DNA methylation at Mu Opioid Receptor gene (OPRM1) promoter predicts preoperative, acute and chronic post-surgical pain after spine fusion

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INTRODUCTION

The incidence of persistent surgical pain after surgery (CP) in children ranges between 13 to 68.8%. Genetics and recently epigenetics together have been implicated in the transition of acute to chronic postsurgical pain1,2. DNA methylation, a common epigenetic mechanism (addition of a methyl group to the 5' position of cytosine at CpG dinucleotides) is a potent repressor of gene expression. In this study, we evaluated DNA methylation at the promoter of the mu opioid receptor gene (OPRM1) which plays a key role in opioid-pain pathways, as a predictor of preoperative, acute postoperative pain and CP.

RESULTS

Demographics of the cohorts and description of covariates

<table>
<thead>
<tr>
<th>Variable</th>
<th>Acute Postoperative Pain (N=128)</th>
<th>Chronic Postoperative Pain (N=129)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>14.49 ± 1.91 (95% CI 12.59-16.40)</td>
<td>14.78 ± 1.67 (95% CI 12.59-16.40)</td>
</tr>
<tr>
<td>Sex</td>
<td>0.23</td>
<td>0.54</td>
</tr>
<tr>
<td>Male</td>
<td>35 (26%)</td>
<td>9 (21%)</td>
</tr>
<tr>
<td>White</td>
<td>111 (87%)</td>
<td>66 (88%)</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>54.00 (46.00-61.90)</td>
<td>54.00 (46.00-61.90)</td>
</tr>
<tr>
<td>Maternal OPRM1 (Child)</td>
<td>4.40 (3.60-5.80)</td>
<td>3.60 (3.00-5.20)</td>
</tr>
<tr>
<td>Maternal Anxiety (Parent)</td>
<td>5.05 (4.00-6.00)</td>
<td>5.00 (4.00-6.00)</td>
</tr>
<tr>
<td>Preoperative pain score</td>
<td>0.00 (0.00-0.00)</td>
<td>0.00 (0.00-0.00)</td>
</tr>
<tr>
<td>Number of vertebral levels fused</td>
<td>12.00 (11.00-12.00)</td>
<td>12.00 (11.00-12.00)</td>
</tr>
<tr>
<td>Surgical times (hours)</td>
<td>0.87 ± 0.67</td>
<td>0.87 ± 0.67</td>
</tr>
<tr>
<td>Pain AUC P0D1/2</td>
<td>198.66 ± 88.18</td>
<td>226.84 ± 80.44</td>
</tr>
<tr>
<td>Morphine dose P0D1/2 mg/kg</td>
<td>1.10 (0.67-1.62)</td>
<td>1.10 (0.67-1.62)</td>
</tr>
</tbody>
</table>

a: data exhibited normal distribution; shown as mean ± SD and compared using t-tests for CP.
b: data exhibited frequency proportion; and compared using Chi-squared tests for CP.
c: data did not exhibit normal distribution; shown as median (IQR) and compared using Wilcoxon rank sum tests for CP.

a. modelled using logistic regression adjusted for preoperative pain score and morphine consumption over postoperative days 1 & 2.
b. modelled using linear regression adjusted for preoperative pain score and morphine consumption over postoperative days 1 & 2.
c. Odds ratio (OR) represents the odds of chronic postoperative CP (CPS) with 1% increase in DNA methylation level.

CONCLUSIONS

• We have found novel biomarkers in blood (DNA methylation at several CpG sites of the OPRM1 promoter region) associated with preoperative, acute postoperative and CP.
• Since DNA methylation is influenced by multiple modifiable factors (diet, exercise, parental upbringing, stress), understanding the role of epigenetic regulation of OPRM1 in pain is critical in better understanding pain pathways.
• Future studies need to map methylation changes in the OPRM1 promoter over the course of persistent post-surgical pain.

METHODS

After IRB approval and appropriate consent, a prospective study was conducted in 135 adolescents with idiopathic scoliosis who underwent posterior spine fusion under general anesthetic and monitored in protocols.

Exclusion criteria: Obesity, developmental delay, significant renal, hepatic, respiratory abnormalities, recent opioid use.

Data collection:
- Preoperative pain score, baseline questionnaires to assess anxiety, functioning in child (CASI, FDI) and catastrophizing (PCS) in child and parent.
- Opioid consumption and pain scores (48 hours postop)
- Follow-up at 2-3 months post-surgery for pain descriptors, CASI, FDI, PCS-C.
- Preoperative blood samples were analyzed for DNA methylation by pyrosequencing of 22 CpG sites at the OPRM1 gene promoter.

Outcomes: preoperative pain, acute postoperative pain (measured by Area under Curve (AUC) for pain scores) and CP (NRS=3/10-23/10 months post-surgery).

Analysis: The association of each pain outcome with the methylation% of each of the CpG sites was assessed using a multi-variable regression model, adjusting for significant (p < 0.05) non-genetic variables.

Functional genomics evaluation included interrogation of 4,953 datasets from various sources (ENCODE, Roadmap Epigenomics, Cistrome, and ReMap-Chip).

Functional Genomics

• This region is located in open chromatin marked by H3K27ac, H3K4me1 and H3K4me3 (indicative of active regulatory regions) in non-cancer brain cells from the caudate-putamen, temporal, frontal lobes and angular gyrus.
• This region contains CREBP-seq peaks for binding of multiple transcription factors, including REST, RAD21, SP1, YY1, and ZNF263 in various tissues. In particular, REST and RAD21 binds the OPRM1 promoter region in three cell lines (SK-N-SH, FPSK1, SK-N-SH cell lines) derived from brain tissue where the opioid receptors responsible for analgesia are found.
• DNA methylation at these sites potentially decreases OPRM1 gene expression via alteration of the binding of transcription factors, leading to decreased response to endogenous and exogenous opioids, and increased pain sensitivity.

REFERENCES