Altered Hippocampal DNA Methylation, Gene Transcription, and RNA Editing in Response to Early Life Environmental Insults in Two Independent Studies of Cognitive Development

Kyle M. Schachtschneider[1,2], Laurie A. Rund[1], Ole Madsen[2], Rodney W. Johnson[1], Martien A.M. Groenen[2], Lawrence B. Schook[1]

[1]Department of Animal Sciences, University of Illinois at Urbana-Champaign, Illinois, USA
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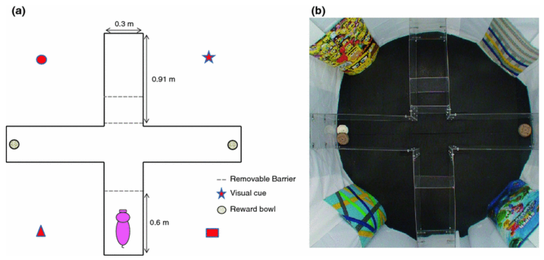
Abstract

This study investigated DNA methylation and gene expression patterns in hippocampus samples from two studies observing reduced hippocampal-based spatial learning and memory in response to early life environmental insults (iron deficiency and PRRSv infection). Genes involved in neurodevelopment and function were differentially expressed, including 2 genes associated with differential methylation in both studies. Altered *HTR2C* RNA editing was also observed in both studies.

Introduction



(Rytych et al., 2012)

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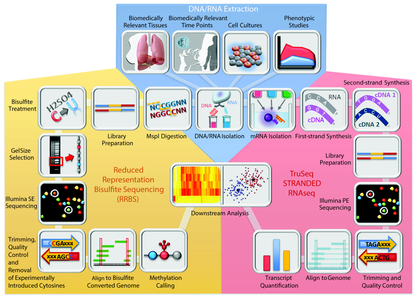

Abstract

DNA methylation is an epigenetic mark that occurs at cytosines throughout the genome, is involved in regulating gene expression, and is altered in response to environmental signals. This study investigated DNA methylation and gene expression patterns in hippocampus samples from two studies observing reduced hippocampal-based spatial learning and memory in response to early life environmental insults (iron deficiency and PRRSv infection) in porcine biomedical models of cognitive development (Rytych et al. 2012; Elmore et al. 2014). Reduced representation bisulfite sequencing and RNA-seq were performed on 16 hippocampus samples (iron deficiency - 3 deficient, 4 control; PRRSv infection - 4 infected, 5 control). In total 192 and 455 differentially expressed genes (DEGs) were detected in the iron deficient and PRRSv infected groups, respectively. Of these, 53 were differentially expressed in both studies, including genes involved in neurodevelopment and function, such as *GRP126*, *SEMA3G*, *CARTPT*, *NTNG1*, *PRSS12*, *GABRE*, and *HTR2C*. Differential DNA methylation was assessed at over 600,000 CpG and 2.4 million non-CpG sites in both studies, identifying 853 differentially methylated (DM) CpG and 99 DM non-CpG sites in the iron deficient group, 12 of which were associated with 9 DEGs. 1,857 DM CpG and 153 DM non-CpG sites were identified in the PRRSv infected group, 26 of which were associated with 19 DEGs. Increased expression of *VWF* (log2 fold change > 1.8) and *HTR2C* (log2 fold change > 1.0) was associated with hypomethylation of the same genomic regions in the iron deficient and PRRSv infected groups. In addition, as *HTR2C* undergoes adenosine-to-inosine (A-to-I) RNA editing at 5 sites known to affect *HTR2C* receptor activity and brain function in humans, the editing frequency of *HTR2C* was determined. Increased editing was detected at site A in both groups, although the difference was only significant for the iron deficient group (P = 0.019). In addition, one RNA isoform (IAAAI) and one protein isoform (V-S-I) were expressed exclusively in both the iron deficient and PRRSv infected groups. Together, these results provide evidence for altered hippocampal DNA methylation, gene expression, and *HTR2C* RNA editing in response to early life environmental insults in two independent studies of cognitive development.

References:
Rytych et al. 2012. Early life iron deficiency impairs spatial cognition in neonatal piglets. J. Nutr. 142(11):2050-2056

Elmore et al. 2014. Respiratory viral infection in neonatal piglets causes marked microglia activation in the hippocampus and deficits in spatial learning. J. Neurosci. 34(6):2120-2129

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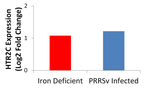

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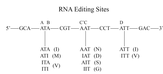

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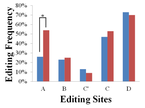

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Differential methylation and altered gene expression was identified in two independent studies reporting reduced cognitive development in response to early life environmental insults. Increased expression of the serotonin receptor *HTR2C* was associated with hypomethylation of a region ~2,400 bp upstream of the TSS in both studies.

