UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Genética e neuroimagem no TDAH e fenótipos relacionados

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Tese submetida ao Programa de Pós-Graduação em Genética e Biologia Molecular da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do Grau de Doutor em Genética e Biologia Molecular.

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Porto Alegre

Dezembro 2019

Instituições e Fontes Financiadoras

Este trabalho foi desenvolvido principalmente no Laboratório de Genética Humana Molecular do Departamento de Genética do Instituto de Biociências da Universidade Federal do Rio Grande do Sul (UFRGS). A aluna recebeu bolsa de estudos concedida pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Para a realização desse trabalho, o financiamento do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - CNPq/MS/SCTIE/DECIT nº 26/2014) também foi de suma importância e possibilitou a aquisição de imagens cerebrais dos pacientes.

Parte dessa Tese foi desenvolvida no *Radboud Medical Center (Radboudumc)* e *Donders Institute for Brain and Cognition*, em Nijmegen, na Holanda (fomentado pelo Programa de Doutorado Sanduíche CAPES PDSE Processo nº 27/2017 – 6 meses, e pela *International Brain Research Organization* (IBRO) através do programa *IBRO LARC Exchange Fellowships* – 1 mês).

Esse trabalho também contou com a colaboração do laboratório de neurociência cognitiva do desenvolvimento da Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS).

Agradecimentos

Tenho muito a agradecer a todos que de alguma forma fizeram parte desse crescimento comigo. Primeiramente gostaria de agradecer ao meu orientador, **Claiton**, pela ajuda, disponibilidade e confiança que sempre demonstrou em mim. Foi uma jornada que começou ainda na graduação e que só tenho a agradecer.

Aos colegas e amigos do laboratório (nossa "matilha"). À **Nina** que faz parte de muito que eu sou hoje, amiga e inspiração para mim. À **Jaque** e à **Djeni**, outra companheira de choros e risos nessa jornada, por toda a ajuda no trabalho e na vida. À **Bruna,** parceira de trabalho, de churrasco e cerveja. Ao **Diego**, comigo desde o início, ainda na graduação, mais uma inspiração a ser seguida. À **Duda**, essencial na execução desse projeto, sempre pronta pra ajudar. À **Diana**, sempre disposta a ajudar, pelos diversos e-mails que me mandou com arquivos ou anotações esquecidas. À **Cibele**, que participou do início ao fim desse trabalho comigo, me apoiando e ajudando sempre, não tenho palavras para agradecer! Aos mais recentes integrantes, **Pamela**, **Robson** e **Eduarda**.

A todos os membros do **PRODAH**, na coleta de dados, discussões dos dados, que propiciam esse trabalho. Em especial ao **Eugenio** e ao **Vitola**, com discussões e análises incessáveis e fundamental contribuição.

À minha coorientadora, **Barbara**, e todos os que me acolheram e ajudaram durante minha estada na Holanda. Em especial à **Emma,** por todo o apoio e ensinamentos. Aos amigos que essa experiência me proporcionou, **Louise, Karine, Rafael, Zsofie.**

Ao **PPGBM** e todas instituições que possibilitaram esse trabalho.

Porque mesmo o trabalho exige momentos de descontração, aos "**polônicos**", que me acolheram no grupo e tornaram esses anos mais leves, com muita dança e risadas. Dziękuję Ci!

À **família** que sempre me apoiou de todas as formas possíveis. As meus pais, **Kátia** e **Chê,** que propiciaram que eu chegasse até aqui, me apoiando e incentivando, entendendo minhas ausências e meus desesperos e celebrando comigo todas conquistas. Aos meus irmãos, **Júlia** e **Pedro,** que mesmo que não sempre próximos sei que posso contar.

Aos participantes que fizeram esse trabalho possível doando um pouco do seu tempo e paciência.

A todos, o meu MUITO OBRIGADA, foi uma caminhada longa na qual vocês foram essenciais! Vocês são incríveis!

"Se eu vi mais longe,
Foi por estar no ombro de gigantes"
Isaac Newton

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Lista de Abreviaturas

AD – *Axial diffusivity* (difusividade axial)

ATR - *Anterior Thalamic Radiation*

BAIAP2 - *Brain-specific angionesis inhibitos 1-associated protein 2*

BDNF - *Brain-derived neurotrophic factor*

BOLD - *Blood-Oxygen-Level-Dependent*

COMT – Catecol O-metiltransferase

CHARGE – *Cohorts of Heart and Aging Research in Genomic Epidemiology*

CPF – Córtex Pré-Frontal

CING - *Dorsal cingulate gyrus*

CST - *Corticospinal tract*

DMN – *Default Mode Network*

DRD4 – Receptor de dopamina D4

DRD5 - Receptor de dopamina D5

DSM –*Diagnostic & Statistical Manual of Mental Disorders* (Manual Diagnóstico e Estatístico de Transtornos Mentais)

DTI– *Diffusion Tensor Imaging* (Imagem por tensor de difusão)

DUSP6 - *Dual specificity phosphatase 6*

ENIGMA - *Enhancing Neuro Imaging Genetics through Meta-Analysis*

FA – *Fractional Anisotropy* (Anisotropia fracionada)

FOXP2 - *Forkhead box P2*

FOV – Field of View

FSL - FMRIB *Software Library*

GRAPPA - *GeneRalized Autocalibrating Partial Parallel Acquisition*

GWAS - *Genome-wide association studies* (Estudo de associação por varredura genômica)

HTR1B – Receptor de serotonina 1B

HIPPCING - *Ventral cingulate gyrus*

ICV – I*ntracranial volume* (volume total intracraniano)

IFOF - *Inferior longitudinal fasciculus*

ILF - *Inferior longitudinal fasciculus*

IMpACT - *International Multi-centre persistent ADHD CollaboraTion*

JHU - John Hopkins University

LINC00461 - *Long intergenic non-protein coding RNA 461*)

MD –*Medial diffusivity* (Difusividade média)

MEF2C - *Myocyte enhancer factor 2C*

MPRAGE - *Magnetization Prepared Rapid Acquisition Gradient Recalled Echo*

NIMH - *National Institute of Mental Health*

PRODAH-A - divisão de adultos do Programa de Déficit de Atenção-Hiperatividade

PRS – *Poligenic Risk Score* (Escore de risco poligênico)

PUCRS - Pontifícia Universidade Católica do Rio Grande do Sul

RD – *Radial diffusivity* (Difusividade radial)

RDoc - *Research Domain Criteria Iniciative*

Ricopili - *Rapid Imputation and COmputational PIpeLIne*

RMN – Ressonância Magnética Nuclear

ROI – *Region of interest* (Região de interesse)

SEM6AD - *Semaphorin 6D*

SLC6A3 - Solute Carrier Family 6 Member 3 / Transportador de dopamina

SLC6A4/5HTT – Solute Carrier Family 6 Member 4 / Transportador de serotonina

SLF - *Superior longitudinal fasciculus*

SLFTEMP - *Temporal part of superior longitudinal fasciculus*

SNAP25 - *Synaptossomal-associated protein 25*

SNP - *Single Nucleotide Polymorphism* (Polimorfismo de nucleotídeo único)

ST3GAL3 - ST3 beta-galactoside alpha-2,3-sia

TBSS – *Trac-Based Spatial Statistics*

TDAH - Transtorno de Déficit de Atenção/Hiperatividade

TDM - Transtorno Depressivo Maior

TFCE - *Threshold free cluster enhancement*

TUS – Transtorno por Uso de Substâncias

UF - *Uncinate fasciculus*

VNTR - *Variable Number Tandem Repeats* (variante de repetição em tandem)

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Resumo

O Transtorno de Déficit de Atenção/Hiperatividade (TDAH) é altamente prevalente e leva a prejuízos em diversos domínios. Compreender mais sobre sua etiologia pode romper estigmas que o acompanham e fornecer novas perspectivas para o tratamento. Avanços genéticos recentes têm contribuído substancialmente para isso, embora a maioria dos fatores genéticos envolvidos permaneça desconhecida. Endofenótipos, como aspectos de neuroimagem, constituem em uma estratégia promissora na compreensão da fisiopatologia e da arquitetura genética de transtornos psiquiátricos. Nesse sentido, a presente Tese busca compreender aspectos de neuroimagem e genética do TDAH na vida adulta através de métodos estruturais de ressonância magnética utilizando desde métodos clássicos, abordagens *data-driven* e técnicas de difusão (conectividade estrutural). Além disso, a Tese explora variantes genéticas envolvidas no TDAH e outros fenótipos relacionados por meio de abordagens *single-SNP* e *gene-wide* além de escores de risco poligênico. A Tese inclui ainda dados clínicos de estudo de seguimento em TDAH em adultos, procurando relacionar diferentes desfechos a bases biológicas. O conjunto geral de resultados contribui na compreensão da neurobiologia do TDAH, demonstrando, por exemplo, associações entre regiões fronto-estriatais e tratos de substância branca e o TDAH em duas amostras independentes. Além disso, utilizando abordagens mais clássicas, estendemos para adultos associações já robustamente demonstradas em crianças entre o TDAH e volumes subcorticais e áreas corticais. A presente Tese também mostra em uma abordagem *gene-wide* a associação de uma variante do gene da Synaptotagmina com a integridade estrutural da substância branca, além da associação de uma variante específica com o Transtorno por Uso de Crack. Dada a alta complexidade do fenótipo TDAH, a presente Tese é parte do início dos esforços científicos no sentido de uma avaliação integrada das bases genéticas e neurobiológicas do transtorno.

Abstract

The Attention-Deficit Hyperactivity Disorder (ADHD) is highly prevalent and leads to impairment in several domains. The understanding about its etiology can break stigmas and provide new perspectives for treatment. Recent genetic advances have contributed substantially to this, although most of the genetic factors involved remain unknown. Endophenotypes, as neuroimaging features, constitute a promising approach for understanding the pathophysiology and genetic architecture of psychiatric disorders. In this sense, the present Thesis sough to understand neuroimaging and genetics aspects of ADHD in adulthood through structural magnetic resonance imaging, using classical methods, data-driven approaches and diffusion techniques (structural connectivity). In addition, the Thesis explores genetic variants involved in ADHD and other related phenotypes through single-SNP and gene-wide approaches, as well as polygenic risk scores. This Thesis also include clinical follow-up data of adults with ADHD, aiming to investigate biological basis underlying different clinical outcomes. The overall results contributes to the understanding of ADHD neurobiology, demonstrating, for instance, associations between fronto-striatal regions and subjacent white matter tracts and ADHD in two independent samples. Also, using a more classical approach, we have extended into adults previously robustly associations between ADHD and subcortical volumes and cortical areas in children. The present Thesis also shows in a gene-wide approach the association of Synaptotagmin gene variants with structural integrity of the white matter, as well as the association of a specific variant with Crack Use Disorder. Given the high complexity of the ADHD phenotype, this Thesis is part of the beginning of scientific efforts towards an integrated assessment of the genetic and neurobiological basis of the disorder.

Capítulo I

Introdução Geral

1. Transtorno de Déficit de Atenção/Hiperatividade (TDAH)

O Transtorno de Déficit de Atenção/Hiperatividade (TDAH) é um dos transtornos psiquiátricos mais prevalentes no mundo todo (aproximadamente 5% das crianças e 2,5%-4.4% dos adultos - Biederman, 2003; Kessler et al., 2006; Polanczyk et al., 2007; Simon et al., 2009), sendo caracterizado por sintomas de desatenção e/ou hiperatividade. O diagnóstico, de acordo com o Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM, do inglês *Diagnostic and Statistical Manual of Mental Disorders),* exige a presença de ao menos seis (para crianças e adolescentes) ou cinco (para adultos) sintomas **(Tabela 1)** em pelo menos um dos domínios (desatenção ou hiperatividade/impulsividade). Estes, devem ser frequentes nos últimos seis meses e acarretar prejuízo em pelo menos dois contextos da vida (e.g. situações de lazer, familiares, trabalho) (American Psychiatric Association 2013). Indivíduos com TDAH podem apresentar uma grande variabilidade nos sintomas e na gravidade desses. A apresentação clínica pode variar de acordo com os sintomas experienciados pelo indivíduo: predominantemente desatenta, predominantemente hiperativa/impulsiva ou combinada (American Psychiatric Association 2013).

Indivíduos com TDAH frequentemente apresentam prejuízos em múltiplos domínios, principalmente de funções cognitivas e executivas, como vigilância, atenção sustentada, planejamento e memória de trabalho (Willcutt et al. 2005; Mostert et al. 2015). Muitos desses prejuízos associados ao TDAH podem ser observados mesmo em populações subclínicas (i.e., que apresentam sintomas de TDAH, mas não o diagnóstico em si) (Brown and Casey, 2016; Groen et al., 2018). Nas palavras de Thomas Brown "a função executiva pode ser comparada a sinfonia de uma orquestra composta com bons músicos, mas sem um condutor

Tabela 1. Sintomas do TDAH de acordo com a quinta versão do DSM (DSM-5).

1. Desatenção
a. Frequentemente não presta atenção em detalhes ou comete erros por descuido em tarefas escolares, no trabalho ou durante outras atividades (e.g. negligencia ou deixa passar detalhes, o trabalho é impreciso).
b. Frequentemente tem dificuldade de manter a atenção em tarefas ou atividades lúdicas (e.g. dificuldade de manter o foco durante aulas, conversas ou leituras prolongadas).
c. Frequentemente parece não escutar quando alguém lhe dirige a palavra diretamente (e.g. parece estar com a cabeça longe, mesmo na ausência de qualquer distração óbvia).
d. Frequentemente não segue instruções até o fim e não consegue terminar trabalhos escolares, tarefas ou deveres no local de trabalho (e.g. começa as tarefas, mas rapidamente perde o foco e facilmente perde o rumo).
e. Frequentemente tem dificuldade para organizar tarefas e atividades (e.g. dificuldade em gerenciar tarefas sequenciais; dificuldade em manter materiais e objetos pessoais em ordem; trabalho desorganizado e desleixado; mau gerenciamento do tempo; dificuldade em cumprir prazos).
f. Frequentemente evita, não gosto ou reluta em se envolver em tarefas que exijam esforço mental prolongado (e.g. trabalhos escolares ou lições de casa, preparo de relatórios, preenchimento de formulários, revisão de trabalhos longos).
g. Frequentemente perde coisas necessárias para tarefas ou atividades (e.g. materiais escolares, lápis, livros, instrumentos, carteiras, chaves, documentos, óculos, celular).
h. Com frequência é facilmente distraído por estímulos externos ou pensamentos não relacionados.
i. Com frequência é esquecido em relação a atividades cotidianas (e.g. realizar tarefas, obrigações, retornar ligações, pagar contas, manter horários agendados).
2. Hiperatividade e Impulsividade
a. Frequentemente remexe ou batuca as mãos ou os pés ou se contorce na cadeira.
b. Frequentemente se levanta da cadeira em situações em que se espera que permaneça sentado (e.g. sai do seu lugar em sala de aula, no escritório ou em outro local de trabalho ou em outras situações que exijam que se permaneça em um mesmo lugar).
c. Frequentemente corre ou sobe nas coisas em situações em que isso é inapropriado (ou, em adolescentes ou adultos, sensações de inquietude).
d. Com frequência é incapaz de brincar ou se envolver em atividade de lazer calmamente.
e. Com frequência "não para", agindo como se estivesse "com o motor ligado" (e.g. não consegue ou se sente desconfortável em ficar parado por muito tempo, como em restaurantes, reuniões; outros podem ver o indivíduo como inquieto ou difícil de acompanhar).
f. Frequentemente fala demais.
g. Frequentemente deixa escapar uma resposta antes que a pergunta tenha sido concluída (e.g. termina frases dos outros, não consegue aguardar a vez de falar).
h. Frequentemente tem dificuldade para esperar a sua vez (e.g. aguardar em uma fila).
i. Frequentemente interrompe ou se intromete (e.g. mete-se nas conversas, jogos ou atividades; pode começar a usar as coisas de outras pessoas sem pedir ou receber permissão; intrometer-se em ou assumir o controle sobre o que outros estão fazendo).

para organizá-los e integrá-los. O problema com o TDAH não está em partes individuais do cérebro que corresponderiam, *per se,* aos músicos individualmente, mas no sistema que controla e integra as atividades momento a momento" (traduzido de Brown, 2002).

A memória de trabalho permite o armazenamento temporário, manutenção e manipulação de informações sensoriais e cognitivas, constituindo, dessa forma, em uma interface entre percepção, atenção, memória e ação (Osaka et al. 2012). O prejuízo na memória de trabalho tem sido relacionado ao TDAH, especialmente em relação a desatenção (Martinussen et al. 2005; Willcutt et al. 2005; Lui and Tannock 2007; Rapport et al. 2008), visto que diferenças na memória de trabalho parecem estar relacionadas a capacidade de focar em informações pertinentes (Conway et al. 2001; Kane et al. 2001). Pior desempenho em tarefas que exigem atenção sustentada também são frequentemente associados ao TDAH (Huang-Pollock et al. 2012).

O TDAH também está relacionado ao prejuízo em respostas de reforço, referidos como *delay aversion* (i.e. escolha de recompensas menores e recebidas mais rapidamente, ao invés de recompensas maiores, porém que demorariam mais tempo a ser obtidas) (Solanto et al. 2001; Sonuga-Barke 2003; Bessette and Stevens 2019). Modelos propõem que no intuito de evitar a espera, o indivíduo pode tornar-se mais ativo, para que a percepção de tempo se altere, ou buscar recompensas mais imediatas (impulsividade) (Sonuga-Barke 2003). Assim *delay aversion* estaria principalmente relacionado a hiperatividade e impulsividade. Traços de impulsividade no TDAH também podem ser relacionados a déficits no controle inibitório (Nigg 2001; Desman et al. 2008; Bessette and Stevens 2019).

Tendo em vista os diferentes domínios implicados no TDAH, modelos de prejuízo cognitivo sugerem o transtorno como um construto maior, capaz de englobar múltiplos perfis cognitivos independentes (Castellanos and Tannock 2002; Nigg et al. 2005; Sonuga-Barke 2005). A ampla gama de sintomas e os

diversos domínios cognitivos envolvidos no TDAH ilustram a grande heterogeneidade desse transtorno (Luo et al. 2019).

Além disso, a heterogeneidade do TDAH é marcada pelo grande número de comorbidades, como transtornos de aprendizagem e linguagem, transtornos de humor e ansiedade, e transtornos disruptivos, do controle de impulsos e da conduta (Sobanski 2006; Fatseas et al. 2012). Aproximadamente 60% das crianças e 80% dos adultos com TDAH apresentam alguma comorbidade (Jensen et al. 1997; Gillberg et al. 2004; Sobanski 2006). Essa grande heterogeneidade clínica dificulta no diagnóstico, tratamento e compreensão da etiologia do TDAH (Barkley and Brown 2008; Mao and Findling 2014).

Indivíduos com TDAH possuem mais chances de desenvolver problemas por abuso ou dependência de substâncias, como álcool, nicotina, maconha, cocaína ou outras (Molina and Pelham 2003; Saules et al. 2003; Ohlmeier et al. 2007; Charach et al. 2011; Lee et al. 2011; Ilbegi et al. 2018). Buscando compreender essa relação, alguns estudos sugerem por exemplo que o uso de álcool, drogas e nicotina poderia ser iniciado tanto como uma forma de automedicação (Ohlmeier et al. 2007) ou como resultado da desinibição comportamental que acompanha a hiperatividade e impulsividade (Molina and Pelham 2014; Rooney et al. 2015).

Efeitos do TDAH também se estendem a problemas na condução de veículos, com um maior número de acidentes com automóveis e autuações em indivíduos afetados (Barkley et al. 2002; Amiri et al. 2011); bem como a uma maior morbidade, refletido em visitas hospitalares e internações mais frequentes do que em controles (Dalsgaard et al. 2015), possivelmente decorrentes de um comportamento de risco (*risk taking behavior).* Além disso, comparados a população geral, adultos com TDAH apresentam maiores dificuldades interpessoais e problemas legais (Rasmussen et al. 2001; Barkley et al. 2004; Biederman et al. 2006; Flory et al. 2006; Hoza 2007; Ginsberg et al. 2010; Garcia et al. 2012; Hosain et al. 2012; Klein et al. 2012; Gudjonsson et al. 2013; Ros and Graziano 2018; Owens and Hinshaw 2019), com mais chances de ser preso, de

comportamentos sexuais de risco (não utilização de métodos contraceptivos, mais gestações indesejadas, maior prevalência de doenças sexualmente transmissíveis), de se divorciar, além de possuírem menos amigos.

Reconhecer e tratar o TDAH é fundamental, visto que o transtorno está associado a prejuízos em diversos âmbitos da vida do indivíduo, afetando não só a sua própria qualidade de vida, mas a família e a sociedade como um todo (Swensen et al. 2003; Wilens and Dodson 2004; Barkley et al. 2006; Able et al. 2007; Watters et al. 2018; Thorell et al. 2019). Dificuldades acadêmicas são comumente observadas em indivíduos com TDAH desde a pré-escola ao ensino médio e faculdade, como por exemplo habilidades básicas de leitura, notas mais baixas, maior número de repetências e suspensões/expulsões, necessidade de aulas extras, abandono da escola, menores chances de se formar na faculdade (Wilens and Dodson 2004; Barkley et al. 2006; Biederman et al. 2006; Loe and Feldman 2007; DuPaul et al. 2009; Daley and Birchwood 2010). Quando adultos, encontram problemas para administrar o tempo e se organizar, resultando muitas vezes em dificuldades para conseguir emprego e atingir uma estabilidade financeira (Nadeau 2005; Biederman and Faraone 2006; Das et al. 2012; Kotsopoulos et al. 2013). Quando o TDAH é reconhecido e adequadamente tratado, problemas relacionados ao baixo desempenho acadêmico tendem a diminuir (Daley and Birchwood 2010; Molina and Pelham 2014).

1.1 TDAH ao longo da vida

Apesar de relatos anteriores de características conhecidas hoje como TDAH, o transtorno em si foi descrito primeiramente como um diagnóstico clínico nos anos 1930s sob o nome de “doença hipercinética da infância” (Lange et al. 2010) e incorporado ao DSM em 1968 (American Psychiatric Association 1968), na sua segunda edição sob o nome de “Reação Hipercinética da Infância”. Nessa edição, o transtorno era caracterizado por hiperatividade, inquietação, distração e

falta de atenção, e enfatizava que "o comportamento geralmente diminui na adolescência" (American Psychiatric Association 1968). Essa visão do TDAH como um transtorno exclusivo da infância permaneceu por muito tempo (Lange et al. 2010), até que se mostrasse convincentemente que os sintomas poderiam persistir em adultos (Wood et al. 1976). Atualmente é estimado que entre 15 a 65% das crianças continue a ter TDAH na vida adulta (Faraone et al. 2006c). No entanto, o transtorno em adultos só foi oficialmente incluído no DSM na sua última versão (DSM-5), publicada em 2013 (American Psychiatric Association 2013). A maioria dos estudos na área é baseado em crianças e adolescentes, com um aumento de conhecimento a respeito das diferenças em adultos principalmente nos últimos anos (Ramos-Quiroga et al. 2014). O DSM-5 precisou sofrer algumas adaptações decorrentes de observações clínicas e epidemiológicas de aspectos que diferiam no TDAH em adultos, já que inicialmente havia sido baseado em estudos em crianças. O TDAH na vida adulta parece ter um perfil clínico ainda mais heterogêneo, incluindo um amplo espectro de desregulações emocionais e prejuízos funcionais, não observado em amostras pediátricas (Katzman et al. 2017).

A quinta versão do DSM também trouxe outras mudanças em relação ao diagnóstico de TDAH. A versão anterior do Manual exigia um início de sintomas antes dos sete anos de idade (critério B – implementado a partir do DSM-III), o qual foi muito questionado (Barkley and Biederman 1997; Applegate et al. 1997; Brown 2002; McGough and Barkley 2004; Barkley and Brown 2008; Todd et al. 2008; Kieling et al. 2010). Estudos mostraram que adultos que preenchiam todos outros critérios diagnósticos, a exceção desse, não diferiam clinicamente de forma significativa daqueles que relataram o início dos sintomas anterior aos sete anos (Faraone et al. 2006a; Faraone et al. 2006b; Faraone et al. 2009; Karam et al. 2009; Polanczyk et al. 2010; Guimarães-da-Silva et al. 2012; Lin et al. 2015). Assim, na versão atual essa idade foi alterada para 12 anos,

porém esse critério continua sendo debatido (Chandra et al. 2016), principalmente com o argumento de que muitas vezes os sintomas de TDAH só são reconhecidos na adolescência ou início da vida adulta, com o aumento da demanda cognitiva e organizacional (Barkley and Biederman 1997; Brown 2002); ou do possível viés de memória gerado por uma pergunta retrospectiva (Henry et al. 1994; Barkley and Biederman 1997; Todd et al. 2008; Miller et al. 2010). A respeito disso, inclusive o DSM-5 pondera que "As lembranças dos adultos sobre sintomas na infância tendem a não ser confiáveis, sendo benéfico obter informações complementares". Além disso, o TDAH é o único transtorno com início na infância para o qual se exige uma idade máxima de início dos sintomas como critério diagnóstico. Barkley e Biederman em 1997 já questionavam "Em virtude de estudar crianças em idade escolar, a idade de início teria, necessariamente, que ser observada na infância. [...] Se o transtorno tivesse sido estudado primeiro em adultos, como muitos transtornos do humor e ansiedade, provavelmente não teria que ser especificado um critério de idade de início, mesmo que a maioria dos adultos com transtornos de humor e ansiedade possam traçar o aparecimento dos primeiros sintomas na infância ou adolescência." (traduzido de Barkley and Biederman, 1997).

Evidências dos últimos cinco anos sugerem que o TDAH na vida adulta possa não ser necessariamente uma continuação do TDAH na infância já que grande proporção dos adultos com TDAH parecem não ter um histórico do transtorno quando crianças (Moffitt et al. 2015; Agnew-Blais et al. 2016; Caye et al. 2016a). As primeiras evidências de um início de sintomas do TDAH na vida adulta vieram de um estudo longitudinal da Nova Zelândia publicado em 2015 incluindo 1037 indivíduos nascidos entre 1972 e 1973 e acompanhados até os 38 anos de idade (Moffitt et al. 2015). Esse estudo observou, surpreendentemente, que os grupos de crianças e de adultos diagnosticados não se sobrepunham, ou seja, os adultos com TDAH, na sua maioria, não tinham manifestado sintomas na infância. Os autores questionam, então, se o TDAH em adultos seria o mesmo

transtorno que em crianças, com seu aspecto neurodesenvolvimental e início na infância. Muito debatido, observações semelhantes foram feitas em duas coortes independentes: *Environmental-Risk Longitudinal Twin Study* (Reino Unido – 2232 gêmeos acompanhados até os 18 anos) (Agnew-Blais et al. 2016; Agnew-blais et al. 2018), Coorte de Pelotas (Brasil – 5269 indivíduos acompanhados até os 18 anos) (Caye et al. 2016a). Além disso, Chandra et al. (2016) descrevem um grupo de casos de TDAH na vida adulta que apesar de uma sintomatologia mais leve apresenta os mesmos prejuízos funcionais do TDAH persistente da infância (Chandra et al. 2016). Contudo a visão empírica de um TDAH de início tardio (na vida adulta) tem sido contestado por inconsistências metodológicas entre os estudos e com argumentos de que esses casos estariam apenas subdiagnosticados ou sublimiares na infância (ou seja, já existiam sintomas) ou de que os 'sintomas de TDAH' seriam na verdade decorrentes de outras comorbidades ou lesões cerebrais (Sibley et al. 2017; Solanto 2017; Franke et al. 2018). Assim, as trajetórias do TDAH ao longo da vida, e especialmente o início dos sintomas da vida adulta, têm sido muito debatidas por pesquisadores do mundo inteiro (Faraone and Biederman 2016; Caye et al. 2016b; Caye et al. 2017; Sibley et al. 2017; Franke et al. 2018; Solanto 2018).

Estudos sugerem que os sintomas de TDAH tendem a diminuir com a idade, em geral destacando-se a dimensões de hiperatividade e impulsividade (Biederman et al. 2000; Faraone et al. 2006c; Francx et al. 2015), embora Biederman et al., (2010) tenham demonstrado a mesma taxa de redução para desatenção. Nesse estudo, Bierderman et al. sugerem ainda que essa redução dos sintomas após os 21 anos possa estar relacionada a transição de um ambiente educacional para um ocupacional, e que dessa forma indivíduos com TDAH possam escolher carreiras que tenham uma baixa exigência de atenção e assim diminuir a sua percepção dos sintomas. Outra possível explicação para essa redução de sintomas é a insensibilidade de critérios diagnósticos ao

desenvolvimento, considerando que com o amadurecimento os sintomas se tornariam menos perceptíveis (Faraone et al. 2006c). Conforme pode ser visto na **Tabela 1**, a quinta edição do DSM, primeira a incluir oficialmente o TDAH na vida adulta, ao citar os sintomas, exemplifica situações abrangendo adultos.

Através do primeiro estudo de seguimento de indivíduos diagnosticados com TDAH na vida adulta, nosso grupo observou que uma considerável proporção dos pacientes com TDAH não mantinham o diagnóstico sete anos após sua primeira avaliação, independentemente da idade (Karam et al. 2015). Apesar dos sintomas de TDAH terem diminuído na maioria dos casos avaliados, alguns pacientes tiveram um inesperado aumento de sintomas (Karam et al. 2017). Resultados semelhantes foram observados por outro grupo (Edvinsson and Ekselius 2017).

Manifestações dos sintomas de TDAH podem variar ao longo do desenvolvimento. Crianças mais novas apresentam um perfil mais externalizante, com comportamentos hiperativo-impulsivos, enquanto sintomas de desatenção tendem a surgir mais tarde na vida, sendo predominantes (em relação a hiperatividade) no fim da adolescência e idade adulta. Além disso, também se observam diferenças na proporção entre homens e mulheres com TDAH. Geralmente, em crianças e adolescentes aproximadamente 80% dos casos são meninos, enquanto em adultos essa proporção é de aproximadamente 50% (Franke et al. 2018).

1.2 Neurobiologia do TDAH

1.2.1 Aspectos cerebrais do TDAH

Por muito tempo o TDAH foi considerado uma disfunção da circuitaria fronto-estriatal ignorando o possível envolvimento de outros circuitos (Castellanos

and Proal 2012). Avanços tecnológicos possibilitaram o desenvolvimento de modelos da fisiopatologia do TDAH em maior escala, considerando o transtorno como resultado do mal funcionamento de múltiplos sistemas neurais, e não apenas de um único domínio neurofisiológico (Nigg and Casey 2005; Sonuga-Barke 2005; Casey and Durston 2006; Castellanos et al. 2006; Castellanos and Proal 2012). Modelos simples, centrado no mau funcionamento inibitório ("*A simple cognitive dysfunction model"*) propunham uma desregulação no circuito fronto- dorsal-estriatal (Barkley 1997), que liga o córtex pré-frontal (CPF) ao estriato dorsal, especialmente núcleo caudado (Eagle and Robbins 2003). Enquanto modelos alternativos consideram os déficits no sistema de recompensa (*delay aversion)* relacionados como reflexos de alterações em processos motivacionais (*motivation-based dysfunction models*) (Sagvolden et al. 1998), envolvendo o circuito fronto-ventral-estriatal, o qual conecta o estriato ventral, particularmente o núcleo accumbens, com regiões frontais, especialmente cíngulo anterior e córtex orbitofrontal. O TDAH envolve alterações nos dois domínios (função executiva/controle inibitório e *delay aversion*) e ambos possuem elementos comuns, de forma que não são exclusivos e parecem apontar, pelo menos em parte, para algumas das vias envolvidas na etiologia do transtorno (Sonuga-Barke 2005). Apesar disso, esses modelos são limitados, no sentido de não 'explicar' todo o conjunto de prejuízos envolvidos no TDAH. Sonuga-Barke (2005) destaca a importância de modelos envolvendo múltiplas vias, combinando elementos cognitivos e motivacionais (Sonuga-Barke 2005).

Os estudos de ressonância magnética nuclear (RMN) permitem avaliar, de maneira não invasiva, aspectos cerebrais. A RMN apresenta diferentes modalidades, as quais permitem estimar a anatomia e função. A RMN estrutural fornece medidas anatômicas/morfológicas, em que diferentes tecidos (substância branca e substância cinzenta) e estruturas corticais e subcorticais podem ser mapeadas, e aspectos da estrutura cerebral podem ser quantificados e

comparados. Estudos utilizando RMN estrutural tem focado em regiões de interesse (ROI, do inglês *Region Of Interest)* específicas definidas *a priori* e segmentadas automaticamente, ou explorado alterações no volume e medidas corticais através de métodos univariados contemplando todo o cérebro (e.g. *voxel-based analysis*).

A conectividade estrutural pode ser avaliada utilizando métodos baseados em difusão como o DTI (do inglês, *Diffusion-Tensor Imaging)*, que permite traçar as conexões físicas entre diferentes regiões do cérebro com base em propriedade de difusão da água (Beaulieu 2002). O DTI fornece medidas da direcionalidade da difusão da água ao longo das membranas neuronais, permitindo identificar tratos de substância branca no cérebro. Dessa forma, é possível se inferir sobre a integridade e orientação desses tratos. Dentre as medidas obtidas nessa técnica, a anisotropia fracionada (FA, do inglês *Fractional Anisotropy)* é uma das principais medidas utilizadas, com variação de zero a um. Anisotropia indica que a difusão ocorre de maneira direcional (oposto a isotropia que indica a difusão em todas as direções). Dessa forma, um alto FA indica uma maior direcionalidade dentro de determinado voxel. Variações no FA estão associadas a diferentes fatores, incluindo mielinização, densidade axonal, organização das fibras e integridade (Albajara Sáenz et al. 2019). Além do FA, através da técnica de DTI pode se obter valores de difusividade média (MD, do inglês *Mean Diffusivity*), difusividade radial (RD, do inglês *Radial Diffusivity*) e difusividade axial (AD, do inglês *Axial Diffusivity*). Estudos de DTI podem utilizar análises envolvendo uma seleção manual ou automatizada de ROI ou análises baseadas no cérebro inteiro (*whole-brain)*, como o TBSS (do inglês, *Tract-Based Spatial Statistics).*

A função cerebral pode ser avaliada por RMN funcional, através de imagens sensíveis a alterações locais nos níveis de oxigênio no sangue (BOLD, do inglês *Blood-Oxygen-Level-Dependent)* na substância cinzenta decorrentes da

ativação cerebral. A RMN funcional pode ser realizada com tarefa (isto é, nas quais o indivíduo executa alguma tarefa ou está recebendo algum estímulo sensorial) ou sem tarefa/repouso (*resting state*). A primeira avalia regiões ativadas em determinada tarefa (*task-positive networks)*, e a segunda, a conectividade funcional, identificando sincronizações temporais espontâneas entre diferentes regiões do cérebro no estado de repouso, como o *Default-Mode-Network* (DMN). Modelos neurocognitivos do TDAH têm focado em padrões de conectividade alterados entre redes funcionais cerebrais. A hipótese do DMN (Sonuga-Barke and Castellanos 2007) postula que flutuações e variabilidades cognitivas e atencionais relacionadas ao TDAH resultem de uma regulação inadequada do DMN pelo *task-positive networks,* aumentando pensamentos espontâneos não-relacionados ao processo cognitivo em questão.

Apesar de estudos de neuroimagem já terem identificado diversas variações estruturais e funcionais no cérebro associadas ao TDAH, a sua neurobiologia ainda não é totalmente compreendida, com muitos achados inconclusivos ou resultados contraditórios entre estudos. Os achados mais concretos dizem respeito à diminuição no volume total intracraniano (ICV, do inglês *intracranial volume*) em indivíduos com TDAH (Valera et al. 2007; Nakao et al. 2011; Greven et al. 2015; Hoogman et al. 2017; Albajara Sáenz et al. 2019) e a volumes subcorticais, especialmente os núcleos da base (estriado – caudado e putâmen, globo pálido e substância negra) (Valera et al. 2007; Ellison-Wright et al. 2008; Nakao et al. 2011; Frodl and Skokauskas 2012; Norman et al. 2016; Hoogman et al. 2017; Albajara Sáenz et al. 2019). Hoogman et al. (2017), na maior mega-análise de TDAH e volumes subcorticais publicada até o momento (1713 casos com TDAH e 1529 controles), encontraram volumes significativamente menores do accumbens, amigdala, caudado, hipocampo e putâmen no grupo com TDAH (Hoogman et al. 2017). Nesse estudo, análises estratificadas mostraram que esse efeito era mais forte em crianças, e

não significativo quando só adultos (maiores de 22 anos) eram avaliados. No entanto, essa observação pode ser decorrente de um tamanho amostral muito menor de adultos do que crianças.

O volume cortical é definido como o produto da espessura cortical (i.e. a distância entre as fronteiras de substância branca e cinzenta) e área da superfície, ambos relacionados a características de dobramento do córtex cerebral – *gyrification* (Hogstrom et al. 2013). Estudos já demonstraram diferenças na espessura cortical entre indivíduos com e sem TDAH, especialmente nas regiões frontais, pré-frontais, parietais, temporoparietais e occipitais (N. et al. 2007; Shaw et al. 2007; Narr et al. 2009; Almeida et al. 2010; Proal et al. 2011; Silk et al. 2016), entretanto alguns estudos não encontraram essas diferenças (Wolosin et al. 2009; Ambrosino et al. 2017). Diferenças na área de superfície também foram observadas nas mesmas regiões (Wolosin et al. 2009; Shaw et al. 2012; Silk et al. 2016; Ambrosino et al. 2017). Recentemente, Hoogman et al (2019) demostraram, em uma amostra incluindo 2246 casos e 1934 controles, que crianças com TDAH apresentam um redução na área de superfície cortical, especialmente nas regiões frontal, cingulada e temporal; bem como uma redução na espessura cortical no giro fusiforme e polo temporal (Hoogman et al. 2019).

