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Research Article

The Link Between Pregnancy-Related Complications and Changes in Brain Metabolites in Children with Attention Deficit Hyperactivity Disorder

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Abstract

Background: ADHD is a prevalent neurobiological condition with neurometabolite changes. Pregnancy-related complications are risk factors in ADHD. However, the link between these two factors remains unknown. This study aims to assess the relationship between pregnancy-related complications and alterations in neurometabolites in children with ADHD.

Methods: From June 2007-December 2010, children with ADHD (*n* = 27) and their unaffected siblings (control, *n* = 33), 5-12 years old, underwent proton magnetic resonance spectroscopy sessions to measure ratio to creatine of N-acetyl-aspartate (NAA/Cr), glutamate (Glu/Cr), glutamate-glutamine (Glx/Cr), and myo-inositol (mI/Cr) in left cerebellum and in bilateral prefrontal, and striatal brain regions. The Kinney Medical and Gynecological Questionnaire was used to report pregnancy complications.

Results: The pregnancy-related complications of the third trimester were higher in the ADHD group (t=2.4;p=.02). Children with ADHD had higher Glu/Cr (p=.001), Glx/Cr (p=.004) and NAA/Cr (p=.007) in left prefrontal cortex compared to controls, higher NAA/Cr (p=.03), Glx/Cr (p=.025), and Cho/Cr (p=.021) in right striatum, and increased Glx/Cr (p=.013) in left cerebellum. A positive correlation was observed between the number of third trimester complications and NAA/Cr in left (p=.009; R^2=.092) and right (p=.001; R^2=.17) striatum, and mI/Cr in left cerebellum (p<.001; R^2=.19). Additionally, there was a significant association between the number of complications throughout pregnancy and mI/Cr in left cerebellum (p=.003; R^2=.12).

Limitations: Our study was limited by modest sample size, potential recall bias concerning the report of pregnancy complications, and use of metabolite ratios.

Conclusions: This study suggests the contribution of pregnancy-related complications in ADHD, particularly their possible impact during third trimester. Their association with neurometabolite changes suggests this trimester as a potential window of vulnerability for ADHD development. If confirmed, addressing prevention programs during this period would be helpful for clinicians.

Keywords: *ADHD; Perinatal; Pregnancy Complications; Risk Factors; Magnetic Resonance Spectroscopy; Glutamate; Prefrontal Cortex; Striatum; Cerebellum; Brain Metabolites*

Introduction

Attention-deficit hyperactivity disorder (ADHD) is a highly prevalent disorder, affecting approximately 5% of children worldwide [1]. The core clinical features are symptoms of inattention, hyperactivity, and impulsivity that result in functional impairments such as academic failure, low self-esteem, poor peer relationships, family difficulties, anti-social behaviors, and accidents [2]. ADHD has been identified as a serious health problem by the Center for Disease Control given its high prevalence, morbidity, and chronic outcomes [3].

It is now recognized that ADHD is a multifactorial condition with complex etiologies. Epidemiological studies support the neurobiological basis of this disorder, with the involvement of both environmental and genetic factors. Family studies have identified a 4 to 5.4 increase in the risk for ADHD in parents and siblings of children with ADHD [4]. Based on twin studies, the mean of heritability estimation of ADHD is approximately 75% [2]. These studies also indicate that the rest of the variance in the ADHD phenotype (10-25%) is accounted for mostly by individual environmental factors (i.e. experienced only by one member of the family). In contrast, shared environmental factors (shared by all the individuals in the same family) appear to play only a minimal role [5].