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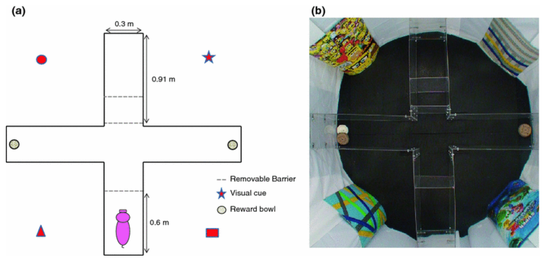

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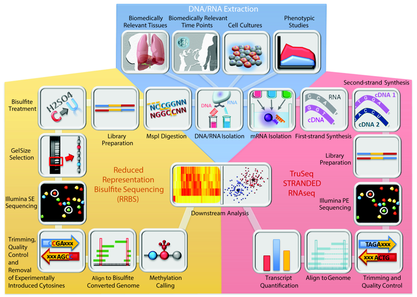
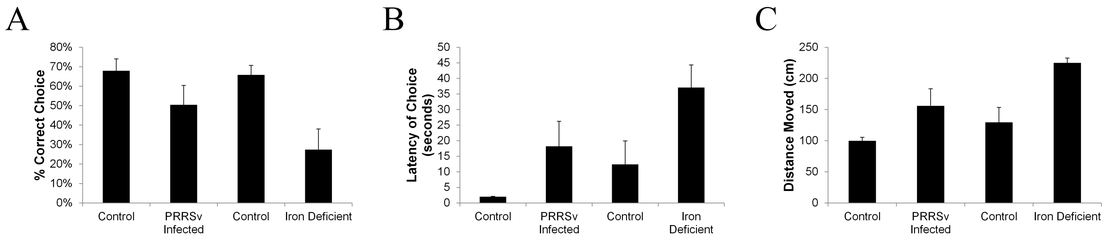


Introduction

- Altered DNA methylation levels are associated with aberrant gene transcription and represent a link between genetics and environmental signals that has been reported to play an important role in environmentally induced human pathologies including cancer and neurological disorders.
- Both iron deficiency and viral infection are common issues during childhood, and have been linked to cognitive impairments.
- Previous studies have observed reduced hippocampal-based spatial learning and memory in piglets suffering from neonatal iron deficiency (Rytych et al., 2012) and porcine reproductive and respiratory syndrome virus (PRRSv) infection (Elmore et al. 2014).
- Spatial learning and memory was assessed using a clear plastic T-maze with visual cues.
- Both iron deficient and PRRSv infected piglets had (a) fewer correct choices, (b) took longer to locate the reward, and (c) covered more distance in the maize, all indicative of reduced cognitive performance



- What are the epigenetic mechanisms responsible for reduced cognitive performance in response to early life environmental insults?

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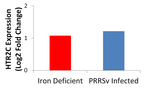

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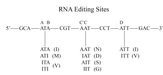

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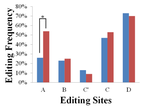

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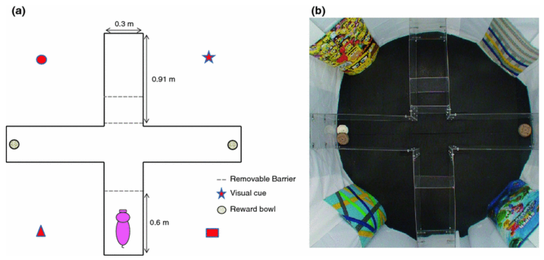

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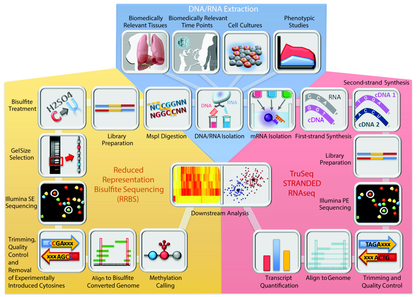