Diferenças na integridade da substância branca sugerem que déficits estruturais no TDAH também incluam a conectividade estrutural. Três meta-análises avaliaram estudos de DTI no TDAH (van Ewijk et al. 2014; Chen et al. 2016; Aoki et al. 2018), os resultados em sua maioria são contraditórios, mostrando FA tanto aumentada quanto diminuída em diversos tratos. Van Ewijk et al (2014) apontam déficits na substância branca relacionados ao TDAH observados principalmente no *cingule bundle*, corona radiata, corpo caloso, fascículo longitudinal inferior, capsula interna, pedúnculo cerebelar, e fascículo longitudinal superior. No entanto, os resultados mesmo nessas regiões são inconsistentes, sendo mais robusta a diminuição da integridade da substância

branca no fascículo longitudinal quando presentes sintomas de desatenção. Estudos utilizando métodos de tractografia, os quais tentam reconstruir tratos a partir de dados de DTI, têm mostrado alterações em conexões pré-frontal-estriatais (Hong et al. 2014) e frontal-accumbens (Cha et al. 2015) em crianças com TDAH.

Devido ao fato de que a maioria dos estudos incluem apenas, ou majoritariamente, crianças, em que há predominância masculina, mulheres/meninas estão frequentemente pouco representadas. Alguns estudos observaram diferenças entre TDAH em homens e mulheres em relação ao volume cerebral (corpo caloso, caudado, córtex cingulado anterior) (Hutchinson et al. 2008; Onnink et al. 2014; Villemonteix et al. 2015) e microestruturas da substância branca (King et al. 2015), sugerindo déficits neurológicos subjacentes ao TDAH distintos entre os sexos. Alguns estudos não encontraram essas diferenças (Greven et al. 2015; Van Rooij et al. 2015; Hoogman et al. 2017).

Diversos estudos longitudinais acompanhando crianças com TDAH até a adolescência ou início da vida adulta têm buscado compreender mecanismos envolvidos na remissão de sintomas. Sudre et al (2018) revisa esses achados e destaca três possíveis modelos relacionados a remissão em infância para a vida adulta: 1) convergência para função e estrutura cerebral normal, na qual no TDAH persistente as anomalias continuam, enquanto na remissão ocorre a 'normalização' - nesse caso o cérebro do indivíduo que remitiu (*remitter*) seria igual, ou próximo, ao dos controles; 2) remissão se deve ao recrutamento de novas regiões cerebrais que ajudam o indivíduo a superar os sintomas do TDAH (compensação/reorganização neural), nesse caso o cérebro de *remitters*, controles e TDAH seria diferente; 3) *fixed anomaly*, no qual as anomalias persistem, independente do curso clínico do TDAH, nesse caso o cérebro de pacientes e *remitters* seria igual, diferente do dos controles. Esses modelos não são incompatíveis entre si e precisam ser avaliados.

1.2.2 Aspectos genéticos do TDAH

A etiologia do TDAH é fortemente influenciada por fatores genéticos, sendo um dos transtornos psiquiátricos com maior herdabilidade estimada (70-80% - (Brikell et al. 2015; Faraone and Larsson 2018). Apesar dessa grande herdabilidade, poucos genes envolvidos no desenvolvimento do transtorno já foram identificados, provavelmente devido à grande heterogeneidade do TDAH e ao seu caráter poligênico, no qual múltiplas variantes genéticas de pequeno efeito contribuem na sua etiologia (Faraone and Larsson 2018). Estudos buscando identificar essas variantes têm utilizado tanto abordagens baseadas em hipóteses, como estudos de gene-candidato, quanto livre de hipóteses, como varreduras genômicas de ligação (*linkage*) e de associação (GWAS). Os estudos de ligação buscam identificar no genoma regiões que co-segregam com o transtorno em famílias. No entanto, essa técnica forneceu poucos resultados na psiquiatria por ser mais adaptada ao mapeamento de traços monogênicos.

Na psiquiatria a abordagem mais eficaz é a dos estudos de associação. Inicialmente foram realizados estudos de gene-candidato, baseados em hipóteses, buscando associar genes que *a priori* seriam plausíveis de ser envolvidos na etiologia do transtorno. Dessa forma, estes estudos focaram primeiramente em genes relacionados à neurotransmissão dopaminérgica e noradrenérgica por serem vinculados a hipóteses etiológicas e de sistemas alvos de fármacos utilizados no tratamento do TDAH. Outros estudos incluíram genes relacionados a outros sistemas de neurotransmissão e ao desenvolvimento, manutenção e plasticidade neuronal. Meta-análises apontam como mais consistentemente associados ao TDAH os genes dos transportadores de serotonina (*5HTT/SLC6A4*) e dopamina (*DAT1/SLC6A3),* receptores de dopamina D4 e D5 (*DRD4* e *DRD5*) e de serotonina 1B (*HTR1B*), o gene codificador de uma proteína envolvida na liberação de neurotransmissores (*SNAP25 – synaptossomal-associated protein 25*), o gene codificador da enzima catecol O-metiltransferase (*COMT*) e do *brain-*

derived neurotrophic factor (*BDNF*) (Gizer et al. 2009; Lee and Song 2018) e, exclusivamente em adultos, um gene envolvido em proliferação, sobrevivência e maturação neuronal (*BAIAP2 - brain-specific angionesis inhibitor 1-associated protein 2*) (Bonvicini et al. 2016). A principal variante associada no gene *DAT1/SLC6A3* é uma variante de repetição em tandem (VNTR, do inglês *Variable Number Tandem Repeats*) em que as variantes mais comuns são 9 e 10 repetições (9R e 10R, respectivamente). Curiosamente, o alelo 10R foi associado ao TDAH em crianças, enquanto o 10R foi associado em adultos (Franke et al. 2010).

Estudos de GWAS "varrem" o genoma inteiro em busca de variantes associadas ao transtorno. A genotipagem em escala genômica é feita através de um chip capaz de identificar centenas, milhares ou até milhões de variantes, na sua maioria polimorfismos de nucleotídeo único (SNP, do inglês *Single Nucleotide Polymorphism*) mas também pequenas inserções/deleções. Em virtude do grande número de variantes avaliadas, a significância estatística a nível genômico é de $p < 5x10^{-8}$. Por muitos anos estudos de GWAS na área de psiquiatria não tiveram sucesso (Sullivan et al. 2012). Através de consórcios mundiais foi possível obter um maior tamanho amostral, o que se mostrou essencial em estudos de GWAS envolvendo fenótipos psiquiátricos (Bergen and Petryshen 2012; Nishino et al. 2018). Só esse ano foi publicado o primeiro GWAS a encontrar *loci* significativamente ($p < 5x10^{-8}$) associados ao TDAH (Demontis et al. 2019), com 12 *loci* associados com TDAH em uma amostra de 20183 casos e 35191 controles. Dentre os genes implicados estão vários genes relacionados previamente a fenótipos psiquiátricos ou com papeis biológicos relevantes, como o *FOXP2* (*Forkhead box P2*), *DUSP6* (*Dual specificity phosphatase 6*), *SEMA6D* (*Semaphorin 6D*), *ST3GAL3* (*ST3 beta-galactoside alpha-2,3-sia*), *LINC00461* (*Long intergenic non-protein coding RNA 461*) e *MEF2C* (*Myocyte enhancer factor 2C*).

1.2.3 Ligando bases genéticas a aspectos cerebrais

Fenótipos intermediários, também chamados de endofenótipos, são características do transtorno (1) que se relacionam mais intimamente com vias neurológicas do que os sintomas clínicos (Doyle et al. 2005) e (2) compartilham fatores genéticos de susceptibilidade com o próprio transtorno (Gottesman and Gould 2003). O uso de endofenótipos é considerado uma estratégia promissora na compreensão da arquitetura genética de fenótipos complexos, como transtornos psiquiátricos (Faraone et al. 2014; Bogdan et al. 2017). Klein et al (2017) avaliaram achados de neuroimagem em relação aos principais genes associados ao TDAH e concluíram que o entendimento a respeito do transtorno pode se beneficiar de estudos de neuroimagem e genética no TDAH, apesar de ainda não ser possível tirar muitas conclusões devido ao estágio inicial da área. Dentre perspectivas para futuras contribuições, eles destacam a importância de abordagens avaliando o efeito combinado de múltiplas variantes genéticas em um único teste estatístico. Segundo eles, esses modelos, como *gene-wide* ou *set-based,* reduzem o número de testes estatísticos e, por explicarem uma maior variância fenotípica possibilitam descobertas de efeitos genéticos não detectáveis em abordagens avaliando uma única variante (Klein et al. 2017), como a abordagem utilizada no Capítulo II (*"The broad SYT1 role in psychiatry disorders in light of neuroimaging: a DTI study in adults with ADHD")* . Outra estratégia apontada são abordagens *in silico* buscando integrar achados através de ferramentas de bioinformática, possibilitando predizer variantes genéticas relacionadas a expressão e regulação (Klein et al. 2017).

Os aspectos cerebrais associados ao TDAH através de estudos de neuroimagem têm uma herdabilidade moderada a alta e estão, dessa forma, sobre grande influência genética (Hulshoff Pol et al. 2006; Jahanshad et al. 2013). A herdabilidade estimada em estruturas cerebrais parece variar de acordo com a região, por exemplo volumes do lobo frontal tem uma herdabilidade estimada de

90 a 95%, enquanto para o hipocampo ela varia de 40 a 70% (Peper et al. 2007). A medida cortical com maior herdabilidade estimada é a espessura cortical (entre 34 a 64%) (McKay et al. 2014). Em relação a DTI, valores globais de FA tem uma herdabilidade estimada de aproximadamente 55% (McKay et al. 2014; Kochunov et al. 2016) e entre 40 a 90% em tratos específicos (Kochunov et al. 2016; Sudre et al. 2017).

Assim como no caso do TDAH, as bases genéticas por trás da estrutura e função cerebrais ainda não são conhecidas. Grandes consórcios, como o ENIGMA (*Enhancing Neuro Imaging Genetics through Meta-Analysis,* http://enigma.ini.usc.edu/), ou coortes como o UK *Biobank* (Sudlow et al. 2015), têm fornecido insights sobre a arquitetura genética do cérebro. Estudos do ENIGMA já encontraram diversos *loci* associados a volumes do hipocampo e ICV (Stein et al. 2012; Adams et al. 2016), volumes subcorticais (Hibar et al. 2015a; Satizabal et al. 2019) e estruturas corticais (Grasby et al. 2018). Por exemplo, um estudo envolvendo *datasets* do ENIGMA, CHARGE (*Cohorts of Heart and Aging Research in Genomic Epidemiology)* e UK Biobank, encontrou 25 *loci* associados a volumes subcorticais, incluindo 62 genes implicados no neurodesenvolvimento, sinalização sináptica, transporte axonal, apoptose e previamente associados à susceptibilidade a transtornos psiquiátricos (Satizabal et al. 2019). Além disso, um estudo do UK *Biobank* avaliando medidas de neuroimagem provenientes de diferentes modalidades (i.e. morfológica, difusão e anatômica) mostrou o envolvimento de genes relacionados ao desenvolvimento cerebral e plasticidade, bem como genes que contribuem para o transporte de ferro, nutrientes e minerais (Elliott et al. 2018).

Klein et al., (2019) demostraram que TDAH e ICV parecem estar negativamente correlacionados (análise de correlação genética), onde variantes de risco ao TDAH estão relacionadas a um menor ICV. Além disso, esse estudo encontrou evidencia de variantes pleiotrópicas afetando tanto o TDAH quanto o

volume de regiões subcorticais (amigdala, caudado e putâmen) e ICV (Klein et al. 2019). Através de técnicas de escore de risco poligênico (PRS - explicada em maior detalhe na próxima seção), foram observadas bases genéticas compartilhadas entre TDAH e volume do caudado (Alemany et al. 2019) e valores de FA (Albaugh et al. 2019; Sudre et al. 2019; Zhao et al. 2019). Além disso, Sudre et al. 2019 sugeriram que aspectos neurais (medidas de FA e anatomia cortical) possam ser mediadores da associação observada entre a arquitetura genética do TDAH (avaliada pelo PRS de TDAH) e sintomas de hiperatividade/impulsividade (Sudre et al. 2019). As medidas volumétricas do caudado também parecem mediar a associação entre o PRS de TDAH e problemas atencionais em meninos (Alemany et al. 2019).

2. Etiologia compartilhada entre os transtornos psiquiátricos

Muitos transtornos psiquiátricos apresentam sintomas e outros traços fenotípicos em comum (Nigg and Casey 2005; Young et al. 2009; Liu et al. 2018), além de frequentemente co-ocorrerem (Thapar et al. 2001; Sobanski 2006), como mencionado anteriormente. Dessa forma é plausível uma etiologia compartilhada entre transtornos. Algumas variantes em genes codificadores de canais de cálcio, por exemplo, apresentam um efeito pleiotrópico (i.e., conferem risco para vários fenótipos distintos) (Graves and Hanna 2005; Haan et al. 2008; De Kovel et al. 2010).

Utilizando resultados de diferentes estudos de GWAS é possível computar correlações genéticas, indicando em que grau a arquitetura poligênica desses transtornos se sobrepõe. A correlação genética sugere fatores genéticos de risco compartilhados, e uma possível fisiopatologia em comum, entre diferentes transtornos. Também é possível testar o compartilhamento de bases genéticas entre dois fenótipos diferentes avaliando sua arquitetura poligênica através de PRS. Esse método parte de resultados de GWAS de uma amostra *discovery* num dado fenótipo, por exemplo TDAH. Resultados desse GWAS incluem o alelo de risco, tamanho de efeito e valor de P da associação com o fenótipo testado. Com base nesses resultados é calculado um escore genômico de risco em uma amostra independente (*target*). O escore é calculado para cada indivíduo da amostra *target* como uma soma dos alelos de risco que leva em conta seu tamanho de efeito na amostra *discovery*. Os SNPs a serem incluídos nessa "conta" dependem do valor de P limiar (*threshold;* P_T) definido, sendo incluídos somente SNPs com um valor de P da associação na amostra *discovery* menor do que o P_T (quanto maior o P_T, mais SNPs serão incluídos). Os fenótipos das amostras *discovery* e *target* podem ser diferentes (*cross-phenotype analyses).* Por exemplo, pode-se calcular o escore

utilizando dados de uma amostra *discovery* de TDAH em uma amostra *target* de esquizofrenia, fornecendo *insights* sobre fatores genéticos compartilhados entre os dois transtornos.

Estendendo resultados anteriores (Cross-Disorder 2013), os consórcios *Brainstorm* e *Cross Disorder* observaram uma alta correlação genética entre os transtornos psiquiátricos, especialmente TDAH, esquizofrenia, transtorno bipolar, transtornos de ansiedade e Transtorno Depressivo Maior (TDM), enquanto doenças neurológicas parecem ser geneticamente mais distintas (Consortium et al. 2018; Cross-Disorder 2019). A nível de variantes, o grupo *Cross Disorder* ainda mostrou uma existência substancial de pleiotropia, em que aproximadamente 75% dos 146 SNPs significativos influenciavam mais de um transtorno. Esse efeito pleiotrópico foi particularmente alto (afetando quatro ou mais transtornos) em 23 *loci*, compreendendo principalmente genes envolvidos no neurodesenvolvimento (neurogênese, regulação do neurodesenvolvimento e diferenciação neuronal) e expressos em neurônios.

Estudos utilizando PRS já mostraram fatores genéticos em comum entre TDAH e neuroticismo, depressão, ansiedade, comportamentos de risco (*risk taking behavior*), consumo de bebidas alcoólicas, dependência de álcool, tabagismo e *verbal-numeric reasoning* na população geral (Du Rietz et al. 2018) e desenvolvimento de comportamentos externalizantes (Li 2019). Comportamentos de risco (*risk-taking behaviors*) constituem um aspecto central em vários transtornos psiquiátricos e comumente levam a transtornos por uso de substâncias (TUS) (Kreek et al. 2005; de Haan et al. 2015), comorbidade frequentemente observada em indivíduos com TDAH.

Genes envolvidos na manutenção da estrutura sináptica, liberação de vesículas sinápticas, bem como a transmissão de neurotransmissores são apontados como possivelmente envolvidos em mecanismos moleculares comuns

nos transtornos psiquiátricos, já que são implicados em disfunção neuronal e prejuízos cognitivos (Van Spronsen and Hoogenraad 2010; Torres et al. 2017; Bosiacki et al. 2019).

Capítulo II

Justificativa e Objetivos

3. Justificativa

Os transtornos psiquiátricos estão entre as principais causas de incapacidade em todo o mundo, com forte prejuízo para o indivíduo, familiares e para a sociedade como um todo. O TDAH por muito tempo foi considerado um transtorno exclusivo da infância, de forma que o conhecimento sobre o transtorno em adultos ainda é limitado. Na última década as trajetórias do TDAH têm sido muito debatidas, com resultados inesperados e que ressaltam o quanto ainda temos a aprender sobre esse transtorno.

O TDAH é um transtorno de etiologia complexa, onde diversos fatores genéticos e ambientais estão implicados. O forte componente genético envolvido é evidenciado pela alta herdabilidade. Com recentes achados a níveis genômicos no TDAH tivemos um grande avanço na identificação de variantes específicas, contudo a maior parte da herdabilidade ainda não foi explicada no nível molecular. Aspectos estruturais do cérebro são considerados bons endofenótipos, e assim uma estratégia promissora para uma melhor caracterização fenotípica permitindo assim maior sucesso em estudos genéticos. Esse entendimento é de suma importância, podendo contribuir no diagnóstico, identificação e, futuramente, em novas abordagens de tratamento. Além disso, dada a provável etiologia compartilhada entre transtornos psiquiátricos, o entendimento do papel de variantes com efeito pleiotrópico pode fornecer novos *insights* sobre mecanismos envolvidos na fisiopatologia dos transtornos.

4. Objetivos

4.1 Objetivo Geral

Buscar compreender aspectos de neuroimagem e genética do TDAH na vida adulta

4.2 Objetivos Específicos

4.2.1 Identificar aspectos estruturais do cérebro envolvidos no TDAH e seus efeitos em diferentes fases da vida eu duas amostras independentes, utilizando uma abordagem *data-driven*;

4.2.2 Investigar o efeito do gene *SYT1* em alterações microestruturais na substância branca em uma abordagem considerando o efeito combinado das variantes do gene;

4.2.3 Avaliar o efeito da *SYT1* sobre o Transtorno por Uso de Cocaína/crack, um transtorno altamente comórbido ao TDAH, em uma abordagem *single-SNP*;

4.2.4 Entender fatores associados a remissão dos sintomas de TDAH na vida adulta;

4.2.5. Explorar aspectos relacionados a integridade da substância branca possivelmente envolvidos no TDAH explorando efeitos sexo-específicos em duas amostras independentes;

4.2.6 Verificar aspectos relacionados a integridade da substância branca possivelmente envolvidos no Transtorno por Uso de Cocaína/crack.

Capítulo III

5. Reduced Fronto-striatal Volume in Attention-Deficit/Hyperactivity Disorder in Two Cohorts Across the Lifespan

Artigo em revisão na revista *Biological Psychiatry*.

Title: Reduced fronto-striatal volume in Attention-Deficit/Hyperactivity Disorder in two cohorts across the lifespan
Short title: Lifespan fronto-striatal volume reduction in ADHD
Authors: Renata Basso Cupertino*[1], Sourena Soheili-Nezhad*[2,3], Eugenio Horacio Grevet[4,5], Cibele Edom Bandeira[1], Felipe Almeida Picon[4,5], Maria Eduarda Tavares[1], Jilly Naaijen[2,3], Daan van Rooij[3], Sophie Akkermans[2,3], Eduardo Schneider Vitola[4], Marcel P Zwiers[3], Diego Luiz Rovaris[4,5], Pieter J. Hoekstra[6], Vitor Breda[4,5], Jaap Oosterlaan[7,8], Catharina A Hartman[7,8], Christian F Beckmann[2,3], Jan K Buitelaar[2,9], Barbara Franke[10,11], Claiton Henrique Dotto Bau**[1,5], Emma Sprooten[2,3]**

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Keywords: ADHD, white matter, fronto-striatal, tensor-based morphometry, independent component analysis

Abstract

Background: Attention-Deficit/Hyperactivity Disorder (ADHD) has been associated with altered brain anatomy in neuroimaging studies. However, small and heterogeneous study samples, and the use of region-of-interest and tissue-specific analyses have limited the consistency and replicability of these effects. We used a fully data-driven multivariate approach to investigate alterations in both gray and white matter simultaneously, and capture neuroanatomical features associated with ADHD in two large, independent, demographically different cohorts. **Methods:** The study comprised two ADHD cohorts with structural magnetic resonance imaging data: the Dutch NeuroIMAGE cohort (n=890, average age 17.2 years, discovery sample) and the Brazilian IMpACT cohort (n=180, average age 44.2 years, cross validation sample). Using independent component analysis of whole-brain morphometry images in the NeuroIMAGE cohort, 375 independent components of neuroanatomical variations were extracted and assessed for their association with ADHD. We cross validated ADHD-associated components in the Brazilian IMpACT cohort. **Results:** In both discovery (corrected-p=0.0085) and validation (p=0.032) cohorts, ADHD diagnosis was significantly associated with reduced brain volume in a component mapping to frontal lobes, striatum, and their interconnecting white-matter tracts. The most pronounced case-control differences were localized in white matter adjacent to the orbitofrontal cortex. **Conclusion:** Independent component analysis is a sensitive approach to uncover neuroanatomical alterations in ADHD and avoid bias attributable to *a priori* region selection part of classical approaches. Current results provide further evidence for the role of the fronto-striatal circuit in ADHD. The fact

that the two cohorts are from different continents and comprising different age ranges highlights the robustness of the findings.

Introduction

Attention-Deficit Hyperactivity Disorder (ADHD) is one of the most common psychiatric disorders worldwide, characterized by age-inappropriate levels of inattention and/or hyperactivity/impulsivity leading to significant impairment. Its prevalence is about 5% in children and about 3% in adults (1, 2). ADHD is clinically highly heterogeneous, and around 70% of the affected individuals present with comorbid psychiatric disorders, further complicating diagnosis and treatment (3–5). The clinical profile of ADHD changes throughout development: while children are more likely to present with symptoms of hyperactivity/impulsivity, adolescents and adults often experience more symptoms of inattention (1, 6). Cognitive functions such as inhibitory control and working memory (7), emotion regulation (8), and motivational processes (9) are also affected in many individuals with ADHD across the lifespan.

Although the underlying neurobiology of ADHD is only partly understood, neuroimaging studies have identified several structural and functional brain changes associated with this disorder (10–13). The most consistent findings in structural magnetic resonance imaging (MRI) studies on ADHD point to a reduction in total brain volume and grey matter in individuals with ADHD compared to controls (11, 12, 14, 15). More specifically, Sáenz et al (11) associated ADHD with structural alterations in the basal ganglia, prefrontal cortex, and the corpus callosum. A mega-analysis comprising over 3,000 individuals found smaller volumes of five out of seven subcortical structures in ADHD, with strongest effects observed in the amygdala, nucleus accumbens, and putamen (14). Age-stratified analyses in that study showed that the effect of ADHD on brain structure was

stronger in children, and no statistically significant effect was seen in adults above the age of 22 years (14). More recently, a coordinated analysis involving 36 centers showed that children with ADHD also have reduced cortical surface area, especially in frontal, cingulate, and temporal regions as well as reduced cortical thickness in fusiform gyrus and temporal pole (16). That study also did not find differences in surface area and cortical thickness in the adolescent and adult groups, again suggesting an age-dependent effect. Evidence from studies using diffusion-weighted imaging (DWI) implicates white-matter microstructural alterations in ADHD (17–19), possibly hampering neural communication amongst and between cortical and subcortical areas. Two meta-analyses found altered fractional anisotropy in widespread regions in patients with ADHD, with the most consistent findings in the corpus callosum, anterior corona radiata, right forceps minor, bilateral internal capsule, and left cerebellum (10, 18).

Previous structural MRI studies in ADHD either focused on *a priori*, automatically segmented, cortical or subcortical areas delimiting specific regions of interest (ROIs) (12, 14), or explored changes in brain volume and cortical area and thickness using mass univariate methods at a voxel or vertex level (20–22). While these methods are primarily sensitive to the detection of grey-matter differences, evidence from diffusion-weighted imaging shows that ADHD may also affect white matter microstructure (10, 17, 19). In the present study, we used a combination of tensor-based morphometry (TBM) and independent component analysis (ICA), allowing us to optimize sensitivity to the detection of local differences in both grey- and white-matter tissue and their spatial covariation, in a whole-brain multivariate analysis.

ICA has been frequently used in functional MRI studies, leading to the identification of several functional brain networks (23, 24). More recently, ICA has also been

successfully applied to whole-brain diffusion imaging data (25, 26). In relation to ADHD, multi-modal linked ICA has been used for data fusion across different MRI modalities (cortical thickness and area, voxel-based morphometry, and diffusion tensor imaging), showing association of ADHD symptom severity with several spatial modes of grey and white matter properties distributed across many brain regions (27–29). However, replication in neuroimaging remains scarce, and the generalizability across populations of different ethnic, social, and cultural backgrounds is unknown. Furthermore, it remains unclear how any potential neuroimaging markers of ADHD, being a neurodevelopmental disorder, vary with age across the lifespan.

The present study aimed to identify structural brain differences in association with ADHD across the lifespan in two relatively large, independent cohorts. Using TBM, we created images capturing local brain volume variation in both grey and white- matter. Subsequently, ICA was used to isolate spatial modes from these images. We assessed association of the independent brain components with ADHD diagnosis and symptom dimensions in a discovery sample of adolescents and young adults (NeuroIMAGE). Further, we appraised the effect of a significantly associated component in an independent and clinically different validation sample of individuals diagnosed with ADHD during adulthood (Brazilian IMpACT cohort).

Methods and Materials

Samples

NeuroIMAGE cohort

The NeuroIMAGE cohort is a Dutch prospective multi-site study aimed to investigate the longitudinal course of ADHD relying on two MRI waves (NeuroIMAGE I/II). Details of this cohort have been described elsewhere (30). Participants were enrolled at two sites, the Vrije Universiteit in Amsterdam and the Radboud University Medical Center in Nijmegen. The study was approved by the local ethics committees, and written informed consent was obtained from all participants and their legal guardians. The cohort included unrelated participants (n=138) as well as those with full sibling relationships in families of different sizes (n=241 sibling pairs, n=70 three siblings, n=15 four siblings). In order to maximize sample size, data from the two longitudinal study waves were combined such that individuals who participated twice were only included at the first wave (NeuroIMAGE I). The ADHD diagnosis was primarily based on a semi-structured clinical interview using a Dutch translation of the Schedule for Affective disorders and Schizophrenia for school-age children - present and lifetime version (K-SADS-PL), based on the fourth version of the diagnostic and statistical manual (DSM-IV). To optimize the diagnostic assessment, the information from the K-SADS-PL interview was combined with information from the Conners Adult ADHD Rating Scale (CAARS R-L), Conners Parent Rating Scale (CPRS R:L), and Conners Wells Adolescent Self-Report Scale: Short Form (CASS:S). For an ADHD full diagnosis, individuals had to have six or more symptoms in the inattention domain and/or in the hyperactivity/impulsivity domain causing impairment in multiple settings, as well as a Conners T-score ≥63. Unaffected individuals had ≤3 symptoms *and* a Conners T-score <63. Participants who did not fulfill criteria for either category were classified as *subthreshold* ADHD. Psychiatric comorbidities, such as anxiety, depression, and oppositional behavior were assessed by the Dutch version of the Strengths and Difficulties Questionnaire (SDQ).

Exclusion criteria were: Intelligence Quotient (IQ) <70, diagnosis of autism, neurological disorders, such as epilepsy, general learning difficulties, brain trauma, or known genetic disorders, such as Fragile X or Down syndrome.

From the NeuroIMAGE I study wave, 807 individuals were enrolled for a scan session. In both sites, 1.5T MRI scanners were employed (Siemens Magnetom SONATA and AVANTO, Erlangen Germany), using 8-channel phased array head coils. T_1-weighted anatomical scans were acquired at an isotropic resolution of 1mm using a 3D magnetization prepared rapid acquisition with gradient echoes (MPRAGE) sequence with 176 slices, flip angle=7°, TE=2.95ms, TR=2,730ms, TI=1,000ms, matrix size=256x256, and parallel acquisition (GRAPPA) with an acceleration factor of 2. From NeuroIMAGE II study wave, 87 subjects were considered, only from one site (Nijmegen); MRI scanner and image acquisition parameters remained the same as the first wave. After quality control, structural brain MRI scans of a total of 890 individuals were considered (359 affected, 98 subthreshold, and 433 unaffected; ages range from 7 to 29 years). Sample characteristics of this cohort are provided in **Table 1**.

Brazilian IMpACT cohort

The Brazilian IMpACT cohort was assessed by the ADHD Outpatient Program – Adult Division at the Hospital de Clínicas de Porto Alegre (PRODAH-A). Further information of this cohort has been described elsewhere (31, 32). All participants were unrelated adults of white (European descent) Brazilian ethnicity aged 18 years or older. ADHD diagnosis was based on DSM-5 diagnostic criteria, using the K-SADS epidemiologic version (KSADS-E) adapted for adults (34). Individuals were recruited when seeking for psychiatric help (cases) or

donating blood (controls). The Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) was used for assessing other lifetime psychiatric comorbidities. Individuals with significant neurological disease, head trauma, history of psychosis, and/or an estimated IQ score below 70 were excluded. Participants signed an informed consent form before the study, which was approved by the Ethics Committee of the hospital. Sample characteristic can be found in **Table 2**.

Participants were re-evaluated on average 13 years after diagnosis and underwent MRI scanning, using a 3.0T Siemens SPECTRA scanner and a 12-channel head coil. A high-resolution structural MRI volume was acquired using a T_1-weighted 3D MPRAGE sequence with 192 slices, flip angle=7°, TE=2.55ms, TR=2,530ms, TI=1,100ms, matrix size=256x256, isotropic resolution of 1mm, and a GRAPPA factor of 2. After quality control procedures, 180 individuals with structural MRI scan were included (118 affected and 62 unaffected; age range from 26 to 74 years).

Imaging preprocessing

All T1-weighted images were corrected for magnetic field bias using the N4 algorithm (33), and the brain field of view was cropped using the FSL standard_space_roi tool. For each cohort separately, images were registered to an average space to create a cohort-specific minimum deformation brain template (**Supplementary Figure 1**). Four iterations of linear registration and five iterations of diffeomorphic SyN registration were used for template creation (35). The nonlinear warps that transformed each subject's native brain volume to the common template were used to derive Jacobian determinant fields that encode local brain volume variation across the study individuals. The Jacobian values were subsequently log-

transformed to symmetrize their distribution around zero to obtain Jacobian determinant maps per participant. The Jacobian values were normalized within a brain mask in the standard template space, hence removing the global brain size effect.

Analysis

The Jacobian determinant maps of the NeuroIMAGE cohort (n = 890) were decomposed into spatially independent components using MELODIC, a probabilistic ICA method (24). Four decompositions were performed with varying dimensionality (25, 50, 100 and 200 components) to allow the identification of independent spatial sources in the Jacobian fields at various levels of spatial specificity. Thus, a total of 375 components were extracted. ICA performs a linear matrix factorization of the Jacobian determinant maps resulting in 2 matrices: a matrix of spatially independent component maps reflecting the contribution of each voxel to each component; and a mixing matrix containing the loading of each individual on each component. The later was used to test case-control differences in each spatial component.

In the discovery cohort (NeuroIMAGE), a permutation-based general linear model (PALM) (36) was used to correct for multiple testing of all 375 brain components loading values with ADHD, while controlling for age, sex, scan site and study wave. To control for family structure in the NeuroIMAGE sample, permutation analysis was used with exchangeability blocks that only allow permutations either within unrelated participants or siblings-blocks separately, or whole-block permutations across families of the same sizes (37). Type I error rate was controlled across all tested brain components using 10,000 random permutations.

To test the significant NeuroIMAGE-based ICA component in the Brazilian IMpACT cohort, the brain component significantly associated with ADHD in the discovery cohort was mapped to the brain template of the Brazilian IMpACT cohort using non-linear SyN transformation. Spatial regression was used to derive the level of brain volume in this spatial component in each subject of the Brazilian IMpACT cohort. The obtained brain volume parameter, now containing the values of the NeuroIMAGE-based ICA-feature for the subjects in Brazilian IMpACT cohort, was used as an imaging feature and its association with ADHD was assessed using linear regression, controlling for sex and age confounders.

Results

NeuroIMAGE cohort

A total of 375 components were obtained by probabilistic ICA showing areas of structural brain covariation across individuals, of them 52 components were nominally associated ($p_{uncorrected}<0.05$) with current diagnosis of ADHD. Only one component spanning frontal lobes and striatum remained significant after multiple testing correction. This component showed reduced loading in individuals with current ADHD (n = 359, participants, mixed model t = -3.61: uncorrected $p = 3\times10^{-4}$, permutation-corrected p = 0.0085) and lifetime history of ADHD (n = 418 participants, mixed model t = -3.19, uncorrected p mixed model = $3\times10^{-4,}$ permutation-corrected p = 0.0015). The maximal focus of brain volume reduction in this component localized to the bilateral fronto-striatal white-matter adjacent to the orbitofrontal cortex - **Figure 1**, also represented in as an **e-component**). This association remained significant after exclusion of individuals with a positive history of ADHD medication (n = 197 drug naïve patients, $p = 9\times10^{-4}$; t = -3.36). Results depicted in **Table 3**.

There was significant correlation of the fronto-striatal component with Conners' ADHD symptom dimensions; inattention $p = 0.012$, $t = -2.53$; hyperactivity $p = 0.003$, $t = -3.01$), and with the number of hyperactivity/impulsivity symptoms ($p = 0.040$, $t = -2.06$) but not with the number of inattention symptoms ($p = 0.21$; $t = -1.26$) assessed by the KSADS. The fronto-striatal component was correlated with subjects' sex (reduced in females compared to males; $p < 0.0001$, $t = 9.35$) and age (greater volume reductions with age; $p < 0.0001$, $t = -5.46$), although no interaction effects with sex or age were observed ($p = 0.92$. and 0.27, respectively). The smaller group of participants with subthreshold ADHD also showed similar trend for reduced brain volume in the fronto-striatal component (current subthreshold diagnosis n = 98 subjects, $p = 0.11$, $t = -1.61$; lifetime subthreshold diagnosis n = 74, $p = 0.13$, $t = -1.54$).

Brazilian IMpACT cohort

The association of the fronto-striatal ICA component was replicated in the Brazilian IMpACT cohort with current ADHD ($p = 0.032$, $t = -2.16$) and with lifetime history of ADHD ($p = 0.021$, $t = -2.33$), also controlling for age and sex (**Table 3** and **Figure 1**). Again, this result remained significant when excluding individuals currently under pharmacological treatment ($p = 0.030$, $t = -2.18$). The same direction of effect was observed as in NeuroIMAGE: adults with ADHD showed smaller brain volume in the fronto-striatal component. The component was also associated with the number of hyperactivity/impulsivity symptoms ($p = 0.046$, $t = -2.011$), where a larger number of symptoms was associated with reduced brain volume. The component was associated with sex ($p < 0.001$, $t = 5.47$), but not

with age (p = 0.75). There was no interaction of lifetime ADHD with sex (p = 0.412) or age (p = 0.076) on the component.

Secondary Analyses

Considering the broad age-range of the two cohorts, and previously reported significant age-by-diagnosis interactions for brain volumes (38) or differences in brain volumes across the lifespan (14, 16), other ICA components were also investigated for possible interactions with age. To reduce the number of tests, only components of the 200-component dimension were investigated. In the NeuroIMAGE cohort, no ICA component showed significant interaction effects after multiple testing correction; however, a nominal effect was observed for 14 components (**Supplementary Figure 2**). The strongest nominal finding was found for a component mapping to bilateral putamen (ICA 17 - **Figure 2**), where participants with ADHD had a slower decrease in regional brain volume with age compared to controls ($P_{uncorrected}$ = 0.0043 - **Figure 2**). The 14 components showing nominal age-by-diagnosis interaction effect in the NeuroIMAGE cohort were also investigated in the Brazilian IMpACT cohort (**Supplementary Figure 2**). In the adult cohort, a nominal interaction effect was observed only for the putamen-related component (ICA 17; $P_{uncorrected}$ = 0.025; **Figure 2**), in the same direction as in the NeuroIMAGE cohort, with the patients showing a less steep rate of decline with age compared to the controls.

Discussion

This study sought to identify structural brain associations with ADHD using a different approach from those used in most structural MRI studies. The data-driven approach

presented herewas sensitive to both grey- and white-matter volume variation, and the multivariate decomposition into independent spatial sources increased sensitivity to detect whole brain variations that are correlated, both spatially and across individuals. The results provide new evidence for the role of the fronto-striatal circuitry and point to the importance of white-matter in ADHD pathophysiology. Importantly, our work shows the robustness of this finding, as it was seen in two independent cohorts, which were geographically distant and comprised different stages of the lifespan. Also, the present study reported another component with a nominal age-by-diagnosis interaction effect in line with age-dependent effects previously observed in structural changes in ADHD.

