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Short communication

RNA-Seq blood transcriptome profiling in familial attention deficit and hyperactivity disorder (ADHD)

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ABSTRACT

We have carried an exploratory study by blood transcriptome to find RNA expression signatures in familial ADHD. Samples were collected from three cases with familial ADHD and their paired controls and evaluated by RNA-Seq. Transcriptome profiling identified 7 differentially expressed transcripts with a FDR <0.05 that were involved in pathways in Huntington's disease or axonal guidance signaling previously implicated in ADHD, and enriched for signal peptide, growth factor binding, and notably the lipid metabolism pathways. These findings show that blood transcriptome can have an associated signature and highlight a potential to use blood transcriptome to identify patterns of ADHD.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is defined as a clinically heterogeneous neurodevelopmental syndrome comprising the triad of inattention, hyperactivity and increased impulsivity (Brahmbhatt et al., 2016). A number of studies have demonstrated clearly that ADHD is driven by genetic drift. The rate of ADHD is significantly higher in the relatives of probands with ADHD (Franke et al., 2012) and twin studies have reported concordance rates significantly higher among monozygotic than dizygotic pairs providing further evidence that ADHD is significantly heritable (Brikell et al., 2015). Nevertheless, the monozygotic twin concordance rates lower than 100% suggests that epigenetic and/or environmental influences play also a role in the etiology of this disorder. Studying blood-based changes in RNA gene expression may present a different strategy for differentiating patients with ADHD from healthy pairs that is independent of the underlying genetic or environmental cause. Here, we present a small-scale study of whole-blood gene expression profiling that generated a blood-based signature mapped to 30 genes possibly related to ADHD biological substrate.

2. Methods

We designed a paired case-control study of pediatric familial ADHD. Cases fulfilled the following inclusion criteria: ADHD according to the diagnostic criteria of the DSM-V (American Psychiatric Association, 2013), male gender, one or both parents with ADHD, one or more siblings affected of ADHD, onset before the age of 7 years via retrospective diagnosis, life-long persistence, a not affected brother in the same age range (± 5 years) and absence of medical or psychiatric comorbidities. Age at recruitment was between 12 and 25 years. The not affected brother was selected for control. The Ethics Committee of the Hospital Ramon y Cajal approved and verified the study. Out of 65 patients screened, the study was carried on in 3 cases and 3 controls fulfilling the aforementioned criteria. 2.5 ml of blood were extracted with PAXGENE tubes and stored at −80 °C until RNA extraction. Total RNA was extracted from the blood samples using the RNeasyKit (Qiagen, Valencia, CA). RNA concentrations were determined by Qubit and RNA quality assessed in a Bioanalyser 2100 (Agilent). Only RNA samples with greater 28S than 18S intensity and no obvious degradation or contamination were used (all samples had 260:280 ratios exceeding 1.8). RNA sequencing (RNA-Seq) libraries were prepared using 1 μg of total RNA. After globin mRNA depletion, each library was sequenced using TrusSeq SBS kit v3-HS, in paired-end mode generating 40-million paired-end reads in a sequencing platform HISEQ2000 (http://www.illumina.com/). Analysis of data was done in a pipeline that included quality check, data preprocessing, alignment, quantification of expression, statistical analysis, and functional annotation.

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cDNAs were aligned to reference human hg19 genome using Hisat2 v.2.1.0 (ccb.jhu.edu/software/hisat2) and counts determined with HTSeq (htseq.readthedocs.io/en/release_0.9.1/index.html) using gencode.v19.gtf as transcript reference file with exon counting in union model. For differential expression (DE) analysis to compare the read counts between healthy and ADHD groups, count data distribution was fitted using the generalized linear model (GLM) with EdgeR (bioconductor.org). We tested both models, a paired design model so that baseline differences between patients are subtracted out and then the dispersion is estimated, adjusting for baseline differences between the patients and a second model for exact test significance of pooled cases and control groups (classic EdgeR exact test based on a negative binomial model)(Kvam et al., 2012). We also looked up for functional enrichment analysis using gene ontology (GO) with the Database for Annotation, Visualization and Integrated Discovery (DAVID v.6.7) (david.ncifcrf.gov) and the Gene Multiple Association Network Integration Algorithm (GeneMANIA) (genemania.org) to detect known interactions among the signature genes and identify additional genes connected to the signature genes. For functional enrichment analysis we included the 30 genes with top DE in the study (the same genes that were included for heatmap calculation). As background genes were used DAVID default population background in enrichment calculations, in this study the human genome genes with at least one annotation in the analyzing categories.