Case-control epidemiologic studies suggest that perinatal (including gestational, labour/delivery, and neonatal) complications can predispose children to ADHD [6]. For instance, during pregnancy, the fetal exposure to tobacco, alcohol, drugs, maternal stress, poor maternal health, toxaemia and eclampsia, maternal bleeding, as well as increased labour duration, foetal distress, low-birth weight, and prematurity are all factors identified as predicting subsequent ADHD in offspring [7,8]. It is believed that these perinatal factors are potential causes of brain function disruption as they occur during crucial periods of fetal development and may induce long-lasting effects on cognition and behaviour [9], although the underlying mechanisms remain somewhat undetermined. Studies on this topic are highly important to our understanding of ADHD, but consensus is still lacking on the role of genetic variability and post-natal environment (e.g., socioeconomic status) because studies have been mostly conducted without genetic background control (e.g., unaffected sibling as controls). Our previous study [10] conducted within-family comparison of children with ADHD to their unaffected siblings and showed that neonatal complications are a risk factor for ADHD, independently of other shared environmental factors.

Dysregulation of the dopaminergic and noradrenergic systems in the fronto-striatal-cerebellar pathway has been found to be implicated in the core symptoms of inattention, hyperactivity, and impulsiveness that characterize this ADHD [11,12]. This circuit is inherent to motor control, executive function, and control of inhibition. Indeed, volumetric studies of brain structures using magnetic resonance or computed tomography scan reported a smaller volume of the total brain, the frontal cortex, the cerebellum, and of striatal subcortical structures in children with ADHD [12,13]. However, significant proportion of these observations may be attributable to genetic factors [14]. In fact, when comparing children with ADHD to their unaffected siblings, only the reduction of the cerebellum volume remains, with no difference in overall brain volume and prefrontal cortex. These findings differ from the results of studies in general population comparing affected and non-affected unrelated children. These differences suggest that brain volume changes in ADHD are region-specific and may be related in part to shared family genetic factors.

While functional brain studies using positron emission tomography or functional magnetic resonance imaging allowed to show abnormalities in fronto-subcortical regions [15], particularly in the striatum of ADHD children (known to be highly vulnerable to neonatal hypoxia [16]), current ADHD neurochemical theories call for other means of investigation.

The magnetic resonance spectroscopy (MRS) is a promising tool in ADHD research. It is a non-invasive approach that uses proton type (i.e., without ionizing radiation) [17] to assess the concentration of brain biochemical metabolites, as well as their profiles (increased vs. decreased), in regions of interest. Interestingly, in ADHD children, alterations in ratios to creatine (Cr) of choline compounds (Cho/Cr), myo-inotisol (mI/Cr), N-acetylaspartate (NAA/Cr) and glutamate-glutamine (Glx/Cr) in the fronto-striatal pathway [17] and the cerebellum [18,19] were observed.

To take into account the family as an environmental and genetic factor, we conducted a within-family study (siblings as controls) in a genetically homogeneous population of Quebec to study the link between pregnancy-related events and changes in the brain metabolites. We compared children with ADHD to their unaffected siblings, which allowed, at the very least, a partial control (50%) of the genetic variability and of some confounding factors (e.g., socioeconomic status, family environment).

Objective

This study aims to assess the relationship between pregnancy complications (as a non-shared environmental risk factor) and the brain metabolites characteristics in ADHD. Specific goals are to compare drug naïve children with ADHD with their non-affected siblings for: (1) the number or perinatal complications and the trimester in which they occurred; (2) the brain metabolite concentrations using MRS

in specific regions of interest; and (3) to assess the relationship between pregnancy complications and brain metabolite spectra among children with ADHD.

Methods

Study design

A case-control study design was chosen to retrospectively determine the relationship between exposure to perinatal complications and brain metabolite concentrations in children with ADHD and a control group. The subjects were recruited through advertisements in the outpatient child psychiatry clinic of Hôtel-Dieu Hospital between June 2007 and December 2010. The ethics committee of the hospital approved this study and written consent and assent were obtained from all participants and their parents.

