PRE, PERI AND POSTNATAL COMPLICATIONS IN CHILDREN WITH ATTENTION DEFICIT HYPERACTIVITY DISORDER:

A FAMILY STUDY

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ABSTRACT

Genetic and non-shared environmental factors (factors experienced by one child to the exclusion of his siblings) have been strongly implicated in the aetiology of Attention Deficit Hyperactivity Disorder (ADHD). Shared environmental factors were not implicated. Pregnancy, labour/delivery and neonatal complications (PLDNC) were often associated to ADHD. However, no investigations aimed at delineating the shared/non-shared nature of these factors were reported. In order to identify PLDNC which are more likely to be non-shared, we recruited 65 children with ADHD and assessed their history for PLDNC. 45 of them had non-affected siblings that were also assessed for PLDNC. Compared to their non-affected siblings, ADHD children had significantly higher neonatal complications ($p = 0.01$). Furthermore, neonatal complications were associated with higher motor activity ($p = 0.04$) and attention deficits ($p=0.03$) in ADHD diagnosed children, suggesting that neonatal complications are more likely to be non-shared environmental factors of etiological relevance to ADHD.

RESUMÉ

Des facteurs génétiques et environnementaux sont impliqués dans le Trouble de l'Attention-Hyperactivité (TAHA). Seuls les facteurs environnementaux non-partagés (touchant un membre à l'exclusion des autres dans une famille), contrairement aux facteurs partagés, seraient incriminés dans le TAHA. En tant que facteurs environnementaux, les complications survenant durant la grossesse, le travail, l'accouchement, et la période néonatale (CGTAN) seraient associés au TAHA, néanmoins aucune précision de leur caractère partagé ou non n'est reportée.

Dans le but de reconnaître parmi ces facteurs ceux qui sont non-partagés, l'histoire des CGTAN chez 65 enfants atteints de TAHA et 45 membres de leur fratrie saine ont été comparés. Les résultats montrent que les enfants atteints de TAHA ont significativement plus de complications néonatales ($p = 0.01$). Ces complications sont associées à un niveau plus élevé d'hyperactivité ($p = 0.04$) et d'inattention ($p = 0.03$), suggérant non seulement l'association de ces facteurs au TAHA mais probablement leur caractère non-partagé, et donc potentiellement étiologique.

INTRODUCTION

Attention Deficit Hyperactivity Disorder (ADHD) is an early-onset, clinically complex disorder characterised by inattention, hyperactivity, and impulsivity. The prevalence of ADHD among school-aged children from varying cultural and socio-economic backgrounds approximates 5 to10 % (Swanson et al., 2000;Paule et al., 2000). ADHD is a disabling disorder associated with impaired cognitive functioning and poor school performance and often leading to poor social skills and dysfunctional familial relationships. Follow-up studies showed that approximately one-third of children with ADHD retain this diagnosis when they become adults. In addition, a substantial proportion of children with ADHD (up to 25%) develop antisocial personality disorder and/or a substance abuse/dependence disorders later in life (Gittelman et al., 1985;Swanson et al., 1998).

Although the exact factors implicated in the aetiology of ADHD are not known, the most satisfactory model of pathogenesis for this complex disorder incriminates a combination of genetic and environmental factors.

It is now overwhelmingly established that ADHD have a high genetic component implicated in its aetiology, as shown by family (Faraone et al., 1994;Hechtman, 1996;Samuel et al., 1999), twin (Eaves et al., 1997;Goodman and Stevenson, 1989;Levy et al., 1997;Nadder et al., 1998;Sherman et al., 1997b;Sherman et al., 1997a;Stevenson, 1992) and adoption (Cadoret and Stewart, 1991) studies.

In particular, twin studies showed that ADHD heritability (the proportion of the total phenotypic variance explained by additive genetic factors) is very high, ranging from 75 to 90%. In addition, these studies indicate that the rest of the variance in the ADHD phenotype (10-25%) is accounted for mostly by non-shared environmental factors (factors experienced by one member of the family to the exclusion of his siblings). In contrary, shared environmental factors (factors shared by all the individuals in the same family) did not seem to be involved in increasing the risk for ADHD (McGuffin and Martin, 1999;Levy et al., 1997).

Case/control epidemiological studies indicate that pregnancy, labour/delivery and neonatal complications (referred to hereafter as PLDNC), increase the risk for ADHD. (Astbury et al., 1987;Milberger et al., 1997f). It is believed that such early trauma/stressors on the foetal brain during crucial periods of development may have long-lasting effects on cognition and behaviours (McGrath et al., 2000).

To our knowledge, all studies investigating PLDNC in ADHD used case/control designs comparing children with ADHD to normal, unrelated controls with regard to their individual history of PLDNC. In addition to the classical limitations of case/control risk studies (Clayton and McKeigue, 2001), such experimental designs are not able to distinguish between the shared and non-shared environmental factors. It is therefore important to design a study that can distinguish, at least in part, between these two types of factors.

In this study we aim at identifying, among PLDNC, those who are potentially non-shared environmental factors by using an intra-familial case/control design. More specifically, our hypotheses and objectives will be:

1. Some of the PLDNC, often reported to be associated with ADHD, may represent shared environmental factors with little or no effect in increasing the risk for this disorder. In contrast, some other PLDNC may represent non-shared environmental factors with a potential role in increasing the risk for ADHD. In order to identify these non-shared PLDNC factors, we will compare children with ADHD to their non-affected siblings with regard to PLDNC. We expect that non-shared PLDNC will be more frequent in ADHD children compared to their non-affected siblings.

2. We hypothesise that if non-shared PLDNC (identified in question 1) play a potential role in increasing the risk for ADHD (causal relationship), they will be correlated to the severity of ADHD symptoms. To test this hypothesis, we will study the relationship between non-shared PLDNC and the severity of the symptoms (evaluated by laboratory measurements) in children diagnosed with ADHD.

BACKGROUND

Risk studies in ADHD have focused on obstetrical, perinatal complications and postnatal trauma as possible predictors of the development of ADHD. It is believed that such adverse events experienced in the early stages of development may affect the foetal brain and results in long lasting behavioural and cognitive effects (McGrath et al., 2000).

In accordance with the stated objectives of this work, the present review of the relevant literature will focus mainly on the most frequently reported environmental factors associated with ADHD during the pregnancy, labour, delivery and neonatal periods of development.

PREGNANCY COMPLICATIONS

A number of studies have linked pregnancy complications or conditions with the development of ADHD (McIntosh et al., 1995). Although a myriad of factors during this developmental period were incriminated, only a minority of them were subjected to particular scrutiny and emerged as having a specifically important role. Of these, maternal smoking and alcohol exposure received an important interest.

Maternal smoking:

Several studies have found evidence of increased rates of ADHD in children of mothers who smoked during pregnancy. Denson et al.(Denson et al., 1975) found that mothers of hyperkinetic children smoked significantly more than

mothers of a group control. In a much larger study, including 2256 children, Weitzman (Weitzman et al., 1992) found that maternal smoking was independently associated to many childhood behavior problems including hyperactivity. Fergusson et al. (Fergusson et al., 1993) conducted a 15-year prospective study on 1265 children, examining the relationship between maternal smoking and several childhood behaviours, including attention deficit disorder (ADD). In this study, several confounding variables such as gender, maternal age, socio-economic status (SES) and parental discord were considered and their effects statistically controlled for. Smoking in excess of 20 cigarettes per day during, but not after, pregnancy was independently associated with higher behavioural problems reported by both teachers and mothers. Maternal smoking during pregnancy was associated with a significant, albeit small, increase in the frequency of all disruptive disorders including ADD.

Milberger et al. examined the association between maternal smoking and the development of ADHD in numerous related studies. The first study (Milberger et al., 1996) included 140 boys with ADHD who were compared to 120 controls with regard to maternal history of smoking during pregnancy. They reported that 22% of ADHD children compared to 8% of controls had a maternal history of smoking during pregnancy. However, this result is difficult to interpret because the prevalence of women smoking in the appropriate general population was comparable the prevalence of smoking in the female general population (27%). Subsequently, these same authors showed that PLDNC were associated with some phenomenological aspects of ADHD (i.e. impaired cognitive

functioning and poor school performance). They also suggested that only complications that reflect chronic exposure, such as smoking, maternal bleeding, family problems, and illicit drug use during pregnancy accounted for these findings (Milberger et al., 1997e).

