Supplementary section

Title: Enrichment of genomic pathways based on differential DNA methylation associated with chronic postsurgical pain and anxiety in children – a prospective, pilot study

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Methods (supplementary)

Anesthesia and Pain protocols

Standardized general endotracheal anesthesia was used for all procedures. This included midazolam, propofol-remifentanil total intravenous anesthesia, anti-emetics, and morphine/hydromorphone titrated at the end of surgery to pain and respiratory rate. Surgeries were conducted mostly (>90%) by two surgeons. Postoperatively, all patients were followed by the perioperative pain service. They received Patient Controlled Analgesia (PCA) with morphine/hydromorphone (standard doses of 20 mcg/kg PCA demand doses for morphine and 4 mcg/kg for hydromorphone) with/without continuous infusions, intravenous acetaminophen, ketorolac, diazepam as needed for muscle spasms and methocarbamol.

Figure legends

Supplementary Figure 1: DARPP32-Dopamine pathway (figure derived from the Ingenuity pathway analysis) in the presynaptic neuron (one of the top canonical pathways shared by chronic postsurgical pain and anxiety outcomes). Ingenuity pathway illustration of the DARPP32-Dopamine pathway for CPSP (Panel A) and CASI (Panel B). Some molecules/genes influenced by methylation changes in CPSP and CASI are common (calcium channel and adenyl cyclase) and some different (DARPP32 protein, dopamine receptor, CAMKK in CPSP; and NMDA, K channel, PKA, CALM, and CREB for CASI). These molecules/genes have varied functions (channels, receptors, second messengers, etc.) and work together to regulate the pathway.

Supplementary Table 1. Top canonical pathways mapped to significantly methylated CpG sites for chronic postsurgical pain and anxiety sensitivity.

Ingenuity Canonical Pathways for Chronic postsurgical pain	**-log(p-value)***
GABA Receptor Signaling	3.78
PKCθ Signaling in T Lymphocytes	3.11
Dopamine-DARPP32 Feedback in cAMP Signaling	2.39
Cellular Effects of Sildenafil (Viagra)	2.26
GPCR-Mediated Nutrient Sensing in Enteroendocrine Cells	1.92
Calcium Signaling	1.87
nNOS Signaling in Skeletal Muscle Cells	1.87
Dopamine Receptor Signaling	1.83
FcγRIIB Signaling in B Lymphocytes	1.80
cAMP-mediated signaling	1.66
Corticotropin Releasing Hormone Signaling	1.55
Fatty Acid α-oxidation	1.53
Tryptophan Degradation X (Mammalian, via Tryptamine)	1.43
Top 20 Ingenuity Canonical Pathways for Childhood anxiety sensitivity	**-log(p-value)***
Cardiac β-adrenergic Signaling	8.61
cAMP-mediated signaling	5.67
CDK5 Signaling	5.54
Protein Kinase A Signaling	5.22
G-Protein Coupled Receptor Signaling	4.58
Dopamine-DARPP32 Feedback in cAMP Signaling	4.47
Axonal Guidance Signaling	4.13
Dopamine Receptor Signaling	4.12
GNRH Signaling	3.98
Androgen Signaling	3.89
Cellular Effects of Sildenafil (Viagra)	3.71
Nitric Oxide Signaling in the Cardiovascular System	3.63
G Beta Gamma Signaling	3.58
mTOR Signaling	3.43
Melanocyte Development and Pigmentation Signaling	3.41
PTEN Signaling	3.35
AMPK Signaling	3.30
GPCR-Mediated Integration of Enteroendocrine Signaling	3.25
GABA Receptor Signaling	3.08
nNOS Signaling in Skeletal Muscle Cells	3.08

*Pathways with -log(p-value) ≥ 1.3 are reported here as statistically significant