Participants

Eligible children were aged 5 years through 12 years, and either met the DSM-IV [20] diagnostic criteria for combined type ADHD (i.e. with both inattentive and hyperactive/impulsive symptoms) or were unaffected siblings of participants with ADHD. The presence or absence of a diagnosis based on DSM-IV [20] was confirmed using (a) a clinical assessment by experienced child psychiatrists (including L. Ben Amor); (b) the Diagnostic Interview Schedule for Children-IV [21]; and (c) the Conner Rating Scale (both parent and teacher versions) [22-24]. Research assistants supervised by experienced clinicians compiled the results of each of these evaluation methods. In total, 27 children with ADHD and 33 unaffected siblings were recruited. Furthermore, all children were psychostimulantnaïve at the time of our study and had to be Caucasian, third-generation French Canadians in order to ensure the general genetic homogeneity of our population. To prevent the presence of other confounding factors known to interfere with ADHD, children were excluded for (1) IQ below 70 (as assessed with the Wechsler Intelligence Scale for Children-IV [25] or the Wechsler Preschool and Primary Scale of Intelligence-III [26], (2) preterm birth (defined as under 37 weeks of gestation), (3) low birth weight (defined as lower than 3500g), or (4) previous brain surgery. As a MRS contraindication, children with body metal implants were also excluded.

Procedure

Measures

Pregnancy and Birth Complications. The *Kinney Medical and Gynecological Questionnaire* [27] was used during an interview with the mothers to collect information on obstetric and neonatal complications. The participants' answers on this questionnaire were scored using the McNeil-Sjöström Scale [28]. This risk scale assesses the severity of complications during pregnancy, labor, delivery, and the neonatal period based on a severity continuum ranging from 1 to 6 (with scores of 3 and higher considered significant).This scale is based on the inferred probability of harm to the offspring, with special focus on the central nervous system. Complications scoring above 3 on the McNeil-Sjostrom Scale were recorded for each trimester as well as for the whole pregnancy and were compared between the two groups. All interviews and scoring were done by a clinician who was blinded to the children's status.

Brain imaging measures. Proton Magnetic resonance spectroscopy [1] (H-MRS), a non-invasive technique increasingly used in ADHD investigations, allows the study of *in vivo* brain metabolites [29]. Imaging and spectroscopy for all participants were performed with a 1.5-T General Electric Signa scanner operating at 63.85 MHz (General Electric Medical Systems, Waukesha, WI). Anatomical images were acquired for spectral localization and the position of the voxels was determined from T1-weighted spin-echo images of an axial plane. Spectra were acquired from 8.0 cm^3 voxels localized in five different regions of interest: left and right prefrontal area; left and right strital area; and left cerebellum. For voxel location, we used predetermined anatomical parameters (striatum area: the posterior portion of the head of caudate nucleus according to the antero-posterior axis; prefrontal area: above the ones determining the striatum voxels; and cerebellum: approximately 3 mm laterally from the ledge of the fourth ventricle). After each region localization, we used the GE PROBE protocol, which comprises a suppression of the water signal using the chemical shift selective (CHESS) method, followed by detection of the proton signal using the point-resolved spectroscopy (PRESS) sequence. The acquisition parameters were as follows: TR, 1500 ms; TE, 30 ms; number of acquisitions, 128; spectral width, 2000 Hz; number of points, 1024; total acquisition time, about 40 min. With these acquisition conditions, the signal/noise ratio was 12 or more for the main signals (ranging from 12 to 28, depending on the region). The MRS data were analysed using the LCModel software, v.6.0 (Provencher, 1993), and metabolite ratios for N-acetylaspartate/creatine

(NAA/Cr), choline/Cr (Cho/Cr), myo-inositol/Cr (mI/Cr), glutamate (Glu/Cr) and glutamate-glutamine/Cr (Glx/Cr) were calculated.

Before undergoing the spectroscopy, children were invited to sessions with a simulator to become familiar with the noises and conditions of the scanner. Nevertheless, a few (6.1%) imaging data from the siblings group were excluded due to technical problems (e.g. the children slightly moved within the scanner).