More recently, this group of investigators explored the role of maternal smoking during pregnancy in ADHD by comparing history of maternal smoking during pregnancy of 174 high-risk non-affected siblings of ADHD children and 129 siblings of non-ADHD controls. Fifteen (47%) of the high-risk siblings had a history of maternal smoking during pregnancy compared with 33 (24%) of the siblings without ADHD ($p = 0.009$). They also reported that maternal smoking was associated with lower IQ in the high risk children (Milberger et al., 1998). The authors concluded that these results extend the previous findings of an association between maternal smoking during pregnancy and ADHD. In fact, other studies of this same group point to a more complex relationship between ADHD and smoking during pregnancy (Milberger et al., 1997c) (Milberger et al., 1997b;Milberger et al., 1997d;Milberger et al., 1997a). Indeed in three different other studies, Milberger et al. reported that ADHD is a predictor of smoking at follow-up into mid-adolescence, and that it is associated with early onset of smoking in children with ADHD. In addition, non-affected siblings of ADHD children showed the same profile of high risk for smoking at an early age. Together, these associations may suggest a common familial factor, possibly genetic in nature, that predisposes to smoking (including during pregnancy) and ADHD.

Recent studies do not replicate the association between ADHD and maternal smoking, and suggest that substance abuse may be a familial trait in relatives of children with ADHD. In a longitudinal study including 150 adolescents, Hill et al. (Hill et al., 2000) studied three main risk factors (family history of alcoholism, child's exposure to prenatal alcohol and cigarettes) on the development of alcoholism as well as other behavioural problems including ADHD. Analyses controlled for each of the risk factors, SES and family history of antisocial personality disorder, when calculating risk of developing psychiatric disorders. ADHD was predicted by family history of alcoholism, but not by exposure to prenatal cigarettes or alcohol consumption.

Maternal Alcohol consumption:

Maternal Alcohol consumption has also been suggested as a risk factor in ADHD. Indeed ADHD is often diagnosed in children with foetal alcohol syndrome or foetal alcohol effect (FAS/FAE) and in children of alcoholics.

Nanson et al, (Nanson and Hiscock, 1990) compared twenty children with FAS/FAE to 20 children diagnosed with attention deficit disorder (ADD) and 20 normal controls. They indicated that although the children with FAS/FAE are significantly more impaired intellectually, their attention deficits and behavioural problems are similar to those of children with ADD. However, these similarities do suggest neither association, nor causality between ADHD and alcohol effect.

Brown et al, (Brown et al., 1991) reported that, children born to mothers who had abused alcohol throughout pregnancy had more behavioural and

attention problems than children born to mothers without alcohol consumption during pregnancy. There was a clear correlation between the occurrence and the severity of neuro-psychiatric disorders, including attention deficit, and the degree of alcohol exposure of the children while they were in-utero. They also compared the effect of alcohol exposure throughout the pregnancy to alcohol consumption that was stopped after educational intervention during the second trimester. Interestingly, they reported that only children exposed throughout the pregnancy showed the attention deficits.

Streissguth et al, (Streissguth et al., 1994) in a longitudinal prospective study, examined the effect of prenatal alcohol exposure on 500 children from birth to the age of 14 years. Prenatal ethanol exposure was significantly associated to attention and memory deficits in a dose-dependant fashion. This association remained significant after controlling for maternal smoking. These dose-dependent effects on neurobehavioral function were not limited to attention deficits but extended also to other disorders including learning problems.

Aronson et al., (Aronson et al., 1997) found that 10 of 24 children born to mothers who had abused alcohol throughout pregnancy had ADHD. They reported a clear correlation between the occurrence and severity of the neuro-psychiatric disorder and the degree of alcohol intra-uterine exposure. Here again, the reported disorders were not limited to ADHD as 3 (out of the 24) children had autistic disorders, 6 had mental retardation and 11 needed special education. Only seven children were attending regular schools without any type of support.

Coles et al, (Coles et al., 1997) studied 149 African-American children of low SES. Eighty-seven had FAE, 27 had ADHD without FAE and 35 were normal controls. They showed that both FAE and ADHD groups performed more poorly than controls in intellectual tests. However, the ADHD children performed more poorly on conventional tests sensitive to attention problems and conduct disorder. This study suggest that the alcohol-affected children do not display the same neurocognitive and behavioural characteristics compared to children with a primary diagnosis of ADHD. The authors suggested that alcohol might not play an important role in the development of ADHD per se, but that ADHD and FAS include attention deficit with distinct patterns.

The study of Hill et al (Hill et al., 2000) mentioned in the previous section does not support the role of maternal alcohol consumption in ADHD development, although it does suggest that family history of alcoholism increases the risk of having ADHD.

In conclusion, the relationship between maternal smoking and/or alcohol use during pregnancy and ADHD may not be a simple one. As cautioned by Barkley (Barkley, 1990), several factors need to be controlled for (SES, learning disabilities, social/familial environment) in order to establish that any of them represent a risk factor for ADHD. This may be a very difficult task in case/control studies, since matching for several factors may be very difficult. The fact that family history of smoking and alcoholism may be related to higher risk of developing ADHD further complicates the problem. This is may be so because it is very difficult, if at all possible, to match case and controls for family

histories. In addition this may indicate that the correlation between these two putative risk factors and ADHD may be secondary to a common underlying factor, such as shared genes that increase the risk for these risk behaviours and ADHD. An intra-familial design, comparing children with ADHD to their non-affected siblings, will have the advantage of controlling for many of the above mentioned confounding factors, since all siblings share the SES, family environment etc, and they also share 50% of their genes.

Other pregnancy related events and ADHD

Numerous other factors occurring during pregnancy have been reported to be over-represented in children with ADHD. For example, Hartsough and Lambert (Hartsough and Lambert, 1985) compared 301 hyperactive to 191 non-hyperactive children and found that young maternal age, poor maternal pregnancy health, eclampsia and pregnancy parity were factors predictive of subsequent ADHD. Chandola et al (Chandola et al., 1992) compared birth records of 129 referred hyperactive children to the remaining 24,656 members of the same geographical birth cohort. Factors associated with increased referral were maternal age younger than 25 years at the time of their child's birth and social class. In one of the Milberger studies that we mentioned earlier (Milberger et al., 1997e), family problems, maternal bleeding and maternal accidents during pregnancy had a positive association with the development of ADHD.

LABOUR AND DELIVERY COMPLICATIONS

Hartsough and Lambert, (Hartsough and Lambert, 1985) showed that foetal distress during birth or delivery (nonspecific distress, head or other birth injuries) was reported by 17% of mothers of hyperactive children, compared to 8% of control mothers.

NEONATAL COMPLICATIONS

Because of the development the techniques of caring for new-borns with very premature birth, concerns about the future cognitive and behavioural development of children with low birth weight (LBW) were raised and subjected to scientific scrutiny.

Breslau et al.1996 (Breslau et al., 1996) examined the association between LBW (< or = 2,500 g) and attention deficit hyperactivity disorder (ADHD) in a total of 823 children, 473 with LBW and 350 with Normal Birth Weight (NBW). LBW was associated with ADHD, and most strongly in the urban than in the suburban population. Data from teachers' ratings revealed an association between LBW and attention problems.

In a study by Botting et al (Botting et al., 1997), one hundred and thirty-seven very LBW (VLBW) children were compared at 12 years with a sample of matched peers (n = 148) on a number of psychiatric symptoms including Attention Deficit/Hyperactivity Disorder. The main psychiatric outcome in the population of VLBW was Attention Deficit Hyperactivity disorders, with 23% of

VLBW children meeting clinical criteria, compared to 6% of controls. More than one quarter of VLBW children (28%) showed a psychiatric disorder of some type compared to only 9% of peers.

Similarly, Szatmari et al. (Szatmari et al., 1990) investigated a cohort of children weighing at birth between 500 to 1000g at five years of age. In 82 of 90 survivors, they found that LBW children were much more likely to experience developmental delay and problems with motor co-ordination. In particular, 16 % of the LBW children had an ADHD, compared to 6.9 per cent of controls. However, it is noteworthy that these findings may be due to failing to take into account the effects of a third variable as controlling for the effects of neurodevelopment problems collapsed this association between LBW and ADHD to statistical non-significance.

Although some other studies are supportive of this risk relationship (Levy et al., 1992), not all studies were able to replicate these findings. For example, Hartsought and Lambert (Hartsough and Lambert, 1985) found no significant difference between ADHD children and normal controls with regard to premature birth or LBW. Consistent with this finding, Milberger et al. (Milberger et al., 1997e) reported that LBW was associated only with lower IQ scores but not with ADHD. O'Callaghan et al. (O'Callaghan and Harvey, 1997) followed prospectively eighty-seven (70%) of 125 LBW children until they reached between 4 to 6 years of age. There was no evidence to suggest an association between ADHD and perinatal adversity in LBW children.

In conclusion, studies of the effects of LBW on cognition and behaviours have robustly and consistently established an association between LBW and intelligence level and learning abilities. However the effect of LBW on attention and motor behaviour remain a subject of controversy (McGrath et al., 2000) (Hack and Breslau, 1986). LBW is also often associated with many other risk factors making it a difficult factor to examine and identify its specific effects as opposed to the effects of the co-morbid conditions that are often associated to LBW. Apart from LBW and premature birth, no other neonatal abnormal conditions were reported to be associated with ADHD.