A significant case-control brain volume difference was observed for a brain component localized to bilateral fronto-striatal white matter, adjacent to the orbitofrontal cortex. Fronto-striatal circuits are implicated in complex behaviors, such as reward processing, emotion regulation, inhibition, and motivational states (39), and the dysfunction of these circuits is implicated in several psychiatric disorders (39–41), including ADHD (42). Previous structural MRI studies have shown volume reductions in frontal lobes and the striatum (11, 14) in individuals with ADHD compared to controls. The present study indicates that this fronto-striatal trait is age-independent across adolescence and adulthood in two independent cohorts of different age ranges and shows for the first time that this fronto-striatal trait alterations generalizes to adults with ADHD up to middle age.

Contrary to most studies that use *a priori* segmented areas, this study used data-driven ICA for separation of raw data into linearly mixed spatial sources. Such decomposition of voxel-wise brain morphometry is useful to discover spatial features that covary beyond *a priori* defined regional boundaries, while avoiding mass univariate voxel wise tests. Extracting

morphometric sources without predefined anatomical boundaries assists in deriving MRI features that may better reflect underlying pathophysiological processes. Moreover, using this approach, the maximal focus of brain volume deficits was detected in the bilateral frontal-striatal white-matter, suggesting the importance of considering both grey- and white-matter in conjunction.

Given that several studies, including the largest ADHD neuroimaging mega-analyses to date (14, 16), suggested age-dependent associations of ADHD with brain anatomy, we also performed an exploratory analysis to identify potentially age-dependent associations of the ICA components with ADHD. We observed one nominal, but replicated, age-by-diagnosis interaction effect. In line with Hoogman et al. (14) and Greven et al. (38), this component showing a nominal age-by-diagnosis interaction effect is mapped in the bilateral putamen, where the age-related volume decline occurred at slower rate in ADHD compared to the control group.

It is important to consider this study in the context of some strengths and limitations. This is a cross-sectional study of adolescents and adults, limiting conclusions with regard to brain structural changes across the lifespan and conclusions about developmental aspects, for which longitudinal imaging data would further increase sensitivity to within-subject longitudinal effects. Nevertheless, considering both cohorts, the study includes individuals of a wide age-range, even overlapping ages, allowing us to infer that the main result observed was independent of age.

In conclusion, using tensor-based morphometry driven by both grey- and white-matter, the present findings reinforce the importance of fronto-striatal circuitry and medial frontal white-matter in ADHD neurobiology. Identification of brain structural differences

between individuals with and without ADHD provides new insights into the biology underlying this disorder and can contribute to improving diagnosis and treatment in the future. For this, the neural substrate observed here might be of particular interest, given its cross-cultural and age-independent validity.

Acknowledgments

The NeuroIMAGE study was supported by NIH Grant R01MH62873 (to Stephen V. Faraone), NWO Large Investment Grant 1750102007010 (to Jan Buitelaar), ZonMW grant 60-60600-97-193, NWO grants 056-13-015 and 433-09-242, and matching grants from Radboud University Nijmegen Medical Center, University Medical Center Groningen and Accare, and Vrije Universiteit Amsterdam. The research leading to these results also received support from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement number 278948 (TACTICS). Brazilian IMpACT was financed by Conselho Nacional de Desenvolvimento Científico e Tecnológico (Grants 476529/2012-3, 466722/2014-1 and 424041/2016-2), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and FIPE-HCPA 160600. Barbara Franke was supported by a personal Vici grant from the Innovation Program of the Netherlands Organization for Scientific Research (NWO; grant 016-130-669). Renata B Cupertino was supported by International Brain Research Organization-Latin America (IBRO LARC) Exchange Fellowship. Emma Sprooten is funded by a Hypatia Fellowship (Radboudumc) and a NARSAD Young Investigator Grant (Brain and Behavior Research Foundation, ID: 25034).

This study is part of the International Multicentre persistent ADHD Collaboration (IMpACT; www.impactadhdgenomics.com). IMpACT unites major research centres working on the genetics of ADHD persistence across the lifespan and has participants in The Netherlands, Germany, Spain, Norway, the United Kingdom, the United States, Brazil and Sweden. Principal investigators of IMpACT are: Barbara Franke (chair), Andreas Reif (co-chair), Stephen V. Faraone, Jan Haavik, Bru Cormand, J. Antoni Ramos-Quiroga, Marta Ribases, Philip Asherson, Klaus-Peter Lesch, Jonna Kuntsi, Claiton H.D. Bau, Jan K. Buitelaar, Alejandro Arias Vasquez,Tetyana Zayats, Henrik Larsson, Alysa Doyle, and Eugenio H. Grevet.

Disclosures

Eugenio Grevet was on the speaker's bureau for Novartis and Shire for the last 3 years. He also received travel awards (air tickets and hotel accommodations) for participating in two psychiatric meetings from Shire and Novartis. Barbara Franke has received educational speaking fees from Medice. Jan K Buitelaar has served as a consultant to / member of advisory board of / and/or speaker for Shire, Roche, Medice, and Servier. He is not an employee of any of these companies, and not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, royalties. All other authors declare that they have no conflict of interest.

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Tables and Figures

Table 1. Demographic and clinical characteristics of the NeuroIMAGE cohort.

	ADHD (N=359)		Subthreshold (N=98)		Controls (N=433)	
	n	*%*	*n*	*%*	*n*	*%*
Sex (male)	247	68.8	56	57.1	206	47.6
Scan site (Nijmegen)	195	54.3	48	49.0	203	46.9
Wave drawn (NeuroIMAGE I)	336	93.6	85	86.7	386	89.1
Age (years)[a]	17.0	3.7	18.1	3.9	17.2	3.8
Comorbidities (lifetime)						
Oppositional Defiant Disorder	109	30.4	7	7.1	7	1.6
Conduct disorder	22	6.1	2	2.0	0	0
Major depressive disorder	4	1.1	0	0	2	0.5
Generalized anxiety disorder	6	1.7	2	2.0	2	0.5
Avoidant/social phobia disorder	3	0.8	0	0	1	0.2
Panic disorder	0	0	1	1.0	0	0
Symptoms (KSADS) [a]						
Inattention	6.6	1.9	3.6	1.4	1.2	0.9
Hyperactivity/Impulsivity	5.2	2.4	2.8	1.5	1.4	1.0

[a] Data represented as mean (standard deviation).

Table 2. Demographic and clinical characteristics of the Brazilian IMpACT cohort.

	ADHD (N=118)		Controls (N=62)	
	n	*%*	*n*	*%*
Sex (male)	51	43.2	33	53.2
Age (years)[a]	46.9	10.5	39.2	9.6
Comorbidities (lifetime)				
Generalized Anxiety Disorder	77	65.3	18	29.0
Oppositional Defiant Disorder	82	69.5	4	6.4
Major Depressive Disorder	53	44.9	23	37.1
Social Phobia	43	36.4	11	17.8
Bipolar Disorder*	36	30.5	3	4.8
Substance Use Disorder	33	28.0	5	8.1
Eating Disorders [c]	25	21.2	8	12.9
Obsessive Compulsive Disorder	26	22.0	2	3.2
Antisocial Personality Disorder	4	3.4	1	1.6
Symptoms (KSADS) [a]				
Inattention	6.3	2.5	1.3	2.3
Hyperactivity/Impulsivity	4.6	2.7	1.2	1.6

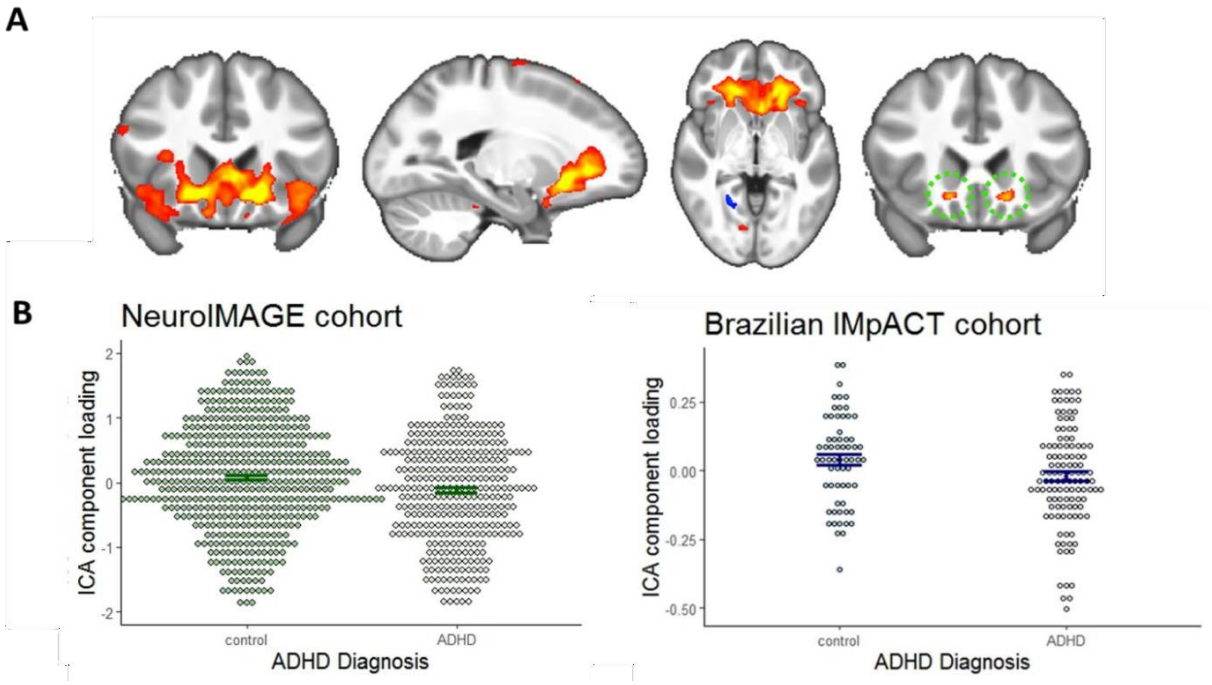
[a] Data represented as mean (standard deviation). [b]Including other specified bipolar and related disorder cases. [c]including anorexia nervosa, bulimia nervosa or binge-eating.

Table 3. Loading of the ICA component spanning frontal lobes and striatum in both cohorts.

	t	P-value
NeuroIMAGE cohort[a]		
ADHD lifetime	-3.20	**0.0015***
ADHD current (at the time of scanning)	-3.62	**0.0085***
ADHD symptoms (KSADS)		
Inattention	-1.26	0.21
Hyperactivity/Impulsivity	-2.06	**0.04**
ADHD symptoms (Conners ADHD)		
Inattention	-2.53	**0.012**
Hyperactivity/Impulsivity	-3.01	**0.003**
Brazilian IMpACT cohort[b]		
ADHD lifetime	-2.33	**0.021**
ADHD current (at the time of scanning)	-2.16	**0.032**
ADHD symptoms		
Inattention	-1.455	0.15
Hyperactivity/Impulsivity	-2.011	**0.046**

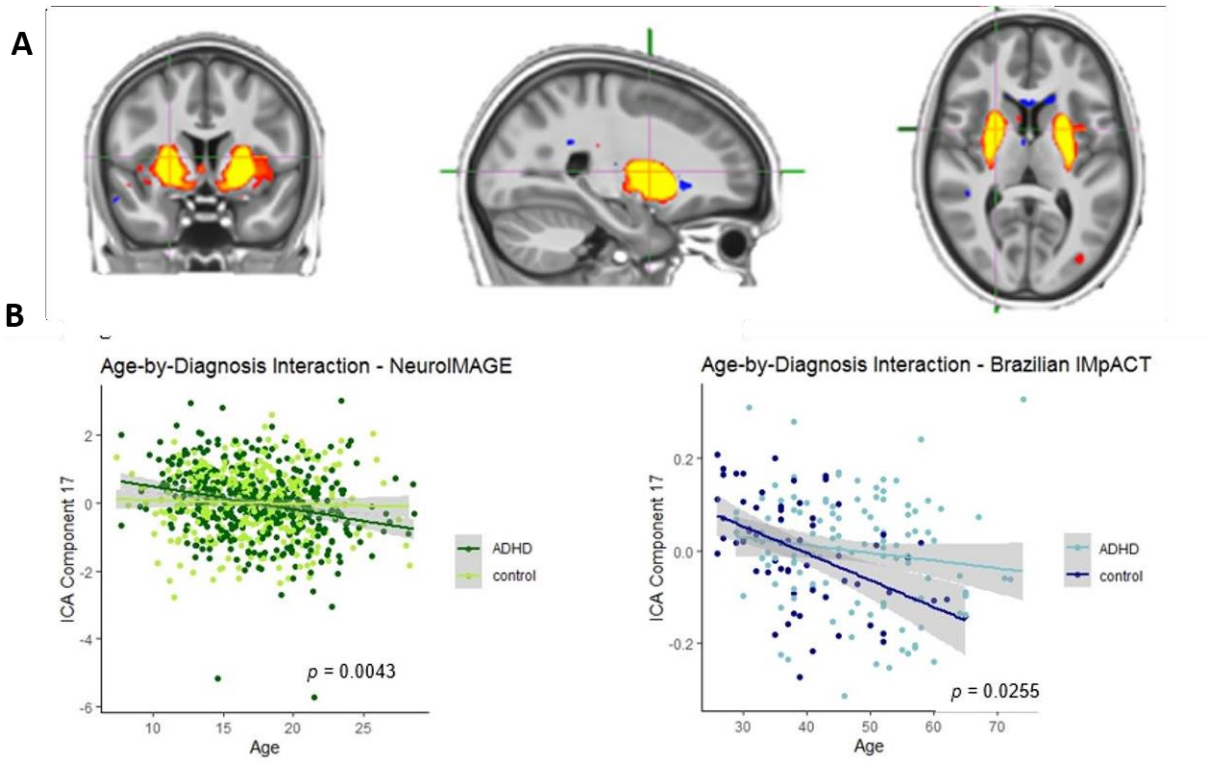
ICA = Independent component analysis; ADHD = Attention-Deficit/Hyperactivity Disorder. [a]Analyses included age, sex, study site and study wave as covariates. [c]Calculated without considering *Permutation-corrected P-value [b]Analysis included age and sex as covariates.

Figure 1. ICA component associated with ADHD.



A. ADHD patients demonstrated reduced loading of a component capturing volume of prefrontal white-matter together with orbitofrontal, striatal, an sular grey-matter (IC z-score>3.6). The focal area of highest component probability (IC z-score>8) is depicted on the right, showing orbitofrontal white-matter volume reduction bilaterally. **B.** Residualized ICA component loading in individuals with and without ADHD in both cohorts. Error bar indicates standard error.

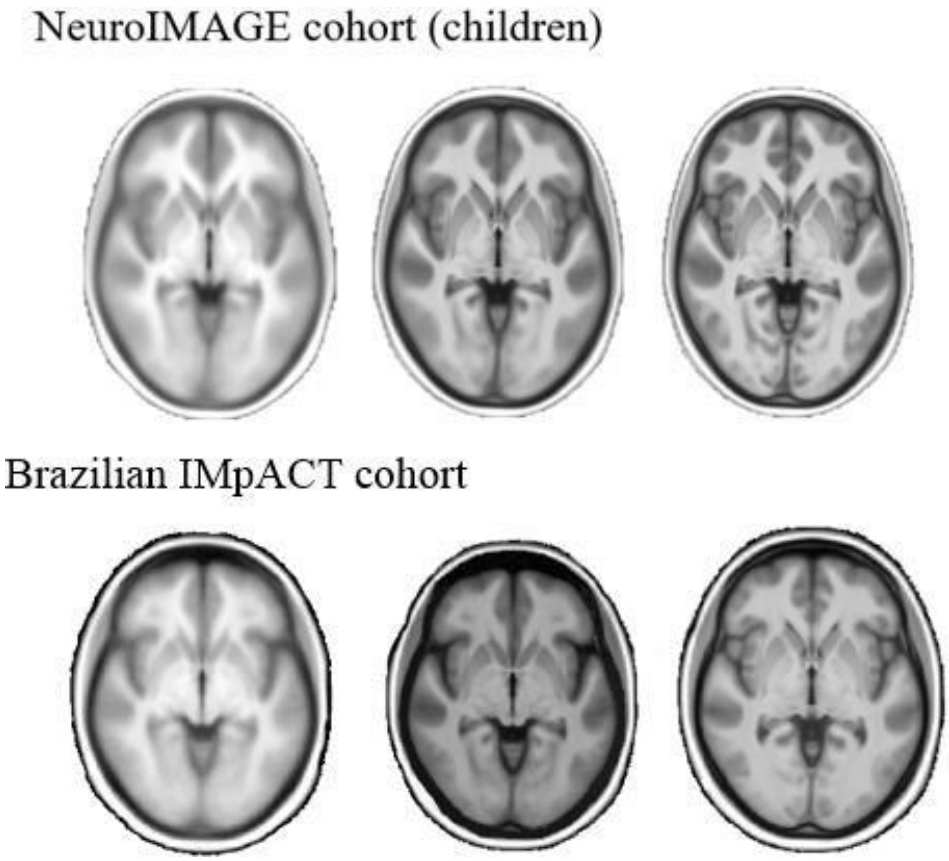
Figure 2. Age-by-diagnosis interaction



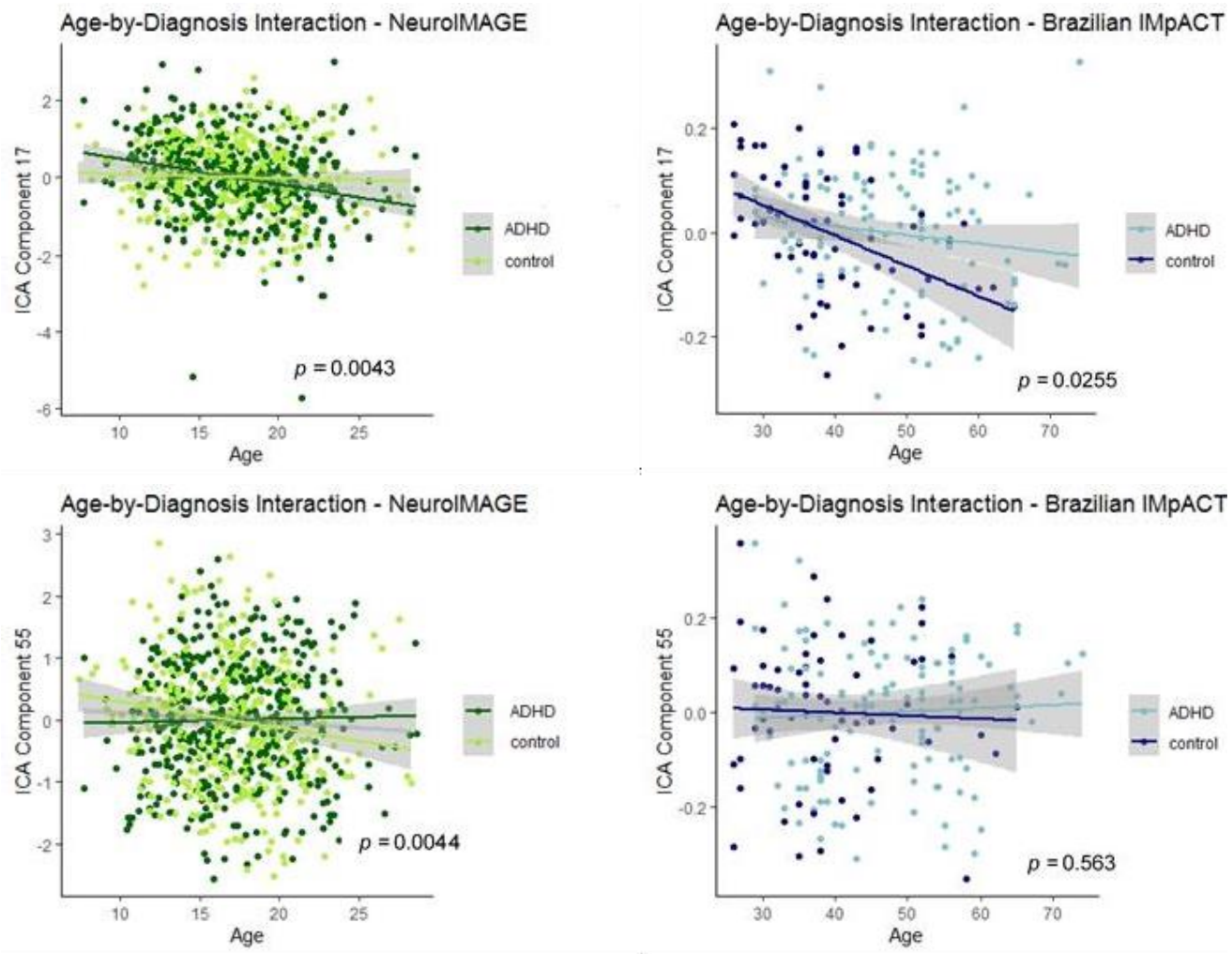
A. ICA component with strongest nominal age-by-diagnosis interaction effect. **B.** Plots of the age-by-diagnosis interaction on ICA component 17 in both cohorts.

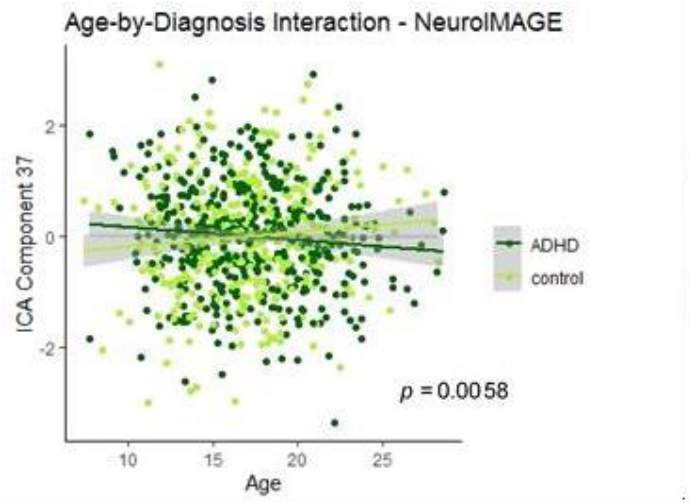
e-component

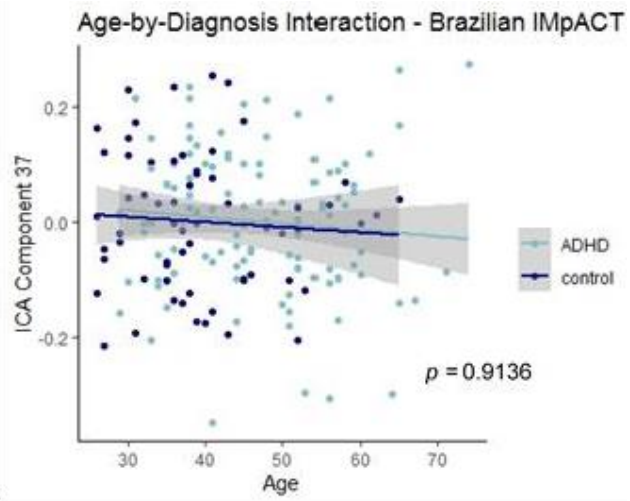
Supplementary Figure 1. Brain templates of both cohorts constructed by iterative registration of all T1-weighted volumes using affine (left) and nonlinear SyN algorithms (middle: first SyN iteration; right: fifth SyN iteration).

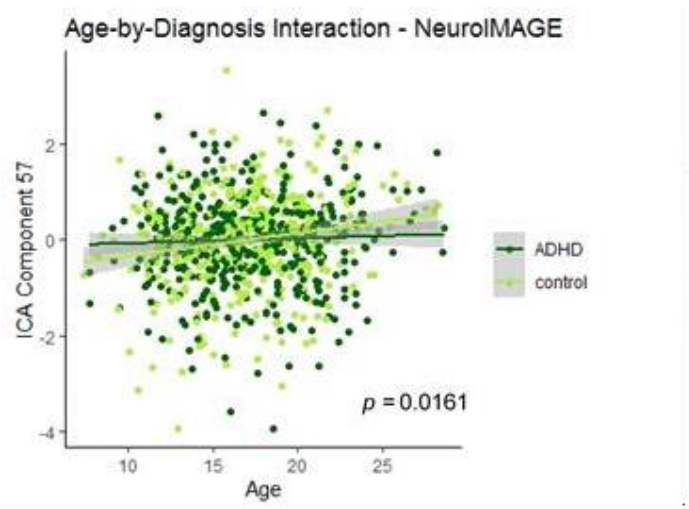


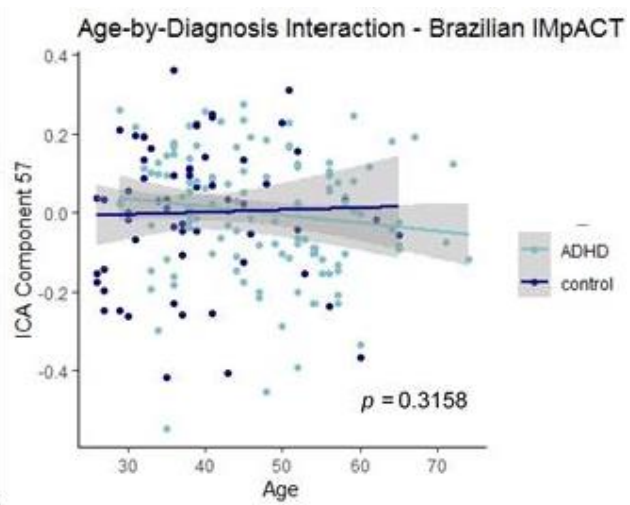
Supplementary Figure 2. Fourteen ICA components with nominal age-by-diagnosis interaction effect in the NeuroIMAGE cohort, investigated also in the Brazilian IMpACT cohort. Component 17 (top), the top-associated component in the discovery sample, was the only component with a significant effect of a consistent direction in both cohorts.

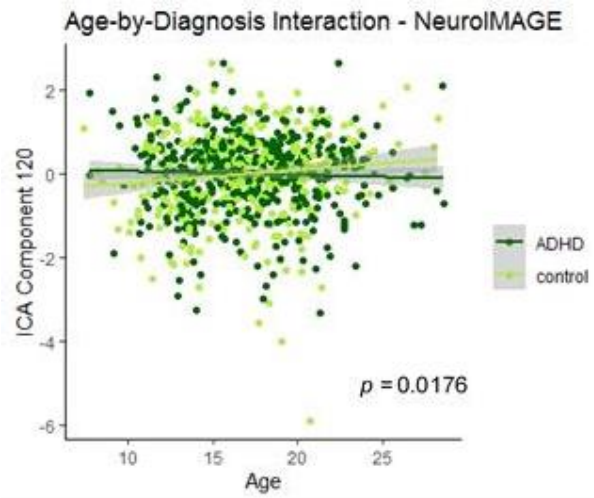


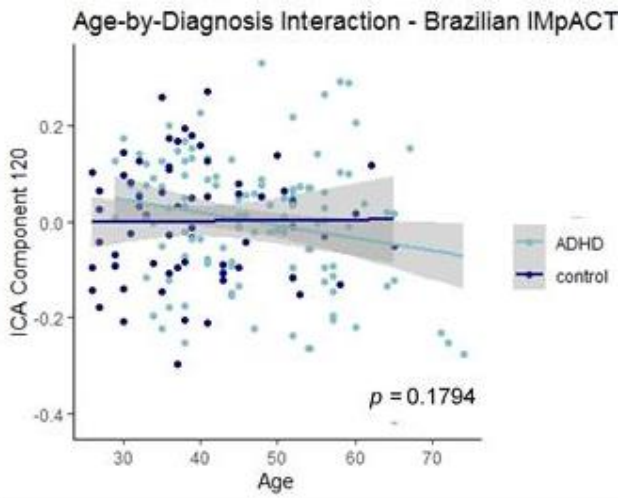

Age-by-Diagnosis Interaction - NeuroIMAGE
ICA Component 37
Age
ADHD
control
p = 0.0058

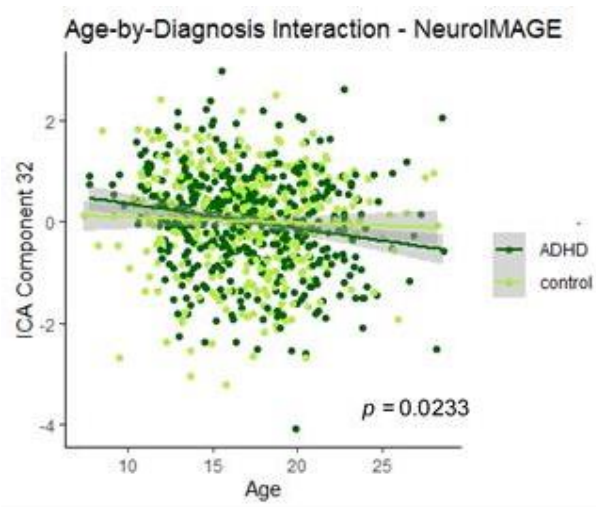

Age-by-Diagnosis Interaction - Brazilian IMpACT
ICA Component 37
Age
ADHD
control
p = 0.9136

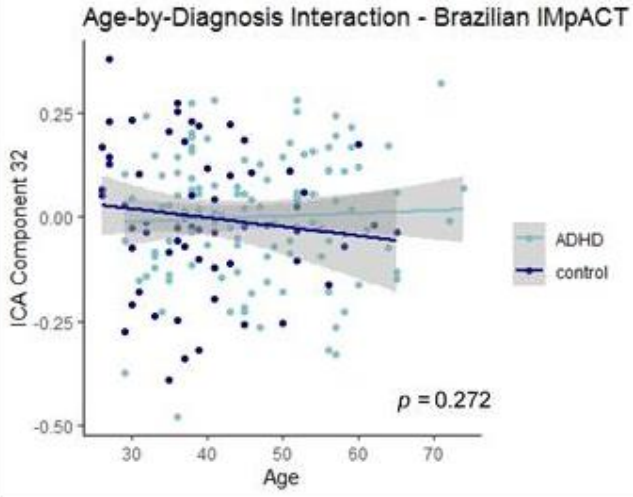

Age-by-Diagnosis Interaction - NeuroIMAGE
ICA Component 57
Age
ADHD
control
p = 0.0161

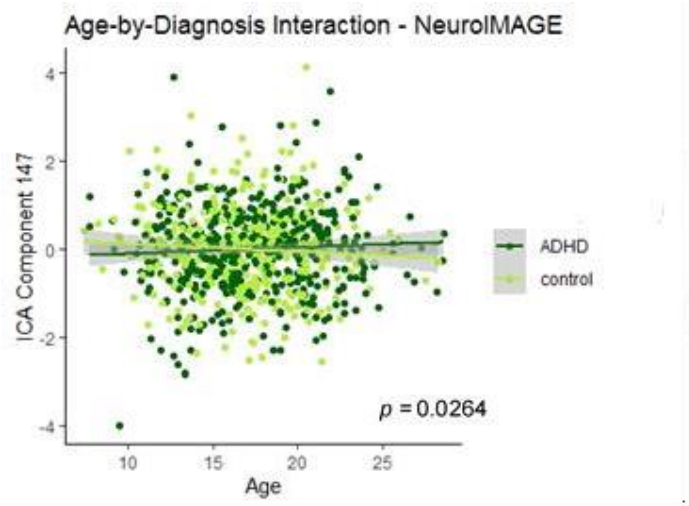

Age-by-Diagnosis Interaction - Brazilian IMpACT
ICA Component 57
Age
ADHD
control
p = 0.3158

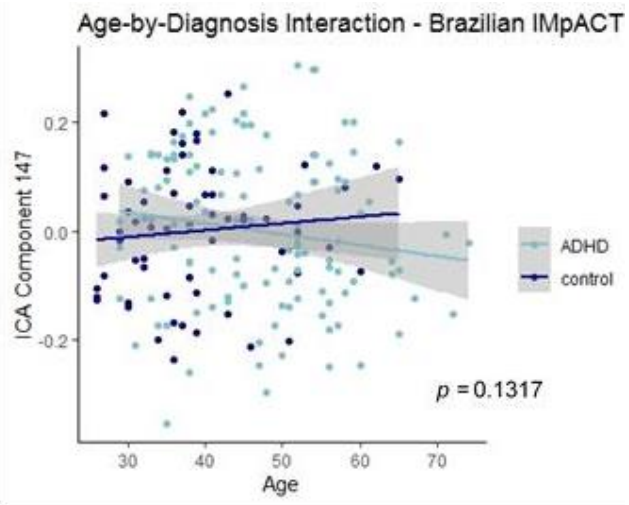

Age-by-Diagnosis Interaction - NeuroIMAGE
ICA Component 120
Age
ADHD
control
p = 0.0176

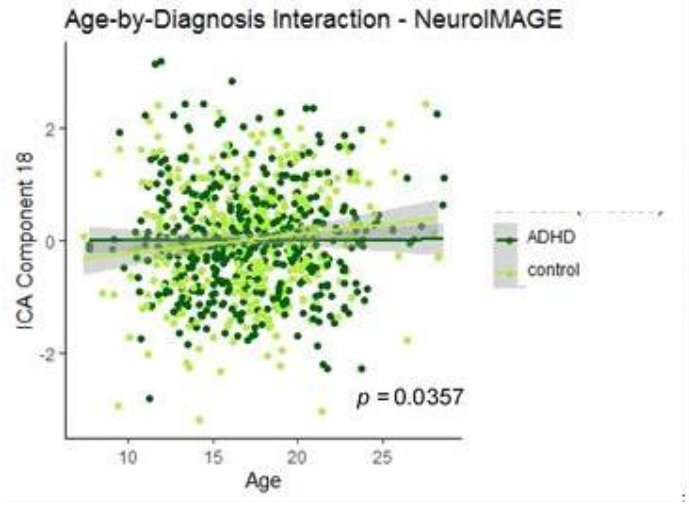

Age-by-Diagnosis Interaction - Brazilian IMpACT
ICA Component 120
Age
ADHD
control
p = 0.1794

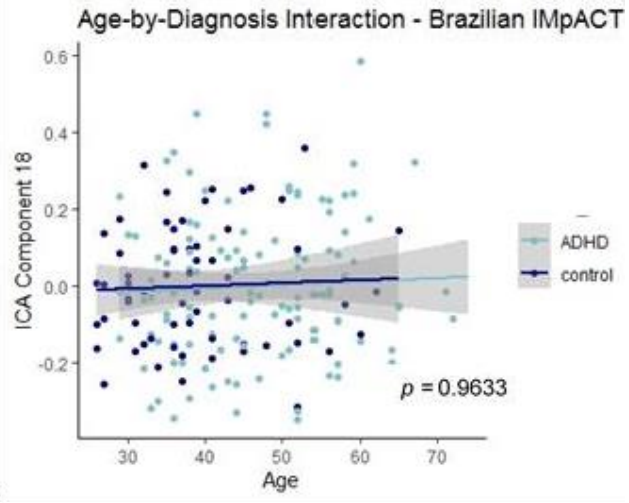

Age-by-Diagnosis Interaction - NeuroIMAGE
ICA Component 32
Age
ADHD
control
p = 0.0233


Age-by-Diagnosis Interaction - Brazilian IMpACT
ICA Component 32
Age
ADHD
control
p = 0.272


Age-by-Diagnosis Interaction - NeuroIMAGE
ICA Component 147
Age
ADHD
control
p = 0.0264


Age-by-Diagnosis Interaction - Brazilian IMpACT
ICA Component 147
Age
ADHD
control
p = 0.1317


Age-by-Diagnosis Interaction - NeuroIMAGE
ICA Component 18
Age
ADHD
control
p = 0.0357