Statistical Analysis

Analyses were performed with SPSS.12.0 (Chicago, Illinois). A chi-square analysis was conducted to compare the proportion of boys vs. girls between groups. Independent t-tests were used to compare the two groups on age and IQ, as well as on the number of perinatal complications and brain metabolite concentrations [1] (H-MRS data). A Pearson regression analysis was used to test the association between the number of perinatal complications and brain metabolite concentrations. The level of statistical significance was set to p = 0.05, two-tailed.

Results

General Characteristics

There were no significant differences regarding age between the ADHD (mean age = 8.9; SD = 2.1) and the siblings (mean age = 8.3; SD 1.9) *(t* (58) = 1.1; p = 0.26) groups. The IQ was also found to be similar in both groups (*t* (58) = -1.4; *p* = 0.17). The proportion of boys was higher in the ADHD group (19/27; 70.4%) than in the siblings group (18/33; 54.5%), although this difference was not statistically significant (χ^2 = 1.6; p = 0.20).

Perinatal Complications

Trimester	Number of complications		*t*	*p*
	ADHD *n* = 27	Siblings *n* = 33		
	M (SD)	*M* (SD)		
Trimester 1	1.3 (1.3)	1.1 (1.3)	0.5	0.608
Trimester 2	1.5 (1.4)	1.2 (1.3)	1.0	0.306
Trimester 3	2.5 (2.3)	1.3 (1.5)	2.4	0.020*
All trimesters	5.3 (3.7)	3.6 (3.6)	1.8	0.077

Table 1: *Number of pregnancy complications reported by mothers.*

** p<0.05*

There were significantly more pregnancy complications in the third trimester for the ADHD patients when compared to their siblings (*t* (58) = 2.4; *p* = 0.02). Although the number of complications was higher for all pregnancy trimesters in the ADHD group, the differences were only significant for the last trimester.

Brain Imaging Results

Table 2 shows the mean brain metabolite ratios resulting from [1] H-MRS measures taken in the five regions of interest studied. The following differences were found: In the left prefrontal cortex, the Glu/Cr, (*t* = 3.4; p = 0.001), the NAA/Cr (*t* = 2.8; p = 0.007), and the Glx/Cr (*t* = 3.0; p = 0.004) ratios were higher in children with ADHD than in their siblings. In the left cerebellum, the Glx/Cr concentration ratio was the only brain metabolite with significantly higher levels in children with ADHD (*t* = 2.6; p = 0.01) than in the siblings. In the right striatum, the concentration ratios of the Cho/Cr (*t* = 2.3; p = 0.025), the NAA/Cr (*t* = 2.2; p = 0.03), and the Glx/Cr (*t* = 2.3; p = 0.025) were high in children with ADHD compared with their siblings. All metabolite ratios studied were comparable between groups for the right prefrontal cortex and the left striatum. Interestingly, the Cho/Cr ratios were comparable between groups in almost all brain regions, aside from the aforementioned right striatum measure