PATHOGENESIS

Biological factors implicated in ADHD

The most compelling neurochemical hypothesis of ADHD postulates a dysfunction of the dopamine (DA) systems causing deficits in sustained attention, over-activity and impulsiveness and underlying most of the behavioural abnormalities observed in ADHD (Sagvolden and Sergeant, 1998). This hypothesis is based mainly on the fact that psychostimulant drugs that increase the synaptic level of DA are effective in treating the symptoms of ADHD. As well, it has been found that cerebrospinal fluid concentration of homovanillic acid, the main metabolite of DA, correlate with behavioural measures of impulsivity/hyperactivity in boys diagnosed with ADHD (Castellanos et al., 1994). Neuroimaging studies also support this hypothesis. For example, Ernst et al. (Ernst et al., 1998) used positron emission tomography to compare the integrity

of the presynaptic dopaminergic function between ADHD adults and healthy controls. They reported decreased Dopa-decarboxylase activity and DA storage in the prefrontal cortex. More recently, it has been shown that patients with ADHD show higher levels of dopamine transporter in their striatum compared to normal controls (Krause et al., 2000).

Furthermore, animal models of ADHD suggest that abnormalities of DA neurotransmission may be pivotal in the pathogenesis of ADHD or some of its symptoms. Indeed, it has been shown that mice lacking the gene encoding the DA transporter show elevated dopaminergic tone and marked hyperactivity (Gainetdinov and Caron, 2001). Interestingly, the hyperactivity of these animals is exacerbated by exposure to a novel environment and decreased in response to psychostimulant drugs (Fumagalli et al., 1998). It is also interesting to note that all known strains of animals that exhibit hyperactivity such as hypertensive rats (Carey et al., 1998) and coloboma mice (Raber et al., 1997) have shown alteration in their brain DA neurotransmission systems.

Although the role of DA may be important in the pathogenesis of ADHD, other catecholamine neurotransmitters have also been implicated. For example, Hanna et al. (Hanna et al., 1996) found urinary concentration of 3,4-dihydroxyphenylacetic acid, a norepinephrine metabolite, and epinephrine to be significantly lower in ADHD subjects compared to normal controls confirming other reports (Ernst et al., 1997) of abnormal metabolism of norepinephrine and epinephrine in ADHD (Shekim et al., 1983).

PLDNC and brain dopamine systems: a mechanistic explanation of the correlation between ADHD and PLDNC.

Correlation between an environmental factor and a specific condition can not be interpreted as a causal relationship unless some other types of evidences are supplemented. Some of the evidence may be a dose-effect relationship whereby incremental dosing of the putative risk factor leads to incremental increase of the risk or the severity of the condition under study. Another important supplementary information that may buttress the causal relationship may be a plausible biological relationship between the putative risk factor and the condition under investigation.

Attempts to explain the biological effects of the PLDNC on the brain of developing children and their possible impact on cognition and behaviours were reported and vary in the extent of their development and explanatory power, depending of the specific factor. While the cellular and molecular mechanistic effects of some factors such as smoking are being actively studied, some other factors, such as LBW remain without even a rudimentary mechanistic explanation.

Smoking was the most studied factor and two main mechanisms were proposed to explain the effects of maternal smoking on the fœtus. Foetal hypoxia induced by carbon monoxide and the nicotine induced disturbances in the brain dopamine systems (Longo et al. 1977; Fung YK 1989; Fung and Lau, 1989; Ernst et al., 2001). Although, the exact chain of events implicated in this process is not

well understood in humans, animal models are shedding some light on these biological pathways. More than 20 years ago, Davis (Davis et al., 1979) found that rat neonates exposed to anoxia showed higher levels of hyperactivity. Similarly, Speiser et al.(Speiser et al., 1983), exposed rats to severe anoxia within 24 h following birth. These rats demonstrated augmented motor activity in ambulation, sniffing and rearing activities in an open field. Activity was significantly increased at 10 days of age, maximal at 20-25 days.

Another example of the effect of perinatal adverse events on behaviour is the study of Vaillancourt and Boksa, (Vaillancourt and Boksa, 1998) in which rats born by caesarean section under general anaesthesia showed increase in dopamine-mediated behaviours including hyperactivity. More recently, Brake et al. (Brake et al., 2000) found that rats born by caesarean section and exposed to intra-uterine anoxia, were hyperactive and had mesocortical dopamine activation impairments in the left prefrontal cortex as measured by dopamine transporter density. All these studies suggest a brain dopamine dysfunction as a result of obstetrical/neonatal hypoxic complications leading to an increase in locomotor activity in animal models. In addition, Berger et al. (Berger et al., 2000) reported that cesarean section has different effects on dopamine-mediated behaviours in two strains of rats that differ in genetic composition. They suggest that the genetic background may modulate birth complications like C-section on brain dopamine function in the rat.

Although these animal models strongly suggest that different insults to the brain during specific developmental windows may disturb the brain dopamine

systems, possibly leading to increased motor behaviour, these animal models have major shortcomings and do not allow a direct extrapolation to humans. In particular, it is well established that rodents are less sensitive to anoxia than humans, which may indicate that hypoxia during birth, may have a more profound effect on human infants. Another issue that needs to be addressed is that the maturity of rodent pups' brain at birth do not correspond to the maturity of the human brain at birth indicating that more research adjusting for this factor is needed.

Because of these limitations in animal models, studies investigating the direct mechanistic relation between PDLNC and ADHD in humans are needed. However, because it is difficult to study, only suggestions of putative mechanisms were reported in the literature. For example, Lou et al. (Lou et al., 1989) studied regional cerebral blood flow (rCBF) in 19 subjects, six patients with pure ADHD and 13 patients with ADHD in combination with other neurological symptoms using xenon ^{133} inhalation and emission computed tomography. They reported a significant hypoperfusion in the right striatum in subjects with pure ADHD. This hypoperfusion was partially reversible with methylphenidate. They suggest that this location of hypoperfused structures is consistent with a role for an early hypoxic-ischemic event in their origin, and that right stiatum hypofunction is specific to this subtype of ADHD.

More interestingly, some studies in humans suggest that hypoxic/ischemic brain damage and intraventricular hemorrhage, secondary to perinatal asphyxia, can result in neurologic and intellectual dysfunction and possibly psychiatric

disorders. (Whitaker et al., 1997;Lou et al., 1979). In a very interesting study, Low et al, (Low et al., 1992) prospectively studied 130 preterm neonates. The infants were evaluated for echosonographically demonstrable cerebral lesions (EDCL) during the neonatal period, at 3 or 6 months. Among the preterm children who experienced hypotension or hypoxemia in early hours of life, 34% showed EDCL, which are intraventricular hemorrhage (21%) ventriculomegaly (18%) and hyperechoic parenchymal lesions (8%). In contrast, preterm children who did not experience hypotension or hypoxemia displayed a significantly lower frequency of EDCL (13%). When hypotension and hypoxemia were both present, the chance of having EDLC surpassed 50%. However, in this study there was no follow-up to establish a link between brain lesions and ADHD symptoms.

Another study that is suggestive of a relationship between early anoxia/hypoxia and ADHD was reported by Chandola et al.(Chandola et al., 1992). In this study, a retrospective analysis of a series of cases found a relationship between referral for ADHD and infants who received Cardiff Bag Resuscitation, had a 1-minute Apgar score below 7 or a 5-minute Apgar score below 9. However, the exact implications of this study are difficult to reach since these predictive factors were strongly correlated with each other and their effect was weakened when other confounding parameters were controlled for.

In a more recent study, Whitaker et al. (Whitaker et al., 1997) examined the relation of neonatal cranial ultrasound abnormalities (reflecting perinatal brain injury) to psychiatric disorder at age 6 years in a regional birth cohort of low-birth-weight children. Twenty-two percent of the cohort had at least 1 psychiatric

disorder, the most common being attention deficit hyperactivity disorder (15.6%). They conclude that neonatal cranial ultrasound abnormalities suggestive of white matter injury significantly increased risk for ADHD at age 6 years in low-birth-weight children. However, this study did not speculate on the relation between anoxia/hypoxia and the observed brain abnormalities.

In conclusion, as many PLDNC (such as prolapsed umbilical cord, placenta previa, multiple pregnancy, abnormal fetal position or prolonged labour) were associated with hypoxia and with an increased risk for ADHD, it is possible that hypoxia/anoxia during pre, peri and post natal periods plays a role in the future development of ADHD. Although animal models are consistent with this hypothesis, direct evidence in humans remains scant. In addition, given the heterogeneous nature of PLDNC, it is possible that other factors play a more direct role in ADHD. For example, it has been suggested that chronic exposures particularly those producing hypoxia rather than acute insults, are more likely to be associated with neuropsychiatric impairment (Milberger et al., 1997f). The literature indicates also that some developmental periods are more likely to be vulnerability windows where the brain is more susceptible to environmental stressors. Difference in genetic backgrounds may also play some role in the susceptibility of the brain, particularly dopamine systems, to environmental stresses. Our research questions aims at answering some of these questions by using an intra-familial case/control study. More specifically, we propose to compare patients affected with ADHD to their non-affected siblings with regard to PLDNC with the assumption that this design will control for several

(environmental as well as genetic) confounding factors that often plagued this field of research. Moreover, this design may point to PLDNC that are more likely to be non-shared factors, which, according to genetic epidemiological studies, are putatively more relevant to the aetiology of ADHD.

