An Ultraconserved Brain-specific Enhancer within ADGRL3 (LPHN3) Underpins ADHD Susceptibility

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Background
Genetic factors predispose to attention deficit/hyperactivity disorder (ADHD). Previous studies have reported linkage and association to ADHD of gene variants within ADGRL3. In this study, we functionally analyzed non-coding variants in this gene as likely pathological contributors.

Methods
In silico, in vitro and in vivo approaches were used to identify and characterize evolutionary conserved elements within the ADGRL3 linkage region (~207 Kb). Family-based genetic analyses on 838 individuals (372 affected and 466 unaffected) identified ADHD-associated SNPs harbored in some of these conserved elements. Luciferase assays and zebrafish GFP transgenesis tested conserved elements for transcriptional enhancer activity. Electromobility shift assays were used to verify transcription factor binding disruption by ADHD risk alleles.

Results
An ultraconserved element was discovered (ECR47) that functions as a transcriptional enhancer. A three-variant ADHD risk haplotype in ECR47, formed by rs17226398, rs56038622 and rs2271338, reduced enhancer activity by 40% in neuroblastoma and astrocytoma cells (P<0.0001). This enhancer also drove GFP expression in the zebrafish brain in a tissue-specific manner, sharing aspects of endogenous ADGRL3 expression. The rs2271338 risk allele disrupts binding of YY1, an important factor in the development and function of the central nervous system. Expression quantitative trait loci analysis of post-mortem human brain tissues revealed an association between rs2271338 and reduced ADGRL3 expression in the thalamus.

Conclusions
These results uncover the first functional evidence of common non-coding variants with potential implications for the pathology of ADHD.