Brain region	Metabolite	Metabolite concentration ratio to creatine (SD)		*t*	*p*
		ADHD *n* = 27	Siblings *n* = 33		
Left prefrontal cortex	Glu	1.51 (0.21)	1.35 (0.15)	3.4	0.001***
	mI	0.79 (0.15)	0.73 (0.09)	1.6	0.113
	NAA	1.46 (0.18)	1.36 (0.11)	2.8	0.007**
	Glx	2.23 (0.33)	2.10 (0.22)	3.0	0.004**
Right prefrontal cortex	Cho	0.29 (0.04)	0.30 (0.03)	-0.4	0.640
	Glu	1.42 (0.18)	1.35 (0.15)	1.5	0.144
	mI	0.80 (0.24)	0.78 (0.12)	0.4	0.660
	NAA	1.44 (0.17)	1.37 (0.90)	2.0	0.051
	Glx	2.14 (0.36)	2.09 (0.33)	0.6	0.564
Left striatum	Cho	0.30 (0.04)	0.31 (0.04)	-0.3	0.75
	Glu	1.26 (0.17)	1.32 (0.17)	-1.2	0.220
	mI	0.61 (0.18)	0.56 (0.12)	1.4	0.171
	NAA	1.24 (0.18)	1.19 (0.15)	1.0	0.300
	Glx	1.97 (0.24)	1.94 (0.20)	0.5	0.619
Right striatum	Cho	0.24 (0.04)	0.23 (0.03)	1.2	0.201
	Glu	1.35 (0.32)	1.26 (0.18)	1.3	0.190
	mI	0.57 (0.17)	0.52 (0.06)	1.4	0.158
	NAA	1.28 (0.22)	1.17 (0.16)	2.2	0.030
	Glx	2.12 (0.55)	1.86 (0.28)	2.3	0.025
Left cerebellum	Cho	0.25 (0.04)	0.23 (0.03)	2.3	0.021
	Glu	1.02 (0.15)	0.97 (0.16)	1.1	0.287
	mI	0.84 (0.14)	0.80 (0.10)	1.3	0.208
	NAA	1.05 (0.13)	1.03 (0.14)	0.4	0.706
	Glx	1.75 (0.31)	1.54 (0.30)	2.6	0.013**
	Cho	Cho	0.25 (0.13)	1.5	0.128

Table 2*: Metabolite concentration ratios measured in five regions of interest of the brain by Resonance magnetic spectroscopy.*

Note: Statistical significance is set at p< .01 because of the multiple comparisons.

NAA: N-acetylaspartate; Cho: choline; mI: myo-inositol; Glu: glutamate; Glx: glutamate-glutamine

*** p< 0.01; *** p< 0.001 p< 0.05*

Pearson linear regressions were computed to assess the relationship between the number of complications reported by the mothers during pregnancy and brain metabolite concentrations among children with ADHD. Significant associations were found between the number of complications in the third trimester of pregnancy and the NAA/Cr ratio in the left ($p = 0.009$; $R^2 = 0.092$) and right striatum ($p = 0.001$; $R^2 = 0.17$), as well as with the mI/Cr ratio in the left cerebellum ($p < 0.001$; $R^2 = 0.19$). In addition, there was a significant association between the total number of complications reported throughout the pregnancy and the mI/Cr ratio in the left cerebellum ($p = 0.003$; $R^2 = 0.12$).

Discussion

The perinatal complications as non-shared environmental risk factors are among the well-known causes of ADHD. However, our study showed that their influence in the development of ADHD is highly related to complications that occurred in the third trimester

of pregnancy; possibly via alterations in the neurometabolite concentration in the ADHD children that is region-specific, particularly in the left prefrontal cortex and the left cerebellum. Finally, it showed for the first time to our knowledge, a link between the number of third trimester pregnancy-related complications and specific alterations of brain metabolite ratios. Indeed, children with ADHD showed higher NAA/Cr and MI/Cr ratios than their siblings in the bilateral striatum and in the left cerebellum, respectively.

Pregnancy-related complications as a cause of ADHD disease. Regarding pregnancy-related complications, our results point the third trimester of pregnancy as a potential critical period for development of ADHD. Grizenko et al. [30] showed that maternal stress during the last trimester of pregnancy is associated with ADHD severity. This may, in part, be explained by the persistence of the stressful situation after birth, thus affecting the relationship between the mother and her child and predisposing the child to developmental delays or behavioral difficulties [30]. Despite the almost universal acceptance that timing of perinatal insults is crucial in later development of the disorder, current theories mostly focus on the type of perinatal stressors, rather than their timing. Even when emphasis is put on the third trimester, it is mainly in the context of a unique stressor and not on the number of insults.