PLDNC IN CHILDREN WITH ADHD AND THEIR NON-AFFECTED SIBLINGS

The present study is a part of an ongoing and more ambitious clinical and pharmacogenetic study of ADHD with two major purposes. The first purpose is to study the putative interactions between PLDNC and genetic factors involved in ADHD, and the second purpose is to identify genetic and clinical predictors/correlates of therapeutic response to methylphenidate.

The first step of the study aiming at the investigation of the interaction between genetic and environmental factors is to identify, among PLDNC, putative non-shared environmental factors with the assumption that these non-shared environmental factors may be more relevant from an etiological stand point. In a second step, these non-shared environmental factors will be used as covariates in genetic studies of ADHD, with the assumption that this will increase our ability to identify genes implicated in ADHD or in modifying therapeutic response to methylphenidate. This thesis is based on the analysis of the data generated from the first step of the clinical and pharmacogenetic study at its current stage of completion.

METHODS

Subjects:

Patients

Children were recruited sequentially from the Disruptive Behaviour Disorders Program (DBDP) at the Douglas Hospital and the outpatient clinics of the Douglas Hospital. Children are referred to these specialised care facilities by school principals, community social workers and paediatricians and generally 80 % of them are diagnosed with ADHD in the DBDP and 50 % in the outpatient clinic.

Inclusion criteria: In order to be included in the study, children had to fulfil the following inclusion criteria (1) age between 6 and 12 years of age, (2) meet DSM-IV diagnosis of ADHD based on a clinical interview including school and parental reports. Parental report is based on a structured interview (Diagnostic Interview Schedule for Children, fourth revision or DISC-IV). In all cases, a best estimate diagnosis of ADHD was made by a panel of two experienced child psychiatrists who reviewed all the collected data.

Exclusion criteria: were excluded from this study all children who had: (1) history of mental retardation with an IQ less or equal to 70 as measured by the WISC-III. (2) History of autism, Tourette syndrome, pervasive developmental disorder or psychosis. (3) Major medical condition or impairment that would interfere with the ability of the child to complete testing. (4) Previous intolerance

or allergic reaction to MPH. (5) Current treatment with any other medication except for MPH (in particular, patients receiving anti-epilepsy drugs were excluded).

Siblings

In order to include non-affected siblings into the study, we choose to interview mother on history of ADHD in the other siblings whenever possible. In order to be systematic in the inclusion of siblings, we decided to match, as much as possible siblings to their affected siblings with regard to gender then to take the closest in age. If the sibling that meets these two matching criteria is diagnosed with ADHD according to the parental report, the next sibling meeting the two selection rules is investigated. This process is repeated until a non-affected sibling is identified or no sibling remains.

Procedures

If subjects met the inclusion criteria, the children and their parents were given all necessary information through means of a detailed consent form (signed by the parents) describing the study. Once a child is included in the study, he was assessed with regard to several dimensions while he was in a week of wash out from all medications he was taking if he was ever medicated. After this one-week washout period, the children were randomised into a double blind crossover one-week study of methylphenidate.

Behavioural assessments:

Diagnostic Interview Schedule for Children, fourth revision (DISC-IV):

The DISC-IV, a structured diagnostic interview with the parent(s) based on DSM-IV criteria, was used as a major source of diagnostic information. This interview provides information on the child's actions and behaviours in the last 4 weeks, 6 months, and 1-year and whole life. Only the Module E of the interview, which screens for ADHD, ODD (Oppositional Defiant Disorder) and CD (Conduct Disorder) was administered. The diagnosis take olso into account information collected from schools and direct observation of the children. In addition to being a major source of diagnostic information, the DISC provides a rough measure of the severity of ADHD symptomatology, at least from parental point of view (NIMH, 1998). This structured interview was shown to be an acceptable instrument for ascertaining a comprehensive range of child and adolescent diagnoses (Shaffer D, 2000).

The Child Behavioral Checklist (CBCL):

The CBCL (Achenbach 1991) is a 113 items parent questionnaire assessing behavioural and emotional problems in children. The CBCL has the advantage of covering several dimensions (8 or 9 depending on the age) of child behaviour. (Conners & Barkley 1985). It yields total and T-scores for internalizing behaviours (withdrawn, social problems), externalizing behaviours (aggressivity, delinquency) and an overall total score (Achenbach, 1991). It has been shown to

provide good construct validity and to be a valid routine procedure in both a clinical and a research setting (Bilenberg N., 1999).

The Conners' Global Index Score (CGIS) parent form:

The C'GIS is a 10 item parent questionnaire which is sensitive to age groups for children 3 to 17 years old. It yields scores for restless-impulsive behavior, emotional-lability, and a total index score (Connors & Barkley, 1985). It has been shown to be a suitable questionnaire for epidemiological studies, with high degree of structural stability and little dependence on socio-demographic factors (Roussos A. et al, 1999).

Assessment of PLDNC history

The Kinney medical and gynecological questionnaire:

This is a 30-45 minute obstetrical and gynecological interview with the mother encompassing any pre, peri or post-natal complications that could have occurred during any of their pregnancies as well as information on factors such as birth weight, length of labour and gestational age. The mothers were also asked to name any medications or drugs (whether prescription, non-prescription or illicit) taken during pregnancy and give the reason why the medications was taken. The questionnaire also evaluated high risk behaviours during pregnancy and emotional and psychosocial factors (McNeil et al, 1994). This questionnaire was complemented with medical file whenever possible. The *McNeil Sjostrom scale for obstetric complications* was used to score this complicated questionnaire. The

scale gives scores of varying degrees of severity for first, second and third trimester complications, total pregnancy complications, labour and delivery complications, neonatal complications (within the first 8 weeks of birth), and total obstetrical risk. The scale consists of six severity levels, reflecting the ordinal degree of inferred potential harm to the offspring. Severity level 1 refers to complications not harmful or relevant for the somatic condition of the offspring. Severity level 2 refers to complications abnormal or non-optimal, but not likely to be somatically harmful. Severity level 3 refers to complications that are potentially but not clearly harmful or relevant. Severity level 4 refers to complications that are potentially clearly somatically harmful, but not as great as in level 5. Severity level 5 refers to complications potentially clearly greatly harmful. Severity level 6 refers to complications of very great harm to or deviation in offspring. This scoring system was shown to be sensitive and valid (McNeil & Sjostrom, 1995).

PLDNC were collected for all living children in the same family, without previous knowledge of the affection status (except for the proband). This indicates that the interviewer does not know which sibling will be included in the study, thus providing a certain degree of blindness of the interviewer with regard to affection status. In addition, the scoring was performed by the applicant using files that were coded.

Laboratory assessments of attention and motor behaviour

In addition to these clinical assessments, the patients (but not their siblings) were assessed with regard to several laboratory tests, which are part of the pharmacogenetic study. These tests were:

The Conners'CPT (Conner's CPT)

Conner's CPT is a 15-min computerised visual and auditory Continuous Performance Test that measures response inhibition and impulse control. The Connor's-CPT has a good reliability and validity as discussed in its manual and its clinical utility for the assessment of ADHD has been well demonstrated.(Conners 1995; Forbes 1998)

The Restricted academic situation scale (RASS):

The RASS was developed by Barkley (Barkley 1990) and provides information about the frequency and severity of ADHD symptoms during performance of independent academic work. The child is placed alone in a clinic playroom with a set of math problems and told to do as much as he (she) can. The situation lasts 15 minutes and the child behavior is scored on five behavioral categories: "off task", "fidgets", "out of seat", "vocalizes", "play with object". The assessment is done for each 30 seconds interval. Data from this method has been found to correlate with teacher's ratings of ADHD symptoms, and to discriminate children with ADHD from their normal peers (McNeil & Wiegerink 1971).

The CPT and the RASS were assessed on four occasions. First during the wash out period while the patient is having his baseline evaluation. The other four evaluations are made during the third day of treatment with placebo (or active medication): before taking placebo (or active medication) and 45 min. after taking placebo (or active medication). The CPT is always administered first followed by the RASS.

Statistical Analysis

Demographics characteristics (age, sex and average income) as well as behavioural scores (DISC, CGIS and CBCL) were compared using simple t-test statistic.

In order to study the relationship between group membership (affected versus non-affected sibling) we conducted a repeated measure within subject analysis of variance (ANOVA) where the subject status (affected versus non-affected sibling) was the independent variable and the total score of PLDNC during the different period of development (pregnancy, labour/delivery, neonatal) were the within subjects repeated measures. We added scores in each period of development (all severity included) to derive a global score that was entered in the ANOVA.

