Childhood onset Neuropsychiatric Disorders

Risk factors in Tourette's Syndrome and co-morbid Attention Deficit Hyperactivity Disorder

Ph.D. theses

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Introduction

In this thesis, our purpose was to find specific genetic and neuropsychological features in child psychiatric disorders such as Tourette's syndrome (TS) and Attention Deficit Hyperactivity Disorder (ADHD). This area needs an interdisciplinary approach, since biological psychiatry, neurology and cognitive neuroscience face questions like genetical vulnerability, co-morbidity or the adaptive aspects of these disorders. Beside the high co-morbidity of these disorders, there is evidence of common prefrontal and basal ganglia (frontostriatal) pathology and the dysregulation of dopamine in these areas of the brain.

The strong genetic components of these disorders are well-known, however, the specific factors affecting TS and ADHD are not yet clearly understood. Most of the neurobiological and pharmacogenetic studies addressed the possible involvement of dopamine gene polymorphisms in these two disorders.

In humans, the prefrontal cortex (PFC) constitutes nearly one-third of the total surface of the neocortex, and its late maturation is related to the late myelination of axonal connections. This correlates with the development of cognitive functions and behavior, attributed to this brain region. Concepts such as anticipation, goal establishment, planning, inhibition, response trials, monitoring results, and use of feedback reflect the functions, in which the prefrontal lobe plays an important role. These terms are collectively known as executive functions (EF), indicating the supervisory role of the cortex. Structural and functional neuroimaging studies found abnormalities in the area of the prefrontal cortex and basal ganglia in TS and ADHD, so it has been proposed that deficient executive functions may be primary cognitive deficits (like the organization of behavior, inhibition of unwanted responses, monitoring activities) in ADHD and TS. There is no consensus about which specific executive deficits are present in TS. It is known that co-morbid disorders such as ADHD can influence cognitive functions, however, in most studies, it was not controlled for. It is more obvious that there are cognitive problems in ADHD, though the heterogenity of the disorder suggests different ADHD-specific executive deficit theories (working memory or motivational problems, disinhibition, etc.).

Objective

1. Genetic hypothesis

The goal of the present study is to evaluate the association between TS, ADHD and the polymorphisms of three dopaminergic genes: DRD4 – 48 bp VNTR, DAT – 40 bp VNTR and COMT Val158Met. These polymorphisms play an important role in pharmacological effects and the dopaminergic transmission of the frontostriatal system. Beside the case-control analysis, we also would like to use a dimensional approach (symptom severity) and to study the effect of methylphenidate in ADHD.

Our hypothesis is that we will find differences in these polymorfisms regarding TS and ADHD in diagnostic categories, when compared to controls.

Our hypothesis is that we find association between symptom severity of TS and ADHD and these three candidate dopaminergic genes.

Our hypothesis is that there is an association between these dopamine polymorphisms and methylphenidate response in ADHD.

2. Neuropsychological hypothesis

In TS and ADHD, there is a common frontostriatal dysfunction which also affects cognitive executive functions. We would like to clarify the neuropsychological deficits in pure TS, since there is not a consensus in this matter. Considering executive functions and ADHD, results are also very heterogeneous.

We selected three well-known neuropsychological test measuring executive domains such as working memory-planning (Digit Span forward and backward), cognitive flexibility (Wisconsin Card Sorting Test) and cognitive inhibition (Stroop test). Neuroimaging studies have shown that brain areas that are active while subjects perform Stroop task are the medial-ventral PFC, including the anterior cingulate, while in tests such as working memory and cognitive flexibility, it is the dorsolateral prefrontal cortex.

Our hypothesis is that we find specific cognitive dysfunction in pure TS by using a battery of commonly used executive function measures.

We hypothesized that common features in the executive profile would point to a common neuropsychiatric and neuroanatomical background, while certain aspects of executive dysfunction would distinguish the two disorders, thereby separating functional or anatomical brain areas of the frontostriatal system.

Methods

1. Participants

A group of 103 TS patients (mean age: 13 ± 4.5 years; 87.4% male and 12.6% female) and 173 ADHD patients (mean age: 9.14 ± 2.6 years; 87.3% male and 12.7% female) from the Vadaskert Child and Adolescent Psychiatry Clinic participated in this study. The sex-matched control (n=284) group was selected from a healthy Hungarian population group. In our neuropsychological study, we carefully controlled for co-morbid conditions, age, gender, intellectual ability and drug effects, therefore, we used the data of only 164 children in the analysis. All children were in the age range of 8-16: 61 diagnosed with ADHD (mean age: 11.26 years ± 1.29; males: 93%), 24 diagnosed with TS (mean age: 12.5 ±1.59; males: 92%), 29 diagnosed with both ADHD and TS (mean age: 11.28 ±1.57; males: 86%) and 50 normal control subjects (mean age: 11.8 ±1.42; males: 86%) matched in age, sex and socio-economic status.