While the first and second trimesters are characterized by neural proliferation and migration, the third trimester is defined by a rapid increase in synapse formation, with the peak period starting around gestational week 34 and continuing well into postnatal life [31]. This period of synaptogenesis is also particularly vulnerable to neuronal death caused by overstimulation of the excitatory amino acid receptors at synapses, also known as excitotoxicity [32]. According to rodent studies, NMDA receptors are the first glutamate receptors to form around new synapses, followed by AMPA and during the third trimester-equivalent, a higher number and greater function characterize both. The excessive activation of these receptors recognizing glutamate as their substrate then leads to calcium flooding and neuronal death [32] in response to stresses such as hypoxia-ischemia in the perinatal period. This theory is particularly interesting in the framework of Todd and Botteron's energy-deficit hypothesis, in which ADHD could be explained by disturbances in the energy metabolism of monoamine, causing excess glutamate, which then cannot be absorbed appropriately by astrocytes [33].

Unfortunately, our study did not specifically highlight the nature of these pregnancy complications associated with ADHD. Indeed, the relatively small sample as well as the large numbers of perinatal complications investigated limited the statistical power to identify specific perinatal risk factors. Further studies are needed in order to better disentangle the relationship between potential perinatal factors that may harm the central nervous system development, and ADHD etiology. In contrast with our previous work [10], no difference was found in the number of complications occurring during the neonatal period between children with ADHD and their siblings. The exclusion of infants born prematurely or with low birth weight (in order to isolate other pregnancy complications) may have contributed to explain the differences from our previous [10] findings

Significances of brain neurometabolite ratios in ADHD children. The neurometabolites described in our study are those currently reviewed in the literature in ADHD and include: glutamatergic metabolites (glutamate and glutamine)-the principal excitatory amino acids in the human brain; choline compounds-main components of cellular membranes and a marker of myelination and membrane degradation; NAA-marker of neuronal density and viability; myo-inositol (mI)- implicated in signal transduction and a putative marker of glial cells and creatine (Cr)-marker of energy metabolism [29].

The alterations in the brain metabolite ratios observed in the prefrontal cortex are consistent with recent advances in research [34,35], which make a strong case for the importance of this area in the pathophysiology of ADHD. Indeed, its major role in planning, weighting the consequences of future actions, regulating feedback and thus guiding behavior and controlling motor and cognitive impulsivity [36] make it a region of interest in investigations. Neuroanatomical studies have since demonstrated the importance of the extensive interconnections in the prefrontal cortex with other brain regions in the capacity of conducting complex processes. In particular, the dorsal region is very well connected with areas involved in cognitive functions such as attention, working memory and executive processes, known to be deficient in ADHD [37].

One of the specific disturbances we found that reached statistical significance for the left prefrontal cortex, higher Glu/Cr et Glx/Cr in the ADHD group, is consistent with previous studies [38-40], and the diminution of Glu/Cr ratios following pharmacological treatment [41,42], suggested that clinical improvement may be associated with lower concentrations of these metabolites in specific brain

regions. Glutamatergic metabolites are almost invariably involved in current theories on ADHD etiologies, as glutamate is the principal excitatory neurotransmitter in the brain and modulate dopamine release in corticostriatal pathways [43]. These studies in conjunction with our observations are thus prime candidates to support Carlsson's hypothesis of a deep glutamate/dopamine interaction, principally in prefrontal circuits [44] and corroborate with Todd and Botteron's energy-deficit hypothesis, previously described [33].

As reported in other studies [39,45], we observed an increase in the NAA/Cr in the prefrontal cortex that could partially be explained by seeing ADHD as a hyper catecholaminergic state increasing mitochondrial metabolism [46]. In contrast, other studies reported lower ratios in the prefrontal cortex [47,48], striatum [49] and left cerebellar [18]. Explanation of these contradictory data remains speculative but could be in part attributed to heterogeneity in methodologies and population samples.