Age-by-Diagnosis Interaction - Brazilian IMpACT
ICA Component 18
Age
ADHD
control
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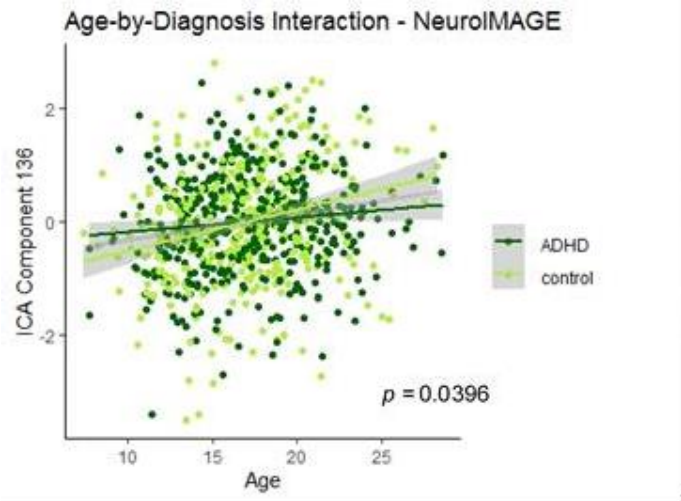
Age-by-Diagnosis Interaction - NeuroIMAGE
ICA Component 136
Age
ADHD
control
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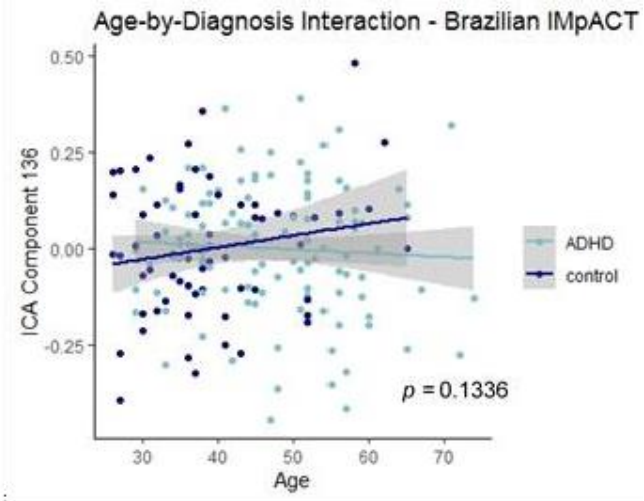
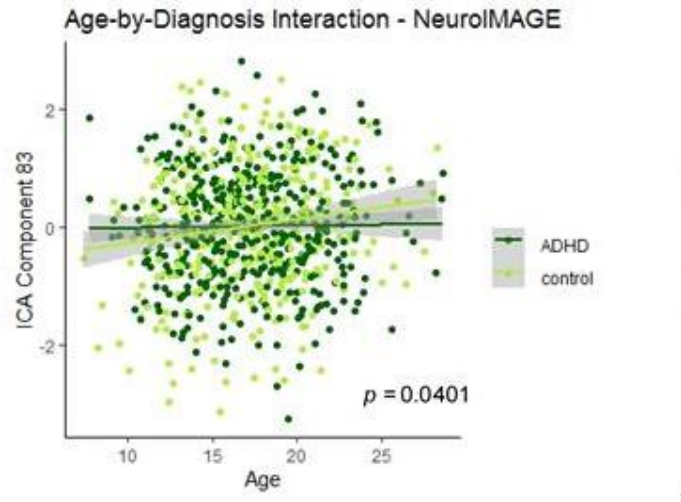
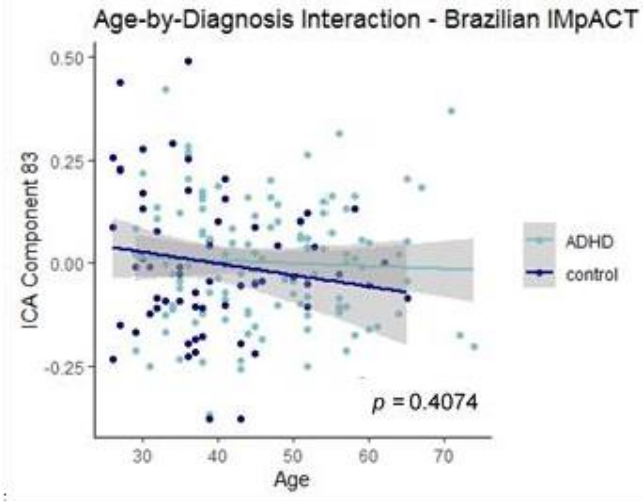
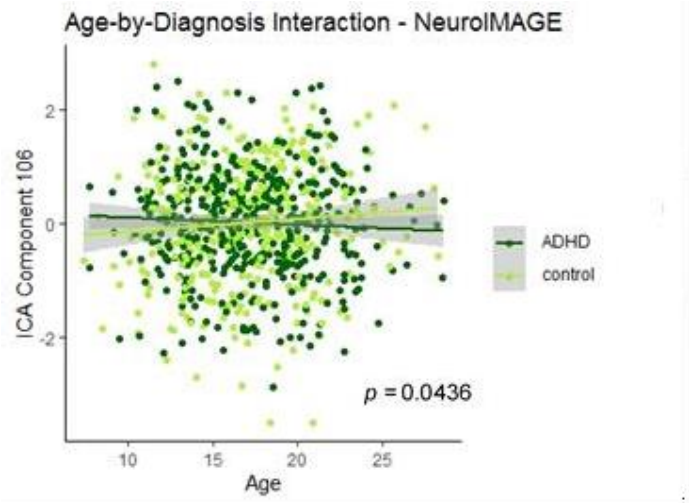
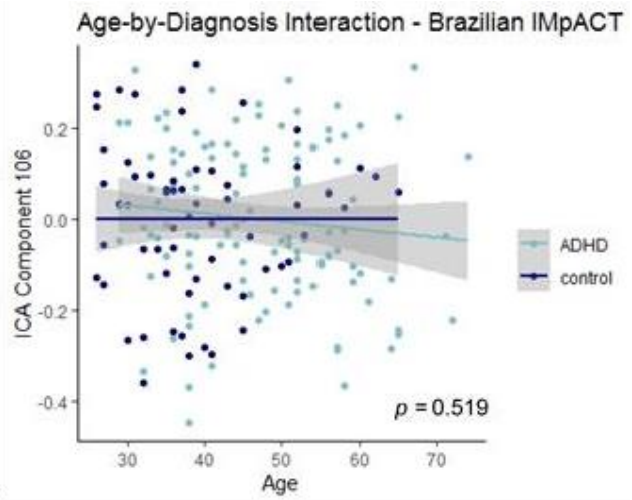
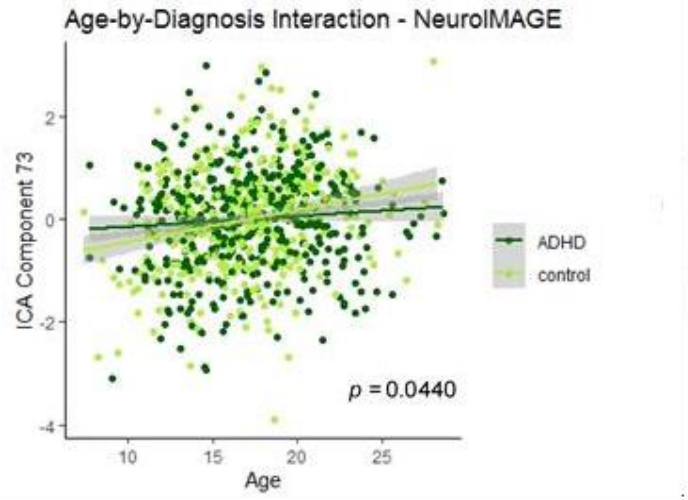

Age-by-Diagnosis Interaction - Brazilian IMpACT
ICA Component 136
Age
ADHD
control
p = 0.1336

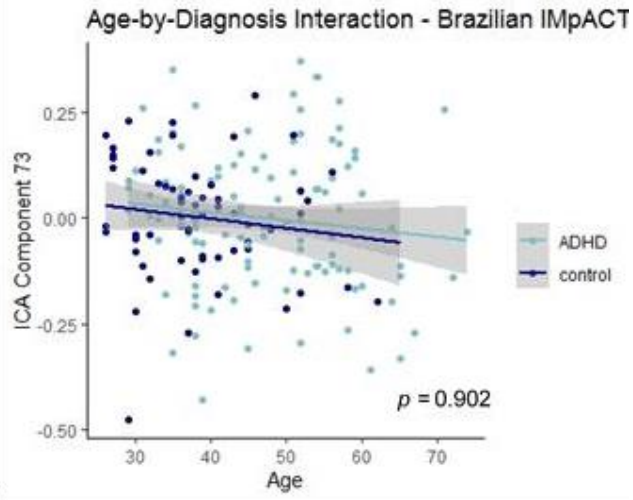

Age-by-Diagnosis Interaction - NeuroIMAGE
ICA Component 83
Age
ADHD
control
p = 0.0401

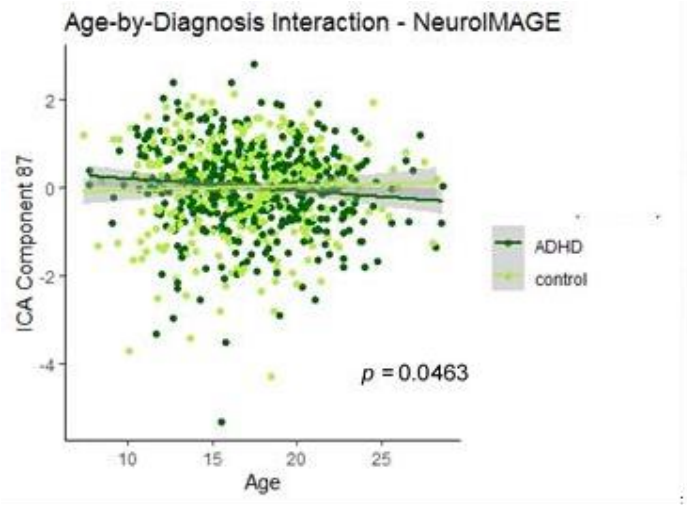

Age-by-Diagnosis Interaction - Brazilian IMpACT
ICA Component 83
Age
ADHD
control
p = 0.4074

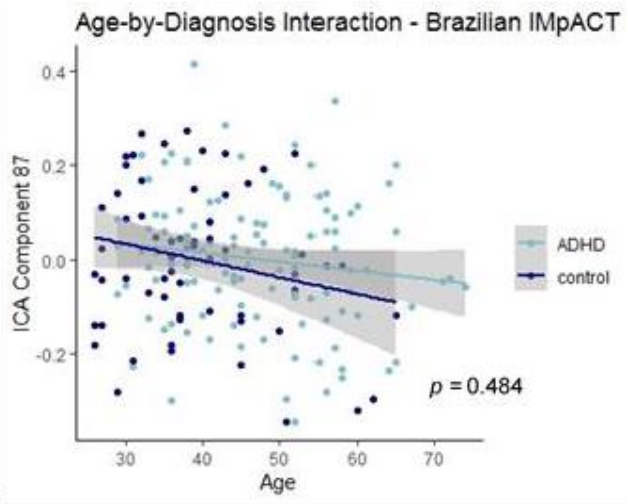

Age-by-Diagnosis Interaction - NeuroIMAGE
ICA Component 106
Age
ADHD
control
p = 0.0436

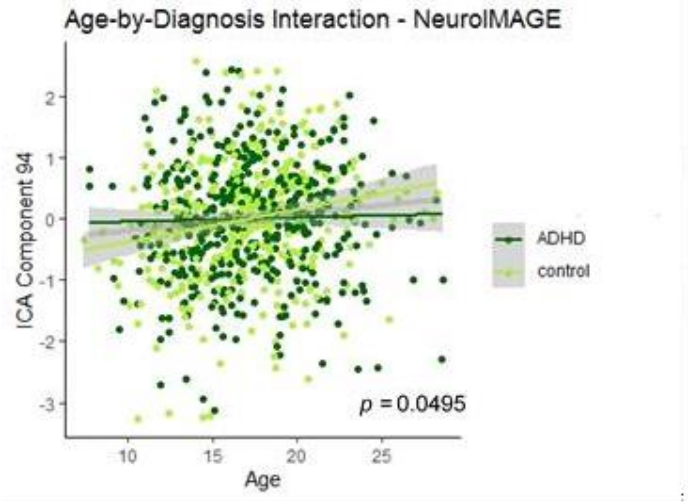

Age-by-Diagnosis Interaction - Brazilian IMpACT
ICA Component 106
Age
ADHD
control
p = 0.519

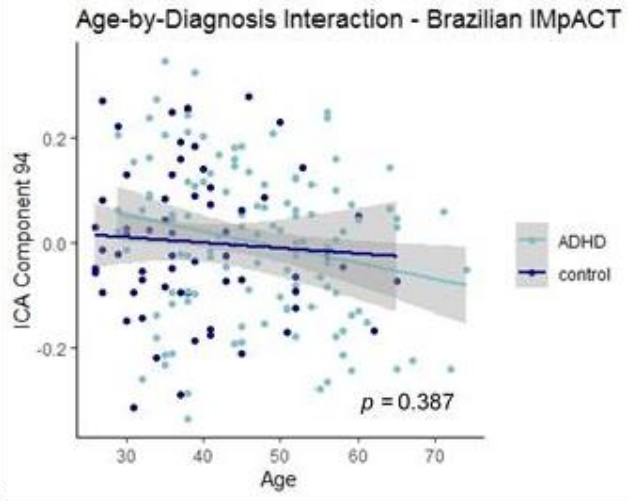

Age-by-Diagnosis Interaction - NeuroIMAGE
ICA Component 73
Age
ADHD
control
p = 0.0440


Age-by-Diagnosis Interaction - Brazilian IMpACT
ICA Component 73
Age
ADHD
control
p = 0.902


Age-by-Diagnosis Interaction - NeuroIMAGE
ICA Component 87
Age
ADHD
control
p = 0.0463


Age-by-Diagnosis Interaction - Brazilian IMpACT
ICA Component 87
Age
ADHD
control
p = 0.484


Age-by-Diagnosis Interaction - NeuroIMAGE
ICA Component 94
Age
ADHD
control
p = 0.0495


Age-by-Diagnosis Interaction - Brazilian IMpACT
ICA Component 94
Age
ADHD
control
p = 0.387

Capítulo IV

6. The broad *SYT1* role in psychiatry disorders in light of neuroimaging: a DTI study in adults with ADHD

Artigo em preparação, a ser submetido na revista Molecular Psychiatry.

Title: The broad *SYT1* role in psychiatry disorders in light of neuroimaging: a DTI study in adults with ADHD

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Abstract

Variants within the Synaptotagmin-1 encoding gene (*SYT1*) have been associated with a wide variety of psychiatric phenotypes, including attention-deficit/hyperactivity disorder (ADHD). ADHD is a complex neurodevelopmental disorder that affects multiple functioning domains, highly overlapping with other psychiatric disorders, and known to involve white matter abnormalities. Inferences about white matter microstructure can be made by diffusion-tensor imaging, which makes this method a promising tool in the understanding of biological changes that underpin cognitive development and brain disorders. Using a set-based analysis, the present study aims to investigate how genetic variants within *SYT1* might be affecting brain white matter at a cellular level using DTI in adults with ADHD (n = 85). The combined effect of all measured variation in the gene (i.e. 468 variants) was evaluated in relation to eleven specific white matter tracts. Set-based analysis was performed in PLINK software and followed by in silico analysis of all variants included in the study. We found *SYT1* associated with white matter changes in two important tracts: the inferior fronto-occipital fasciculus and the forceps major. In this sense, white matter abnormalities may be mediating, at least in part, the broad psychiatric previously observed effects of this gene. In addition, this study reported specific polymorphisms associated with brain features, several of which have already been pointed in GWAS studies.

Introduction

Overwhelming evidence suggests that disturbances in proteins involved in maintenance of the synapse structure, and in the neurotransmitter release may be common molecular mechanisms underlying neuronal dysfunction and cognitive deficits observed in psychiatric disorders and neurologic diseases (Bosiacki et al., 2019; Cupertino et al., 2016; Penzes et al., 2013; Van Spronsen and Hoogenraad, 2010). As evidenced for Attention Deficit/Hyperactivity Disorder (ADHD) and anxiety (Levy, 2004), schizophrenia (Stephan et al., 2006), compulsive behavior (Welch et al., 2007), addiction (Kauer and Malenka, 2007), Parkinson's (Calabresi et al., 2006) and Alzheimer's diseases (Selkoe, 2002).

One of the key regulators of neurotransmitter release is Synaptotagmin-1, which is essential for the correct spatial and temporal regulation of the synaptic vesicle-mediated exocytosis, acting as a calcium sensor to induce the fusion of the presynaptic vesicle with the plasma membrane through the soluble N-ethilmalemide-sensitive factor attachment protein receptors (SNARE) complex binding (Südhof, 2013). Synaptotagmin 1 is abundantly expressed in the whole brain, especially in the frontal cortex. Besides its crucial role in neurotransmitter exocytosis, it is involved in axon branching (Greif et al., 2013), and regulation of the neuronal polarity and axon differentiation (Inoue et al., 2015).

Cellular scale changes in the number of axons, axon diameter, packing density of fibers, axon branching, axon trajectories, and myelination usually reflects in impairment in the white matter (Zatorre et al., 2012), which may lead to disturbances in the neural communication. Disrupted structural connectivity often results in abnormal functional interactions, and such alterations can be detected by diffusion-tensor imaging (DTI) studies

(Cullen et al., 2010; Korgaonkar et al., 2014). DTI is a promising tool in the understanding of biological changes that underpin cognitive development and brains disorders, trough water molecules properties (Alexander, Lee, Lazar, & Field, 2007) it allows inferences about white matter microstructure (i.e. at cellular level). Fractional anisotropy (FA) is the most common measure used in DTI studies, which is based on the water anisotropy along the axon (Beaulieu, 2002), where lower FA values reflects isotropic diffusion, related to reduced white matter integrity.

Abnormalities in the white matter integrity is suggested as an early indicator of brain pathology, with studies suggesting that reduced FA may precede tissue loss (Hugenschmidt et al., 2008; Pfefferbaum and Sullivan, 2002). A study combining post-mortem DTI and histopathology showed that FA abnormalities are associated with axonal degeneration (Preziosa et al., 2019). Imaging studies have demonstrated white matter alterations as a critical substrate in the pathophysiology of ADHD (Chen et al., 2016; Francx et al., 2015; van Ewijk et al., 2014), one of the most common neurodevelopmental disorder worldwide, which is characterized by symptoms of inattention and/or hyperactivity. Such symptomatology is manifested, for instance, by wandering off task, lacking persistence, difficulty sustaining attention, extremely restless and impulsive behavior, talkativeness (American Psychiatric Association, 2013). ADHD is also often accompanied by mild delays in language, motor, or social development, with individuals presenting cognitive problems on tests of attention, executive function, or memory (American Psychiatric Association, 2013).

There is a high genetic correlation among psychiatric disorders, with genes being associated with several phenotypes (Consortium et al., 2018; Cross-Disorder, 2019). Genes involved in the neurotransmitter exocytosis have been extensively investigated in psychiatric

disorders due to their critical role in neurotransmission (Cupertino et al., 2016). Of note, variants within the Synaptotagmin-1 encoding gene (*SYT1*) have been associated with a wide variety of psychiatric phenotypes. An effect of the *SYT1* gene has been pointed in genome wide association studies (GWAS) related with neuroticism (Luciano et al., 2018; Nagel et al., 2018a, 2018b) and its domains of irritability and fed-up feelings (Nagel et al., 2018a, 2018b), cognitive performance and educational attainment (Lee et al., 2018), and schizophrenia (Pardiñas et al., 2018). In addition, a copy number variation study found a segment of the *SYT1* gene implicated in autism spectrum disorder (Szatmari et al., 2007), and candidate gene association studies showed *SYT1* variants implicated with risk to cocaine use disorder (da Silva et al., 2019; Fernandez-Castillo et al., 2012) and cognitive performance variations among women with cocaine use disorder (Viola et al., 2019). Other associations regards ADHD and its related phenotypes, such as susceptibility to ADHD in adults (Cupertino et al., 2017; Sánchez-Mora et al., 2013), early impairment of its symptoms (Cupertino et al., 2017), treatment response (da Silva et al., 2017) and antisocial personality disorders in adults with ADHD (Cupertino et al., 2017). Apart from its broad influence in psychiatric phenotypes, GWAS studies suggest that *SYT1* may also influence the overall intracranial volume in health individuals (Adams et al., 2016).

The overall evidence suggests that *SYT1* may represent a common biological factor underpinning different psychiatric phenotypes, such as ADHD, which is known to involve white matter abnormalities. Therefore, the present study aims to investigate how variants within this gene might be affecting brain white matter at a cellular level using DTI in adults with ADHD. We used a set-based analysis to test the combined effect of all measured variation in the gene for an association with FA in specific tracts.

Material and Methods

Sample

Participants (n = 85, mean age 46.6; 43.5% males) were recruited in the adult division of the ADHD Outpatient Clinic (PRODAH-A) from Hospital de Clínicas de Porto Alegre. ADHD diagnosis was based on DSM-5 diagnostic criteria (American Psychiatric Association, 2013) and performed by experienced psychiatrists using a semi-structured interview (KSADS – E) (Grevet et al., 2005). Exclusion criteria were evidence of clinically significant neurological disease (e.g., delirium, dementia, epilepsy, head trauma) and intelligence quotient (IQ) ≤ 70.

Image acquisition and processing

Diffusion-weighted images were acquired using a 3T Siemens Spectra scanner with an 8-channel head coil using a single-shot echo planar imaging sequence (62 contiguous axial slices, TR/TE=11000/110ms, voxel size=2x2x2mm, slice thickness=2.0mm, FOV=240mm, one b0 image and 64 diffusion-weighted images with gradient directions b=1400s/mm^2). Among those 85 participants, 15 restless or claustrophobic individuals were scanned using an adapted protocol with reduced acquisition time (voxel size=2.4x2.4x2.4mm, slice thickness=2.4mm TR/TE=11000/106ms, 32 diffusion-weighted images, other parameters remained the same). All participants signed an informed consent form approved by the hospital ethical committee (IRB 0000921). Images were corrected for motion and eddy currents and preprocessed using standard FMRIB Software Library (FSL) tools (https://fsl.fmrib.ox.ac.uk/fsl/), followed by visual quality control. Whole-brain FA maps for each individual were calculated using *dtifit* in FSL. Mean FA values within the TBSS skeleton

were extracted for whole brain and for 11 tracts (anterior thalamic radiation - ATR, corticospinal tract - CST, dorsal cingulate gyrus - CING, ventral cingulate gyrus - HIPPCING, forceps minor, forceps major, inferior fronto-occipital fasciculus - IFOF, inferior longitudinal fasciculus - ILF, superior longitudinal fasciculus - SLF, uncinate fasciculus – UF, temporal part of SLF - SLFTEMP) according to the John Hopkins University (JHU) white-matter tractography atlas (Mori et al., 2007; Wakana et al., 2007) (https://identifiers.org/neurovault.image:1403). Evaluated tracts are shown in **Supplementary Figure S1**.

DNA extraction and genotyping

Blood was collected from all participants, and DNA was extracted using salting out. Samples were genotyped using Illumina Infinium PsychArray-24 v1.1. Pre-imputation quality control at individual and SNP level, principal component analyses for ancestry genetic outlier detection, and identification of related and/or duplicated individuals and gender discrepancies were performed using the Rapid Imputation and COmputational PIpeLIne (Ricopili). Phasing of genotype data was performed using SHAPEIT2 algorithm, and imputation was performed with IMPUTE2 considering as reference the European ancestry panels of the 1000 Genomes Project Phase 1 version 3 (v3) (April 2012) from the genome build hg19.

Statistical analysis

In order to simultaneously test all measured variation in the *SYT1* gene (468 SNPs) the *set-based* test was performed with an additive genetic model in PLINK software, performing a standard single SNP analysis and selecting for the set *N* "independent" SNPs

(linkage disequilibrium $R^2 < 0.5$, max 5 SNPs) associated with the outcome ($p < 0.05$); the statistic for the whole set is calculated as the mean of these single SNP statistics (gene-wide analysis). Analyses considered a flanking region of 2kb upstream and 1kb downstream, and were adjusted for age, gender, head motion, and the first ten principal components. Ten thousand permutations were performed, followed by False discovery rate (FDR) to correct set *p-values* per number of tracts evaluated.

In silico analysis and open databases

Predictions tools and open databases were used in order to investigate possible functions and previous associations of polymorphisms within the *SYT1* gene. HaploReg (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php), RegulomeDB (http://www.regulomedb.org/#dbsnp_example), Provean (http://provean.jcvi.org/index.php), Genotype-Tissue Expression (GTex - https://gtexportal.org/home/) and GWASatlas (https://atlas.ctglab.nl) were used for this purpose.

HaploReg v4.1 (Ward and Kellis, 2012) is a tool for exploring annotations of the genome variants, regarding chromatin states (from DNAse and histone ChIP-Seq), sequence conservation across mammals, and the effect of SNPs on regulatory motifs. Chromatin states may indicate a biologically plausible enrichment for cell type-specific enhancers. Regulatory motifs alterations are based on position weight matrices, scored on genomic sequences. RegulomeDB (Boyle et al., 2012) identifies regulatory elements, computing a score ranging from 1 to 7 assessing the evidence for regulatory potential, where lower values means strong evidence for regulatory potential. Provean predicts the effect of amino acid substitution on the biological function of a protein (Choi and Chan, 2015).

GTex is a database to enable the identification of genetic variation that influence gene expression, providing data on quantitative trait locus (eQTLs). In this analysis, a p-value and normalized effect size (NES) of the eQTLs is attributed to each SNP. NES is a slope of the linear regression computed in a normalized space with no direct biological meaning. GWASatlas (Watanabe et al., 2019) is a database of available GWAS summary statistics, it can provide a PheWAS for a SNP, with information regarding all phenotypes associated with this specific SNP.

Results

Gene-wide analysis showed that the *SYT1* gene was associated with three out of eleven evaluated tracts: bilateral inferior fronto-occipital fasciculus (P_{SYT1}= 0.008, P_{SYT1}-corrected 0.038), forceps major (P_{SYT1} = 0.005, P_{SYT1}-corrected 0.038) and nominally associated to superior longitudinal fasciculus (P_{SYT1} = 0.020, P_{SYT1}-corrected = 0.066). Moreover, all evaluated tracts presented associations with individual polymorphisms of *SYT1* gene (permutated $P_{polymorphism} < 0.05$). Association results of overall gene and independent polymorphisms are depicted in **Table 1**, and regional visualization of *SYT1* results for each outcome can be found in **Supplementary Figure S2** (designed at LocusZoom - Pruim et al., 2011)**.** In addition, the effect of each polymorphism within the gene in relation to specific tracts are shown in **Supplementary Table S1**.

In silico analysis

Predicted functions and previous implications in GWAS studies (P<0.0001) for each polymorphism included in this study can be found in **Supplementary Table S1**, with

information based on RegulomeDB, HaploReg, GTex, GWAS atlas. For instance, 24 polymorphisms were predicted to bind to other proteins, 41 to be implicated in acetylation/methylation, 26 eQTLs in brain tissues and several implicated with psychiatric phenotypes in GWAS studies. Provean database identified only one SNP (rs2037743) in a protein coding region, with a neutral or tolerated effect.

These results were from data available up to October 22nd and may change since these platforms are always improving.

Discussion

The present study sought to explore the influence of *SYT1* variation in light of brain changes in adults with ADHD, which may represent a common biological factor underpinning different psychiatric phenotypes. Taking into account all genetic variation within the *SYT1* gene, this study showed *SYT1* associated with white matter microstructure in the inferior fronto-occipital fasciculus and the forceps major, part of the corpus callosum, two important tracts previously implicated in a wide variety of behaviors. Also, although other tracts were not significantly associated in the gene-wide analysis, most of them presented several associated polymorphisms with FA at a single SNP level. Understanding the molecular underpinnings of neuronal networks may provide new insights into the biology behind psychiatric disorders and reveal novel therapeutic targets to treat them.

The corpus callosum is the largest white matter fiber bundle in the human brain, which connects the left and right cerebral hemispheres, having a critical role integrating and transferring information to process sensory, motor, and high-level cognitive signals (Goldstein and Mesfin, 2019). According to histological characteristics, it is divided by the rostrum, genu,

body, and splenium (Goldstein and Mesfin, 2019). The fibers of the splenium run posteriorly and contribute to the forceps major. The forceps major (also known as posterior forceps of the corpus callosum) then connects the occipital lobe and crosses the midline, running in a U-shape. It communicates somatosensory information between the two halves of the parietal lobe and the visual cortex at the occipital lobe (Hofer and Frahm, 2006).

White matter abnormalities in the forceps major have been implicated with a wide range of cognitive behaviors, psychiatric disorders, and neurological diseases. For instance, diffusion parameters in this tract were associated with working memory and IQ scores (Giedd, 2004; Giorgio et al., 2010; Nagy et al., 2004), control over impulses (Stoica et al., 2019), executive function (Bian et al., 2018), visual working memory (Krogsrud et al., 2018), and facial emotion perception in schizophrenia patients (Zhao et al., 2017). White matter microstructures abnormalities in forceps major were also associated with obsessive-compulsive disorder (Watanabe et al., 2018), major depression (Shen et al., 2017; Won et al., 2017), gambling and cocaine use disorder (Yip et al., 2017), post-traumatic stress disorder (Li et al., 2016), ADHD (Lin et al., 2018), childhood trauma in schizophrenia patients (Asmal et al., 2019; Cancel et al., 2019), anxiety in depressive patients (Xia et al., 2018), headache in migraine patients (Chong et al 2019), sleeping problems (Mulder et al., 2019). Moreover, DTI studies reported changes in multiple white matter measures in the forceps major preceding symptom onset in Alzheimer's disease (Caballero et al., 2018), concurring studies have shown lower white matter integrity in this region associated with subjective cognitive decline (the earliest stage on the continuum toward Alzheimer's disease) (Ohlhauser et al., 2019), and mild cognitive impairment (Wen et al., 2019). Changes in this tract were also implicated in Friedreich's ataxia, another neurodegenerative disorder, (Vavla et al., 2018).

Inferior fronto-occipital fasciculus is the longest associative bundle, which is a direct pathway connecting the temporo-occipital lobe and the superior parietal lobe to the frontal lobe, integrating auditorium and visual association cortices with the prefrontal cortex (Schmahmann and Pandya, 2007). Details about its anatomy and function are under debate, but it is thought to play a role in spatial aspects of cognitive processing, such as attention (Mesulam, 1981; Posner et al., 1984) and working memory (Petrides et al., 2012), in language networks of semantic processing (Cocquyt et al., 2019), and in movement planning (Caverzasi et al., 2014). It is noteworthy the high complexity of this tract, a diffusion study suggested that inferior fronto-occipital fasciculus can be segmented into five subcomponents according to distinct anatomical characteristics and functionality (Wu et al., 2016): (I) The polar part of the frontal lobe associated with many aspects of complex cognitive functions, such as social cognition, attention, multitasking, and episodic memory; (II) Connects the orbitofrontal cortex with the parietal and occipital cortices, and is often implicated in obsessive-compulsive disorder, which presents insufficient cognitive-behavioral flexibility, executive function deficits and alteration in decision-making; (III) Runs through the external capsule, connecting occipital, parietal, and posterior temporal regions to the Broca's areas, being linked with language processing issues; (IV and V) Perhaps the most debatable, they play a role in semantic processing of language, visual conceptualization, and recognition.

Studies comparing several major white matter tracts in human and macaque identified the inferior fronto-occipital fasciculus as one of the major differences between the species, suggesting that macaque had no equivalent structure (Martino et al., 2010; Schmahmann and Pandya, 2009; Takemura et al., 2017). In this sense, this bundle may be related to evolutionary changes underlying unique human behavioral repertoire and abilities

(Forkel et al., 2014; Thiebaut de Schotten et al., 2012). This may also be related to the much greater size of the human brain (15 fold the volume of macaque), where the human brain needs longer association tracts to connect distant regions, whereas short association fibers were enough to 'evolutionary old areas' communicate (Deacon, 1990).

Given the broad role of the inferior fronto-occipital longitudinal fasciculus in several domains, it is not surprising that this major associative bundle has been implicated with cognitive performance, psychiatric disorders, and neurological diseases. It has been implicated in reading performance (Cheema and Cummine, 2018; Rollans et al., 2017), verbal fluency (ability to generate words quickly and efficiently according to predefined phonological or semantic criteria) (Blecher et al., 2019), visual working memory (Krogsrud et al., 2018), and cognitive scores (comprehension scores) (Kato et al., 2019). White matter microstructures abnormalities in this structure have also been observed in early Parkinson's Disease (Li et al., 2018), subjective cognitive decline (Ohlhauser et al., 2019), high-function autism (Im et al., 2018) and autism spectrum disorder (Hattori et al., 2019), early adulthood smokers (Wang et al., 2017), depression (Wang et al., 2019), anxiety trait in healthy individuals (Lu et al., 2018), auditory verbal hallucinations in schizophrenia (Zhang et al., 2018), childhood trauma in schizophrenia (Asmal et al., 2019; Cancel et al., 2019) and in healthy young males (Tendolkar et al., 2018).

Although all variants associated in this study are intronic and have no clear functionality described, several have already been pointed in GWAS studies. For instance, the rs1732664 SNP associated with white matter microstructure in inferior fronto-occipital fasciculus in our study, presented previous suggestive evidence of association with cognitive performance ($P = 2.41e^{-7}$) (Lee et al., 2018), verbal-numerical reasoning ($P = 8.32e^{-6}$) (Davies

et al., 2018), educational attainment ($P = 3.34e^{-5}$) (Lee et al., 2018) and schizophrenia ($P = 1.42e^{-4}$ and $1.3e^{-3}$) (Pardiñas et al., 2018; Ripke et al., 2014); and the rs1245829, here associated with dorsal cingulate gyrus, was previously associated with cognitive performance ($P = 1.77e^{-14}$) and educational attainment ($P = 3.51e^{-10}$) (Lee et al., 2018). In addition, most variants associated with forceps major and inferior fronto-occipital fasciculus (significantly associated tracts in the gene-wide analysis) are predicted to affect regulatory motifs, some to be in conserved regions or to act as enhancer histone markers in brain tissues (**Supplementary Table S1**). The region where rs1405492, associated with inferior fronto-occipital fasciculus, is located is thought to bind the protein FOXA2 (Hepatocyte nuclear factor 3-beta), a transcription factor involved in embryonic development and tissue-specific gene expression. In embryonic development, it is required for notochord formation, which in turn plays an integral role in the development of the neural tube. Considering all variants, despite none scored 1 (), 21 SNPs scored more than 3b in RegulomeDB (i.e., 2a-3b), indicating a "likely to affect binding" evidence.

This study has to be considered in light of some strengths and limitations. The sample size of this study is relatively small for genomic analysis; however, the approach applied here has increased statistical power when compared to genome-wide testing, and it is important to consider the difficulties and costs involved in the acquisition of neuroimaging data. Neuroimaging studies often have smaller sample sizes, and evaluate fewer variables, nonetheless, combining the effect of all genetic variation in the gene allows unbiased identification of SNPs. Psychiatry disorders and behaviors are complex phenotypes in which multiple variants are involved. Even so, these findings need further replications to be

confirmed. Also, a specific-tract analysis as performed here restricts the findings to these structures, where a voxel-wise approach would allow to identify other areas. However, such approach would not be feasible to consider all genetic variation within the gene (468 variants). Yet, to affirm that Synaptotagmin-1 affects the white matter microstructure is beyond the scope of this paper, present results suggest the influence of the *SYT1* genetic variations on brain features that are also implicated in psychiatric disorders. Further biochemical and physiological studies can help to understand mechanism underlying this effect.

In a nutshell, our findings showing the association of *SYT1*, considering the combined effect of all genetic variation within the gene, with microstructural changes in two substantial white matter tracts suggests that white matter abnormalities may be underpinning the *SYT1* role in ADHD. In this sense, white matter abnormalities may be mediating, at least in part, the broad effects previously observed of this gene on psychiatric disorders. In addition, this study reported specific polymorphisms associated with brain features, which are good candidates to be investigated in other studies.

Disclosures

Eugenio Grevet was on the speaker's bureau for Novartis and Shire for the last 3 years. He also received travel awards (air tickets and hotel accommodations) for participating in two psychiatric meetings from Shire and Novartis. Barbara Franke has received educational speaking fees from Medice.

Acknowledgments

This study was financed by Conselho Nacional de Desenvolvimento Científico e Tecnológico (Grants 476529/2012-3, 466722/2014-1 and 424041/2016-2), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and FIPE-HCPA 160600. Renata B Cupertino was supported by International Brain Research Organization-Latin America (IBRO LARC) Exchange Fellowship.