2. Genotyping

Non-invasive DNA sampling was performed from buccal cells by the research team of Sasvári-Székely Mária (Department of Medicl Chemistry, Molecular Biology and Pathobiochemistry). Genotyping procedures for the DRD4 48 bp, the DAT1 3' VNTRs and COMT 158Val/Met were carried out using published PCR amplification and electrophoretic separation protocols.

DRD4 48 bp VNTR genotypes were grouped according to the presence of the 7-repeat allele, resulting in the widely used 7-present (7+) and 7-absent (7-) groups. COMT 158Val/Met genotypes were grouped as Val/Val, Val/Met and Met/Met. In

case of the DAT1 3' VNTR, we used the 9/9, 9/10 and 10/10 genotypes, and the rare genotypes were grouped as "others" and excluded from the analyses.

3. Measures

3.1. Neuropsychological assessments

3.1.1. Cognitive inhibition

Stroop test

This test evaluates the subject's ability to actively suppress irrelevant information and to selectively enhance relevant information stored in his/her memory. It is regarded as a measure of EF related to cognitive inhibition. In our analysis we focused on the most important age-corrected measure (interference score) of this test which shows the degree of cognitive inhibition.

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3.1.2. Cognitive flexibility

Wisconsin Card Sorting Test (WCST)

The WCST is one of the most widely used tasks in neuropsychological evaluation of frontal lobe functions. The test measures cognitive flexibility, conceptual thinking, hypothesis testing, cognitive shifting and perseveration. According to its literature and our concept, we used the five most important T-scores of the test in our analysis: number of trials completed, perseverative errors, completed categories, trials to complete first category, failure to maintain set.

3.1.3. Working memory

Digit span forward and backward

Digit span is a common measure of short-term and working memory. Two measures were used: the maximum digit span the child was able to recall forward and backward. While digit forward involves short term memory, backward span requires working memory.

3.2. Measuring symptom severity

3.2.1. YGTSS (Yale Global Tic Severity Scale)

The Yale Global Tic Severity Scale is a clinician-completed rating scale used to assess the variety, frequency, duration, intensity and complexity of motor and vocal tics. In addition, it measures the degree of overall impairment. In the analysis, peak tic severity (most severe condition in tic symptoms) was used.

3.2.2. ADHD-RS (ADHD Rating Scale)

To measure the severity of ADHD symptoms, the DSM-IV-based ADHD-RS score was the primary outcome measure; the 9 inattention and 9 hyperactivity/impulsivity items can be rated from 0 (not present) to 3 (frequent), adding up to a maximum of 27 points for both subscales.

4. Methylphenidate response in ADHD

From the 173 children diagnosed with ADHD, we had data on methylphenidate (0,3 – 0,6 mg/kg/day) response from 122 children. Drug administration was preceded by a diagnostic procedure. The primary outcome measures were the ADHD-RS scores and the Clinical Global Impression Severity of Illness scale (CGI-S), which assesses overall functioning. Children were followed up in every month; the drug efficacy was evaluated six months later: children were evaluated as responders, if they had an at least 25% decrease in the ADHD-RS global score and a maximum score of 2 points in CGI-S in the last two consecutive months. Ninety children were categorized as responders, whereas 32 patients did not achieve the required improvement. Because of methodological problems, we did not have a chance to measure drug response in TS.

Results

1. Genetic risk factors in TS and ADHD

There was no significant difference in the allele- or genotype-frequencies of the investigated polymorfisms between the TS and control groups and between the DAT and DRD4 VNTRs in the ADHD population. However, significant difference was detected both in allele- and genotype-distribution of the COMT Val158Met polymorphism: the Val-allele and the Val/Val

genotype were more frequent in the ADHD group compared to the healthy population ($p = 0.016$, and $p = 0.037$, respectively). In TS, using dimensional approach (severity of illness) in the analysis, the DAT-40 bp VNTR was associated with peak tic severity measured by the Yale Global Tic Severity Scale ($p = 0.010$). We could not detect any biased distribution in the allele- and genotype-frequencies of the investigated polymorfisms and ADHD symptom severity measured by the ADHD-RS, the Val-allele of the COMT polymorphism, on the other hand, showed a significant association with good methylphenidate response ($p = 0.009$).

2. Neuropsychological features in TS and ADHD

Since learning disorders were significantly more frequent in the ADHD group (approximately 50% of ADHD patients had some learning disorder), we controlled that in the analysis

2.1. Cognitive inhibition

In pure ADHD, there is no dysfunction regarding cognitive inhibition, while the TS+ADHD group showed the worst performance (p=0,006). The pure TS group also shows symptoms of cognitive disinhibition, though the result is statistically not significant (p=0,08). After controlling for learning disorders, this neuropsychological profile is very similar.

2.2. Cognitive flexibility

Significant problems regarding cognitive flexibility were only detected in ADHD, but these differences disappeared, after we had controlled for co-morbid learning problems. In pure TS, there is no dysfunction in this cognitive domain.

2.3 Working memory

There is a significant short term memory deficit in ADHD and ADHD+TS, while the performance of the pure TS group is intact. Regarding working memory capacity, only the ADHD group shows a significant deficit compared to the control ($p<0,0005$), and to the pure TS group ($p=0,043$). These results are independent from the co-morbid learning disorder.

