Neural correlates of gene-environment interactions in ADHD
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Chapter 9

Key findings
Key findings

This thesis documents my doctoral research, aimed at advancing our understanding how genetic and environmental factors shape the ADHD phenotype. To this end, we analysed whether common polymorphisms interact with stressors in relation to ADHD severity, and how brain structure and activity may mediate these effects. Table 1 lists the factors analysed, together with the significant results. Below, I recap the findings followed by some speculative statements, per chapter. These are subsequently combined into my general thoughts on the relation between genetic and environmental risk factors, the brain, and ADHD.

Findings per chapter

Chapter 2: We found no evidence supporting previous claims that the ADHD candidate genes \textit{DRD4} and \textit{DAT1} moderate the impact of prenatal exposure to alcohol or smoking on the presence of ADHD symptoms. However, the polymorphisms and prenatal stressors were all individually associated with differences in brain activity during the performance of a response inhibition task. \textit{DRD4-7}R carriers and those exposed to smoking \textit{in utero} had lower superior frontal brain activity during successful inhibition. During failed attempts, 7R carriers showed higher activity than non-carriers in the frontal pole and occipital cortex, whereas exposure to smoking was associated with higher activity in the parietal cortex. \textit{DAT1} homozygotes had lower cerebellar activity, and prenatal exposure to alcohol was associated with higher signal in the orbitofrontal cortex.

- Neuroimaging data is able to capture effects of genetic variation and environmental stressors on neurobiology that are too small to be detected by behavioural measures.
- Brain measures can inform us how a risk factor may contribute to ADHD, based on its association with specific brain regions.

Chapter 3: We replicated a GxE between \textit{5-HTTLPR} and stress, the number of long-term difficulties and stressful live events experienced in the past five years. S-allele carriers had a strong relation between the amount of stress exposure and ADHD symptom count, whereas L-allele homozygotes did not. This effect was independent of symptom count measured five years earlier and was not mediated by internalizing problems.

- A genetic variant can moderate the impact of an environmental stressor on ADHD.
- Interaction effects can contribute to the inconsistency of findings on the relation of a risk factor with ADHD.
Key findings

In Chapters 4, 5 and 6, we went beyond analysing if there is a GxE on behaviour, and attempted to shed light on how this takes place, through the use of whole-brain mediation analyses.

Chapter 4: We found that S-allele carriers exposed to stress had less grey matter volume in the precentral gyrus, superior frontal gyrus, anterior cingulate, and frontal pole than L-allele homozygotes. The two latter regions also contained significant mediation effects, i.e., lower brain volume in the anterior cingulate and frontal pole of S-allele carriers in response to stress contributed to their stronger relation between stress and ADHD severity.

- Including genetic, environmental, and neuroimaging data in one analysis provides direct information on how they come together to affect behaviour.
- Their combination provides better clues on the processes involved.

Chapter 5: Given the important role of the anterior cingulate and frontal pole in cognitive control and regulation of emotion, we followed up on the structural MRI findings with a study of resting-state functional connectivity in the executive control and default mode networks. The GxE corresponded to lower connectivity within the executive control network, specifically in the precentral gyrus, striatum, and the frontal pole, while we found higher connectivity in the default mode network, specifically in the supramarginal gyrus. We found no significant mediation effects in either network.

- The effects of common genetic and environmental factors are unlikely to be restricted to one specific brain network.
- It may be the combination of networks involved that explain the relation of a risk factor with ADHD and related behaviour.

Chapter 6: We included the glucocorticoid receptor gene NR3C1 in our analyses of 5-HTTLPR and stress exposure, based on evidence that the HPA axis is involved in ADHD, and the known influence of 5-HTTLPR on cortisol levels. Individuals carrying a haplotype of NR3C1 SNPs referred to as 9β, thought to convey lower signalling downstream of cortisol, showed a stronger positive relation between stress and symptom count than non-carriers. This effect was stratified by 5-HTTLPR genotype; both carriers and non-carriers of the 9β haplotype showed a strong relation between stress and ADHD severity, whereas this relation was only present in L-allele homozygotes with the 9β haplotype and absent in non-carriers.

Investigation into the neural correlates of the novel GxE between NR3C1 and stress revealed that it is associated with lower grey matter volume in the cerebellum and parahippocampal cortex. Further, L-allele homozygotes carrying 9β were the only ones showing a negative relation between stress and grey matter volume in the intracalcarine cortex and angular gyrus.

- Knowledge about the neurobiology of risk factors, and their relation to behaviour, may aid in the identification of additional risk factors involved.
- While two genetic variants may lead to a similar effect on a behavioural measure, the underlying neural pathways may differ.
Chapter 7: Here, we took a wider view on the genetics of ADHD, exploring the role of thousands of SNPs across 29 genes linked to the HPA axis. The distribution of findings was in line with current thinking that ADHD severity results from the joint contribution of numerous SNPs with small individual effects. The genomic location of the most predictive SNP indicated that telomere length might deserve more attention in GxE research of ADHD. Other high-ranking SNPs were found in or near genes inconsistently coupled to ADHD and comorbid internalizing as well as externalizing disorders.

- Statistical approaches able to handle high-dimensional genetic data may complement conventional candidate gene approaches.
- Inconsistent findings on the effect of genetic variants may reflect the fact that these are relevant only for a more homogenous subgroup, and/or are dependent on other genetic and environmental factors.