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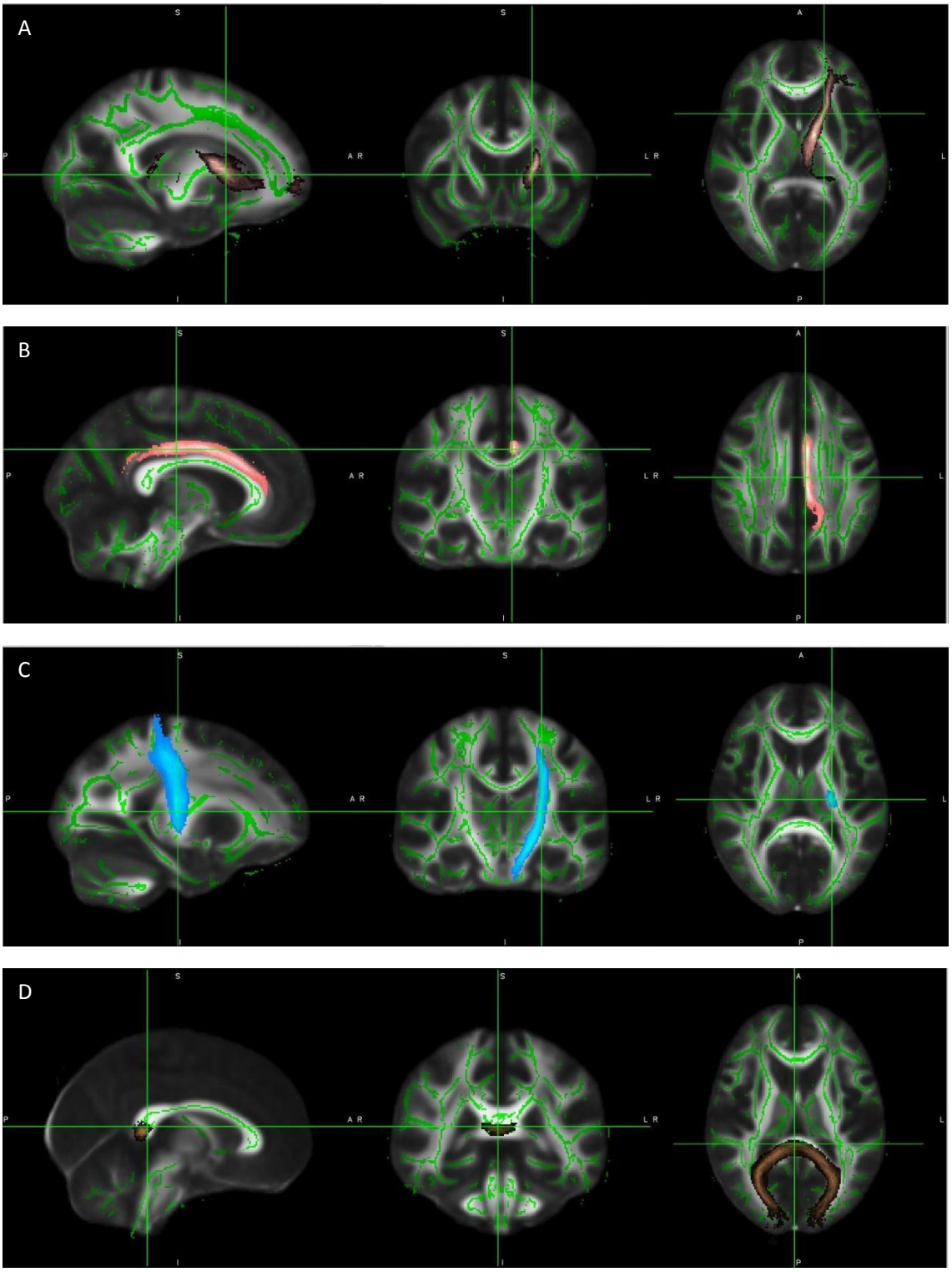
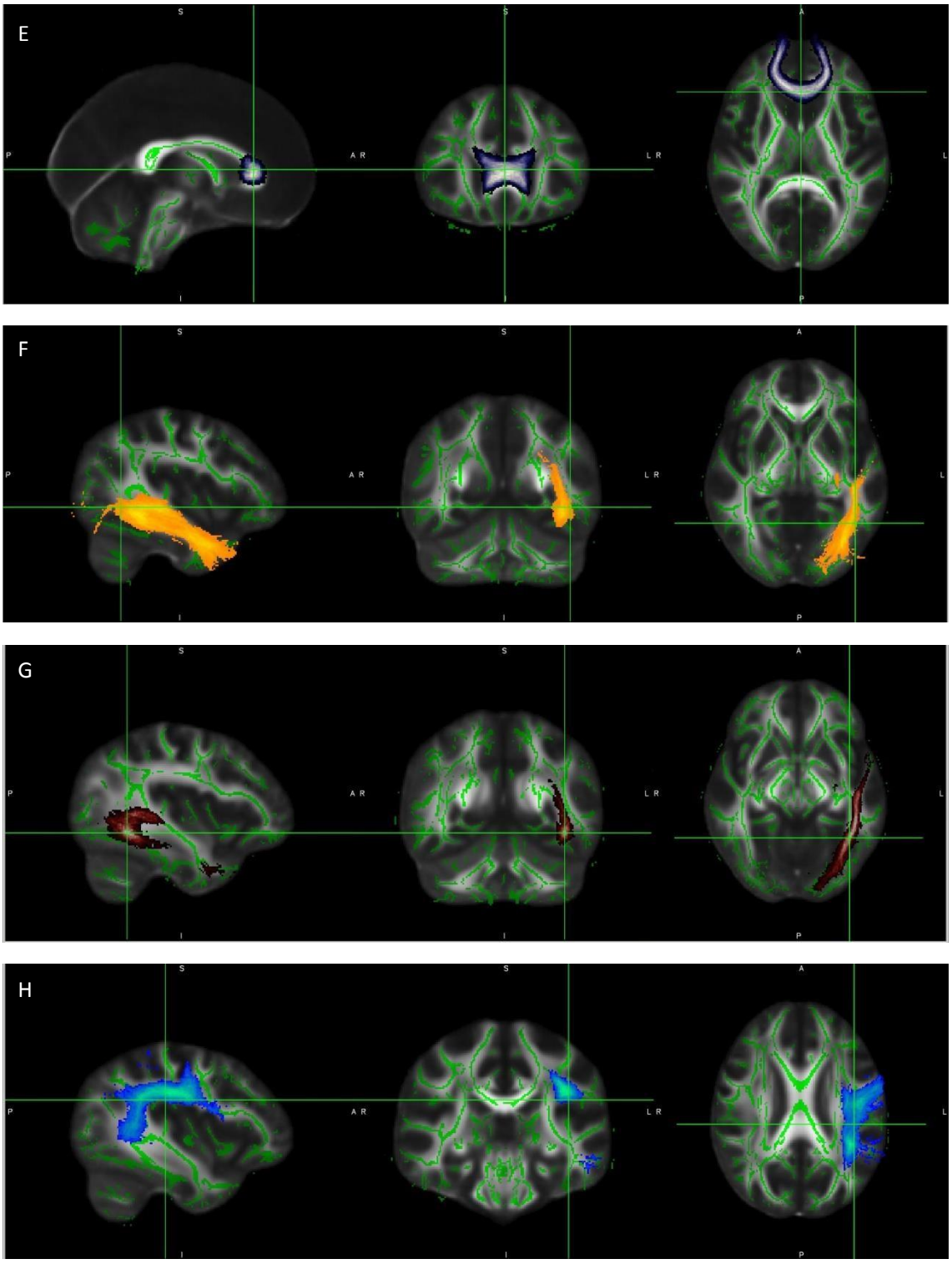
Table 1. SNP-set analysis of the *SYT1* gene (n = 468 variants) on FA values in specific tracts.

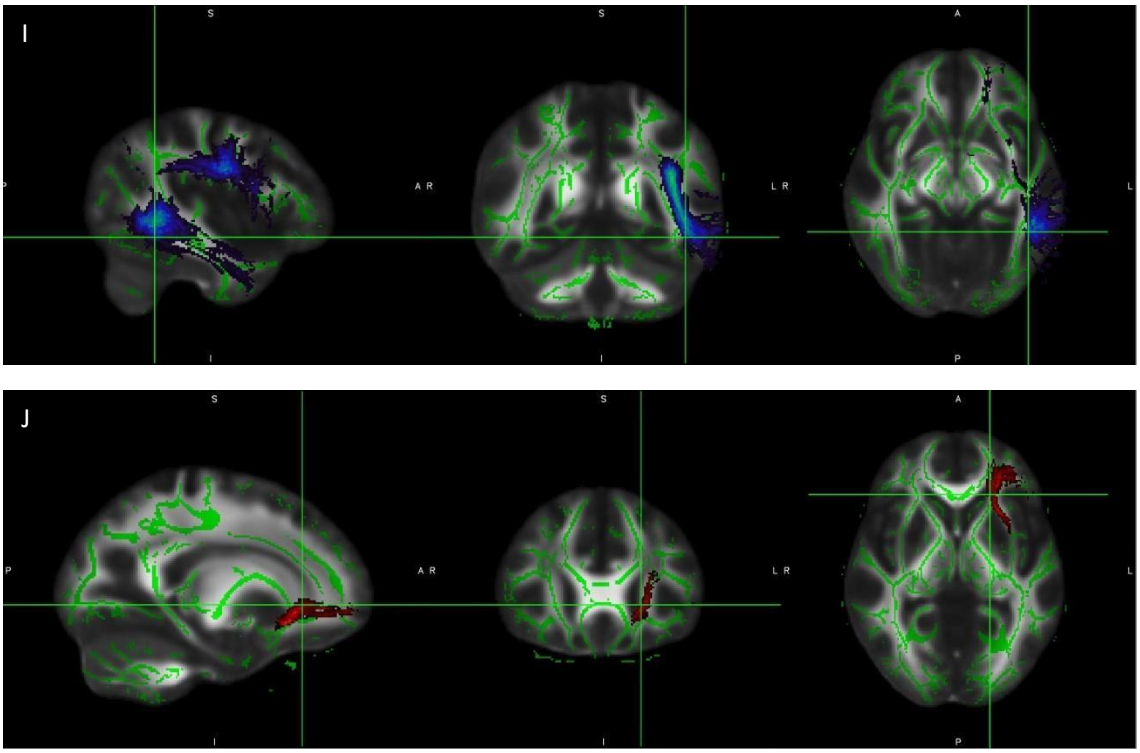
Tracts (bilateral)	N significant polymorphisms	N independent polymorphisms	Independent polymorphisms ID	Polymorphism P-value[a]	Independent polymorphisms location (hg19)	Gene-wide permuted P- value	Gene-wide FDR corrected P-value[b]
Anterior thalamic radiation (ATR)	63	1	rs4445702	**0.008**	12:79806574	0.091	0.253
Corticospinal tract (CST)	24	2	chr12_79341186_D rs7971881	**0.017** **0.022**	12: 79341186 12:79781339	0.319	0.492
Dorsal cingulate gyrus (CING)	67	4	rs7971881 chr12_79341186_D rs1245829 rs7966962	**0.009** **0.017** **0.018** **0.047**	12:79781339 12: 79341186 12:79648170 12:79779417	0.301	0.492
Ventral cingulate gyrus (CINGHIPP)	87	5	rs10778271 rs2895690 rs11608808 rs17046119 rs10861133	**0.009** **0.015** **0.027** **0.031** **0.041**	12:79271359 12:79263070 12:79796144 12:79357962 12:79294033	0.366	0.498
Forceps major	32	1	chr12_79341186_D	**0.001**	12: 79341186	**0.005**	**0.038**
Forceps minor	4	3	chr12_79341186_D rs4278572 rs1514923	**0.027** **0.028** **0.043**	12: 79341186 12:79391433 12: 79443825	0.541	0.531
Inferior fronto-occipital fasciculus (IFOF)	83	3	chr12_79341186_D rs7971881 rs1732664	**7.8e-06** **0.007** **0.044**	12: 79341186 12:79781339 12:79747487	**0.008**	**0.038**
Inferior longitudinal fasciculus (ILF)	15	2	rs1732664 rs1527079	**0.025** **0.028**	12:79747487 12:79341146	0.458	0.531
Superior longitudinal fasciculus (SLF)	96	3	chr12_79341186_D rs7971881 rs4278572	**0.001** **0.002** **0.013**	12: 79341186 12:79781339 12:79391433	**0.020**	0.077
Uncinate fasciculus (UF)	23	2	rs4278572 rs1527079	**0.029** **0.031**	12:79391433 12:79341146	0.502	0.531
Temporal portion of SLF (SLFTEMP)	6	1	rs11114027	**0.013**	12:79775248	0.191	0.433

SNP: Single Nucleotide Polymorphism; FDR: False Discovery Rate. Significant P-values are represented in bold. [a]Permuted P-values (10000 permutations). [b]FDR correction for the multiple phenotypes tested. Analyses adjusted for sex, age, head motion and first 10 principal components.

Supplementary Figure S1. Specific tracts evaluated

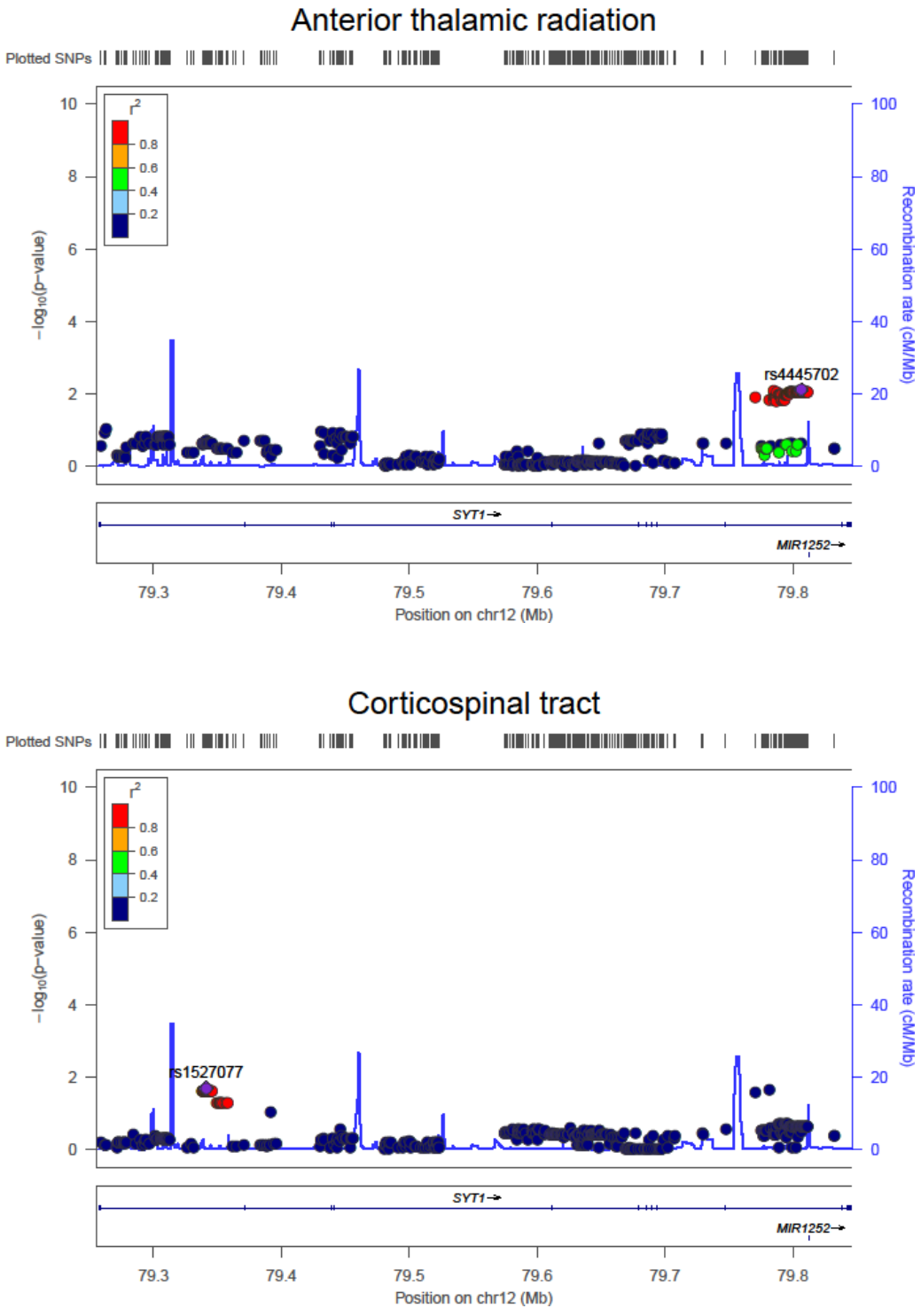


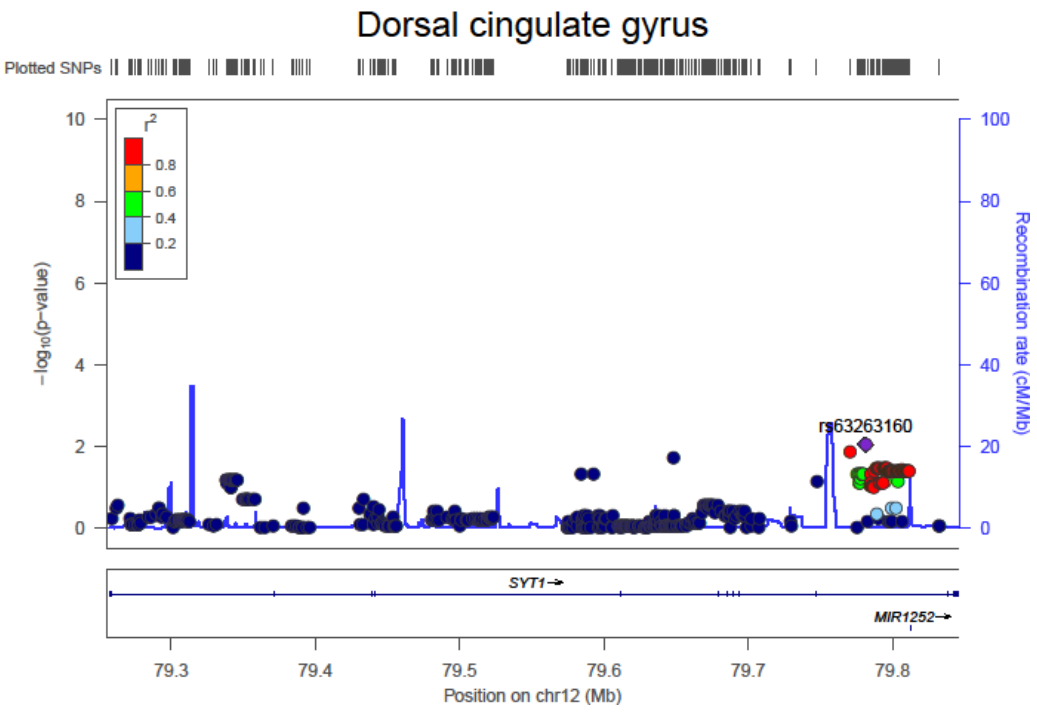

E
F
G
H

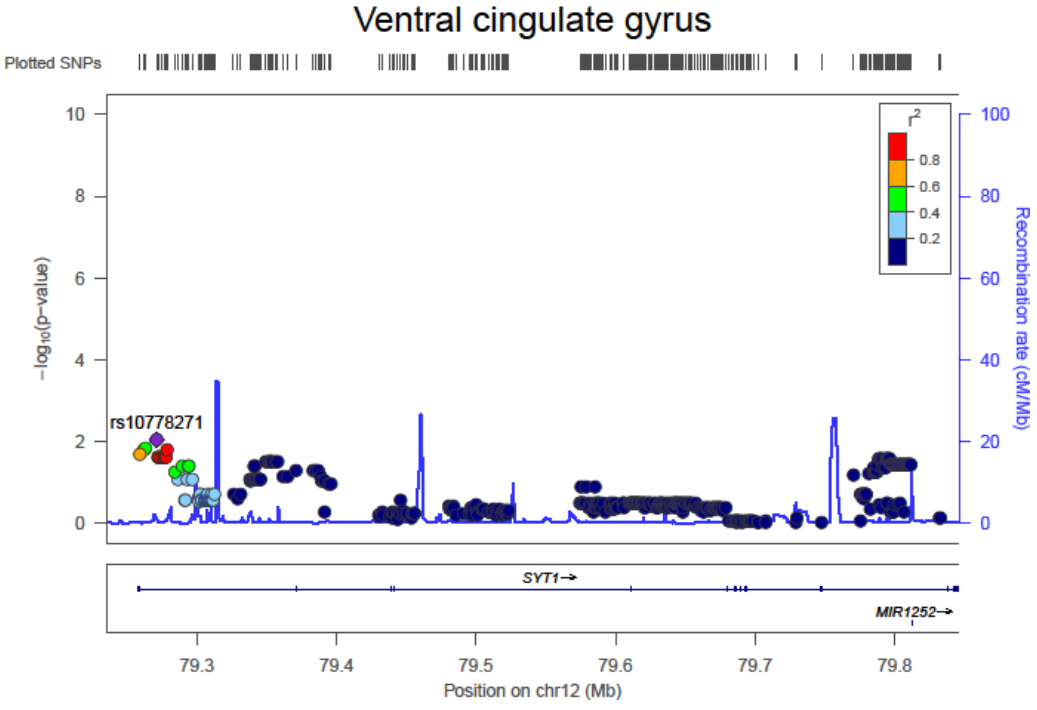


Representation of the evaluated tracts in sagittal (left), coronal (middle) and axial (right) views and the mean fractional anisotropy (skeleton) on top of MNI152 background image. **A.** Anterior thalamic radiation; **B.** Cingulate gyrus (analyzed in ventral and dorsal); **C.** Corticospinal tract; **D.** Forceps major; **E.** Forceps minor; **F.** Inferior fronto-occipital fasciculus; **G.** Inferior longitudinal fasciculus; **H.** Superior longitudinal fasciculus; **I.** Uncinate fasciculus; **J.** Temporal portion of the superior longitudinal fasciculus.

Supplementary Figure S2. Region plots of the *SYT1*gene with the evaluated areas.




Dorsal cingulate gyrus
Plotted SNPs
r²
0.8
0.6
0.4
0.2
rs63263160
−log10(p−value)
Recombination rate (cM/Mb)
SYT1
MIR1252
79.3
79.4
79.5
79.6
79.7
79.8
Position on chr12 (Mb)


Ventral cingulate gyrus
Plotted SNPs
r²
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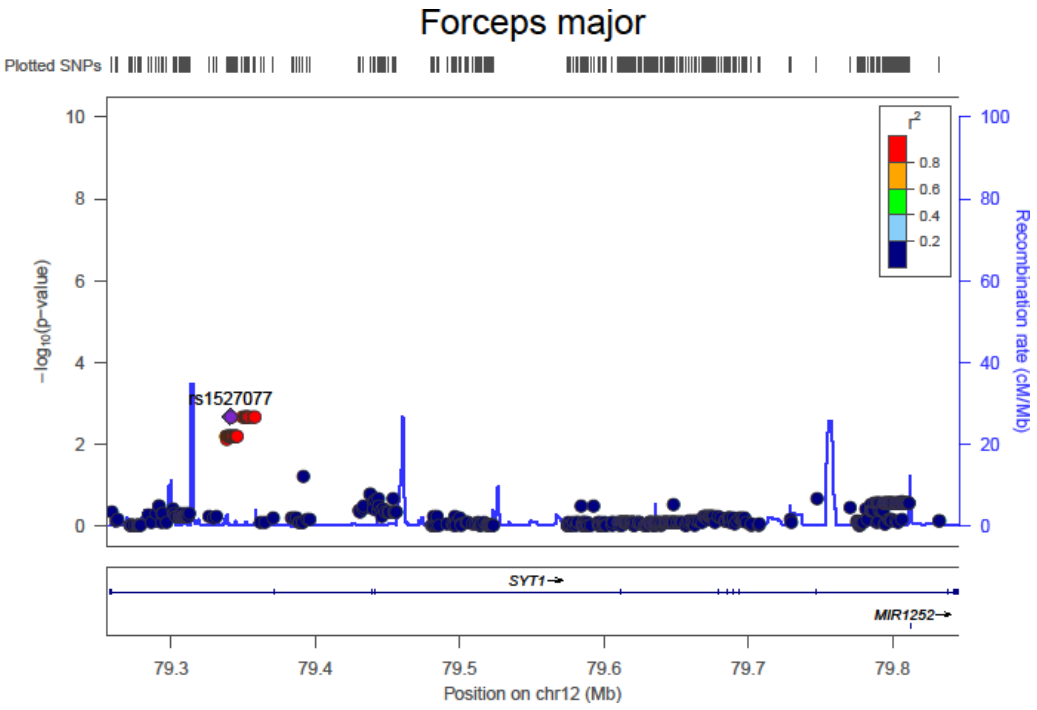
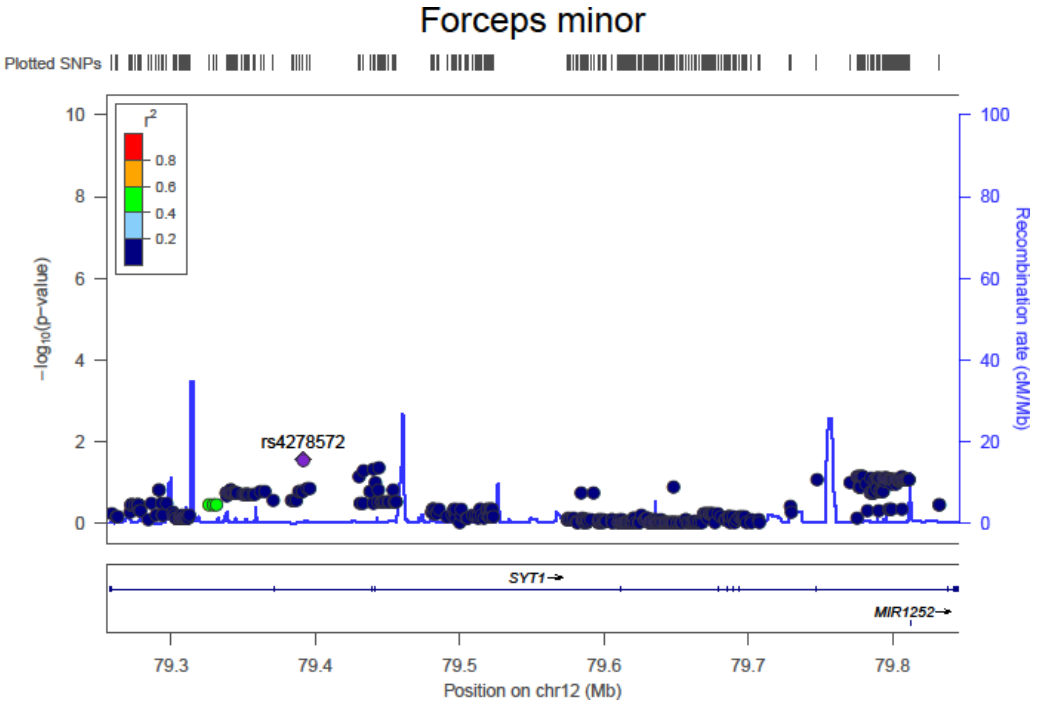
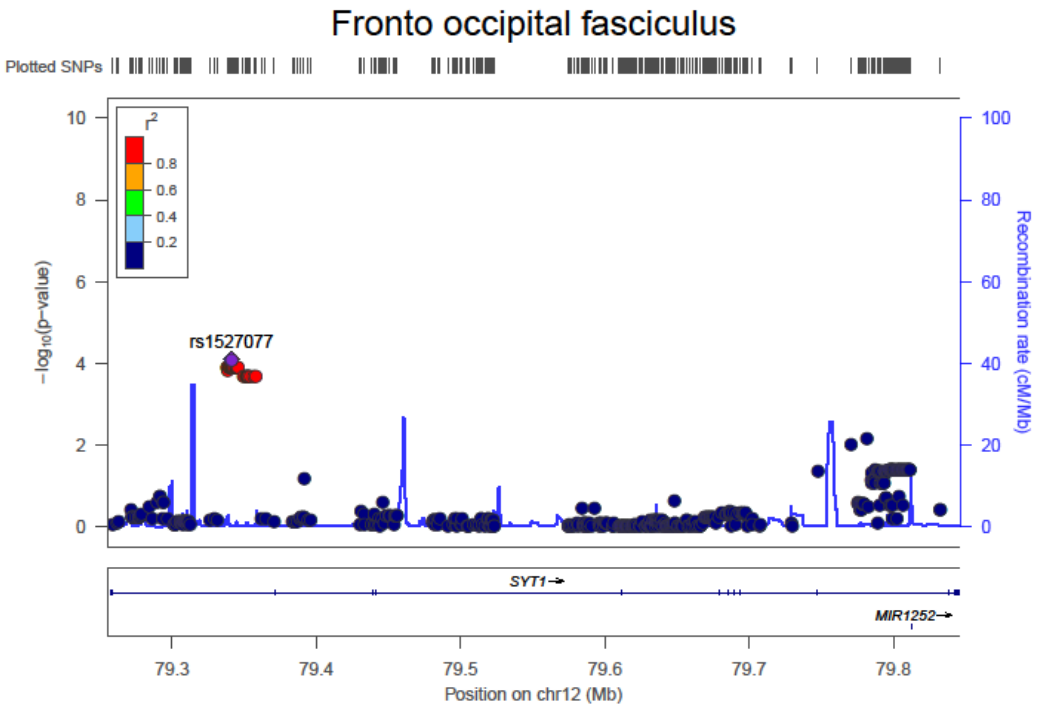
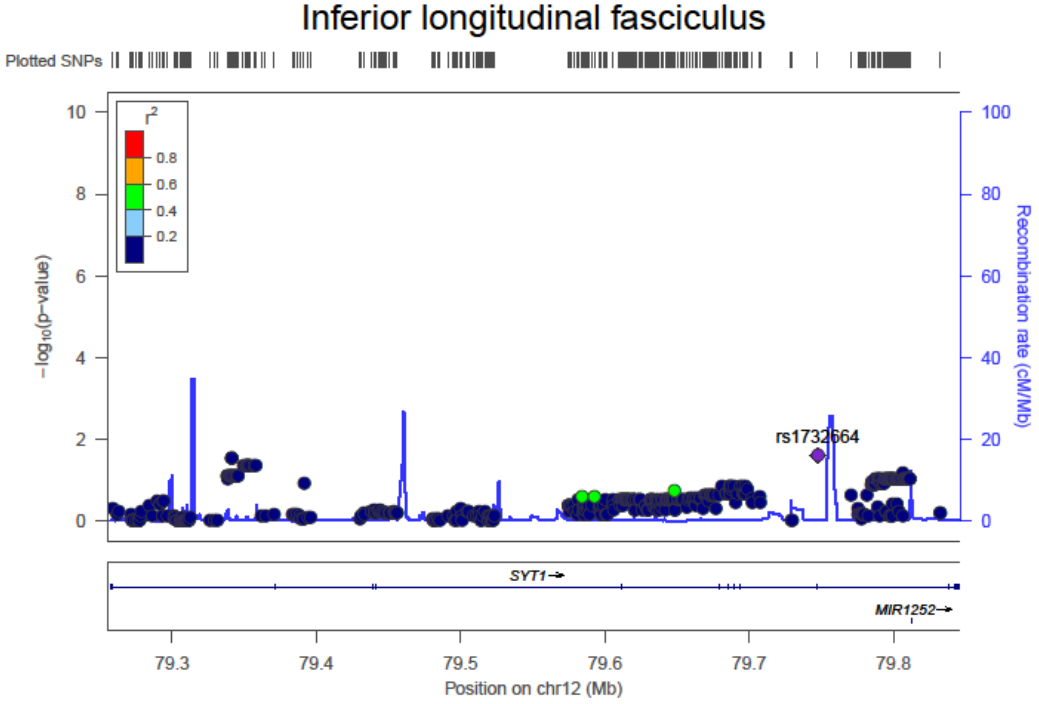
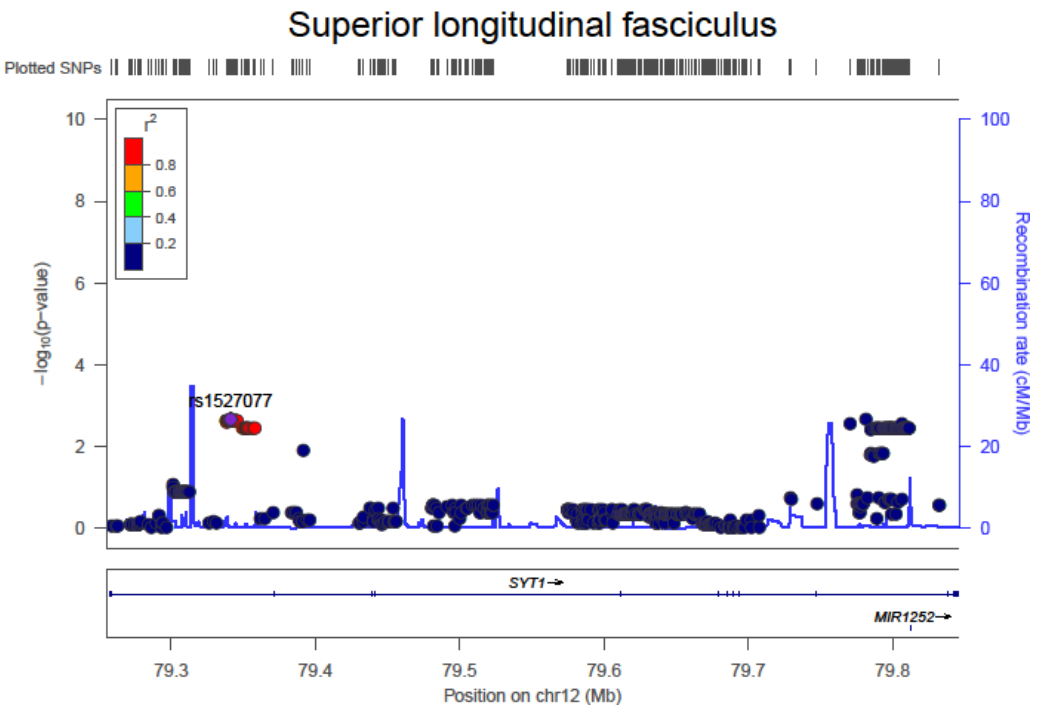
0.6
0.4
0.2
rs10778271
−log10(p−value)
Recombination rate (cM/Mb)
SYT1
MIR1252
79.3
79.4
79.5
79.6
79.7
79.8
Position on chr12 (Mb)

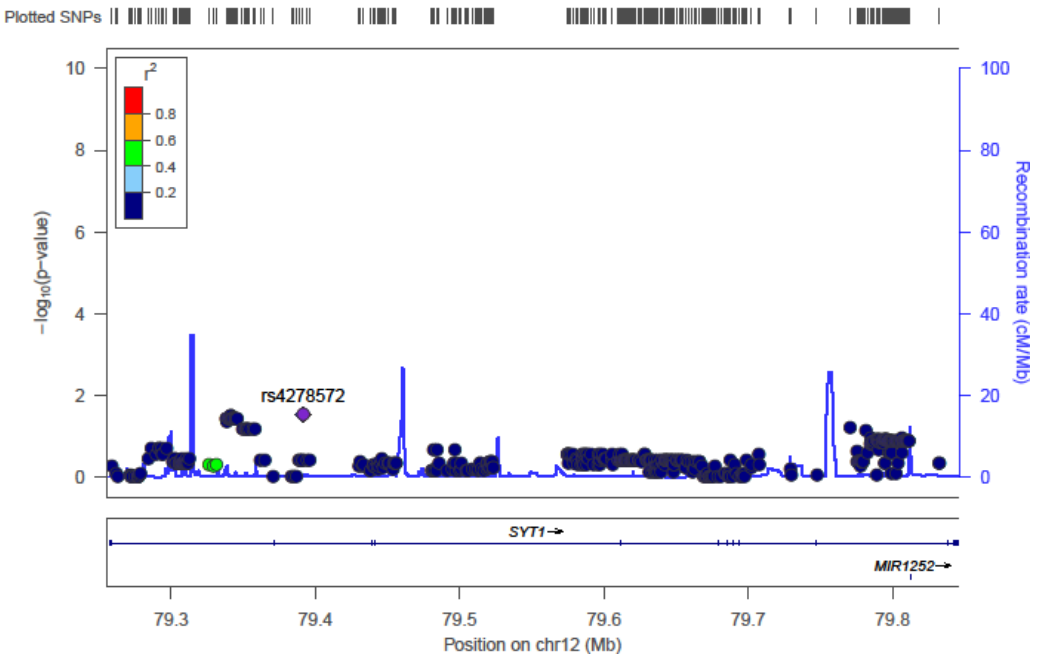

Forceps major
Plotted SNPs
rs1527077
SYT1
MIR1252
Position on chr12 (Mb)
Recombination rate (cM/Mb)

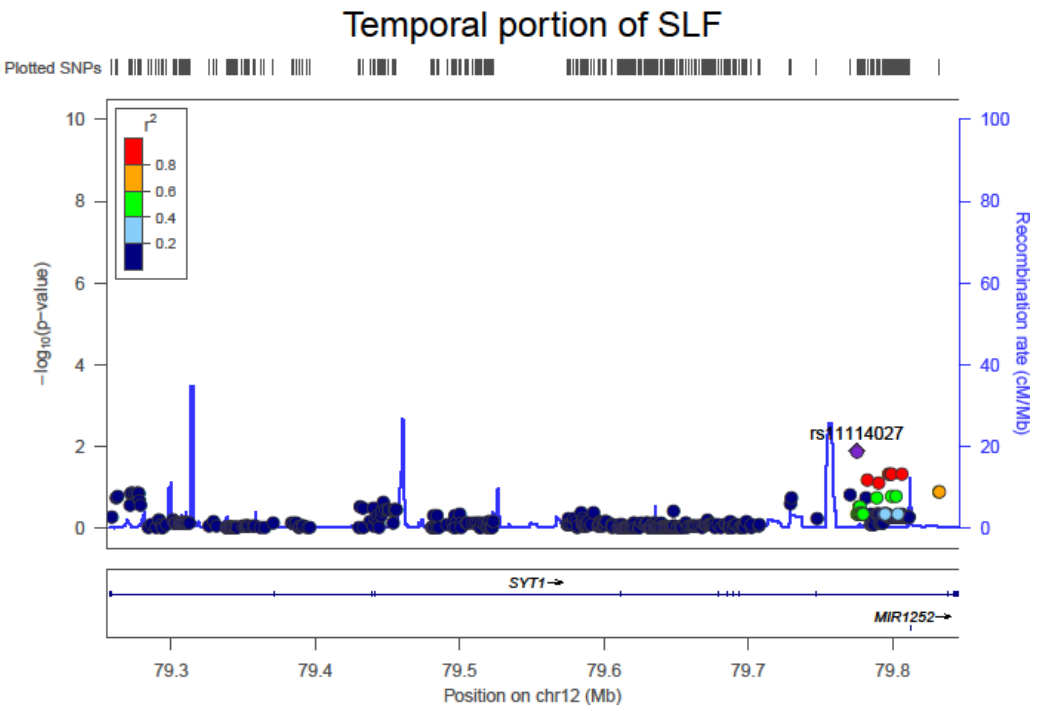

Forceps minor
Plotted SNPs
rs4278572
SYT1
MIR1252
Position on chr12 (Mb)
Recombination rate (cM/Mb)


Fronto occipital fasciculus
Plotted SNPs
r²
0.8
0.6
0.4
0.2
rs1527077
−log10(p−value)
Recombination rate (cM/Mb)
SYT1
MIR1252
79.3
79.4
79.5
79.6
79.7
79.8
Position on chr12 (Mb)


Inferior longitudinal fasciculus
Plotted SNPs
r²
0.8
0.6
0.4
0.2
rs1732664
−log10(p−value)
Recombination rate (cM/Mb)
SYT1
MIR1252
79.3
79.4
79.5
79.6
79.7
79.8
Position on chr12 (Mb)


Superior longitudinal fasciculus
Plotted SNPs
r²
0.8
0.6
0.4
0.2
rs1527077
−log10(p−value)
Recombination rate (cM/Mb)
SYT1
MIR1252
79.3
79.4
79.5
79.6
79.7
79.8
Position on chr12 (Mb)


Uncinate fasciculus
Plotted SNPs
r²
0.8
0.6
0.4
0.2
rs4278572
−log10(p−value)
Recombination rate (cM/Mb)
SYT1
MIR1252
79.3
79.4
79.5
79.6
79.7
79.8
Position on chr12 (Mb)



Regional visualization of *SYT1* results for each outcome. SNP with the lowest P-value is represented in purple, of which linkage disequilibrium with other SNPs are depicted according to R^2.