In addition to this global analysis investigating each developmental period, we also conducted specific comparisons using Fisher's exact tests to explore differences between ADHD patients and their non-affected siblings with regard to a selected number of PLDNC that were often cited as risk factors in the literature

such as cigarette smoking, alcohol abuse, birth weight and premature birth. However, given the high number of comparisons performed with this analysis approach, the results from this data mining approach are considered only for exploratory purposes.

In order to study the relationship between PLDNC and the clinical expression of ADHD, any positive factor identified in the analysis of variance exploring the relation between group membership and developmental period was further explored in the group of children affected with ADHD. This analysis consisted in grouping ADHD patients into those who have positive history of the positive factor(s) that emerged from the previous analysis. Subsequently, analysis of variance was performed, in which the factor under investigation (present/absent) was the independent variable and the clinical measures of severity (CBCL, CGIS) were the dependant variables. Since for the CPT and RASS several measures were taken (baseline, before placebo, 45 min. after placebo, before methylphenidate after 45 min. of methylphenidate) in the same subject, an ANOVA with multiple within subject repeated measures were performed.

RESULTS

The data presented in this thesis is derived from the study sample when it reached 65 patients. These patients were at different stages of the recruitment and evaluation process. Among this population, 45 children had non-affected siblings (20 children have only affected siblings or no siblings at all). Only 47 subjects

had already finished the two-week trial with methylphenidate and did have their code unblinded allowing their inclusion in some analyses of this thesis (analysis of the relation between PLDNC and severity of ADHD phenomenology).

ADHD affected children did not differ from their non-affected children with regard to age ($p = 0.10$). Rank of birth was higher in the affected group compared to the group of siblings (respectively 2 and 1, $p = 0.01$). Also, females were predominant in the group of non-affected siblings (respectively 15/30 and 41/4) (see Table 1 for more details). The over representation of males was present despite our efforts to select non-affected siblings matched to the gender of the child with ADHD. However, this was not possible because, as expected, males were over represented in our study group and many of them had affected male siblings or no siblings at all (which is expected given the sex ration observed in ADHD).

Table 1 shows also that children with ADHD and their non-affected siblings were very distinct with regard to their behavioural profiles as indicated by the highly significant differences in the CBCL and CGIS.

ANOVA analysis comparing children with ADHD and non-affected siblings in different developmental periods (first, second and third trimester of pregnancy, labour/delivery and neonatal periods) revealed no group effect ($F_{1,88} = 2.16$, $p = 0.14$). However there is significant effect of the period of development ($F_{4,352} = 4.90$, $p<0.0000$). The main finding of this study is that the two profiles of PLDNC over the developmental periods in ADHD children and their non-affected

siblings were not parallel as indicated by a significant interaction between group membership and the developmental periods ($F_{4,353} = 3.12$, $p < 0.01$) (See Table 2).

Post-hoc comparisons (LSD method) showed that this differential profile indicated by the significant interaction between developmental period and group membership stemmed from a significant increase in neonatal complications in ADHD children compared to their non-affected siblings ($F_{1,88} = 6.16$, $p < 0.01$) (see Table 3 and Figure 1).

Exploratory analysis of the relationship between specific PLDN events and ADHD was investigated by conducting multiple testing using the Fisher exact test. Non-affected siblings had more frequent history of forceps ($p = 0.04$). In contrast, ADHD children displayed highly significant increase in neonatal hospitalisation and/or therapeutic intervention ($p = 0.003$). It is noteworthy that the factors often reported to be associated with ADHD in case/control studies such as maternal smoking and alcoholism, low birth weight did now show significant differences between patients and their non affected siblings (see Table 4).

Table 1: Demographic and clinical characteristics of children with ADHD (n = 45) and their non-affected siblings (n = 45).

	Subjects	Siblings	p-value
Age yr.	8.8 (1.7)	9.7 (3.7)	0.10
Gender (M/F)	41/4	15/30	0.001
Rank of birth	median = 2	median = 1	0.01
DISC-IV Total score	14.2 (2.9)	2.75 (3.0)	0.000
CBCL Total score	73.5 (7.4)	58.6 (18.2)	0.000
CGIS Total score	76.4 (8.7)	56.7 (18.2)	0.000

CBCL = Child Behavioural Checklist; CGIS = Conners'Global Index Scale, DISC-IV = Diagnostic interview schedule for children, fourth edition. Numbers are mean (SD).

Table 2: ANOVA results showing the effects of group membership, neurodevelopmental periods and the interaction between these two factors.

	df effect	MS effect	df error	MS error	F	p-level
Group effect	1	20.9	88	9.6	2.1	0.14
Period effect	4	25.4	352	5.1	4.9	0.00
Interaction	4	16.2	352	5.1	3.1	0.01

Table 3: PLDNC in children with ADHD and their non-affected siblings in the five developmental periods. Number are mean (SD).

	1st Trimester	2nd Trimester	3rd Trimester	Labor/ delivery	Neonatal	Total
ADHD	2.5 (1.6)	3.1 (2.1)	3.1 (1.9)	3.5 (3.1)	4.1 (3.7)	16.2 (7.5)
Siblings	2.4 (1.9)	2.4 (1.8)	2.8 (2.0)	4.1 (2.8)	2.4 (2.5)	14.2 (6.8)
p-level	0.77	0.08	0.50	0.34	0.01	0.20

Figure 1 : Pregnancy, labor/delivery and neo-natal complications (PLDNC) in children with ADHD (P) and their non-affected siblings (S) during different developemental periods.

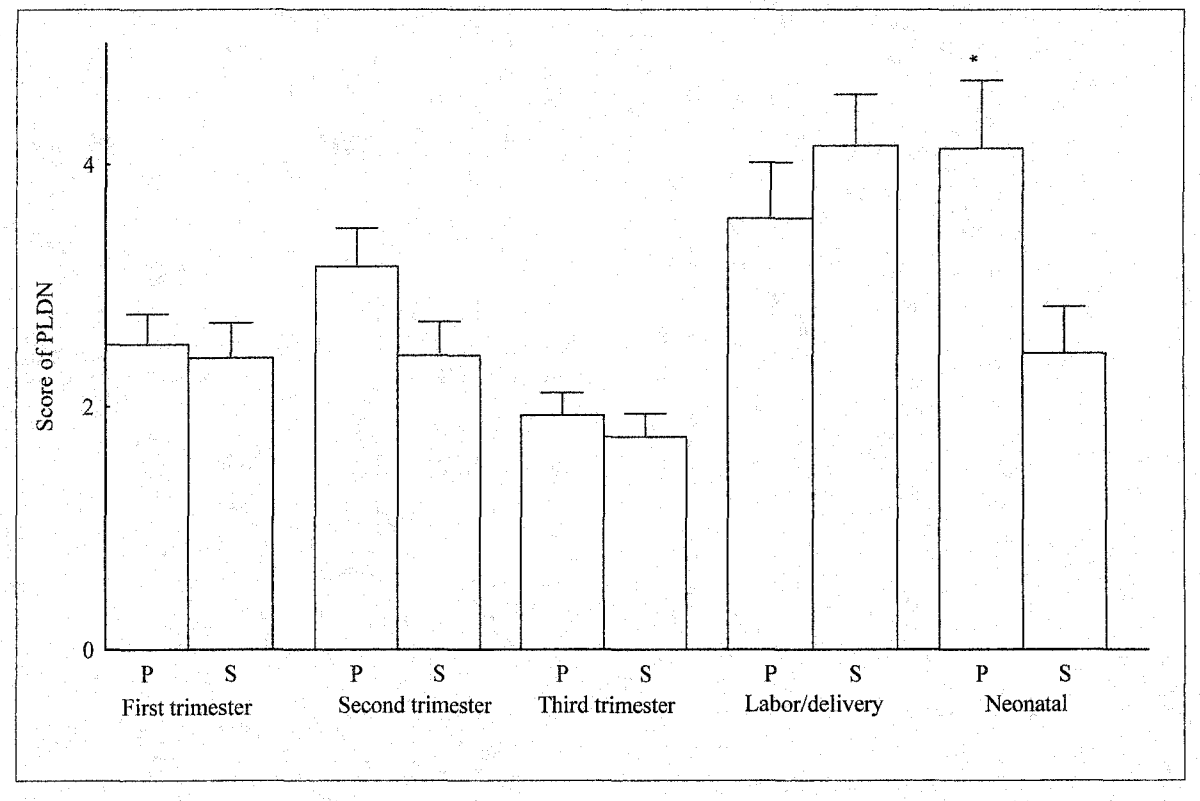


Table 4: prevalence of specific complications (%) in children with ADHD and their non-affected siblings.