Chapter 8: The relation between ADHD severity and brain activity while performing a working memory task appears to be dependent on an individual’s trait anxiety. Specifically, higher anxiety was associated with a more negative relation between ADHD severity and activity in the cerebellum, striatum, and thalamus, whereas those with low anxiety showed a positive relation between ADHD severity and brain activity in these regions.

- Studies into the neural correlates of ADHD may be averaging over individuals that differ widely in brain activity by not taking into account co-occurring internalizing symptoms.
- Co-occurrence with other disorders may have a neurobiological basis that is not explained by either disorder individually.
Key findings

Relationships between risk factors, neurobiology, and ADHD

The statements from each chapter, when combined and phrased more generally, explain the difficulty of studying the relation of individual risk factors with ADHD.

- Most common risk factors have small effects on the brain. One genetic variant or environmental stressor may affect multiple neural pathways. Two or more factors may amplify or dampen each other’s effects on a specific pathway.
- There is no one-to-one relation between neural pathways and behavioural traits. Several neural pathways may contribute to one trait, one pathway may be involved in several traits, and some pathways may compensate for others that are deficient.
- ADHD encompasses many behavioural traits and deficits, no single one is necessary or sufficient for a diagnosis or relates directly to ADHD severity.

Each of these aspects contributes to the large heterogeneity seen in etiological, neuroimaging, and clinical studies of ADHD. It makes it clear that in no way is there a one-to-one relationship between risk factors and ADHD, and that the large number of possible combinations of genetic and environmental factors ensures that each individual diagnosed with ADHD differs from another on specific impairments, course, co-occurring disorders, and response to treatment. These statements also provide clues to what research strategies may prove most effective.

- Neuroimaging measures more directly capture the effects of risk factors. This often makes them more sensitive and informative measures than those based on behavioural data.
- Interpretation of findings can be strengthened by including interactions between factors involved in the same biological pathway, and by analysing their effects on both neuroimaging and behavioural outcome measures.
- The complexity arising from the involvement of many risk factors together calls for statistical approaches that are able to handle high-dimensional data and that take into account any interactions. These may complement focused, hypothesis-driven studies by generating information on sources of heterogeneity that are masking effects.
### Table 1. Results throughout the research chapters

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Polymorphism (risk group)</th>
<th>Stressor</th>
<th>Outcome measure</th>
<th>Predictor</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>DRD4 exon 3 VNTR (7R carriers) DAT1 3' UTR : intron 8 VNTR haplotype (10-6 homozygotes)</td>
<td>Prenatal exposure to alcohol Prenatal exposure to smoking</td>
<td>ADHD symptom count Response inhibition performance Response inhibition brain activity</td>
<td>Exposure to smoking DRD4 DAT1 Exposure to smoking Alcohol</td>
<td>Reaction time variability Superior frontal, parietal cortex Frontal pole, occipital cortex Cerebellum Superior frontal ➖ Parietal cortex Orbitofrontal cortex</td>
</tr>
<tr>
<td>3</td>
<td>5-HTTLPR VNTR (S-allele carriers)</td>
<td>Long-term difficulties and stressful life events composite</td>
<td>ADHD symptom count</td>
<td>5-HTT x stress</td>
<td>S-allele &gt; L-allele</td>
</tr>
<tr>
<td>4</td>
<td>5-HTTLPR VNTR (S-allele carriers)</td>
<td>Long-term difficulties and stressful life events composite</td>
<td>Grey matter volume*</td>
<td>5-HTT x stress</td>
<td>➖ Precentral, superior frontal, anterior cingulate, frontal pole ➖ Cingulate, frontal pole</td>
</tr>
<tr>
<td>5</td>
<td>5-HTTLPR VNTR (S-allele carriers)</td>
<td>Long-term difficulties and stressful life events composite</td>
<td>Executive control network* Default mode network*</td>
<td>5-HTT x stress 5-HTT x stress</td>
<td>➖ Precentral, striatum, frontal pole Supramarginal gyrus</td>
</tr>
<tr>
<td>6</td>
<td>NR3C1 rs6189 : rs6198 SNP haplotype (G:G aka 9β carriers) 5-HTTLPR VNTR (S-allele carriers)</td>
<td>Long-term difficulties and stressful life events composite</td>
<td>ADHD symptom count Grey matter volume*</td>
<td>NR3C1 x stress 5-HTT x NR3C1 x stress NR3C1 x stress 5-HTT x NR3C1 x stress</td>
<td>9β &gt; others (L-allele &gt; S-allele) &gt; (9β &gt; others) Cerebellum, parahippocampus Intracalcarine, angular gyrus</td>
</tr>
<tr>
<td>7</td>
<td>17374 SNPs across 29 genes</td>
<td>Long-term difficulties and stressful life events items</td>
<td>Conners Parent Rating Scale subscale N</td>
<td>12.52% variance explained</td>
<td></td>
</tr>
</tbody>
</table>

#### Severity of ADHD  Severity of anxiety

| 8 | Conners Parent Rating Scale subscale N | Strengths and Difficulties Questionnaire subscale Emotion | Working memory brain activity ADHD ADHD x anxiety | ➖ Superior frontal, frontal pole Cerebellum, thalamus, striatum |

**Note:** * whole-brain mediation analysis, ➖ indicates the directions of a mediation effect: 1) GxE on the brain, 2) Brain measure on ADHD symptom count.

**Abbreviations:** VNTR = variable number tandem repeats, UTR = untranslated region, SNP = single nucleotide polymorphism, aka = also known as.