Supplementary Table S1. Evaluated variants associated with specific tracts and *in silico* predictions (excel file).

Capítulo V

7. The Association Between *SYT1*-rs2251214 and Cocaine Use Disorder Further Supports Its Role in Psychiatry

Progress in Neuropsychopharmacology & Biological Psychiatry 94 (2019) 109642

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

The association between *SYT1*-rs2251214 and cocaine use disorder further supports its role in psychiatry

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ARTICLEINFO

Keywords:
SNARE complex
Cocaine
Addiction
Substance use disorders
Stimulants

ABSTRACT

Synaptotagmin-1 is an essential regulator of synaptic vesicle exocytosis, and its encoding gene (*SYT1*) is a genome and transcriptome-wide association hit in cognitive performance, personality and cocaine use disorder (CUD) studies. Additionally, in candidate gene studies the specific variant rs2251214 has been associated with attention-deficit/hyperactivity disorder (ADHD), antisocial personality disorder and other externalizing phenotypes in adults with ADHD, as well as with response to methylphenidate (MPH) treatment. In this context, we sought to evaluate, in an independent sample, the association of this variant with CUD, a phenotype that shares common biological underpinnings with the previously associated traits. We tested the association between *SYT1*-rs2251214 and CUD susceptibility and severity (addiction severity index) in a sample composed by 315 patients addicted to smoked cocaine and 769 non-addicted volunteers. *SYT1*-rs2251214 was significantly associated with susceptibility to CUD, where the G allele presented increased risk for the disorder in the genetic models tested ($P = 0.0021$, OR = 1.44, allelic; $P = 0.0012$, OR = 1.48, additive; $P = 0.0127$, OR = 1.41, dominant). This is the same allele that was associated with increased risk for ADHD and other externalizing behaviors, as well as poor response to MPH treatment in previous studies. These findings suggest that the neurotransmitter exocytosis pathway might play a critical role in the liability for psychiatric disorders, especially externalizing behaviors and CUD.

1. Introduction

The influence of exocytosis-related variants, including those on Synaptotagmin-1 (SytI) encoding gene (*SYT1*), has been investigated in psychiatric disorders since they play a critical role in neurotransmitter release (for review see Cupertino et al., 2016). *SYT1* gene has been linked to cocaine use disorder (CUD) in a candidate pathway association study (Fernàndez-Castillo et al., 2012), as well as in a recent genome and transcriptome-wide-association study, where it presented a nominal association with CUD in a gene-based analysis and a significant association in a differential expression analysis in postmortem brain tissue (Huggett and Stallings, 2019). A particular SNP on *SYT1* gene - rs2251214 - was associated with adulthood attention-deficit/hyperactivity disorder (ADHD) in two independent clinical samples (Cupertino et al., 2017; Sánchez-Mora et al., 2013), as well as with antisocial personality disorder (ASPD) and other externalizing phenotypes in patients with ADHD (Cupertino et al., 2017). This SNP was also associated with methylphenidate (MPH) treatment response in adults with ADHD, being involved with both symptom response and treatment persistence (da Silva et al., 2018). Therefore, this variant previously implicated in stimulant treatment response is a candidate to be associated with CUD.

SytI acts as a Ca^{2+} sensor to induce the fusion of presynaptic vesicles with the plasma membrane through soluble N-ethylmalemide-

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https://doi.org/10.1016/j.pnpbp.2019.109642
Received 16 February 2019; Received in revised form 2 May 2019; Accepted 2 May 2019
Available online 03 May 2019

sensitive factor attachment protein receptors (SNARE) complex binding, being an important regulator of synaptic vesicle exocytosis (Südhof, 2013). This cascade of events culminates in the release of stored neurotransmitters into the synaptic cleft (Tucker and Chapman, 2002; Wu and Schulten, 2014), and the proper regulation of this process is crucial for the modulation of behavioral responses, including craving for cocaine (Adinoff, 2004; Nestler, 2005). Studies using *in vivo* and *in vitro* models suggest that synaptotagmins could exert a role in stimulants actions. The infusion of low doses of MPH was able to modulate *SYT1* mRNA levels in PC12 cells (Bartl et al., 2010) and treatment with cocaine led to increased *SYT4* mRNA levels in the striatum of rats (Courtin et al., 2006; Denovan-Wright et al., 1998). Moreover, intraperitoneal injection of 3,4-methylenedioxymethamphetamine in adult mice induced increases in SytI and SytIV protein levels in different brain regions (Peng et al., 2002). These alterations of exocytosis regulatory proteins expression induced by stimulant drugs could modulate the efficacy of synaptic transmission and consequently affect functions related to the reward system.

The main mechanism of action of both cocaine and MPH involves the inhibition of the reuptake of neurotransmitters into presynaptic neurons. These stimulants blockade dopamine, norepinephrine and serotonin transporters (DAT, NET and SERT, respectively), leading to increased levels of these neurotransmitters in the synaptic cleft (Han and Gu, 2006; Hannestad et al., 2010). An alternative mechanism has also been proposed to explain cocaine actions (see Heal et al., 2014). Cocaine would act as an inverse agonist of DAT, resulting in reversion of the dopamine transport into the synaptic cleft. This hypothesis is in accordance with the fact that cocaine and MPH are weak dopamine uptake inhibitors and seem to have dopamine releasing effects, differently from other competitive reuptake inhibitors (Heal et al., 2014).

Therefore, since cocaine seems to present a complementary exocytosis-related mechanism of action and can modulate expression of critical elements of neurotransmitter release, it is plausible that *SYT1*-rs2251214 might also influence the susceptibility and severity to CUD. Considering the overall evidence, including an intriguing set of findings involving *SYT1*-rs2251214 and externalizing behaviors (Cupertino et al., 2017; Sánchez-Mora et al., 2013) and MPH treatment response (da Silva et al., 2018), we sought in an independent sample to extend the association to CUD, a phenotype that shares common biological underpinnings with the previously associated traits (Gurriarán et al., 2018).

2. Material and methods

2.1. Sample

The sample was composed by crack cocaine addicted patients ($N = 315$) who voluntarily sought specialized treatment in hospital addiction units from the metropolitan region of Porto Alegre, Southern Brazil, and by healthy non-addicted volunteers ($N = 769$) recruited at a blood donation center (Hospital de Clínicas de Porto Alegre) and at a community from the same city. All individuals self-reported themselves as of European descent, a measure that is significantly correlated with genomic estimates of interethnic admixture in Latin populations (Ruiz-Linares et al., 2014). Moreover, in the specific region where the sample was collected, the population presents nearby 90% of European ancestry (Ruiz-Linares et al., 2014). This project was carried out in accordance with the Declaration of Helsinki. The procedures had been clarified to all subjects, who then signed an informed consent approved by the Research Ethics Committees of the participating institutions.

2.2. Diagnosis

Diagnosis of CUD followed the diagnostic and statistical manual of mental disorders, 4th edition (DSM-IV; APA, 1994) criteria, confirmed through the structured clinical interview for DSM-IV axis I disorders (SCID-I; First et al., 2002) or Mini-International Neuropsychiatric Interview (MINI; Sheehan et al., 1998). Information on the potential problem areas (severity) within the sample of crack cocaine addicted patients was assessed through the sixth version of addiction severity index (ASI-6; Cacciola et al., 2011; Kessler et al., 2012; McLellan et al., 2006). ASI-6 is a semi-structured interview that besides gathering information about substance use problems, it provides composite scores for the severity of problems in other life domains that potentially contribute for negative outcomes (e.g., legal, education/employment, medical, etc.). The higher the scores computed, the greater the severity of the problems the interviewee has for that domain. The severity of general psychiatric symptoms and drug use were evaluated in this study. The calculation of the composite score for the Psychiatry domain combines information about different manifestations (e.g., suicidality, delusions, hallucinations), overall frequency of such symptoms, impairment caused by those symptoms, distress caused and need for treatment specifically for those symptoms. The calculation of composite scores for the Drugs domain is based on data related to drugs use, including information on frequency of use and time since last use, problems due to drug use, how often these problems occur, how much trouble these problems cause and how much treatment is needed. Healthy non-addicted participants screened negatively for crack and/or cocaine use through the SCID-I/P screening module (First et al., 2002) or the alcohol, smoking, and substance involvement screening test (ASSIST; Henrique et al., 2004; WHO ASSIST Working Group, 2003). The exclusion criteria for both cases and controls were presence of schizophrenia and other psychotic disorders and/or severe cognitive deficit that would impair the capacity of answering the instruments.

2.3. Polymorphism selection and genotyping

The *SYT1*-rs2251214 (Chr12:79430071) SNP was selected based on its previous association with ADHD, ASPD and other externalizing phenotypes within ADHD subjects, as well as with MPH treatment response (Cupertino et al., 2017; da Silva et al., 2018; Sánchez-Mora et al., 2013). It was genotyped using a Taqman allelic discrimination assay, according to the manufacturer's instructions (Step One Plus, Applied Biosystems, Foster City, CA, USA).

2.4. Statistical analysis

The influence of *SYT1*-rs2251214 on the susceptibility of CUD was evaluated by logistic regression analysis, while its effects on the quantitative measures of the problem areas of ASI-6 (i.e. drugs and psychiatric scales) were evaluated through linear regression. Different genetic models (allelic, additive, and dominant) were tested using PLINK software v1.07 (Purcell et al., 2007). Sex and age were included as covariates in all analyses, except for the allelic model that runs under the command –assoc at PLINK, and this is not compatible with adjustment for covariates. The significance level was set at 0.05.

2.5. Public databases analyses

HaploReg v4.1, RegulomeDB, and the SNP Function Prediction of the SNPinfo were used to evaluate the possible role of rs2251214 in regulatory mechanisms. HaploReg is a tool that examines annotations of the noncoding genome at disease-associated loci by genome-wide association studies (GWASes) (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php Ward and Kellis, 2011). RegulomeDB annotates SNPs with known and predicted regulatory elements in the intergenic regions of the human genome (http://www.regulomedb.org/ Boyle et al., 2012). The SNPinfo web server is a set of web-based tools to predict functional characteristics of both coding and noncoding SNPs (https://snpinfo.niehs.nih.gov/ Xu and Taylor, 2009).

Table 1
Sample characteristics and genotypic frequencies.

	Cases (n = 315)	n	Controls (n = 769)	n
	MEAN (SD)		MEAN (SD)	
Age (years)	30.9 (8.0)	315	29.2 (8.6)	769
Drugs[a]	53.3 (6.0)	283	–	
Psychiatric[a]	49.7 (8.5)	283	–	
Age of first cocaine use	18.5 (5.7)	259	–	
Years of regular cocaine use	5.8 (6.2)	259	–	
Age of first crack use	23.0 (8.3)	279	–	
Years of regular crack use	5.6 (5.0)	277	–	
	n (%)		n (%)	
Gender (male)	165 (52.4)	315	435 (56.6)	769
Tobacco use disorder[b]	241 (83.1)	290	69 (9.0)	763
Alcohol use disorder[c]	85 (31.3)	272	5 (0.7)	747
Major depressive disorder[c]	57 (20.8)	274	172 (22.9)	750
Bipolar disorder[c]	97 (35.8)	271	30 (4.0)	748
Anxiety disorders[c,d]	105 (38.2)	275	117 (15.6)	750
SYT1-rs2251214		315		769
GG	204 (64.7)		439 (57.1)	
AG	106 (33.7)		283 (36.8)	
AA	5 (1.6)		47 (6.1)	

[a] Subscales of the sixth version of addiction severity index (ASI-6).
[b] Current information.
[c] Lifetime information.
[d] Anxiety disorders include generalized anxiety disorder, obsessive-compulsive disorder, panic disorder and agoraphobia.

3. Results

Clinical characteristics of the sample and genotype frequencies are presented in Table 1. No deviation from the Hardy-Weinberg Equilibrium was found ($P = 0.483$, $\chi^2 = 0.492$).

3.1. Genetic ASSOCIATION ANALYSES

SYT1-rs2251214 was significantly associated with susceptibility to CUD in our sample, where the G allele presented increased risk for the disorder in all genetic models tested ($P = 0.0021$, OR = 1.44, allelic; $P = 0.0012$, OR = 1.48, additive; $P = 0.0127$, OR = 1.41, dominant). However, *SYT1*-rs2251214 does not seem to influence the severity of CUD, since no association was observed for ASI-6 severity subscales (drugs and psychiatric status) or for age of first use of either smoked or snorted cocaine (Table 2).

3.2. Public DATABASES ANALYSES

Additional analyses in public databases were performed to predict the potential functionality of *SYT1*-rs2251214 and refine our results. RegulomeDB showed minimal evidence of transcription factors binding on this SNP (score of 6). The SNP Function Prediction of the SNPinfo web server did not reveal any involvement of *SYT1*-rs2251214 in splicing regulatory mechanisms. Furthermore, HaploReg did not show differences in chromatin state nor the presence of histone modifications in the brain tissues evaluated, suggesting that this SNP is not involved in gene expression regulatory mechanisms. Besides, *SYT1*-rs2251214 was not in high LD (R^2 and $D' > 0.8$) with other variants in the European population.

4. Discussion

In this study, we observed that *SYT1*-rs2251214 G allele is associated with increased risk for CUD. This result extends preceding findings implicating this SNP on externalizing phenotypes and stimulant responses to a different albeit neurobiologically related phenotype in an independent sample. The previous studies reported that the same allele was associated with increased risk for ADHD, and its externalizing-related phenotypes (Cupertino et al., 2017; Sánchez-Mora et al., 2013), as well as for poor treatment response to MPH (da Silva et al., 2018). These results point towards an idea that although both MPH and cocaine target DAT, other mechanisms possibly involving the release of neurotransmitters are major factors to be considered in the actions of these stimulants.

In line with the hypothesis that exocytosis-related genes could be involved in the mechanisms underlying the susceptibility for CUD, a previous candidate pathway association study sought to evaluate SNPs covering 16 genes involved in the release of neurotransmitters (Fernàndez-Castillo et al., 2012). They found that polymorphisms in *SYT1* and *SYT2* genes were nominally associated to CUD severity and susceptibility (Fernàndez-Castillo et al., 2012). This study also reported a significant association for the *NSF* (N-ethylmaleimide sensitive fusion protein) gene, which encodes a protein involved in the recycling of SNARE complex. Such gene was also associated with CUD in a subsequent study (Cabana-Domínguez et al., 2016). In a recent genome and transcriptome-wide-association study, *SYT1* gene presented a nominal association with CUD in a gene-based analysis and a significant association in a differential expression analysis in the human hippocampus (Huggett and Stallings, 2019). Additionally, *SYT1* gene is a hit in genome-wide association studies (Supplementary Fig. 1) since it was associated in gene-based analyses with neuroticism (Luciano et al., 2018; Nagel et al., 2018a,b) and its domains of irritability (Nagel et al., 2018b) and fed-up feelings (Nagel et al., 2018a,b), educational attainment (Lee et al., 2018) and cognitive performance (Lee et al., 2018).

The fact that *SYT1*-rs2251214 was previously associated with different outcomes of MPH treatment (da Silva et al., 2018), and in the present study with CUD susceptibility, but not severity, suggests that in CUD these effects might not involve a genetic modulation of a pharmacodynamic effect, as observed for MPH (da Silva et al., 2018), but

Table 2
Association between *SYT1*-rs2251214 and cocaine use disorder.

	Allelic model			Additive model[a]			Dominant model[a]		
	OR	CI95%	P-VALUE	OR	CI95%	P-VALUE	OR	CI95%	P-VALUE
Case control status	1.44	1.14–1.82	0.0021	1.48	1.17–1.88	0.0012	1.41	1.08–1.85	0.0127
	BETA	SE	P-VALUE	BETA	SE	P-VALUE	BETA	SE	P-VALUE
Drugs[b]	−0.03	0.08	0.6987	−0.03	0.08	0.6952	−0.03	0.08	0.6957
Psychiatric[b]	−0.09	0.08	0.2770	−0.09	0.08	0.2746	−0.12	0.09	0.1802
Age of first cocaine use	0.01	0.08	0.8904	0.01	0.07	0.8524	0.03	0.08	0.7001
Years of cocaine use	0.06	0.07	0.3755	0.07	0.07	0.3359	0.06	0.07	0.4170
Age of first crack use	0.03	0.08	0.7126	0.04	0.06	0.5263	0.02	0.06	0.7234
Years of crack use	0.09	0.08	0.2806	0.09	0.08	0.2478	0.09	0.08	0.2783

Effect allele = G.
OR = odds ratio. CI = confidence interval. SE = standard error.
[a] Adjusted for age and sex.
[b] Subscales of the sixth version of addiction severity index (ASI-6). *P*-value calculated using linear regression analysis.

rather an influence on susceptibility solely. It is important to mention that the interpretation of the findings in patients with CUD may reflect the fact that the chronic and heavy use of smoked cocaine, which is usual among addicted individuals, could potentially alter the functioning of neurotransmission systems (for example through the down-regulation of dopamine receptors) at a point that the subtler effects of genetic variability on CUD severity might become undetectable.

In addition, the overwhelming recent evidence towards the shared heritability in psychiatric disorders (Brainstorm Consortium et al., 2018; Gurriarán et al., 2018) indicates that *SYT1* may indeed represent a relevant biological factor underpinning all the previously associated phenotypes, including CUD. We suggest that *SYT1*-rs2251214 effects in CUD would emerge similarly to what was reported to a range of externalizing (Cupertino et al., 2017) and internalizing (Luciano et al., 2018; Nagel et al., 2018a) behaviors. Conversely, a mediating relationship between *SYT1*-rs2251214 and other phenotypes such as conduct disorders, ADHD, mood and anxiety disorders could be influencing CUD susceptibility indirectly, since patients with CUD present high rates of psychiatric comorbidities (Daigre et al., 2013; Narvaez et al., 2014; Saunders et al., 2015).

This hypothesis-driven study presents some limitations. The first is the fact that we focused on a single SNP previously associated with externalizing phenotypes and stimulant treatment response in our target population. However, other variants of *SYT1* gene, that are not in LD with rs2251214 (Supplementary Table 1 and Supplementary Fig. 2), recently achieved GWAS significance level in cognitive performance and personality studies in non-Latin American populations. Therefore, the inclusion of our sample in future GWAS will extend the analysis to additional variants and genes related to the neurotransmitter exocytosis pathway to confirm their involvement in CUD. This hypothesis is relevant considering the previous evidence from in vitro/in vivo models and association studies involving psychiatric phenotypes and genes related to this pathway. Second, it is not possible to infer putative mechanisms underlying the *SYT1*-rs2251214 association since this intronic SNP has no described functionality yet, and it might not have a functional effect by itself, but instead is in linkage disequilibrium with other not-studied functional SNP. Third, we should not discard the possibility that other psychiatric comorbidities might be playing a role in the association observed. Finally, the fourth limitation relates to the lack of genome-wide data to estimate principal components for more precise control for population stratification, especially considering the significant but moderate correlation between self-perception and genetically determined ancestry in Latin America. However, this possible bias is minimized by the fact that, among Latin-Americans, individuals that self-identified as of European descent presented a trend for higher European ancestry (≈ 90%), as opposed to other groups (Ruiz-Linares et al., 2014).

5. Conclusion

Our results showing an association between *SYT1*-rs2251214 and CUD, combined with previous evidence of the influence of neurotransmitter exocytosis-related genes on psychiatric disorders and stimulants use/abuse, highlight the importance of such pathway in this context. Although these results still need replication, they indicate that additional studies should further explore the effects of genes involved in neurotransmitter exocytosis pathway in other psychiatric phenotypes.

Contributors

BSS and RBC prepared the first draft of the manuscript. BSS, RBC, JBS and DLR participated in the design of the study and in the statistical analysis. BSS, RBC, DBK and CEB worked on the laboratorial techniques, such as blood extraction and genotyping. BSV, LVD, FHP and RGO were involved with the recruitment of patients and the management of the database for analysis. EHG, RGO, CHDB and DLR reviewed all drafts of the manuscript and actively contributed to the writing of the final version. All authors have read and approved the final version of this article.

Role of the funding source

This study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnologico (CNPq, 476529/2012-3, 466722/2014-1, and 466802/2014-5), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - Finance Code 001), and Secretaria Nacional de Políticas sobre Drogas (SENAD, 82264/2015).

Ethical statement

This project was carried out following the Declaration of Helsinki, and all subjects signed an informed consent form that was previously approved by the Research Ethics Committees of the participating institutions.

Declaration of interest

The author(s) declare the following potential conflict of interest with respect to the research, authorship and/or publication of the present article: Dr. EHG was on the speaker's bureau for Novartis and Shire for the last 3 years. He also received travel awards (air tickets and hotel accommodations) for participating in two psychiatric meetings from Shire and Novartis. All other authors report no financial interests or potential conflicts of interest.

Acknowledgments

We are thankful to the staff of the participating institutions for all their support with data collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2019.109642.

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Capítulo VI

8. Fatores genéticos e de neuroimagem envolvidos na remissão e persistência do TDAH em adultos

Conforme abordado no Capítulo I (1.1 TDAH ao longo da vida), nosso grupo observou pela primeira vez a remissão do TDAH independentemente da idade em um estudo de seguimento no qual os pacientes foram avaliados, em média, sete anos após o diagnóstico. Buscando compreender melhor sobre as possíveis trajetórias do TDAH e fatores envolvidos, esse projeto consiste em uma nova avaliação dos pacientes (em média 13 anos após o diagnóstico), incluindo nesse terceiro tempo também medidas de neuroimagem.

9.1 Introdução

Considerado como um transtorno do neurodesenvolvimento, primeiramente exclusivo da infância e posteriormente acreditado que uma pequena parcela de crianças poderia persistir com os sintomas até a vida adulta (Faraone et al. 2006c), o TDAH em adultos tem se mostrado mais complexo do que inicialmente se pensava e com um perfil clínico diferente da infância (e.g., proporção entre homens e mulheres, comorbidades, manifestação de sintomas) (Sobanski 2006; Mostert et al. 2015). O TDAH ao longo da vida tem sido muito debatido nos últimos anos (Franke et al. 2018) com observações surpreendentes provenientes de estudos de cortes e seguimento (Karam et al. 2015; Edvinsson and Ekselius 2017; Karam et al. 2017).

Karam et al (2015) observaram que a maioria dos pacientes avaliados (69,8%) continuavam a preencher critérios diagnósticos em média sete anos após o diagnóstico de TDAH, enquanto 17,8% dos pacientes passaram a manifestar um TDAH sublimiar e 12.4% remitiram os sintomas (menos de 4 sintomas). Essa remissão foi independente de mudanças nas comorbidades. Já em relação a medidas do *baseline* (i.e., primeira avaliação, momento do diagnóstico) níveis mais altos de sintomas de desatenção e hiperatividade/impulsividade, transtorno de oposição desafiante e fobia social foram considerados preditores de persistência.

Dessa forma, os autores concluem que apesar do estágio de maturação cerebral nos adultos sugerir uma estabilidade, parece haver uma possibilidade de remissão de sintomas independentemente da idade. Uma análise dimensional (Karam et al. 2017) sugere ainda que na maioria dos casos os sintomas de TDAH diminuem ao longo do tempo, embora uma fração significativa apresente também um aumento no número de sintomas.

Esse estudo busca compreender mais sobre as possíveis trajetórias de TDAH ao longo do tempo, e como fatores genéticos e de neuroimagem podem estar envolvidos.

9.2 Materiais e Métodos

Os participantes foram avaliados na divisão de adultos do Programa de Déficit de Atenção-Hiperatividade (PRODAH-A) no Hospital de Clínicas de Porto Alegre. Entre 2003 a 2007 o ambulatório diagnosticou 344 adultos com idade entre 18 e 68 anos (49,9% homens) (*baseline*). Como mencionado, após aproximadamente sete anos esses indivíduos foram reavaliados (T2), com uma taxa de retenção de 66% (Karam et al. 2015). O presente estudo avaliou pela terceira vez (T3) esses pacientes, agora aproximadamente 13 anos após o diagnóstico. Dos 225 avaliados no T2, 18 foram considerados inelegíveis e excluídos devido a parentesco, a fim de evitar possíveis vieses por ambiente ou genética compartilhada. Dessa forma, 207 indivíduos foram considerados elegíveis para o estudo, dos quais não foi possível contatar 22 (10,6%). Dos 185 pacientes contatos, 25 não quiseram participar do estudo e 10 apesar de manifestarem vontade de participar não puderam ser avaliados por questões logísticas (e.g. residência em outro estado ou país). Assim, 150 pacientes foram efetivamente contatos no T3, com uma taxa de retenção de 72.5% (150/207 elegíveis). Quatro desses pacientes morreram entre o T2 e T3, de forma que a avaliação final incluiu

146 indivíduos (idade média 47,63±10,70; 45,2 homens). O fluxograma pode ser visualizado na **Figura 1** e a frequência das principais comorbidades em cada avaliação na **Tabela 2**.

Além da avaliação clínica em relação ao número de sintomas, comorbidades, tratamento e dados demográficos, foram adquiridas imagens de RMN desses indivíduos. As imagens foram adquiridas no *scanner* de 3.0 T Siemens SPECTRA, com uso de bobina de crânio de doze canais para recepção do sinal. Imagens de alta resolução baseadas em T1 foram adquiridas com uma sequência MPRAGE (*Magnetization Prepared Rapid Acquisition Gradient Recalled Echo*) com resolução espacial isotrópica de $1mm^3$, 192 fatias contíguas com uma matriz de imagem de 256x256mm, TR=2,530ms; TE=2.55ms; TI=1.100ms; Flip Angle=7^o e aquisição paralela (GRAPPA) com fator de aceleração de 2. As imagens foram processadas utilizando o software FreeSurfer (https://surfer.nmr.mgh.harvard.edu/) e controle de qualidade de acordo com protocolos do ENIGMA (http://enigma.ini.usc.edu/protocols/) (Stein et al. 2012; Hibar et al. 2015b).

Foi coletado ainda amostra de sangue periférico de todos os participantes para extração de DNA pelo método de *salting out*. A genotipagem das variantes foi feita pelo Illumina Infinium PsychArray-24 v1.1. Através do *Rapid Imputation and COmputational PIpeLIne* (Ricopili) foi realizado o controle de qualidade pré-imputação a nível de indivíduo e de SNP, análises de componentes principais para detecção de *outliers* para ancestralidade genética, identificação de relações de parentesco e/ou indivíduos duplicados. Além disso, os algoritmos SHAPEIT2 e IMPUTE2 foram utilizados para faseamento dos genótipos e imputação, respectivamente, considerando o painel de ancestralidade do Projeto 1000 Genomas (fase 1 – versão 3), do *genome build* hg19.

Para fins de comparação, foi incluído um grupo (i.e., nas medidas de neuroimagem: diagnóstico atual no T3 (controle *vs* casos *vs remitters* no T3; no escore de risco poligênico: controles, *remitters* em ao menos uma das avaliações – T2 ou T3, e indivíduos que preencheram os critérios diagnósticos nas três avaliações). Os controles também tiveram dados genômicos (n = 433) e de neuroimagem (n = 72) analisados.

Análises de variância (ANOVA) foram utilizadas para comparar medidas de neuroimagem entre os três grupos (diagnóstico atual – controles, *remitters* no tempo T3 e TDAH), seguido de análises *post hoc* quando observada alguma diferença significativa. As análises foram ajustadas para idade, sexo e ICV. Foram avaliadas medidas de neuroimagem associadas ao TDAH em grandes meta-análises conduzidas pelo ENIGMA de volumes subcorticais (Hoogman et al. 2017) e medidas corticais (Hoogman et al. 2019). Em relação à genética, foram calculados escores de risco poligênico com base na amostra da meta-análise de TDAH mais recente (Demontis et al. 2019). Os escores poligênicos foram calculados no software PRSice v2.2.11 (Choi and O'Reilly 2019), utilizando P_T de 0,05, 0.5 e 1 e comparados através de ANOVA entre três grupos (controles, indivíduos que remitiram (*remitters)* em ao menos uma das avaliações – T2 ou T3, e indivíduos que preencheram os critérios diagnósticos nas três avaliações) e ajustadas por idade, sexo e os cinco primeiros componentes principais.

9.3 Resultados

Considerando as três avaliações, todos os tipos de trajetórias possíveis foram observadas: indivíduos que remitiram no T2 e se mantiveram como *remitters*, que voltaram a preencher diagnóstico; indivíduos que tinham preenchidos diagnósticos no T2 e permaneceram com diagnóstico ou ainda os que remitiram no T3. Além dos classificados como sublimiares (3 ou mais sintomas em

pelo menos um contexto e presença de prejuízo). As trajetórias podem ser observadas na **Figura 2**.

Apesar de não ter observado nenhum efeito significativo em medidas de espessura cortical, a grande maioria das áreas corticais e volumes subcorticais avaliados tiveram diferenças significativas entre os grupos (**Tabela 2**). Análises *post hoc* evidenciaram uma diferença principalmente entre casos (com TDAH em todas avaliações) e controles e entre *remitters* e controles. Nos giros fusiformes e supramarginais, além da diferença entre casos e controles, foi observada uma diferença significativa entre *remitters* e casos, com os *remitters* apresentando tamanhos da estrutura intermediários entre casos e controles. A **Figura 3** ilustra a diferença observada entre os grupos. Nenhum dos escores de risco poligênico calculados (P_T 0,05, 0,5 e 1) tiveram resultados significativos.

9.4 Conclusões preliminares e perspectivas

As diferenças observadas entre casos e controles tanto em volumes subcorticais quanto em áreas de superfície cortical corroboram achados anteriores (Hoogman et al. 2017; Hoogman et al. 2019). Embora em ambos os estudos os resultados observados foram considerados exclusivos ou majoritários em crianças, nossos achados apontam para um efeito também em adultos com TDAH, sugerindo que talvez o efeito maior ou exclusivo em crianças se deva principalmente ao tamanho amostral significativamente menor do grupo adulto.

Em relação a remissão na vida adulta, na maior parte das regiões analisadas os *remitters* apresentaram valores significativamente diferentes dos controles, mas não dos casos mais persistentes. Entretanto, uma diferença entre casos e *remitters* foi observada em algumas áreas, onde os *remitters* não

divergiram do grupo controle, sugerindo que possa haver alguma diferença nesse grupo. O pequeno tamanho amostral desse grupo (n = 35) impossibilita conclusões robustas, ainda que estas devam ser consideradas em estudos posteriores e possam ajudar a esclarecer mecanismos envolvidos na remissão ou persistência do TDAH.

A ausência de significância na comparação dos escores de risco poligênico pode ser devido ao pequeno tamanho amostral já que ao mesmo entre casos e controles seria esperado alguma diferença.

Análises adicionais em relação a trajetória dos sintomas e comorbidades podem auxiliar a compreender esse cenário e ainda serão realizadas.

Figura 1. Fluxograma dos participantes na terceira avaliação (T3).

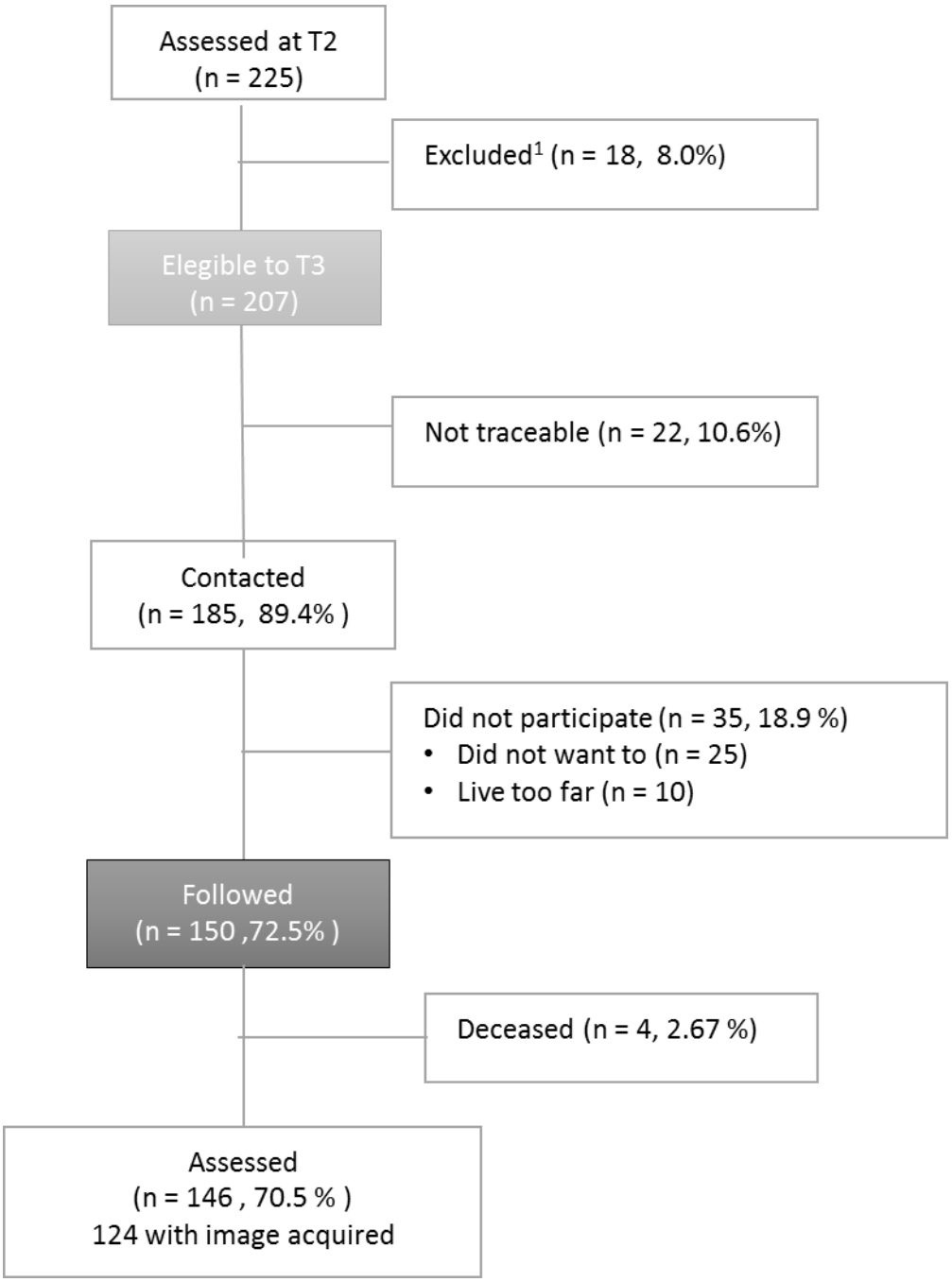


Figura 2. Trajetórias de TDAH nas três avaliações.