	ADHD	Sibling	P
Smoking	60 %	60 %	1.0
Alcohol use	4.5 %	8.9 %	0.6
Drug abuse	6.7 %	8.8 %	1
Hyperemesis	15.6 %	8.8 %	0.5
Bleeding	17.8 %	13.4 %	0.7
Diabetes	13.4 %	8.9 %	0.7
Asthma/Bronchitis	13.4 %	13.4 %	1.0
Maternal problems	55.6 %	46.7 %	0.52
Placenta	6.7 %	2.3 %	0.6
Early contractions	13.4 %	4.5 %	0.2
Drugs against contractions	13.4 %	2.3 %	0.1
Induction of labor	20 %	60 %	0.06
Labor length or inertia	46.7 %	46.7 %	1.0
C-section	31.2 %	26.7 %	0.8
Anesthesia	55.6 %	64.5 %	0.5
Forceps	4.5 %	20 %	-0.04
Ombilical abnormalities	15.6 %	11.2 %	0.7
Other labor/delivery	24.5 %	24.5 %	1.0
Pre-/postmaturity	15.6 %	13.4 %	1.0
Microsomia	4.5 %	4.5 %	1.0
Macrosomia	6.7 %	0 %	0.2
Breeth/cardiac problems	24.5 %	17.8 %	0.6
Jaundice/hyperbilirubin	28.9 %	26.7 %	1.0
Hospitalisation/therapeutic intervention	40 %	11.2 %	0.003
Other neonatal conditions	51.2 %	37.8 %	0.2

Forty-seven subjects completed the pharmacogenetic study in which behavioural and therapeutic response to methylphenidate were assessed using a double blind cross-over design. Among these patients, 34 children had history of neonatal complications and 13 did not. Here, we investigated the relation between the presence of neonatal complications and the phenomenological characteristics as measured by laboratory and ecological clinical measurements of ADHD dimensions.

Table 5 shows that ADHD children with neonatal complications and those without neonatal complications did not differ with regard to average family income, age at the time of evaluation and both included a majority of males without differences in the sex ratio composition ($p= 0.45$).

Table 5 shows also that ADHD children with neonatal complications and those without neonatal complications did not differ with regard to any of the ecological measures of the clinical severity of ADHD. This was true for the total scores of the CBCL and the CGIS and well as for the sub-scores of these two scales (data not shown).

As shown in Figure 2, patients with neonatal complications showed a poorer performance on the Conners'CPT overall index as indicated by a significant group effect ($F_{1,42} = 4.7$, $p = 0.03$). As expected, there is significant effect of the testing time ($F_{3,126} = 4.36$, $p < 0.005$), resulting from the therapeutic effect of methylphenidate. No interaction between group membership and testing time was observed ($F_{3,126} = 0.92$, $p=0.42$). (See Figure 2 and Table 6). Interestingly, the same pattern of results was observed with the total score on the RASS. Indeed, a group effect ($F_{1,45} = 4.43$, $p=0.04$) was observed as well as a significant effect of the testing time ($F_{3,135} = 11.20$, $p <0.000$),

but no interaction between group and testing time ($F_{3,135} = 0.42$, $p=0.75$). (See Figure 3 and Table 7)

Table 5: Demographics and clinical characteristics of ADHD children with and without neonatal complications.

	With neonatal complications (N = 34)	Without neonatal complications (N = 13)
Age (SD)	9.13(1.70)	9.73(1.38)
Gender (M/F)	31/3	11/2
Average Income	3-4	3-4
CBCL total	72.27(6.54)	69.3(10.6)
CGIS total	71.92(12.14)	73(12.65)

M= males, F= females, CBCL = Child Behavioural Checklist; CGIS = Conners'Global Index Scale.

Table 6: Conners' CPT Overall Index scores in children with and without neonatal complications before and 45 min after administration of either placebo or methylphenidate.

	N	CPT OI Before placebo	CPT OI After Placebo	CPT OI Before Ritalin	CPT OI After Ritalin
With NC	31	15.89	15.92	16.61	12.68
Without NC	13	11.19	13.99	13.28	10.94
All groups	44	14.50	14.50	15.63	12.16

CPT OI= Continuous Performance Test Overall Index, NC = Neonatal complications.

Table 7: RASS scores in children with and without neonatal complications before and 45 min after either administration of placebo or methylphenidate.

	N	RASS Before Placebo	RASS After Placebo	RASS Before Ritalin	RASS After Ritalin
With NC	34	48.12	51.80	48.86	23.52
Without NC	13	31.00	36.92	30.76	15.07
All groups	47	43.38	47.68	43.86	21.18

RASS = Restricted academic situation scale, NC = Neonatal complications.

Table 8: Summary of ANOVA of Conners' CPT Overall Index scores in children with and without neonatal complications before and 45 min after administration of either administration of placebo or methylphenidate.

	df effect	MS effect	df error	MS error	F	p-level
NC effect	1	313.61	42	66.61	4.70	**0.03***
Repeat effect	3	81.61	126	18.69	4.36	**0.005***
Interaction	3	17.34	126	18.69	0.92	0.42

Figure 2: CPT OI in children with ADHD stratified according to their history of neonatal complications before and 45 min. after administration of either placebo or methylphenidate (lower scores indicate better performances).

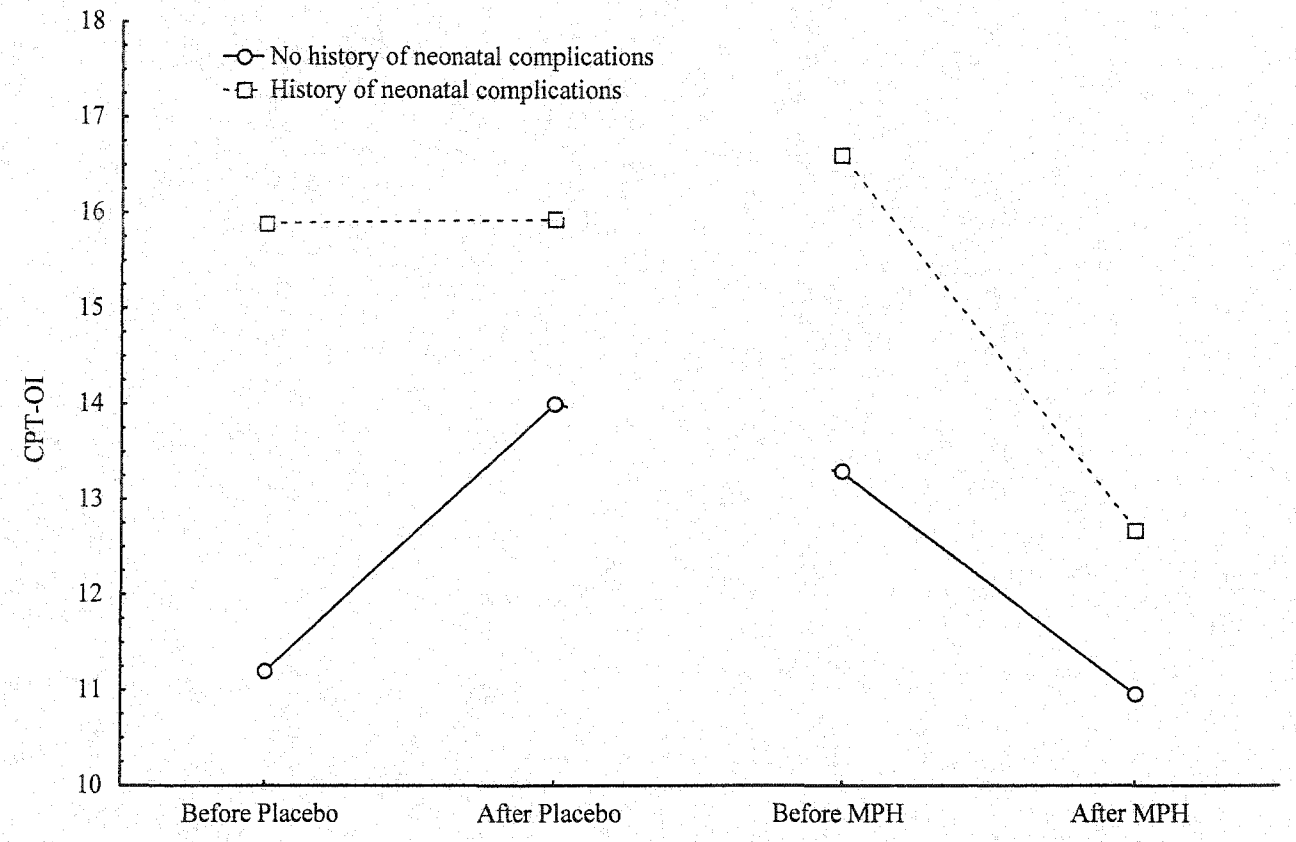
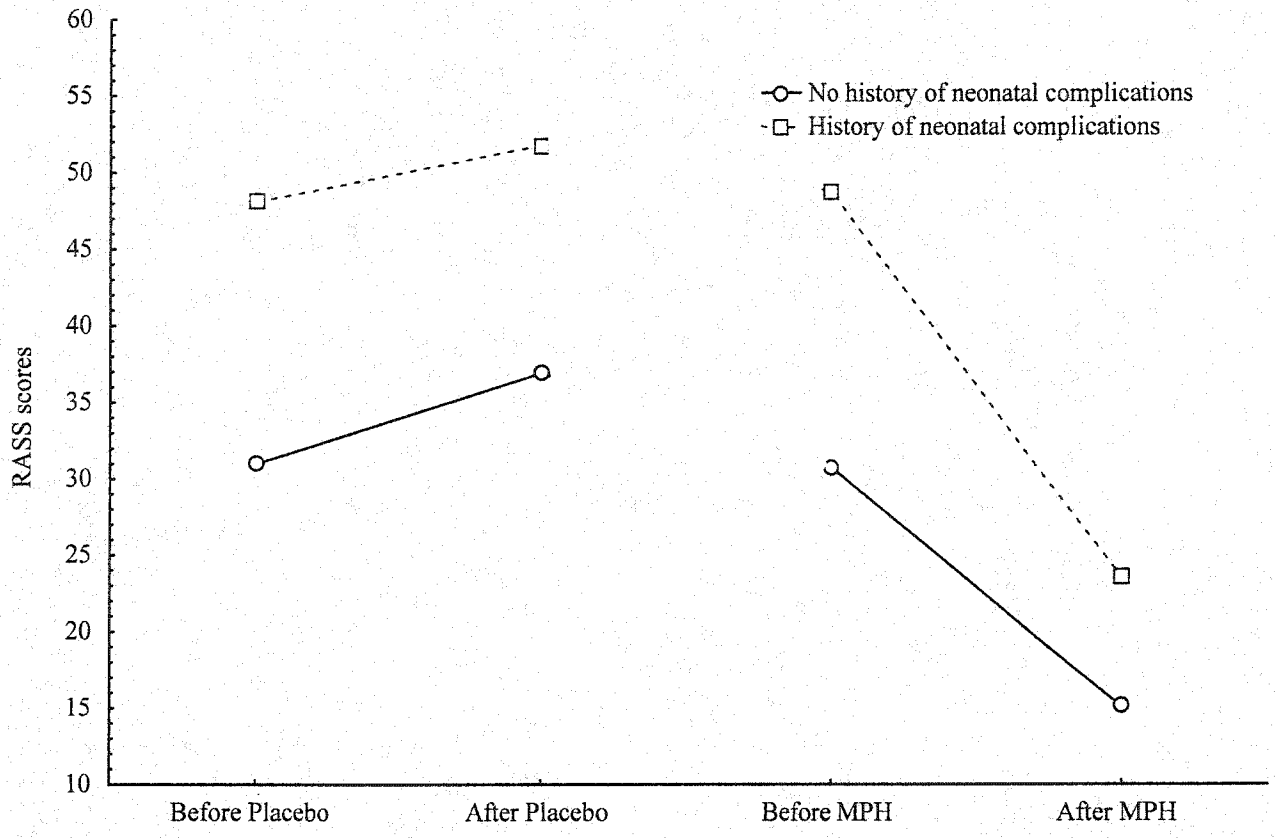