While the cerebellum is best known for its involvement in motor coordination, many studies, both structural and functional, have disclosed its role in cognitive and emotional processing [19]. Lower cerebellar volumes, reduced activation and functional interconnectivity in the fronto-striato-parieto-cerebellar networks, are only a few of cerebellar abnormalities found in ADHD [50]. Spectroscopic findings reported by Perlov [19] (although in adult population) corroborate our results and support the Carlsson dopaminergic/glutamatergic model previously mentioned [44].

The striatum, along with the prefrontal cortex, is part of the fronto-striato-thalamo-frontal circuits of critical importance for attention processes and is generally accepted as a region of great interest concerning ADHD-related anomalies [34]. It is now a focus of spectroscopic investigations and other studies reported results in agreement with our findings regarding Glx/Cr [37,51,52] and Cho/Cr [34,53]. However, results are mitigated for NAA/Cr, as most studies either found no differences [51] or even reduced levels [53]. While current information on this region is still somewhat lacking, findings do support hyper activation of cortical-striatal pathway and alterations in glial function [53].

Positive link between pregnancy and brain metabolite concentrations in ADHD children. We found that the number of pregnancy-related complications of the third trimester of pregnancy was significantly associated with the level of NAA/Cr in the left and right striatum, as well as with the level of mI/Cr in the left cerebellum. The total number of complications throughout the pregnancy was also significantly associated with mI/Cr ratio in the left cerebellum. To our knowledge, only two other studies used 1H-MRS to evaluate the consequences of perinatal complications on brain neurometabolites in ADHD children. One study [54] found that exposure to nicotine during pregnancy alters the mI level in the basal ganglia while decreasing the Cr level but the authors did not find a link between the number of cigarettes and the Cr level, a particularly interesting result, as prenatal tobacco exposure is a well-documented risk factor for ADHD. On the other hand, exposure to alcohol [55] during pregnancy increased the NAA/Cr in the caudate nucleus of children with foetal alcohol spectrum disorders while it decreased the Cho levels in the reflecting alcohol-induced white matter deficits [56]. Further studies are needed to better disentangle the effects of specific perinatal insults on metabolites.

Strengths and limitation of the study

The strength of our results, although obtained from a small population of children, resides in our use of an intra-familial case-control study. This significantly lowered the influence of confounding factors related to the genetic variability, and allowed to have similar groups regarding potentially confounding factors, such as socioeconomic status, maternal age, and family environment, all of which are recognized as shared factors involved in ADHD etiology [14]. General genetic variability was also decreased by the selection of a third-generation French Canadian sample. Additionally, our participants were all medication-free, an important point considering the proven influence of drugs on the level of brain metabolites levels [57]. In the same spirit, we excluded both the preterm and the low birth weight children, both known as risk factors for ADHD development. Finally, we excluded ^{1}H-MSR data where the participant moved during the procedure.

We acknowledge however that our study was conducted on a small number of children, and while we did obtain statistical power for several of the brain metabolites differences between the ADHD and siblings, we did not adjust for multiple comparisons, which could

lead to type-1 error (false positive). Secondly, the Kinney Medical and Gynecological Questionnaire is a retrospective measure, which is subject to recall bias, albeit is commonly used in similar studies [30]. Another limit was the use of creatine as a denominator in our metabolite ratios, instead of using absolute quantities. However, in the brain, creatine is widely regarded as stable and as metabolite ratios are used in most studies, it allowed for better comparisons of our results to other findings [35].

Conclusion

Our results point out that among the already confirmed role of perinatal complications in the etiology of ADHD, the third trimester of pregnancy could be a critical period for the development of ADHD. It also further characterizes the specific brain metabolite profiles of affected children as compared to their unaffected siblings. Our results provide additional support for the hypothesis of an alteration of the fronto-striato-cerebellar pathway in children with ADHD as compared to their unaffected siblings, and suggest that more attention toward the well-being of the pregnant women during the third trimester of pregnancy would be beneficial in families with high risk of ADHD. Our results may allow a better understanding of ADHD intra-familial risk factors.

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