNB1

GPCRs, G protein–coupled receptors; SIDS, sudden infant death syndrome.

(e.g., prenatal diagnosis of long-QT syndrome). Such personalized medicine approaches are likely to be required given the apparent genetic heterogeneity and penetrance of SIDS. As demonstrated by the analysis of colon cancer genotypes, pathway-based prioritization of disease variants is a potent strategy for developing new diagnostic models to understand how seemingly heterogeneous genetic elements can contribute to a common biological endpoint (68). There are several challenges in the development of a pathway model for SIDS, including the unclear role of genetic factors, multiple implicated organ systems, limited and week associations based on few cases, and investigator bias in gene evaluation.

The SIDS WikiPathway resource described herein represents an important starting point from which to begin evaluating next-generation genomic profiles in a focused way, to identify events that may not immediately seem to be related. As an example, we analyzed brainstem proteomics data from infants who died of SIDS or other causes. From these data, we identified a large number of genes that could be connected to previously implicated SIDS genetic loci based on known biological pathway interactions. More importantly, we identified a number of proteins that regulate key biological processes associated with SIDS pathophysiology, such as brainstem gliosis, hypoxia, and inhibition of synaptic vesicle fusion. We subsequently used

both sets of results to improve our SIDS WikiPathway model. Finally, through the restricted analysis of established SIDS genetic loci, we discovered key regulatory factors and potential crosstalk mechanisms between distinct SIDS gene associations that would have been difficult without a pathway-based model. Among these, FEV, REST, HNF4A, and SP1 require additional attention as transcription factors that could drive control elements for established SIDS genes. SP1 and HNF4A were enriched in both literature-associated SIDS genes and among SIDS brainstem differentially expressed proteins.

Potential crosstalk between the serotonin and vasopressin pathways will also require additional exploration. Several of the genes identified through our proteomics re-analysis directly impinge upon cell types associated with the gross phenotypic alterations observed in infants with SIDS (69). One of the most intriguing alterations we observed was in the regulation of factors associated with oligodendrocyte differentiation and myelin deposition. Three of these genes were upregulated in SIDS (PLP1, TPPP, and ATP1A3) and three were downregulated (GAP43, MBP, and TUBB4A). An early discovery into the pathology of SIDS was the finding that infants with SIDS have a significantly increased number of glial cells within the brainstem, which may act to disrupt neuronal growth (69,70). GAP43 expression is known to be downregulated during

oligodendrocyte differentiation and is likely to be a marker of earlier-stage progenitor cells (71). Differentiating oligodendrocytes are believed to be among the most metabolically active cell types in the brain, owing to the energy required for filopodial extension and myelin ensheathment of axons (72,73). In line with this observation, we saw the coordinate and relatively large upregulation of factors promoting glycolysis and the tricarboxylic acid. We also observed the upregulation of the filopodia-associated gene moesin (MSN) in SIDS cases (*$P < 0.05$). These factors represent interesting and possibly causative regulators of increased glial cell production the SIDS brainstem.

Another important observation from this proteomics dataset was the coordinate downregulation of factors involved in synaptic transmission, particularly the synaptic vesicle–associated proteins SNAP25 and VAMP2. Botulinum spores cleave both SNAP25 and VAMP2, and exposure to this toxin is responsible for a subset of documented SIDS cases (74). However, this downregulation could also happen through a regulatory mechanism independent of external pathogens. In addition, the increased expression of a number of HIF1-alpha targets suggests that chronic hypoxia may be a contributing pathology in the SIDS brains analyzed in this study. HIF1-alpha expression is induced during hypoxia, to increase the expression of target genes believed to promote oxygen delivery or facilitate adaptation to hypoxic conditions. Chronic hypoxia is believed to be a common trigger of SIDS (75).

It is important to note that the genes observed in these proteomics analyses have insufficient statistical evidence to definitely associate these with SIDS, because of the small number of samples analyses, relatively small number of peptides detected, and the potential effect of differing postmortem interval. The same is true for the majority of genetic studies reported in this review, which are typically not validated on an independent cohort of sufficient size to definitely associate a given polymorphism with SIDS. Hence, it is critical for researchers to be able to evaluate existing findings within a coherent framework as well as identify *in silico* predicted regulators for focused validation. We believe the SIDS pathway model described in this review will provide an important reference for such future studies and allow for additional connectivity analyses to novel factors. Such studies will inevitability improve this community *in silico* model as a result.

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