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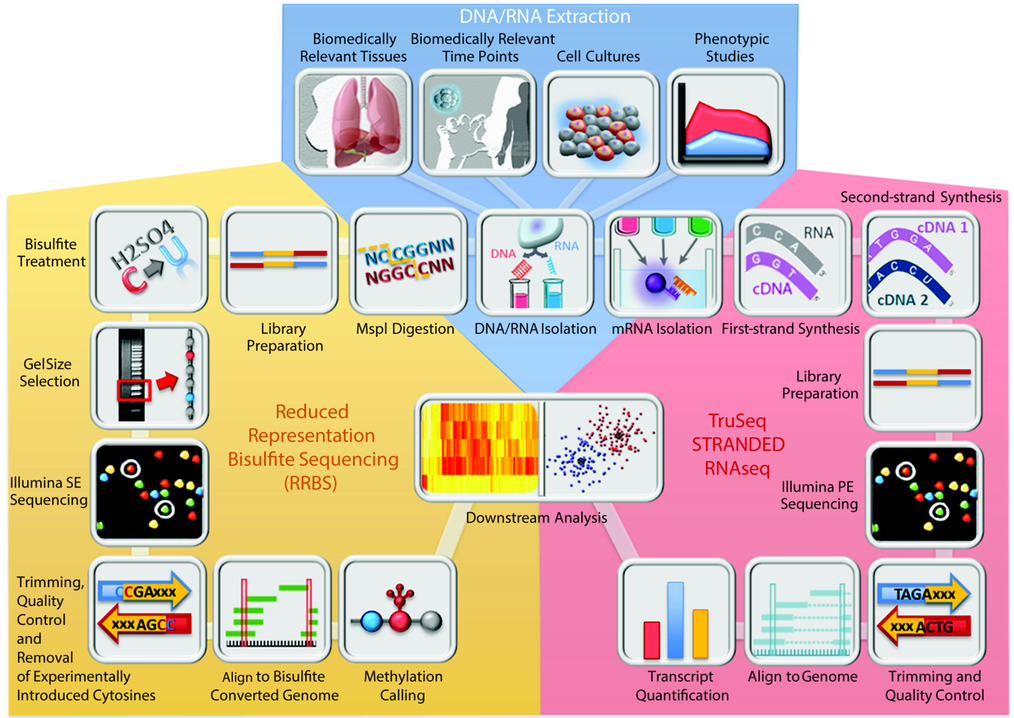
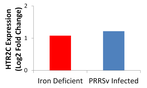


- Piglets from both studies were euthanized at 4 weeks of age
  - DNA and RNA was extracted from hippocampus samples
- RRBS and RNA-seq performed on Illumina HiSeq 2000
- RRBS Analysis:
  - 30 – 160 bp fragments selected
  - Alignment and methylation calling performed using BSseeker2 (Guo et al., 2013)
  - Differential methylation analysis was using the R package methylkit (Akalin et al., 2012)
- RNA-seq Analysis:
  - Alignment performed using Tophat2 (Kim et al., 2013)
  - Differentially expressed gene (DEG) analysis performed using Cufflinks (Trapnell et al., 2013)

Results

Both Studies	
# DEGs	53
# DEGs Same Directional Change	45
# DEGs associated with DM Sites	2

Figure 1: Summary of DM and DEG Results



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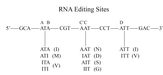

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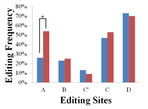

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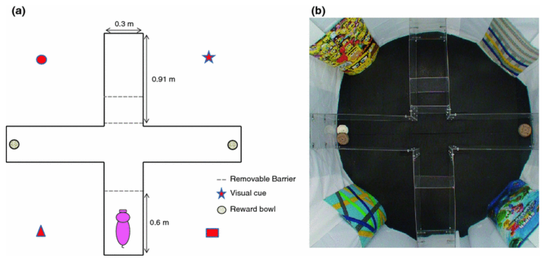

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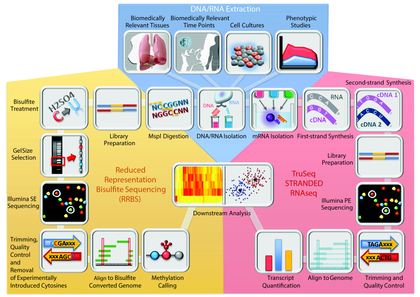


Results

Figure 1: Summary of Differential Methylation (DM) and Differentially Expressed Gene (DEG) Results

