# WHOLE GENOME METHYLATION STUDY OF 48 MONOZYGOTIC PAIRS CONCORDANT OR DISCORDANT FOR ATTENTION PROBLEMS

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## Introduction

Attention Deficit/Hyperactivity Disorder (ADHD) has been demonstrated to be highly heritable, but so far molecular genetic studies have explained only a small percentage of the heritability. Here, we consider the role of epigenetics – specifically examining gene methylation – in the etiopathology of longitudinally-persistent attention problems (AP), a quantitative phenotype related to ADHD.

## Sample

Subjects were fifty monozygotic (MZ) twin pairs selected from the Netherlands Twin Registry and for whom longitudinal information was available on attention problems. 22 concordant affected (CA), 17 concordant unaffected (CU), and 11 discordant pairs participated. Whole genome methylation scans failed in 2 of the CU twins, resulting in a final sample size of 48.

## Measures

The Child Behavior Checklist (CBCL) Attention Problems (AP) scale was completed by parents at ages 7, 10 and 12. Individuals were selected as affected if they had a T-score > 65 for AP on at least one occasion ***and*** a T-score > 60 for AP ***at all three time points.***

## Analyses

•Buccal DNA was isolated, restriction digested, ligated, and incubated with antibodies to 5-methylcytosine

•Antibody-bound DNA was separated from non-antibody bound DNA using the Methylamp Methylated DNA Capture Kit

•Methylated DNA was fragmented, end-labeled, and hybridized to the Affymetrix Human Promoter Array

•Custom-written software program extracted, quantile normalized, and median polished the raw signal values from all of the interrogated CpG islands

•The end result was a single methylation signal for each individual at each of 16,441 annotated gene promoter regions

•Discordant pairs were compared using within-pair t-tests, Concordant pairs were compared with ANOVA

## IPA Analysis

•Genes with p < 0.01 were placed into Ingenuity Pathways Analysis

•Only genes associated with cellular function, neurological function, or from a CNS cell line were entered into the network and pathways analysis

## Results

*Environmental Effects:*
156 promoter methylation sites were significantly different between the discordant twins. Three canonical pathways were significant following adjustment for multiple comparisons, IL-6 signaling (4 molecules, p<0.03), G-protein coupled receptor signaling (5 molecules, p<0.03), and relaxin signaling (5 molecules, p<0.03). The calcyon neuron-specific vesicular protein (CALY) stood out as significant ($p=7.45 \times 10^{-5}$).

*Genetic Effects:*
359 promoter methylation sites were significantly different between the CA pairs and the CU pairs. The neuregulin signaling canonical pathway came very near to significance following adjustment for multiple comparisons (6 molecules, p = 0.0537). The GDNF family receptor alpha 1 (GFRA1) stood out as significant ($p=2.68 \times 10^{-5}$).

## Conclusions

This is the first study of differential methylation in attention problems using twins. There may be separate mechanisms for “genetic” methylation pathways and “environmental” methylation pathways.