3. Results

According to inclusion criteria, cases and controls were male gender, aged between 12 and 23 year-old, and without co-morbidities. We obtained an average of 22,499,930 reads per sample that have a Phred-like quality value higher than 30 in 99.99%. Average mapping to reference human hg19 genome was 96% and average unique mapping rate was 81.1%. After the alignment and assembly the study contained 57,820 transcripts that were reduced to 10,535 after filtering keeping only those with counts higher than 100 in at least a 25% of samples. Total number of counts was 17,816,942, 17,582,911, 16,451,342, in the cases and 19,244,227, 19,187,533, 16,408,872 in the controls. The DE study using the paired design model identified 7 differentially expressed transcripts with a false detection rate (FDR) of less than 0.05 (2 up-regulated and 5 down-regulated) in patients with ADHD compared to their matched control possibly related to disease activity (Fig. 1a). The EdgeR exact test showed similar results but FDR values were remarkably higher than with the paired design. Using a higher FDR cutoff value, 2 additional genes were found. Gene Ontology (GO) analysis was performed to identify functions that are involved in the ADHD differential expression, showing enrichment of genes in biological processes, molecular features or pathways associated with RNA binding, lysophospholipase activity, phospholipid binding, signal transducer activity, G-protein coupled receptor activity, glucose transmembrane transporter activity, epidermal growth factor receptor binding, voltage-gated calcium channel complex postsynaptic density, integral components of membrane, ion binding, oxidoreductase activity and lipopolysaccharide binding were found to be enriched (Supplemental material, Table 1 and 2). Notably, the transcriptomic analysis showed that genes associated lipid metabolism such as PLB1, CYP4F12, SPON2 and FADS2, were differentially expressed downregulated in ADHD cases. A heatmap (Fig. 1b) built with hierarchical clustering of 30 differentially expressed genes with lowest FDR value and fold change ≥ 2 showed the relatedness of the ADHD samples in the clustering of the top of the figure, discriminating between patients and control cases.

4. Discussion

This study provides a first pilot study of the transcriptome of ADHD in human beings and a number of genes and gene pathways were identified. As expected, fold changes were modest, but we distinguished genes that were differentially expressed (FDR < 0.05) in blood in the paired sample model analysis. Despite significant progress in ascertaining genetic risk variants, there is currently no unified pathological mechanism explanation for ADHD (Lenzi et al., 2018). None of the genes previously recognized in ADHD have shown changes in this study probably because practically all of them have been inconsistently replicated (Gallo and Posner, 2016) so very likely these genes appear to account for a relatively small proportion of the variance in ADHD heritability, suggesting that they will not be picked up in a small sample. Genetic studies in ADHD have focused primarily on genes that influence dopamine, norepinephrine, and serotonin pathways due to evidence that these neurotransmitters may play a role in the pathophysiology of ADHD or other connected psychopathology. The dopamine transporter gene (DAT1) and the dopamine D4 receptor gene (DRD4) are the candidate genes that have been investigated most frequently in relation to ADHD (Faraone et al., 2014) but there are more than a dozen of candidate genes where a significant association with ADHD has been reported in one study at least (Wood and Neale, 2010). The less than complete heritability of ADHD, and the difficulty confirming susceptibility genes, indicates that the prevalence of ADHD cannot be explained by genes alone. Several environmental factors have been identified as potential risk factors for ADHD, including prenatal exposure to alcohol, cigarette smoke, and environmental pollutants such as pesticides and polychlorinated biphenyls (PCBs) (Bonvicini et al., 2016).

Three other transcriptome studies have been conducted in animal models of ADHD (Sazonova et al., 2011; dela Peña et al., 2014; Orsini et al., 2016). There are several pathways shared by these studies with the present study, such as oxidative stress response, Huntington disease signaling, ribosome binding and glutamate receptor. While none of the genes identified in our study have been previously established as a ADHD susceptibility genes, Huntington disease signaling and glutamate receptor genes is one of the most consistent findings in transcriptome studies of animal models of ADHD (Sazonova et al., 2011). Replicating the Huntington diseases signaling genes identified here in a further study in larger cohort is an objective of future studies in this regard.

In the study of Sazonova et al. (2011) conducted in a model of ADHD of rats with developmental exposure to PCB, the peroxisome proliferator activated receptor a (PPARa)/retinoid X receptor, alpha was upregulated, a finding that is related to the association of ADHD with essential fatty acid deficiency. Our work has found downregulation of several genes involved in lipid metabolisms, supporting evidence linking ADHD to genetic or environmental fatty acid deficiencies. This finding supports research of the role of fatty acids in ADHD, and further suggests that another potentially therapy target pathway, oxidative stress, may play a function in ADHD (Agostoni et al., 2017).