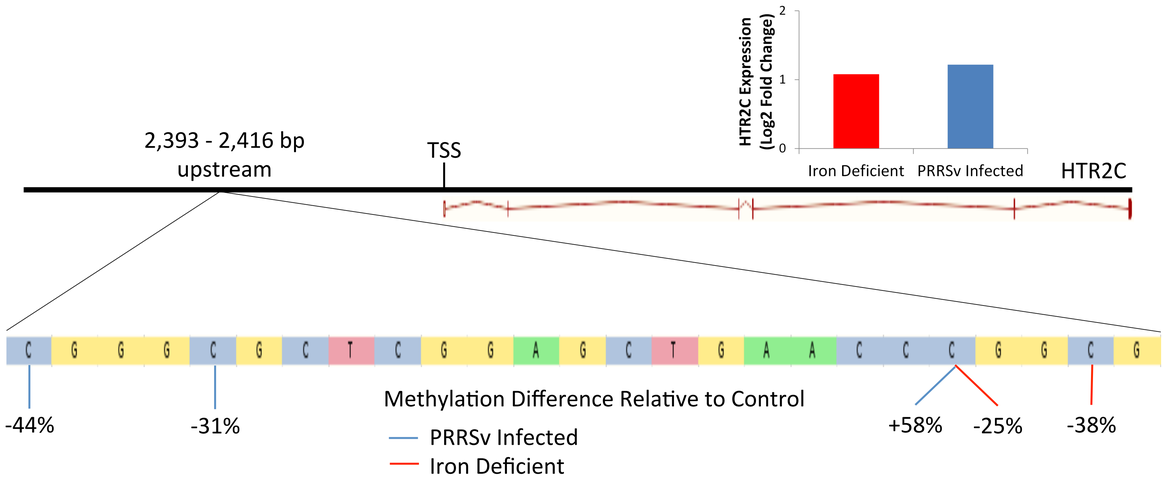
Iron Deficiency	
# DEGs	192
# DM CpG Sites	853
# DM non-CpG Sites	99
# DEGs associated with DM Sites	9

Both Studies	
# DEGs	53
# DEGs Same Directional Change	45
# DEGs associated with DM Sites	2

PRRSv Infection	
# DEGs	455
# DM CpG Sites	1857
# DM non-CpG Sites	153
# DEGs associated with DM Sites	19

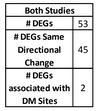
Number of DEGs, DM sites, and DEGs associated with DM sites in the iron deficient and PRRSv infected hippocampus. Many DEGs in both studies involved in neurodevelopment and function.

Figure 2: DM Sites Associated with Increased *HTR2C* Expression



Differential methylation associated with increased expression of the serotonin receptor *HTR2C*. Altered *HTR2C* expression is associated with behavioral disorders in humans.

Results



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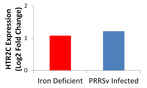

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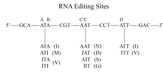

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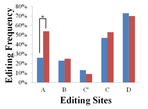

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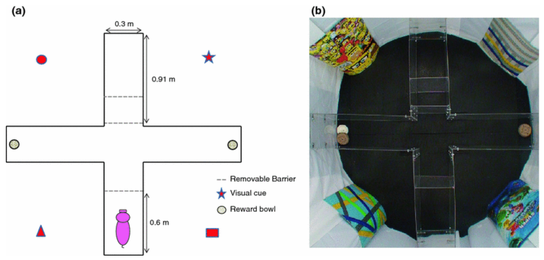

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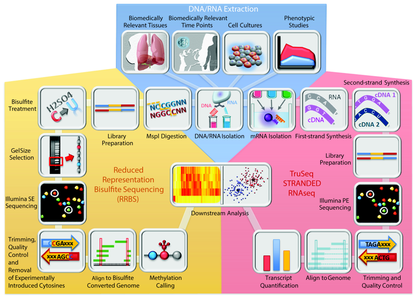

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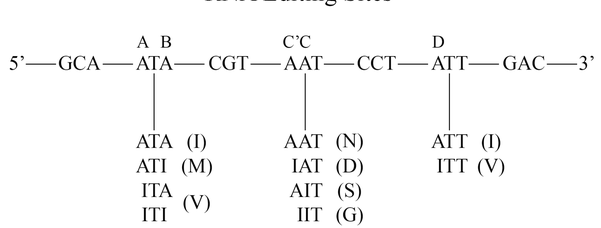

Altered *HTR2C* editing is associated with altered functional and behavioral disorders in humans.