Table 9: Summary of ANOVA of RASS scores in children with and without neonatal complications before and 45 min after administration of either placebo or methylphenidate.

	df effect	MS effect	df error	MS error	F	p-level
NC effect	1	8060.38	45	1816.65	4.43	0.04
Repeat effect	3	4713.23	135	420.65	11.20	0.000
Interaction	3	177.28	135	420.65	0.42	0.73

Figure 3: RASS scores in children with ADHD stratified according to their history of neonatal complications before and 45 min. after administration of either placebo or methylphenidate (lower scores indicate better performances).



3. DISCUSSION:

ADHD is a complex disorder where both genetic and environmental risk factors play a major role. Identifying genes that are increasing the susceptibility for this disorder or modulating the therapeutic response to psychostimulant drugs may be an interesting avenue of research since this will lead to a better understanding of this disorder and possibly improved preventive and therapeutic approaches. Some interesting phenomenological aspect of ADHD are interesting to note and may be an important asset to researcher in the genetics of this disorder. First, although the current DSM-IV definition of this disorder is categorical, it is clear from clinical practice that the two major dimensions (inattentiveness, hyperactivity/impulsivity) of this disorder are manifested along two continuums of behaviours. In addition to its high heritability, this characteristic may allow the use of quantitative genetic analyses approaches that may increase the power to detect genes involved in this condition. Second, there is a number of converging information from both human studies and animal models that indicate that dopamine modulation is an important culprit, when disturbed, in the pathogenesis of ADHD. This, indicate that genes coding for proteins involved in the brain dopamine modulation are important candidate genes as already shown by several molecular studies. Second, ADHD is the only psychiatric condition where the therapeutic effects are observed within a very short time after drug administration and may be well quantified. This also makes the pharmacogenetic studies of psychostimulant drugs in ADHD very attractive. In order to capitalise on these advantage, it may be very interesting to further control for other sources of variance of the ADHD phenotype: this is the general purpose of the current study.

The design used in this study is an intra-family case/control design aimed to identify PLDNC that are specifically observed in affected children to the exclusion to their non-affected siblings. We choose to study PLDNC, but not over environmental factors because these were the most studied factors and they have received some validity from animal studies. The choice of the intra-familial design was decided after our review of the literature indicated that many factors (SES, maternal IQ, family history of mental disorders) may be confounding factors that can limit greatly the interpretation and the conclusions that can be drown from population based control studies. Interestingly, the intra-familial case/control study may circumvent many of these biases since it provides excellent to good matching between cases and controls for several factors, including genetic background. In addition, we were intrigued by the fact that genetic epidemiological studies incriminated only non-shared environmental factors, whereas population based case/control studies identified factors that are intuitively more likely to be common to all children in the same family. For example, smoking which has been often reported to be associated with ADHD, is often a persistent behaviour that often starts in adolescence or early years of adult life and that is likely to be carried on several pregnancies. As such, it may be a shared environmental factor according to the genetic epidemiological partition of the variance. One therefore may speculate that the association between maternal smoking and ADHD in children is possibly a familial communality that may be supported by genetic resemblance in the same family rather than a causal relation between ADHD and smoking. The same reasoning may be proposed for alcohol consumption. Other PLDNC may intuitively be conceived as non-shared factors. For example a placenta previa, a protruded cord or kernicterus secondary

to maternal medicine intoxication may be viewed as a non-shared environmental factor. However, it is to be reminded that the partition into genetic, shared and non-shared factors operated by genetic epidemiology is a statistical one and does not imply that there are identifiable shared and non-shared environmental factors. For example, Rhesus incompatibility may impart its effects into three components: genetic (since the maternal Rh^- genotype is required) shared environment (since all siblings Rh^+ share a toxic environment) and non-shared environment (since siblings who are Rh^+ and those who are Rh^- do not share a toxic environment). Therefore, the fact that a certain type of PLDNC are more frequent in ADHD children compared to their non-affected siblings does not imply that this factor is entirely a non-shared environmental but it simply suggest that its aggregate effect may be more on the side of non-shared effects, hence that it is more likely to be involved in the chain of event leading to ADHD. In other words, we believe that the intra-familial design, previously never used in this field of research, have several advantages mostly providing for ideal controls but also suggesting, but far from confirming a causal role of the identified risk factor.

This study has two major finding. First we found that the profile of PLDNC along five developmental periods in children with ADHD and their non-affected siblings are not parallel. This differential profile was mainly due to an increased level of neonatal complications in children with ADHD compared to their non-affected siblings. This result may bring several comments.

First, compared to the pregnancy and labour delivery events, events experienced in the neonatal period, when the child acquires more independence from his mother, are

conceivably more likely to be specific to each individual, that is to be non-shared factors according to the genetic epidemiological terminology. Hence, this result is consistent with the fact that most of the non-genetic variance of the ADHD phenotype was found to be non-shared in nature. Consequently, this result may suggest that neonatal complications may be a risk factor with putative causal link to the development of ADHD. A closer scrutiny of the neonatal complications identified in this study and distinguishing patients from their non affected siblings were medical conditions that required hospitalisations. This observation gives some confidence that these neonatal complications are of the non-shared type. In contrast to neonatal complications, smoking and alcohol consumption, two environmental factors which are conceivably shared environmental factors did not show difference between patients and their non-affected relatives. For example, smoking was equally high (60%) in pregnancies leading to affected and non-affected children. Remarkably, this rate is much higher that the prevalence observed in the general population of Montreal. This may indicate that smoking in this population is a trait associated with but not causal of ADHD. Had we done a population based association study and compared smoking during pregnancy of mothers of these ADHD patients and mothers of control subjects representative of the general population, we would have reached the spurious conclusion that smoking is a risk factor for ADHD. This same line of reasoning may be also valid for other maternal medical conditions such as diabetes or hypertension, previously incriminated in literature as risk factors.