2.4. Effects of symptom severity on executive functions

The YGTSS scores were uncorrelated with any of the test measures, which means that tic severity did not affect these

cognitive functions. In ADHD, the severity of hyperactivity/impulsivity positively correlated (p=0,025) with one measure of the WCST (trials to complete first category), which means that faster performance in the beginning of the test is related with higher degree of hyperactive symptoms. However, this better performance is characteristic only at the beginning of the test; they were unable to keep the good performance until the end of the test.

Conclusion

1. Genetic risk factors in TS and ADHD

In the case-control analysis, neither the DRD4-48bp VNTR, nor the COMT Val158Met polymorphisms were associated with TS, confirming previous results in this field. A different picture emerged when we analyzed tic severity of the TS patients. The DAT-40 bp VNTR of the dopamine transporter gene was associated with peak tic severity measured by the Yale Global Tic Severity Scale. Assessing the total tic severity score, subjects with a 9-repeat variant had 25% higher scores than those possessing the long allele only. Most studies showed increased dopamine transporter density in the striatum of TS patients compared to controls, but studies are generally inconclusive regarding the role of this allele. However, based on the results of the largest SPECT study (n = 96), where the 9-repeat allele was linked to higher dopamine transporter density, we may speculate that this allele is a risk factor for Tourette's syndrome and/or for tic severity. Still, we need to be cautious in interpreting our result of the association between the DAT- 40 bp VNTR and tic severity until independent replication.

Our results show a positive association between ADHD and the high activity Val-allele of the COMT gene. This finding raises the possibility that the COMT polymorphism might be important in the development of certain ADHD-symptoms connected with prefrontal cortex (PFC) functions.

The COMT Val158Met polymorphism has been widely studied in relation to cognitive functions: those with the Val/Val genotype perform worse in working memory tasks, but after amphetamine administration, the efficiency of PFC-functions increases in this group. Our results confirm this hypothesis, since those children with at least one Val-allele benefited from MPH-treatment to a larger extent. This observation is in agreement with the suggested inverted U-shaped correlation

between cognitive function and PFC dopamine level: the high activity Val/Val group having a lower, suboptimal dopamine level, which can be shifted to the optimal range with a dopamine transporter blocking agent. Our result confirm the hypodopaminergic hypothesis of ADHD, since the COMT Val-allele, which is connected to lower dopamine level in the PFC has been associated with ADHD.

2. Neuropsychological features in TS and ADHD

The high prevalence of learning disorder in ADHD suggests different neurobiological background between the two syndromes regarding complex, mainly dominant hemispheric dysfunctions (e.g. phonological and visual-perceptual organization) in ADHD. Considering cognitive inhibition, there is also a difference between the disorders, since the pure TS and the ADHD+TS groups were worse than controls in Stroop inhibition; an indication of the ability to suppress irrelevant stimuli. On this measure, performance of the pure ADHD group was as good as that of controls. Neuro-imaging studies have shown that brain areas that are active - while subjects perform Stroop-like tasks -, include the anterior cingulate cortex of the medial-ventral PFC. Our study measured only cognitive inhibition, however, other aspects of inhibition (e.g. behavioral) could be also related to the phenomenology of TS or ADHD.

Regarding short term and working memory functions, there is a definite difference between TS and ADHD, since the performance of the ADHD group was not only worse than controls, but also worse than the pure TS group. These results confirm the hypotheses that working memory problem is a core neuropsychological deficit in ADHD. It is connected to the fact, that there is a catecholamine dysregulation in ADHD, since dopamine and noradrenalin modulate short term and working memory processes in the dorsolateral PFC.

We have not found any evidence regarding working memory problems in TS, though other aspects of memory dysfunction have been published: implicit memory dysfunction which is connected to the ventral pathway of the frontostriatal system has been already identified.

Cognitive flexibility is intact in TS and we could detect problems in ADHD only when there was co-existing learning disorder. This result is important, because most studies report cognitive inflexibility in ADHD, but very few are controlled for learning disorders.

Verbal working memory task demanding executive processing produce more dorsolateral prefrontal activation in the PFC, which is consistent with the findings of the role of these areas in ADHD. Dopamine activity in these dorsolateral prefrontal connections allow maintenance of working memory required for goal completion, which can be facilitated by administration of methylphenidate to children with ADHD. However, the heterogenity of the syndrome (high prevalence of learning disorder, inconsistent performance even within the same test, etc.) suggests dysfunctions in the reward and motivational systems and other aspects of inhibition as well, besides working memory impairments.

In our neuropsychological study, we found mainly differences between these two disorders: problems in working memory are connected with ADHD, while disinhibition of suppressing irrelevant stimuli is more characteristic of TS. The role of the dorsolateral prefrontal cortex might be more pronounced in ADHD, while the dysfunction of the medial-ventral PFC seems to be more expressed in TS. Although there is not a significant deficit in pure TS, we could assume that co-morbid conditions (ADHD, learning disorder) work as risk factors regarding executive dysfunction.

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