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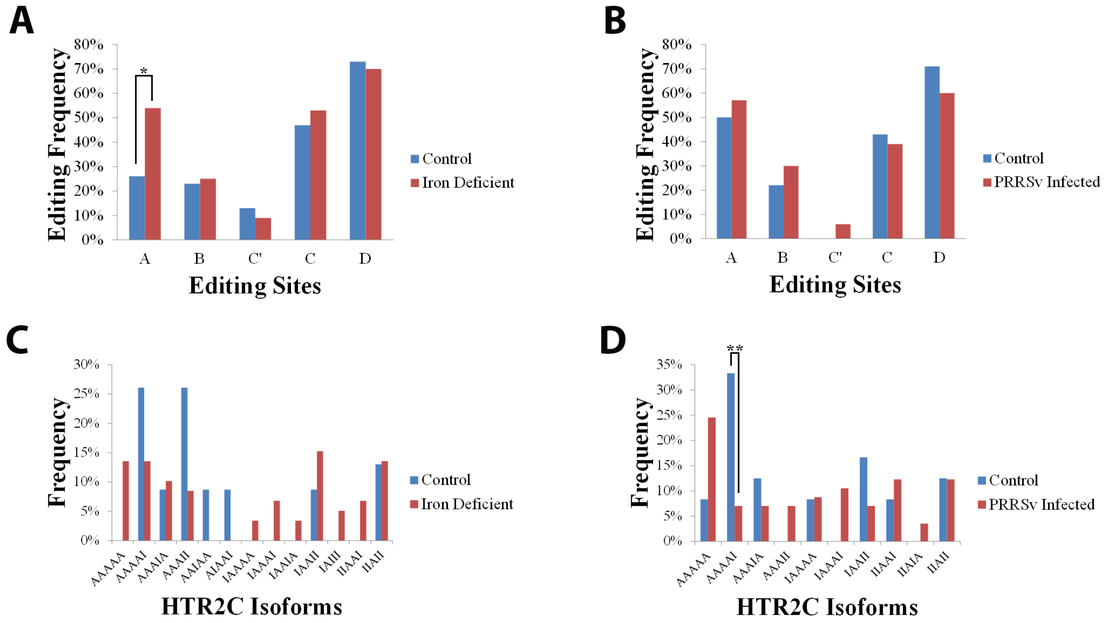

(a, b) Editing frequencies and (c, d) mRNA isoform frequencies in the iron deficient and PRRSv infected hippocampus, respectively. * denotes p-value < 0.05. ** denotes p-value < 0.005.

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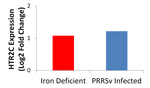

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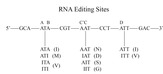

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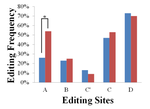

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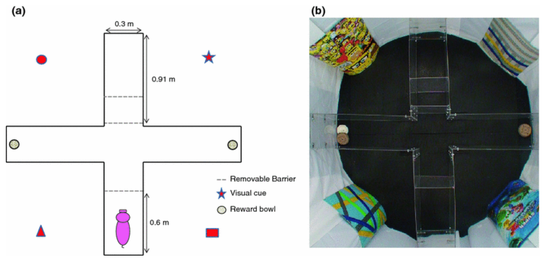

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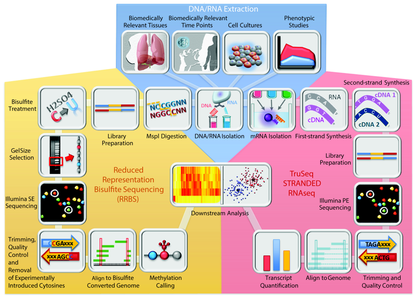
Methods



Conclusions

- Two different early life environmental insults result in altered hippocampal expression and methylation of genes involved in neurodevelopment and function, leading to reduced cognition phenotypes
- Increased expression of the serotonin receptor *HTR2C* was associated with hypomethylation of multiple CpG sites located between 2,390 and 2,420 bp upstream of the TSS in both studies
- Altered *HTR2C* RNA editing frequencies and *HTR2C* isoforms unique to the reduced cognition groups were identified in both studies
  - Altered expression and editing of *HTR2C* is observed in the brains of individuals suffering from behavior disorders including depression.
- Possible role for *HTR2C* in cognitive development
  - Future studies required to determine the importance of *HTR2C* regulation and editing on cognitive development

Results

Both Studies	
# DEGs	53
# DEGs Same Directional Change	45
# DEGs associated with DM Sites	2

Figure 1: Summary of DM and DEG Results

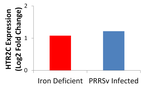


Figure 2: DM Sites Associated with Increased *HTR2C* Expression

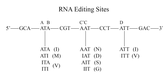


Figure 3: Known *HTR2C* RNA editing sites and resulting amino acid substitutions

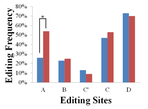


Figure 4. *HTR2C* RNA editing frequencies and resulting isoforms.

Conclusions

Differential methylation and altered gene expression was identified in two independent studies reporting reduced cognitive development in response to early life environmental insults. Increased expression of the serotonin receptor *HTR2C* was associated with hypomethylation of a region ~2,400 bp upstream of the TSS in both studies.

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