Third, because of our analytical approach grouped several complication in particular developmental periods, the present result is an average of different

complications that might be heterogeneous in nature, some of them being possibly important to the development of ADHD and some others being completely irrelevant. Although the subdivision into different developmental periods has a rational -different stage of the brain development and brain vulnerability to environmental stresses- more detailed analysis of the risk factors, one by one, needs to be performed. Unfortunately, this type of analyses can not be performed on a small sample without increasing the risk of the inflation of type I statistical errors. However, in this study group, neonatal complications that were more frequent in ADHD children included several possible events occurring during the first two months of life. Some of them were significantly different between probands and siblings. Of these, neonatal hospitalisation, being in incubator, needing oxygen therapy, general anesthesia or surgery were the most frequent. Although these findings do not point to single event that may explain brain injury leading to behaviour or cognitive problems, it does support the past research in that children with ADHD have a higher prevalence of early-life stressful events. Moreover, these factors are more likely to be chronic rather than acute which is also consistent with previous research suggesting that prolonged stresses are more likely to be associated with ADHD. Also, in line with the results of animal models, it is interesting that at least some of these factors are clearly associated with hypoxia (for example needing oxygen therapy, incubator) during neonatal period.

The second main finding of this study is that neonatal complication were associated with inattention as measured by the Continuous Performance Task overall index (CPT-OI) and the level of motor activity as measured by the restricted academic situation scale. Interesting this observation was made on four different measurement times giving this

result a good repeatability overtime, and hence reducing its chance to be a false positive result. In contrast, clinical ecological measure of the severity of ADHD symptoms were independent from history of neonatal complications. Here again several remarks need to be made.

First, the presence of an association between neonatal complications and the severity of the ADHD manifestation on the two major dimensions of this disorder, combined with the putative non-shared nature of these complications suggest that indeed, these complications may be causally related to the pathological condition.

Second, the fact that neonatal complications were related to measures made in the laboratory but not with clinical measures of severity is not surprising. Indeed, the laboratory measures are made with very high level of standardisation and by trained experimenters with similar training and high inter-rater reliability whereas clinical assessment are made by different person (mothers), with no training and can have several biases resulting in an important between raters variability. This indicates that highly structured laboratory measure are very important in behavioural studies of ADHD, and other psychiatric conditions. Of course, ecologically valid assessments are also important but very high sample sizes might be need to achieve a projected purpose.

Although the mechanism for the positive association between neonatal complications and ADHD are not well established, this finding can be interpreted as consistent with the dopaminergic hypothesis postulated for this disorder. Indeed, several studies linked postnatal insults with alteration in dopaminergic circuits such as prefrontal

cortex, basal ganglia, corpus callosum and cerebellum, structure that are invoked in the development of ADHD.

Brain resonance-imaging studies showed that total brain volume is slightly but significantly decreased in ADHD, the total and regional growth curves for this group run parallel to normal growth curves (Castellanos et al., 2001). This suggests that brain abnormalities in ADHD appear to be fixed rather than an ongoing process and probably occurred early in neurodevelopment. The most striking and consistent finding in these studies is the significant reduction of the volume of the posterior cerrebellar vermis in ADHD subjects. This region of cerebellar vermis is highly dopaminergic and appears like most brain volumetric measures to be highly heritable but also influenced by various factors. Even a subtle injury during this vulnerable process of neurodevelopment in utero (second or third trimester) can affect the brain development and size globally explaining the changes seen in ADHD (Rapoport and Castellanos 2001). This report is interesting because it suggest not only that the neonatal period of brain development may be implicated in the brain abnormalities observed in ADHD children, but also point to the importance of pregnancy adverse effects. In our study, although non significant, we found that affected children tended to have higher score of total obstetrical complications during the second trimester of pregnancy, a developmental period reported to be at risk for the disturbance of dopaminergic related behaviors such as motor activity, compared to their non-affected siblings.

Several limitations should be kept in mind while interpreting the results of this study. First, our conclusions are based on a sample of 45 children ADHD and 45 of their

non-affected siblings. This is of course a small sample size for risk studies. Indeed, some epidemiological studies were based on several hundreds of patients and controls. However, in these large epidemiological studies, the relation between ADHD and risk factors related to pregnancy was not addressed directly but was rather a side question, which limit the strength of the conclusions that may be drawn from these large studies. The largest study addressing the specific question of PLDNC and ADHD reported on less than 200 patients. However, as discussed earlier, this was a case control study where the major limitations, recognized by the authors, is the difficulty of matching cases and controls with regard to several confounding factors. Therefore, our study has the advantage of controlling for these biasing factors. Nevertheless, a small sample size may be a serious limitation, particularly with regard to type II errors, that is the inability to declare two samples not different while they are in fact. For example, in this sample, the total number of pregnancy complications in the second trimester was higher in ADHD children but this was short of reaching statistical significance. If this difference is true, increasing the sample size may lead to a significant difference in this period of development. Given that co-morbidity is very high in ADHD, it would have been very informative to study PLDNC in subgroups of patients stratified according to the existence or not of comorbid disorders. However, the small sample size precluded us from performing this type of analysis. One of our future purposes is to increase our sample size and to answer these types of questions.

The second limitation of our sampling is that subjects were recruited from a tertiary and secondary psychiatric facilities which may limit our ability to extrapolate

these results to the general population of children with ADHD. Hence, replicating these results in a more representative sample of ADHD children will be important.

Another important limitation of this study is that males were over-represented in the groups of ADHD children. However, given the fact that females may be more resilient to developing the disorder even with higher level of causative factors (under a multifactorial model) it is expected that some females may have a high level of PLDNC and yet they do not express the disease. This bias may therefore be conservative that is unlikely to result in false positive findings.

For example in this study, we have observed that non-affected siblings did have more occurrences of forceps interventions. Although this difference may be related to the fact that non-affected siblings were on average more first born compared to their affected siblings, its interpretation in the context of this study is difficult because of the gender difference between the two groups and the putative higher resilience of females to brain insults.

The fourth limitation of this study is the difference in birth rank. However, here again, the fact that most of the affected children were born from a second pregnancy, usually considered as the lowest at risk pregnancy indicates that the observed differences are probably not secondary to such a bias.

In our study, diagnosis of ADHD in affected children was based on different sources of information such as clinical evaluation, parental report and teachers reports. However in siblings the absence of ADHD diagnosis was mainly based on parent reports. Siblings were systematically met at least once during the study by an experienced

member of the research team and none of them showed behaviors consistent with the ADHD diagnosis. The absence of school reports on siblings would have resulted in the inclusion of subjects with ADHD symptoms present in the school setting but not at home or other settings. It is therefore possible the selection procedures of non affected siblings may have contributed to reduce the contrast between affected children and their non-affected siblings with regard to the symptom profile. This may have resulted in an increased type II error.

Another limitation of this design is it reliance on a maternal retrospective interview. However, we compared the maternal report to the neonatal files in a number of patients with ADHD (58 %) and found that mothers tend to under report these type of complications , a bias that have been already reported in the literature (Buca et al. 2000). It is therefore unlikely that this method resulted in a major bias, and if ever there is bias, it is rather conservative. Consistent with this observation, we have scrutinized the list of neonatal complication reported by the mothers and most of them were unlikely to result from false recall, though omission might have occurred. However, there is no indication on whether mother may be prone to a differential bias of recall, possibly remembering more event from the pregnancy of the affected children. To our knowledge, this question was not previously addressed in the literature and may be one of our future questions of investigation. Although the interviewer was not completely blind to the group membership (affected subject or non affected sibling), the scoring of PLDNC was blinded with regard to group status. In addition, the interviewer collected information on all siblings without prior knowledge of the identity of the sibling to be included in the study.

CONCLUSION & SUMMARY

To our knowledge, this is the first risk study using an intra-familial design to address the question of pregnancy, labour/delivery and neonatal complication in ADHD. This design has the important advantage of controlling for many confounding factors, including genetic ones, that often plagued case control risk studies. In addition, this type of design may point to non-shared environmental risk factors which, according to genetic epidemiological studies, are more etiologically relevant to ADHD.

The results of this study, at its current stage of development, suggest that neonatal complications are more frequent in ADHD children compared to their non-affected relatives. The severity of these complications and their prolonged character, combined to the fact that they are conceivably of a non-shared nature, suggest that these factors may play a role in the etiology of ADHD. Furthermore, the fact that neonatal complications were associated with the severity of attention deficit and motor activity consolidates the idea that they may have a direct relation to the causation of ADHD.

However encouraging and interesting, the interpretation of these results is limited by the relatively small sample sizes and the poor gender match between patients and siblings. Future studies will address these limitations and extend the current work to investigate the interaction between the putative non-shared environmental factors and specific genes involved in the dopamine system, thus bridging the three main streams of research in this pathology.

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