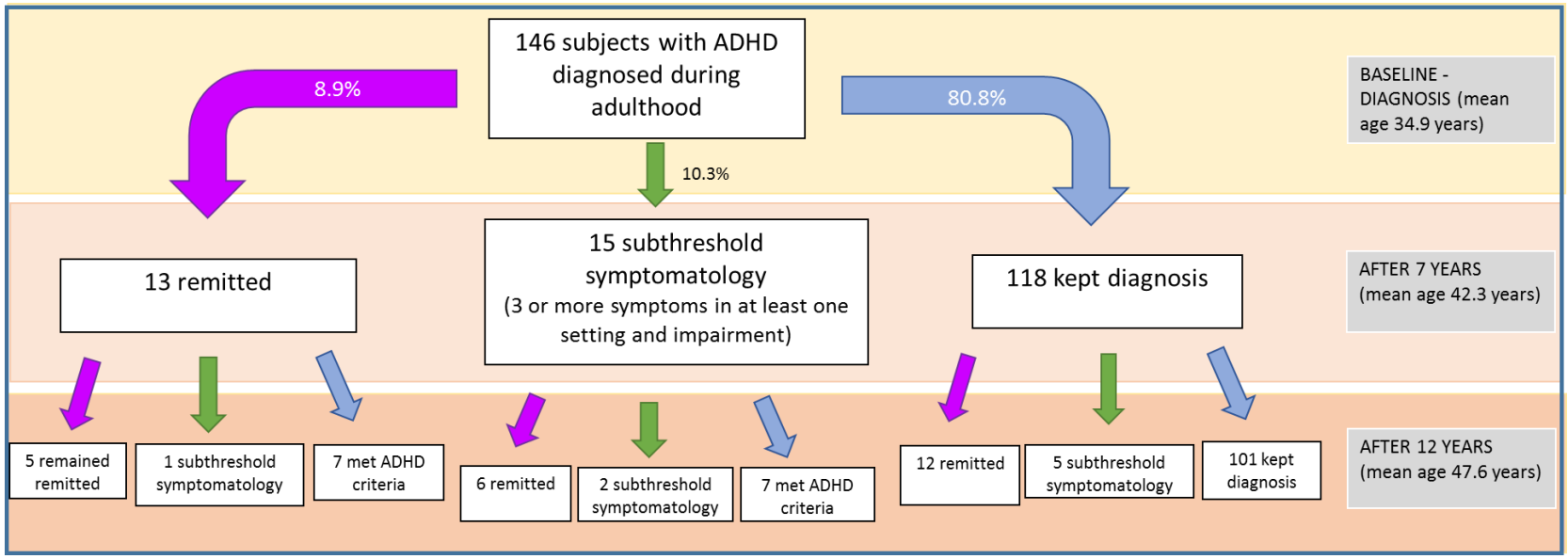
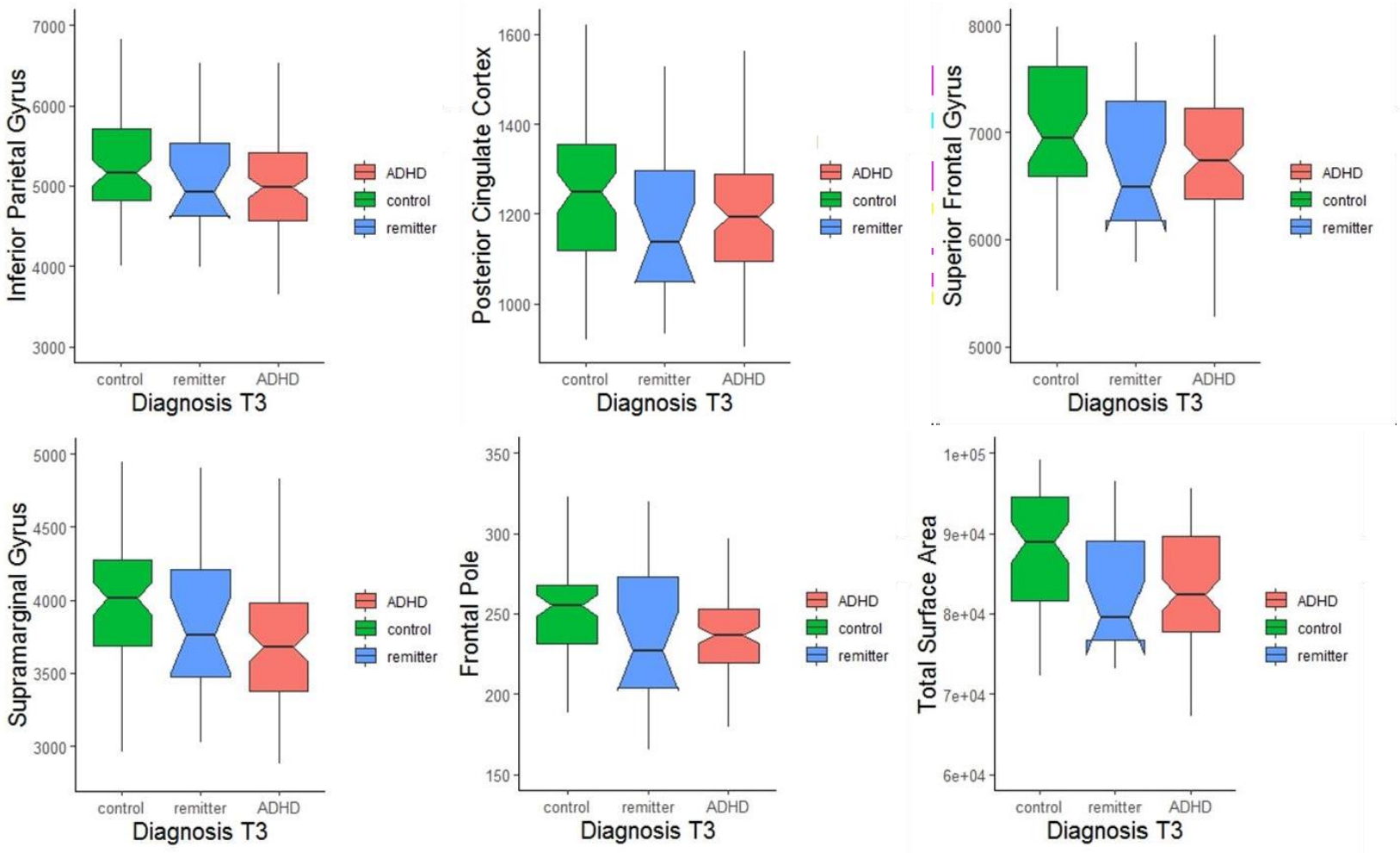
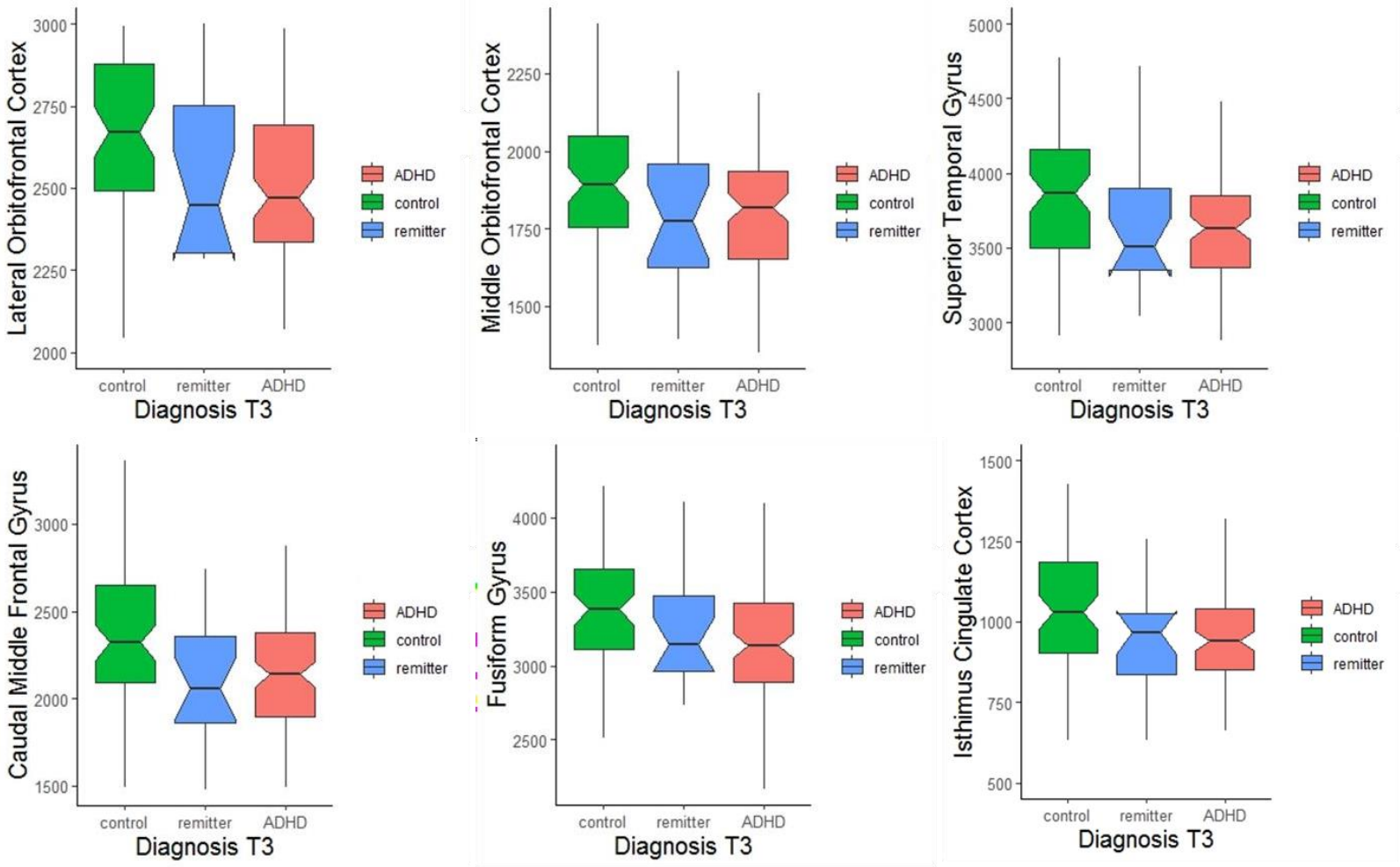
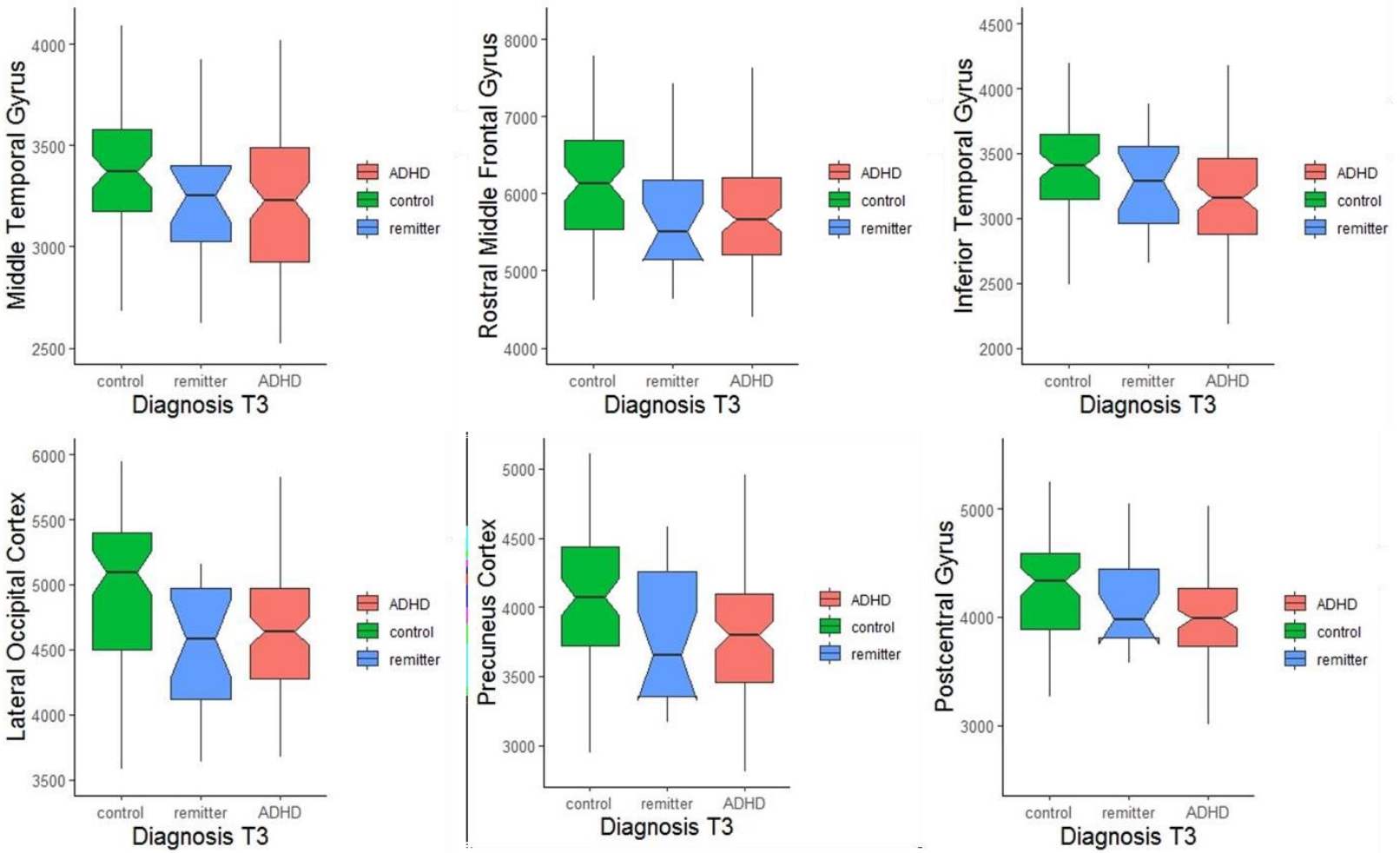
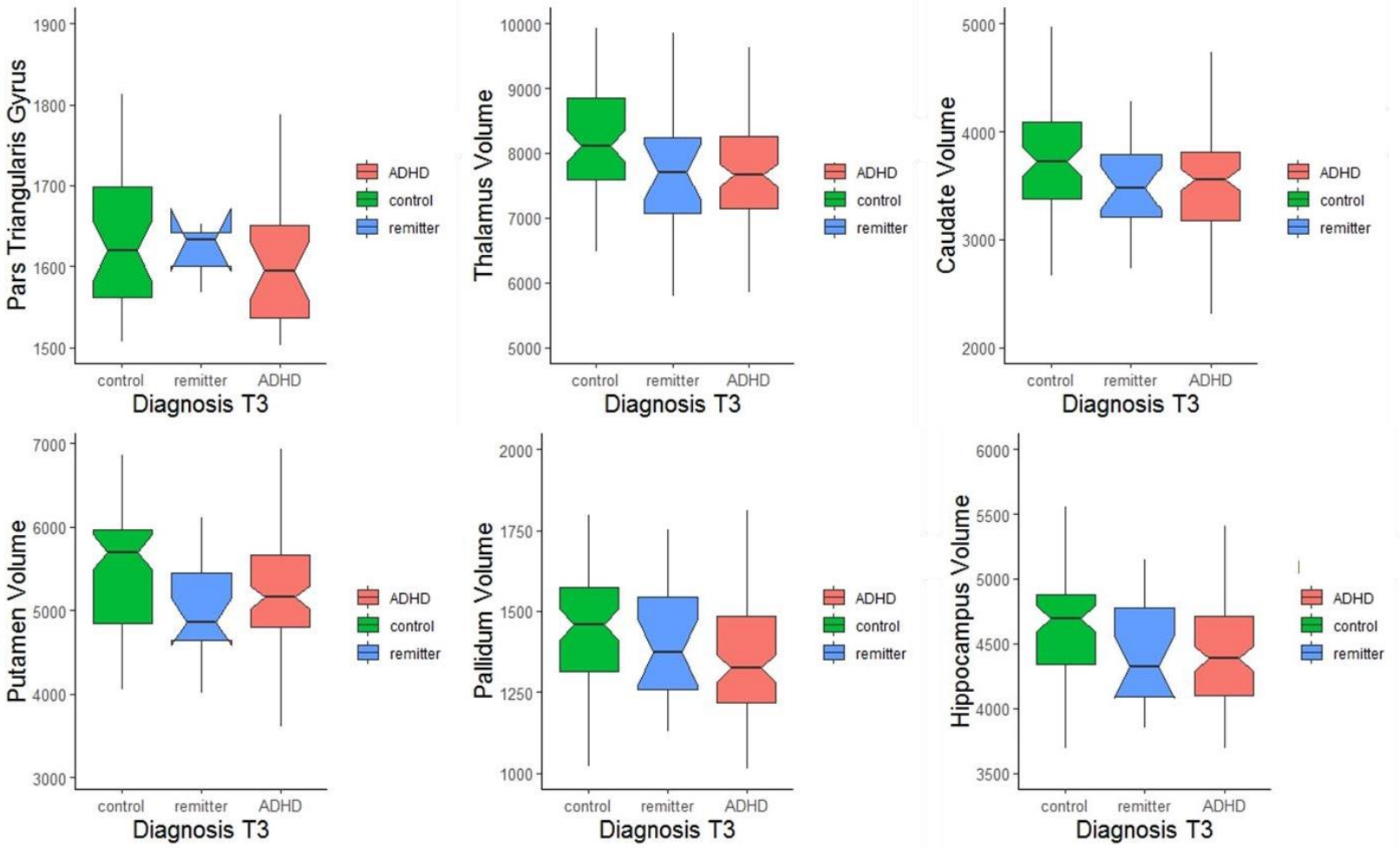


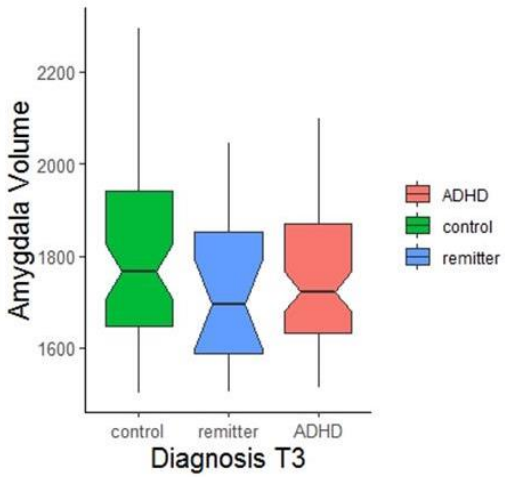
Figura 3. Plots representando as diferenças entre os grupos avaliados.

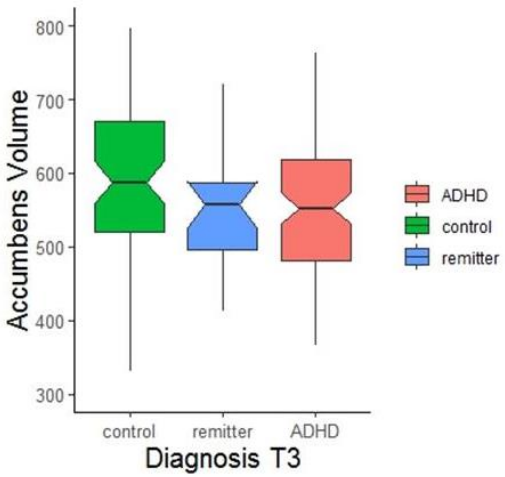



Lateral Orbitofrontal Cortex
Middle Orbitofrontal Cortex
Superior Temporal Gyrus
Caudal Middle Frontal Gyrus
Fusiform Gyrus
Isthimus Cingulate Cortex
control
remitter
ADHD
Diagnosis T3


Middle Temporal Gyrus
4000
3500
3000
2500
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter
Rostral Middle Frontal Gyrus
8000
7000
6000
5000
4000
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter
Inferior Temporal Gyrus
4500
4000
3500
3000
2500
2000
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter
Lateral Occipital Cortex
6000
5500
5000
4500
4000
3500
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter
Precuneus Cortex
5000
4500
4000
3500
3000
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter
Postcentral Gyrus
5000
4000
3000
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter


Pars Triangularis Gyrus
1900
1800
1700
1600
1500
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter
Thalamus Volume
10000
9000
8000
7000
6000
5000
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter
Caudate Volume
5000
4000
3000
2000
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter
Putamen Volume
7000
6000
5000
4000
3000
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter
Pallidum Volume
2000
1750
1500
1250
1000
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter
Hippocampus Volume
6000
5500
5000
4500
4000
3500
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter


Amygdala Volume
2200
2000
1800
1600
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter


Accumbens Volume
800
700
600
500
400
300
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter

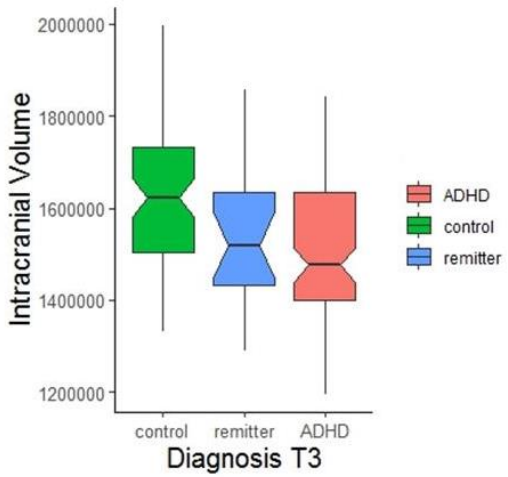

Intracranial Volume
2000000
1800000
1600000
1400000
1200000
control
remitter
ADHD
Diagnosis T3
ADHD
control
remitter

Tabela 2. Tabela descritiva considerando os três tempos de avaliação (n = 146).

	T1(baseline)	**T2**	**T3**
Age	34.9 (10.42)	42.3 (10.59)	47.6 (10.70)
Remitters (partial/full)	-	12(8.2) / 17(11.6)	23(15.8) / 8(5.5)
MDD	56 (38.6)	22 (15.4)	30 (21.6)
Tabagism	39 (26.9)	29 (19.9)	18 (12.3)
Social Phobia	26 (17.9)	12 (8.2)	29 (19.9)
OCD	9 (6.2)	12 (8.3)	8 (5.8)
Alcohol Use Disorder	19 (13.1)	10 (6.9)	6 (4.3)
SUD (other drugs)	12 (8.3)	11 (7.6)	4 (2.9)
Bipolar	6 (4.1)	6 (4.1)	9 (6.2)
GAD	28 (19.3)	46 (31.5)	18 (12.3)
ASPD	4 (2.8)	2 (1.4)	1 (0.7)
ODD	58 (39.7)	41 (28.1)	18 (12.5)

MDD: Major Depressive Disorder; OCD: Obsessive Compulsive Disorder; SUD: Substance Use Disorder; GAD: Generalized Anxiety Disorder; ASPD: Antisocial Personality Disorder; ODD: Oppositional Defiant Disorder. Data expressed by number of individual (frequency), except for age (mean (standard deviation)).

Tabela 3. Associação de medidas de neuroimagem com o diagnóstico atual.

MRI measures	P-value	Post-hoc
Cortical Thickness		
Precentral gyrus	0.299	-
Temporal pole	0.446	-
Fusiform gyrus	0.503	-
Parahippocampal gyrus	0.642	-
Surface Area		
Inferior parietal gyrus	**0.001**	**Controls > ADHD (P < 0.001)**; Remitter = ADHD (P = 0.49); Remitter = Controls (P = 0.11)
Posterior cingulate cortex	**0.032**	**Controls > ADHD (P <=0.014);** Remitter = ADHD (P = 0.74); Remitter = Controls (P = 0.10)
Precentral gyrus	0.2990	-
Superior frontal gyrus	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.41); **Remitter < Controls (P < 0.001)**
Supramarginal gyrus	**<0.001**	**Controls > ADHD (P < 0.001); Remitter > ADHD (P = 0.03);** Remitter = Controls (P = 0.21)
Frontal pole	**0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.94); **Remitter < Controls (P = 0.03)**
Total surface area	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.63); **Remitter** < **Controls (P < 0.001)**
Lateral Orbitofrontal Cortex	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.75); **Remitter < Controls (P = 0.004)**
Middle Orbitofrontal Cortex	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.96); **Remitter < Controls (P = 0.012)**
Superior temporal gyrus	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.74); **Remitter < Controls (P = 0.002)**
Caudal middle frontal gyrus	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.45); **Remitter < Controls (P = 0.001)**
Fusiform gyrus	**<0.001**	**Controls > ADHD (P < 0.001); Remitter > ADHD (P = 0.03);** Remitter = Controls (P = 0.13)

Isthmus cingulate cortex	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.67); **Remitter < Controls (P = 0.011)**
Middle temporal gyrus	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.97); **Remitter < Controls (P = 0.015)**
Rostral middle frontal gyrus	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.82); **Remitter < Controls (P = 0.002)**
Inferior temporal gyrus	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.26); Remitter = Controls (P = 0.071)
Lateral occipital cortex	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.16); **Remitter < Controls (P < 0.001)**
Precuneus cortex	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.96); **Remitter < Controls (P < 0.001)**
Banks of the superior temporal cortex	0.2210	-
Postcentral gyrus	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.10); **Remitter < Controls (P =0.044)**
Pars triangularis gyrus	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.86); **Remitter < Controls (P < 0.001)**
Volume		
Thalamus	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.91) **Remitter < Controls (P < 0.001)**;
Caudate	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.88); **Remitter < Controls (P < 0.001)**
Putamen	**0.002**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.19); **Remitter < Controls (P < 0.001)**
Pallidum	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.12); Remitter = Controls (P = 0.36)
Hippocampus	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.98); **Remitter < Controls (P = 0.03)**
Amygdala	**0.013**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.36); Remitter = Controls (P = 0.35)

Accumbens	**0.021**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.75); Remitter = Controls (P = 0.193)
ICV	**<0.001**	**Controls > ADHD (P < 0.001);** Remitter = ADHD (P = 0.22) **Remitter < Controls (P = 0.036)**

MRI: Magnetic Resonance Imaging; ICV: Intracranial volume;

Capítulo VII

9. Dados complementares

Alterações na integridade microestrutural da substância branca em transtornos psiquiátricos

Primeiramente, esse trabalho busca analisar aspectos de neuroimagem utilizando dados de DTI relacionados à susceptibilidade de TDAH, levando em consideração efeitos sexo-específicos. Para aumentar a confiabilidade do estudo, duas amostras independentes (brasileira e holandesa) serão utilizadas, como fruto do trabalho em colaboração com a Professora Dr. Barbara Franke (*Radboud Universisity Medical Center*) e apoio fundamental da Dr. Emma Sprooten (*Donders Institute*). A discussão desses dados ainda está em andamento, de forma que apenas o embasamento e resultados iniciais serão abordados nessa seção.

Em seguida, dada a alta prevalência de comorbidade entre TDAH e TUS, inclusive transtorno por uso de cocaína/crack e achados anteriores sugerindo uma etiologia, parcialmente, compartilhada, avaliamos também esses aspectos em uma amostra de dependentes de cocaína/crack, fruto da colaboração com o Professor Dr. Rodrigo Grassi-Oliveira (Pontifícia Universidade Católica do Rio Grande do Sul - PUCRS). Sua equipe foi responsável pela aquisição de dados clínicos e das imagens e o aluno Lucca Pizzato Tondo pelo processamento e análises de imagens, junto comigo. Essas análises estão em andamento e fazem parte de um projeto maior, envolvendo ainda dados epigenômicos e outras variantes a serem analisadas. Assim, apenas análises preliminares, mostrando o efeito do uso de crack sobre a integridade microestrutural da substância branca serão mostradas.

9.1 No TDAH

9.1.1 Introdução

Conforme mencionado no Capítulo I dessa Tese, estudos de neuroimagem têm demonstrado que alterações na substância branca são pontos centrais na

fisiopatologia do TDAH (Aoki et al. 2018). Essas alterações podem ser inferidas a nível celular através de técnicas de imagem por difusão, as quais se baseiam em propriedades de difusão da molécula da água ao longo do axônio (Beaulieu 2002), sendo a anisotropia fracionada (FA) a medida mais comumente utilizadas nos estudos com *diffusion-tensor imaging* (DTI).

Evidências de diferenças na estrutura cerebral de homens e mulheres têm sido constantemente reportadas, onde homens teriam um maior volume tanto de substância cinzenta quanto branca (Gur et al. 1999; Good et al. 2001; Chen et al. 2007), apesar mulheres teriam tratos de substância branca mais difusos (Witelson et al. 1995; Zaidi 2010; Gong et al. 2011; Menzler et al. 2011). Além disso, mulheres parecem apresentar mais substância cinzenta comparada a branca (Allen et al. 2003). A respeito de microestruturas da substância branca, diversos estudos avaliaram diferenças sexo-específicas (Kanaan et al. 2012; O'Dwyer et al. 2012; Kanaan et al. 2014), com diferenças no corpo caloso sendo as mais evidenciadas, apesar de ainda não haver um consenso em relação à direção do efeito. Kanann et al (2012), em uma análise *whole-brain* demonstrou um dimorfismo sexual significativo na substância branca, com áreas tanto com FA aumentada em homens em relação a mulheres quanto o oposto (Kanaan et al. 2012). Recentemente, utilizando técnicas de *deep learning*, um estudo confirmou as diferenças entre homens e mulheres em FA *maps*, bem como em regiões específicas (Xin et al. 2019).

Diferenças sexuais também são observadas em transtornos psiquiátricos (Rutter et al. 2003; Gobinath et al. 2017; Riecher-Rössler 2018). Em relação ao TDAH, diferenças em taxas de prevalência são observadas ao longo de toda a vida; enquanto até 80% de crianças com TDAH são meninos, em adultos a taxa homem:mulher é quase equivalente (Rucklidge 2010). O perfil clínico do TDAH também é diferente entre homens e mulheres, por exemplo, enquanto mulheres apresentam mais sintomas de desatenção, homens/meninos são mais propensos a

sintomas de hiperatividade; diferindo também em comorbidades e déficits executivos (Skogli et al. 2013). Além disso, estudos de RMN demonstraram diferenças sexuais relacionadas ao TDAH na conectividade funcional (Poissant et al. 2016; Rosch et al. 2018), morfologia cortical (Dirlikov et al. 2015; Wang et al. 2018) e em alterações microestruturais na substância branca (Jacobson et al. 2015). Utilizando técnicas de DTI, Jacobson et al (2015) investigaram diferenças sexo-específicas relacionadas ao TDAH, e encontraram que meninos tem mais alterações em regiões responsáveis pelo controle de ações básicas, enquanto meninas são mais afetadas em regiões responsáveis por um controle de nível superior.

Considerando diferenças sexuais no cérebro e no TDAH, bem como as já observadas diferenças no TDAH sexo-específicas, esse estudo tem como objetivo investigar o efeito de interações sexo-diagnóstico em adultos com TDAH em abordagens *voxel-wise* (TBSS - *tract-based spatial statistics*), seguida por análises de tratos específicos.

9.1.2 Materiais e métodos

Esse estudo inclui indivíduos avaliados por dois grupos do *International Multicentre persistent ADHD Collaboration* (IMpACT; www.impactADHDgenomics.com), do Brasil e da Holanda. No Brasil, 176 adultos foram avaliados da no PRODAH-A do Hospital de Clínicas – 103 diagnosticados com TDAH (idade média 47.4±1.0; 43.6% homens e 73 controles (idade média 38.2±1.0; 53.4% homens). O diagnóstico de TDAH foi baseado na 5ª edição do DSM (DSM-5) (American Psychiatric Association 2013) através de entrevista semi-estruturada (KSADS). Foram excluídos do estudo indivíduos com doença neurológica significativa (e.g. delírios, demência, epilepsia, trauma encefálico), ou QI menor que 70. As imagens de difusão foram adquiridas utilizando o *scanner* de corpo inteiro 3.0T Siemens SPECTRA com uma bobina de crânio de 8 canais,

usando uma sequência *single-shot echo planar* (62 axial slices, TR/TE-11000/110ms, voxel isotrópico de 2mm^3, espessura da fatia = 2.0mm, *Field Of View* (FOV) – 240mm^3, uma imagem com b0 e 64 imagens com direções de gradiente b=1400s/mm^2). Um protocolo adaptado, com menor tempo de aquisição, foi utilizado para 25 indivíduos inquietos ou com sintomas de claustrofobia (voxel isotrópico de 2.4mm, espessura da fatia = 2.4mm TR/TE = 11000/106ms, 32 imagens com gradiente de difusão, os demais parâmetros foram os mesmos). Todos os participantes assinaram um termo de consentimento livre e esclarecido aprovado pelo comitê de ética do hospital.

Na Holanda, 116 pacientes (idade média 33.9±0.9; 43.1% homens) foram recrutados do departamento de Psiquiatria do Centro Médico da Universidade Radboud, em Nijmegen e 121 controles (idade média 35.6±1.1; 39.7% homens) voluntários recrutados através de anúncios. O diagnóstico de TDAH foi baseado no DSM-IV-TR, utilizando entrevista semi-estruturada (DIVA). Foram considerados critérios de exclusão: psicose, adição de álcool ou outras substâncias nos últimos seis meses, transtorno depressivo maior no momento da avaliação, QI menor que 70, doenças neurológicas, uso de outras medicações que não psicoestimulantes ou atomoxina. Esse estudo foi aprovado pelo comitê de ética regional (*Centrale Commissie Mensgebonden Onderzoe CMO Regio Arnhem — Nijmegen; protocol number III.04.0403*) e todos os participantes assinaram um termo de consentimento. A aquisição de imagens foi através do *scanner* 1.5T Magnetom Avanto Siemens com uma bobina de crânio de 8 canais, usando uma sequência *single-shot echo planar*. Dois protocolos diferentes foram utilizados para a aquisição das imagens. Vinte e oito participantes pelo seguinte protocolo: FOV= 320x320x160mm, TR/TE=10200/95ms, voxel isotrópico de 2.5mm, quatro imagens b0 e 30 com gradientes de difusão b=900s/mm^2, 34 direções. Os outros 209 foram escaneados utilizando um protocolo adaptado para reduzir artefatos relacionados ao movimento: diferindo apenas nos parâmetros TR/TE=67000/85ms e FOV=220x220x140mm^3.

As imagens de ambos os grupos foram corrigidas para movimento e *eddy-currents*, e processadas usando ferramentas padrões do FMRIB *Software Library* (FSL) (https://fsl.fmrib.ox.ac.uk/fsl/), seguidas por um controle de qualidade visual. FA *maps* para cada indivíduo foram calculados através do *dtifit* no FSL. Análises *voxel-wise* foram conduzidas usando TBSS com um FA *threshold* >0.2 (Smith et al. 2006), na qual a escolha de uma região de interesse *a priori* não se faz necessária. A significância estatística foi avaliada usando 5000 permutações para inferências não-paramétricas e *threshold free cluster enhancement* (TFCE; Smith and Nichols 2009) utilizando *randomise* no FSL. Essas análises foram conduzidas separadamente em cada grupo e ajustadas por idade, sexo (quando aplicável) e movimento.

Para melhor explorar o efeito de interação entre sexo e diagnóstico, a média dos valores de FA provenientes do *skeleton* foram extraídas do FA médio e de 11 tratos (*anterior thalamic radiation* - ATR, *corticospinal tract* - CST, *dorsal cingulate gyrus* - CING, *ventral cingulate gyrus* - HIPPCING, *forceps minor*, *forceps major*, *inferior fronto-occipital fasciculus* - IFOF, *inferior longitudinal fasciculus* - ILF, *superior longitudinal fasciculus* - SLF, *uncinate fasciculus* – UF, *temporal part of* SLF - SLFTEMP) de acordo com o atlas de tractografia da John Hopkins University (JHU) (https://identifiers.org/neurovault.image:1403) e analisadas usando modelos de regressão linear. Para obter um maior poder estatístico, as duas amostras foram analisadas juntas, e dessa forma ajustadas também para *site*.

9.1.3 Resultados

Análises voxel-wise não mostraram efeito significativo entre casos e controles em nenhuma das amostras. Um efeito significativo de sexo, independentemente do diagnóstico, foi observado nas duas amostras (P = 0,024 e 0,023 nos grupos do Brasil e da Holanda, respectivamente). A interação do sexo e

diagnóstico não foi significativa, mas demonstrou uma tendência nas duas amostras (P = 0,11 e 0,058 nos grupos do Brasil e da Holanda, respectivamente – mostrados em P < 0.1 e P > 0.2 na **Figura 4).**

Através de análises de tratos específicos foi identificado um efeito de interação significativo no ATR (P = 0,011), ILF (P = 0,028), SLF (P = 0,007) esquerdo; ILF bilateral (P = 0,028), fórceps major (P = 0,03) (**Figure 5**). Além disso, uma tendência foi observada em relação aos valores globais de FA (P = 0,053). Após a correção por FDR apenas o efeito no SLF esquerdo se manteve significativo.

9.1.4 Conclusão Preliminar e Perspectivas

Resultados preliminares indicam um efeito sugestivo de interação entre sexo e diagnóstico no TDAH sobre valores de FA, indicando a necessidade de considerar o sexo nesse tipo de análises. Esse efeito parece ocorrer principalmente no hemisfério esquerdo, em geral mais relacionado a funções de linguagem, lógica, escrita, matemática. No entanto esses resultados devem ainda ser confirmados. Ainda, homens e mulheres com TDAH apresentam um diferente perfil de comorbidades, e dessa forma análises considerando outros transtornos também são necessárias para melhor compreender esse efeito.

Figura 4. Análises TBSS mostrando uma tendência de interação entre sexo e diagnóstico.