This study was done in blood transcriptome not in brain tissue so some or all of the findings may be an effect of environmental factors such as medications or disease state and not causative. However, the observed correlations to other experimental ADHD transcriptome studies suggests that blood transcriptome analysis may be useful for identifying exposures, causative genes and pathological changes. Even though sample size was a limitation of this study due to the strict selection criteria that is necessary in this sort of analysis, this results demonstrate that a large dataset will be value to identify a blood-based ADHD signature and to provide insights into ADHD pathophysiology.

We have no conflicts of interest to disclose.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2018.10.025.

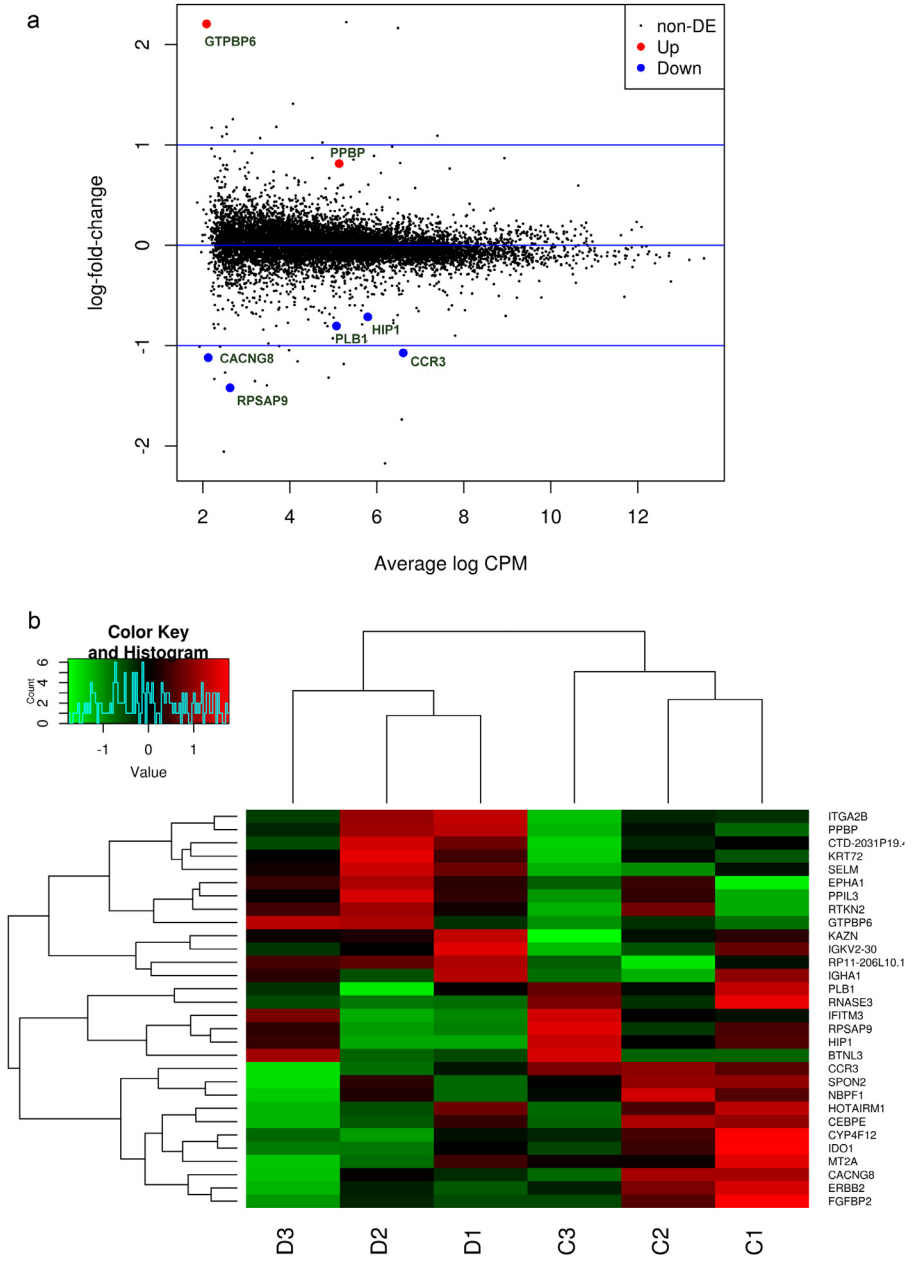

Fig. 1. (a) Plot of log-fold change against log-counts per million, with DE genes highlighted. (CPM:counts per million); (b): Heat map of the hierarchical clustering based on 30 differentially expressed genes with lowest FDR value and fold change ≥2. This plotting was able to graphically cluster the samples in group D (disease) and C (control) pointing that the two groups are different.

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