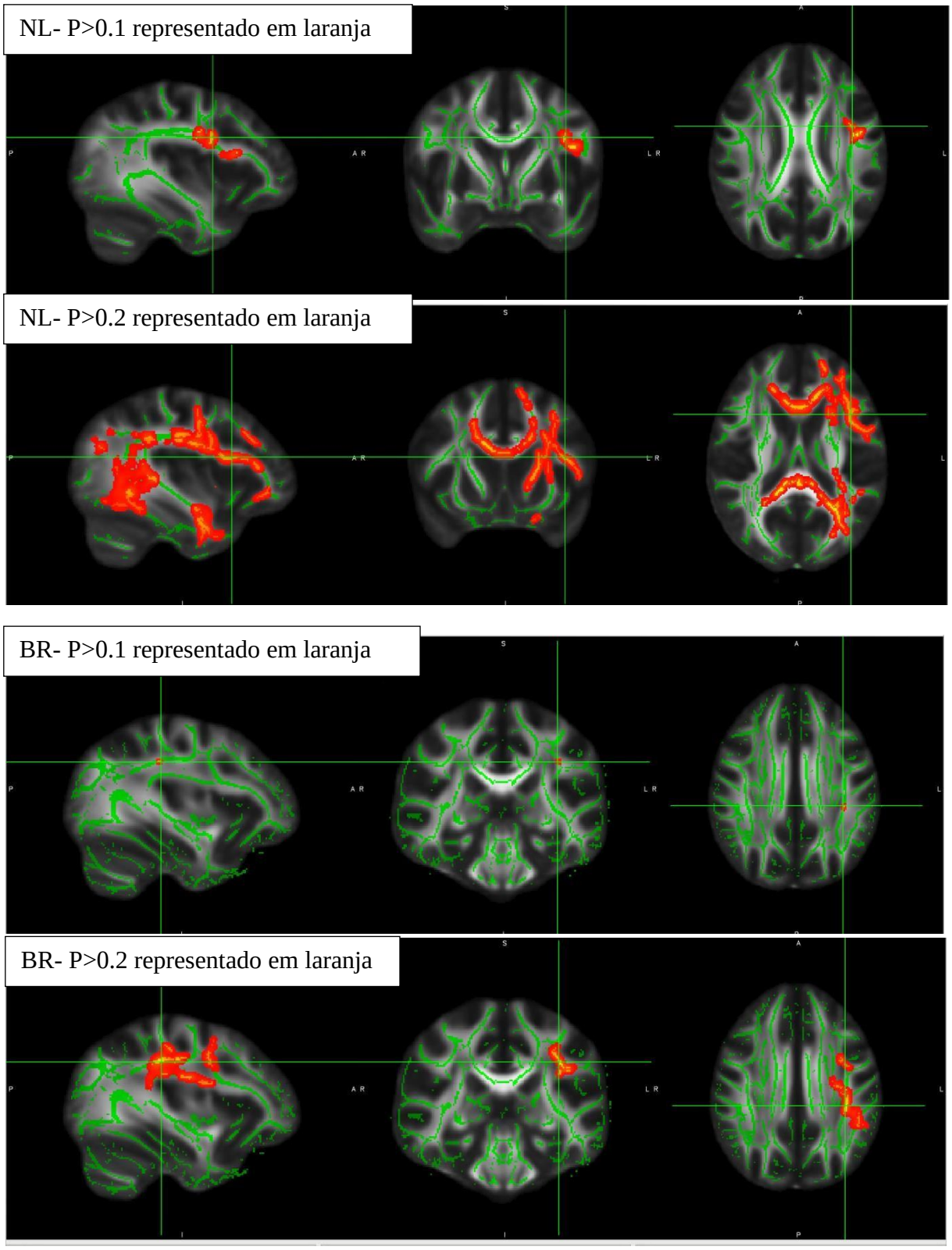
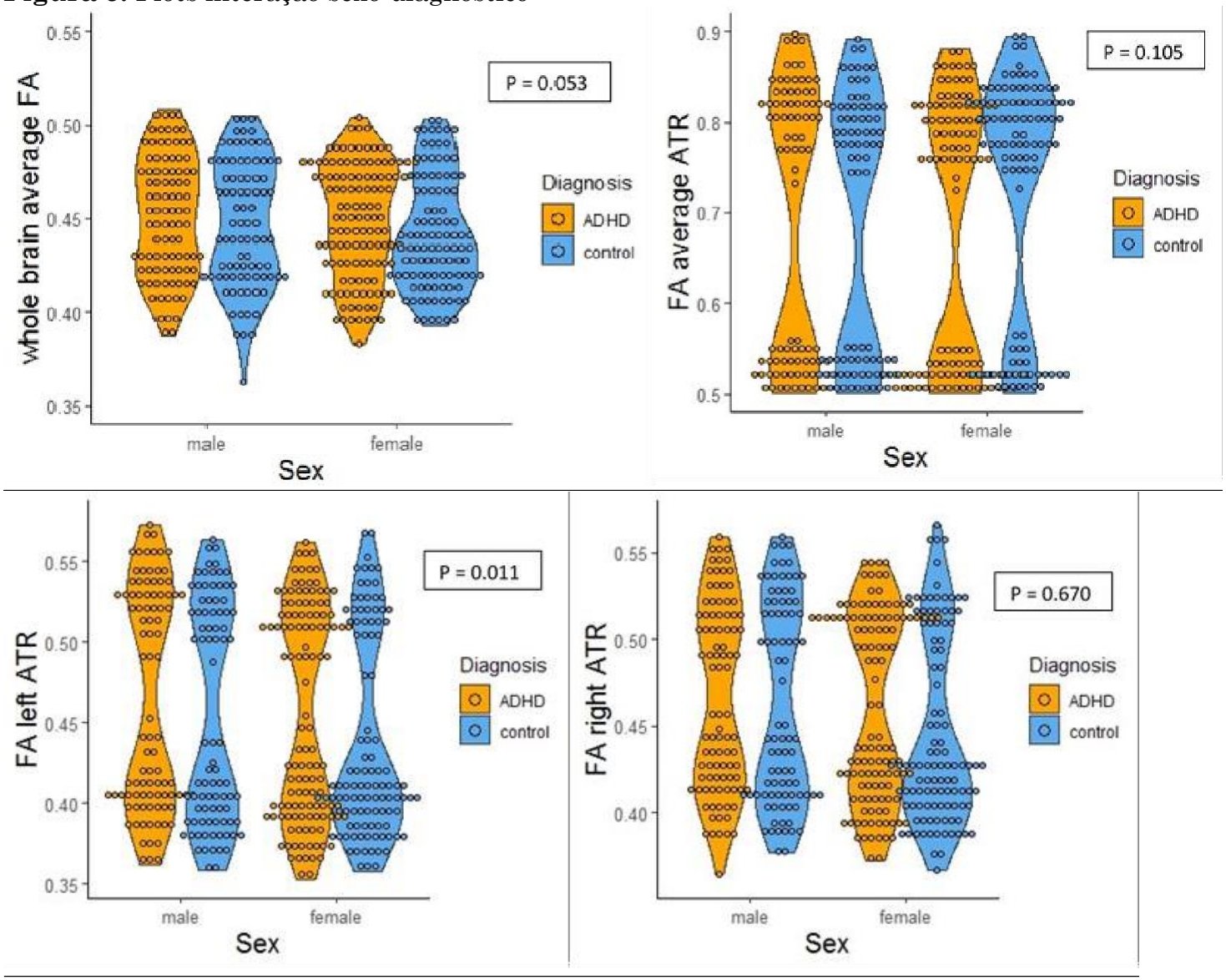
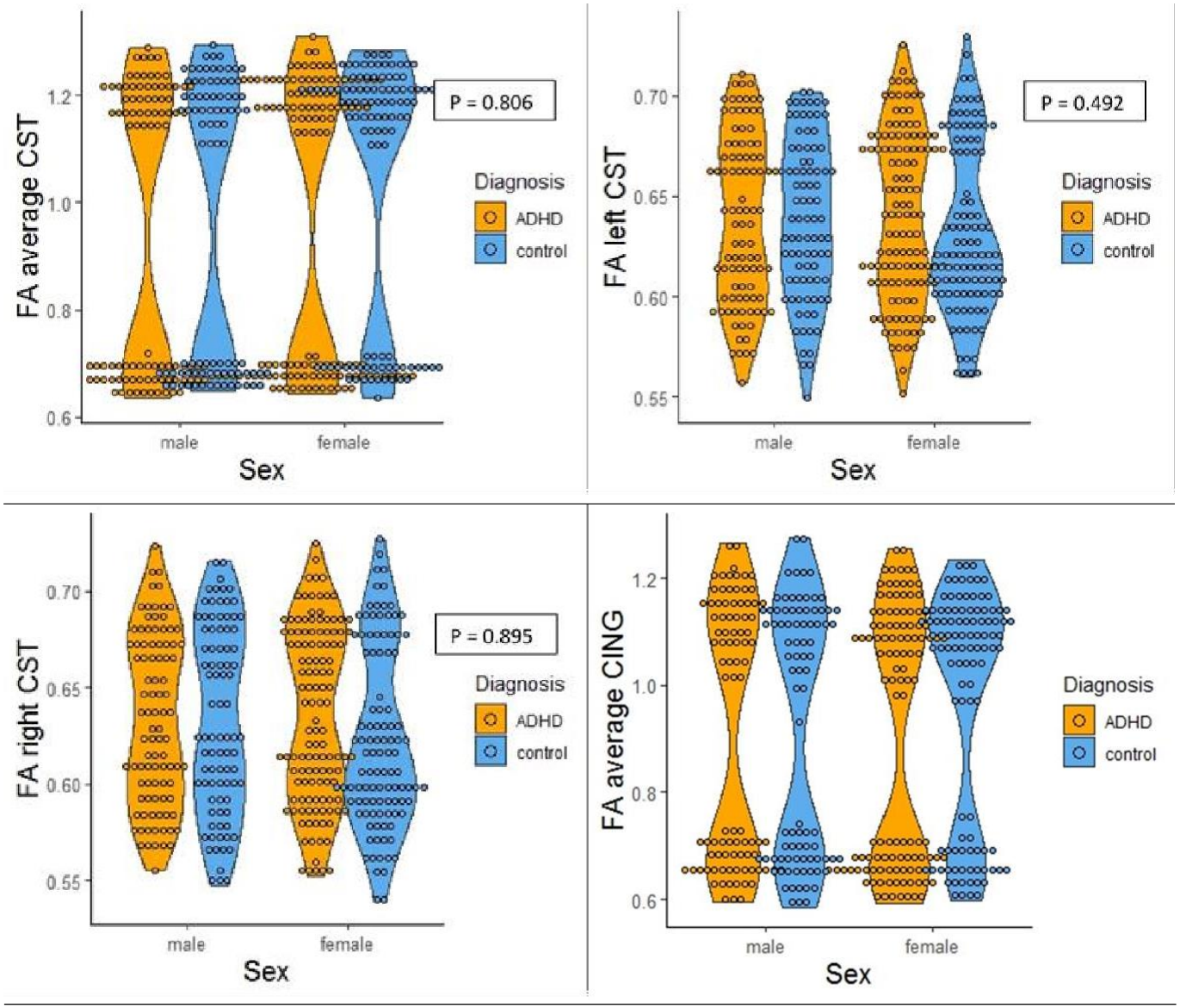
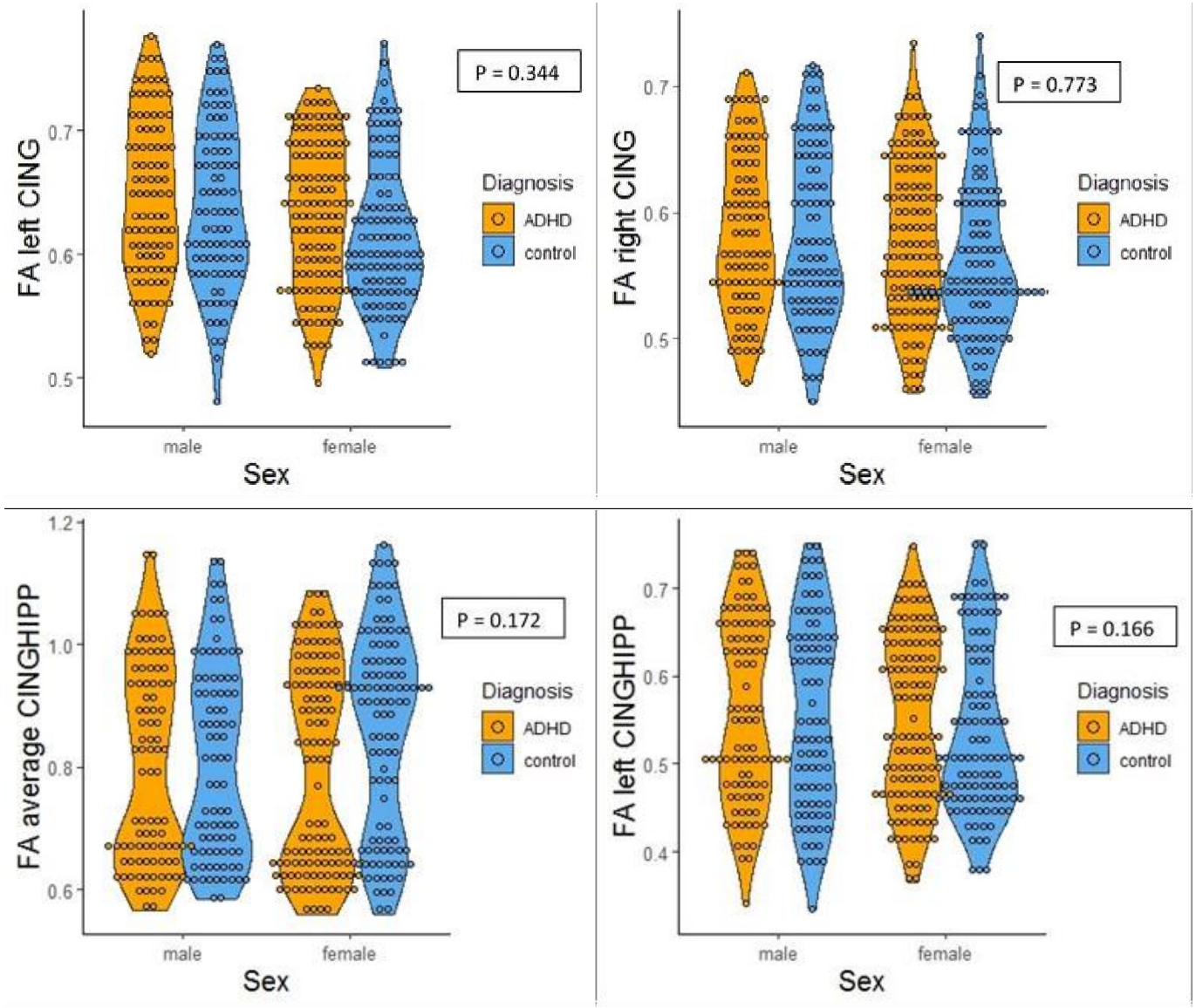
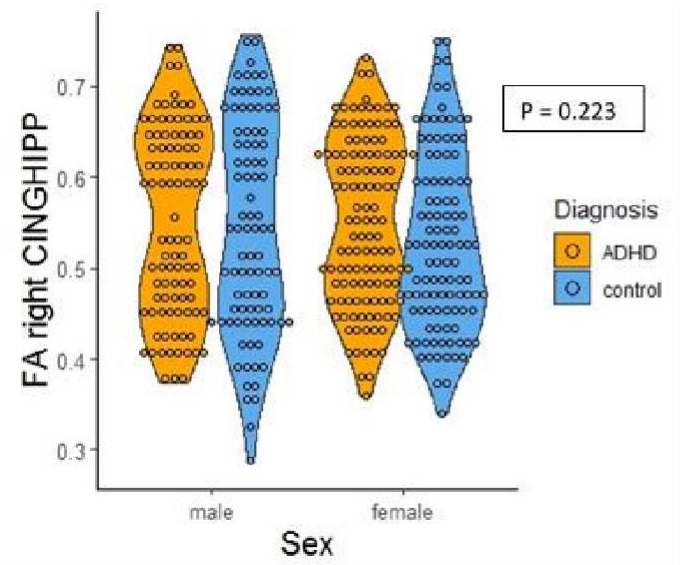


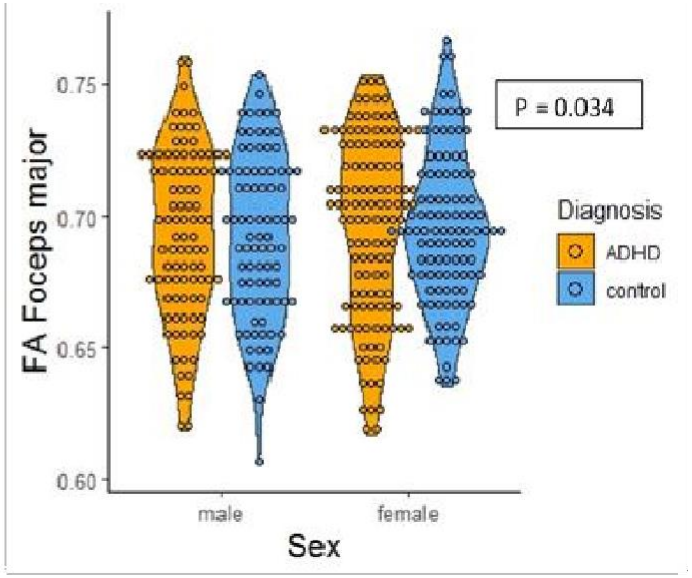
Figura 5. Plots interação sexo-diagnóstico

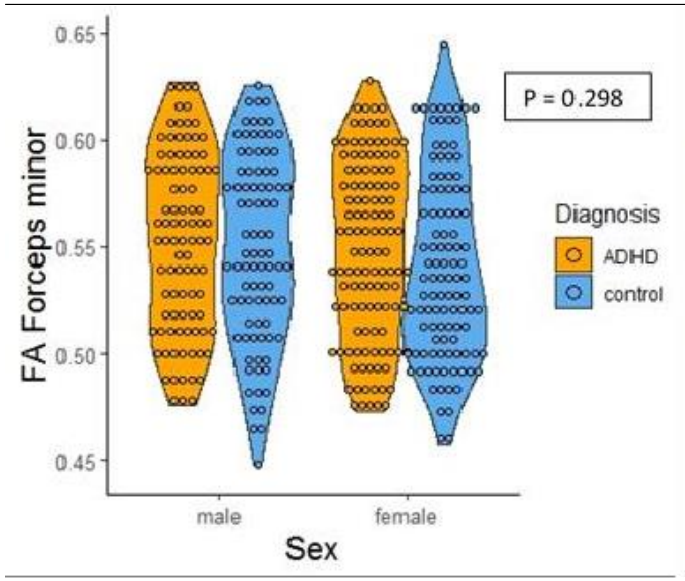


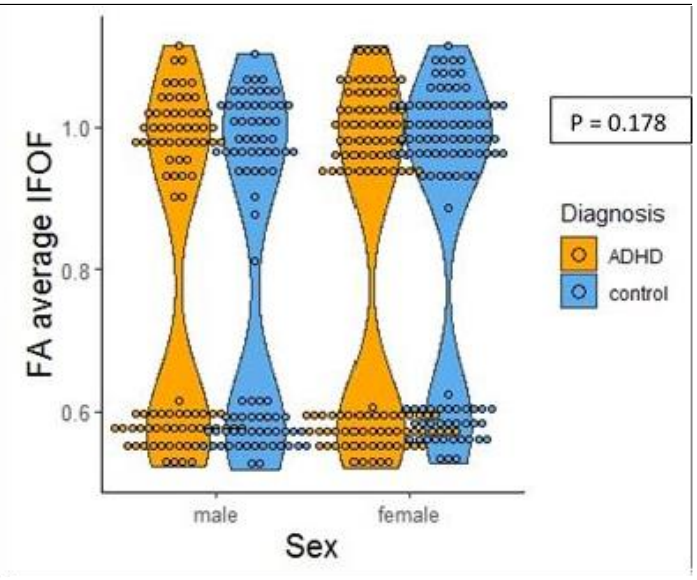

FA average CST
1.2
1.0
0.8
0.6
P = 0.806
Diagnosis
ADHD
control
male
female
Sex
FA left CST
0.70
0.65
0.60
0.55
P = 0.492
Diagnosis
ADHD
control
male
female
Sex
FA right CST
0.70
0.65
0.60
0.55
P = 0.895
Diagnosis
ADHD
control
male
female
Sex
FA average CING
1.2
1.0
0.8
0.6
Diagnosis
ADHD
control
male
female
Sex

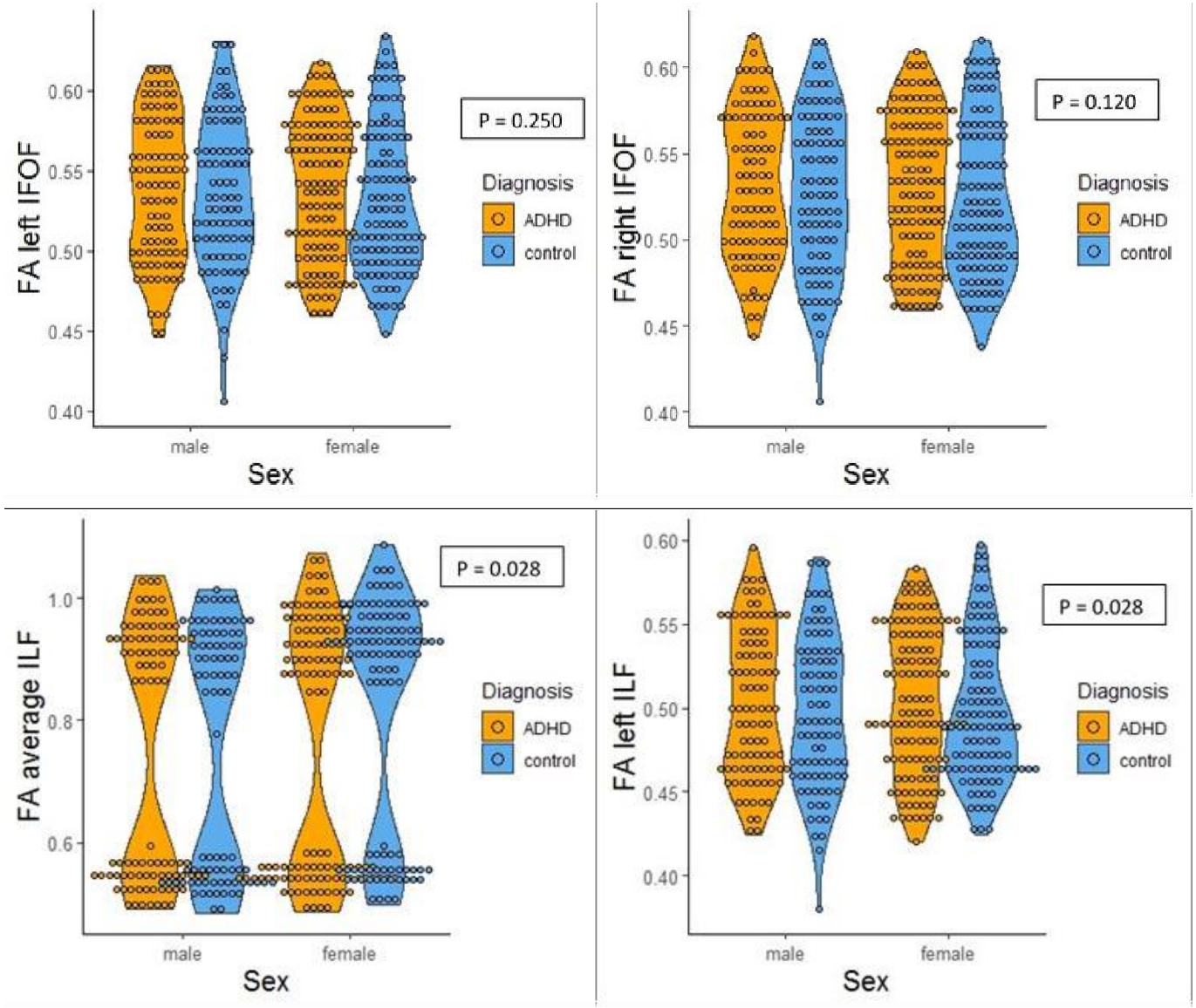

FA left CING
0.7
0.6
0.5
male
female
Sex
P = 0.344
Diagnosis
ADHD
control
FA right CING
0.7
0.6
0.5
male
female
Sex
P = 0.773
Diagnosis
ADHD
control
FA average CINGHIPP
1.2
1.0
0.8
0.6
male
female
Sex
P = 0.172
Diagnosis
ADHD
control
FA left CINGHIPP
0.7
0.6
0.5
0.4
male
female
Sex
P = 0.166
Diagnosis
ADHD
control

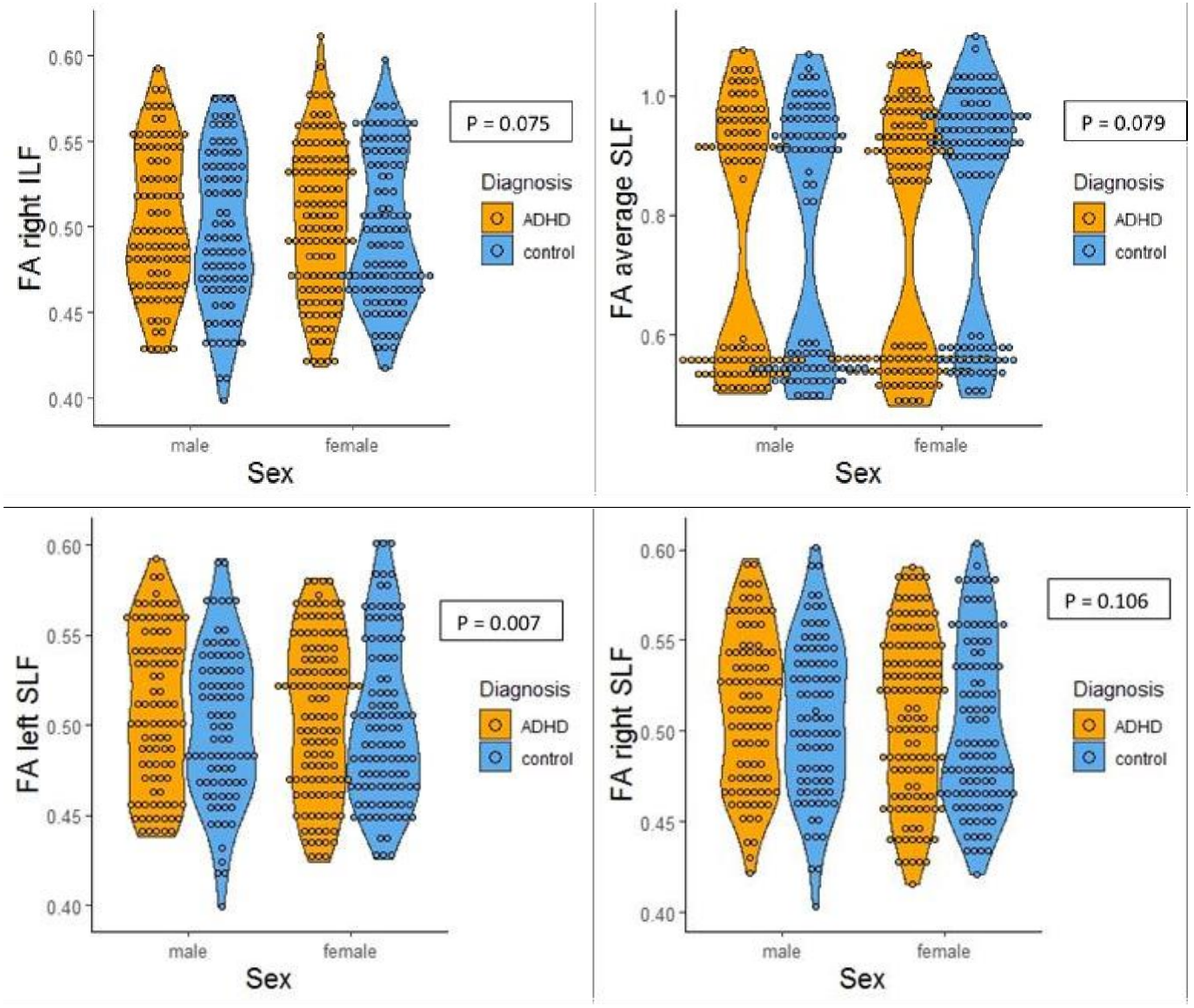

FA right CINGHIPP
0.7
0.6
0.5
0.4
0.3
male
female
Sex
P = 0.223
Diagnosis
ADHD
control

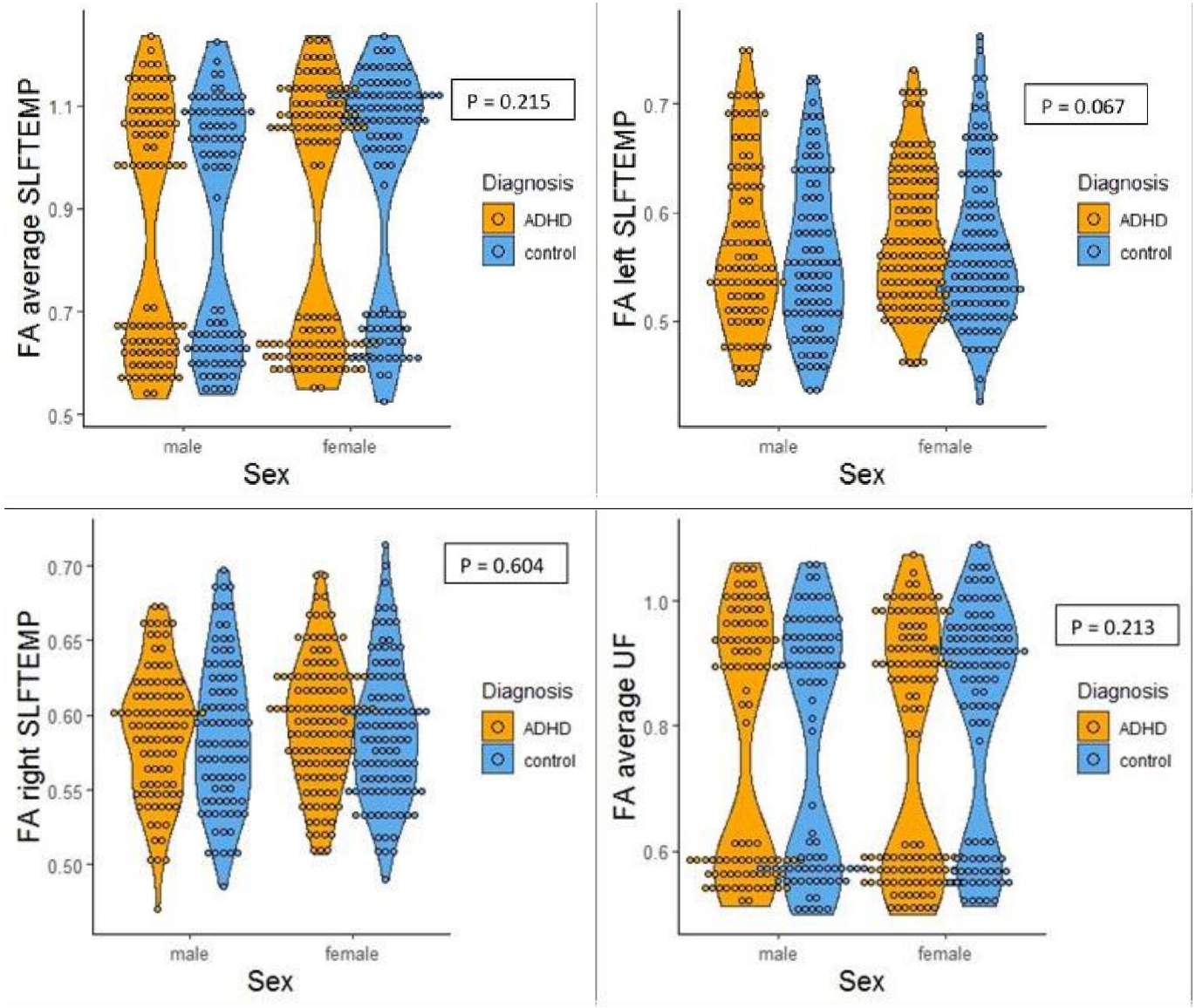

FA Foceps major
0.75
0.70
0.65
0.60
male
female
Sex
P = 0.034
Diagnosis
ADHD
control

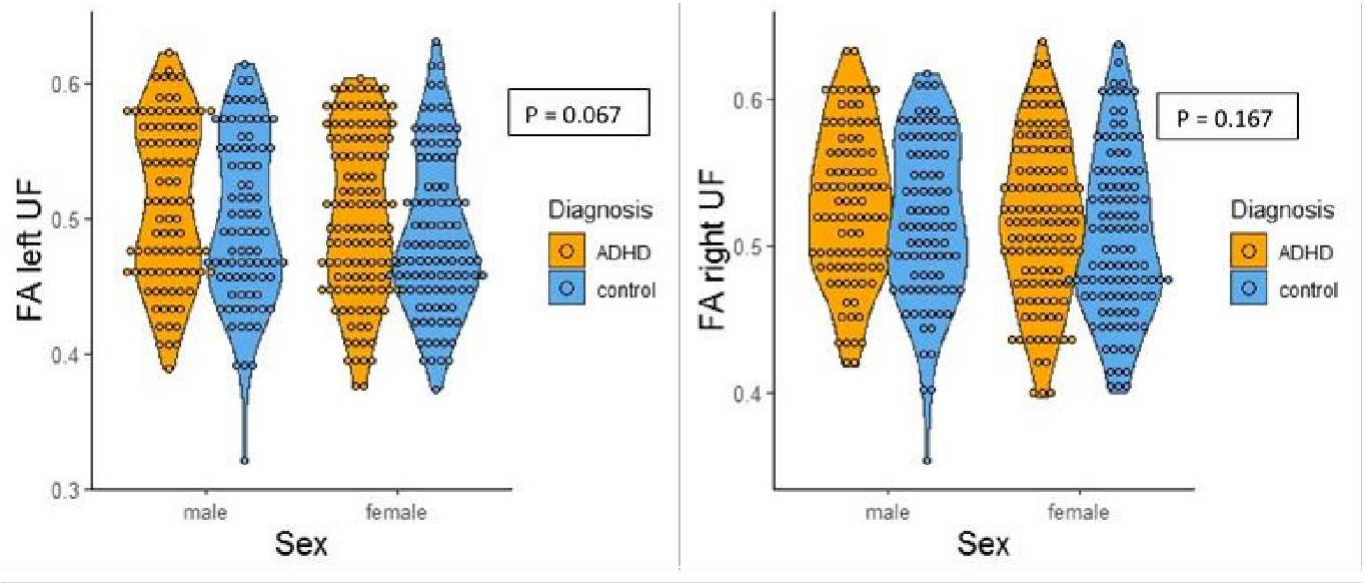

FA Forceps minor
0.65
0.60
0.55
0.50
0.45
male
female
Sex
P = 0.298
Diagnosis
ADHD
control


FA average IFOF
1.0
0.8
0.6
male
female
Sex
P = 0.178
Diagnosis
ADHD
control


FA left IFOF
0.60
0.55
0.50
0.45
0.40
male
female
Sex
P = 0.250
Diagnosis
ADHD
control
FA right IFOF
0.60
0.55
0.50
0.45
0.40
male
female
Sex
P = 0.120
Diagnosis
ADHD
control
FA average ILF
1.0
0.8
0.6
male
female
Sex
P = 0.028
Diagnosis
ADHD
control
FA left ILF
0.60
0.55
0.50
0.45
0.40
male
female
Sex
P = 0.028
Diagnosis
ADHD
control


FA right ILF
0.60
0.55
0.50
0.45
0.40
male
female
Sex
P = 0.075
Diagnosis
ADHD
control
FA average SLF
1.0
0.8
0.6
male
female
Sex
P = 0.079
Diagnosis
ADHD
control
FA left SLF
0.60
0.55
0.50
0.45
0.40
male
female
Sex
P = 0.007
Diagnosis
ADHD
control
FA right SLF
0.60
0.55
0.50
0.45
0.40
male
female
Sex
P = 0.106
Diagnosis
ADHD
control


FA average SLFTEMP
1.1
0.9
0.7
0.5
male
female
Sex
P = 0.215
Diagnosis
ADHD
control
FA left SLFTEMP
0.7
0.6
0.5
male
female
Sex
P = 0.067
Diagnosis
ADHD
control
FA right SLFTEMP
0.70
0.65
0.60
0.55
0.50
male
female
Sex
P = 0.604
Diagnosis
ADHD
control
FA average UF
1.0
0.8
0.6
male
female
Sex
P = 0.213
Diagnosis
ADHD
control


FA left UF
0.6
0.5
0.4
0.3
male
female
Sex
P = 0.067
Diagnosis
ADHD
control
FA right UF
0.6
0.5
0.4
male
female
Sex
P = 0.167
Diagnosis
ADHD
control

9.2 No transtorno por uso de cocaína/crack

9.2.1 Introdução

O TUS ocorre frequentemente em comorbidade com o TDAH (Molina and Pelham 2003; Saules et al. 2003; Ohlmeier et al. 2007; Charach et al. 2011; Lee et al. 2011; Ilbegi et al. 2018), incluindo o transtorno por uso de cocaína, principalmente em homens (Solberg et al. 2018). A cocaína é uma das drogas ilícitas mais consumidas no mundo (UNODC 2017), associada com aspectos de vulnerabilidade social. O crack é a forma fumada da cocaína, derivada da mistura do pó de cocaína com bicarbonato de sódio e outras substâncias (Hatsukami and Fischman 1996). Assim como o TDAH, o transtorno por uso de cocaína tem sido associado a alterações na microestrutura da substância branca (Lim et al. 2008; Ma et al. 2009; Lane et al. 2010; Bell et al. 2011; Narayana et al. 2014).

9.2.2 Materiais e Métodos

Foram avaliados 77 (idade média 32.78±6.66; 50.6% mulheres) usuários de cocaína provenientes de unidades de desintoxicação em Porto Alegre. Esse estudo faz parte do projeto "Alvos de proteção à mulher usuária de crack" da PUCRS coordenado pelo Prof. Rodrigo Grassi-Oliveira. Os critérios de inclusão foram: Diagnóstico de transtorno por uso de cocaína pela entrevista estruturada *The Structured Clinical Interview for DSM-IV Axis I Disorders* (SCID-I, (First and Gibbon 2004); QI maior do que 80; auto relato de crack/cocaína como substância de abuso de preferência; destros. Foram excluídos participantes com alguma restrição a RMN ou que apresentassem sintomas psicóticos. Além disso, 57 controles (idade média 27.98±7.13; 63.8% mulheres) foram recrutados através de anúncios. Os critérios de inclusão foram: histórico negativo de uso de drogas ilícitas e não diagnosticados para nenhum transtorno psiquiátrico, permitindo

apenas transtorno por uso de nicotina.

As imagens foram adquiridas no Instituto do Cérebro do Rio Grande do Sul, através do *scanner* 3.0T GE com uma bobina de crânio de 8 canais, usando uma sequência *single-shot echo planar* (TR/TE-13000/81.1ms, espessura da fatia = 2.4mm, uma imagem com b0 e 33 imagens com direções de gradiente b=750s/mm^2). O processamento das imagens foi o mesmo utilizado na amostra de TDAH, utilizando ferramentas do FSL. As análises também foram conduzidas pelo TBSS, utilizando sexo, idade e movimento como covariáveis.

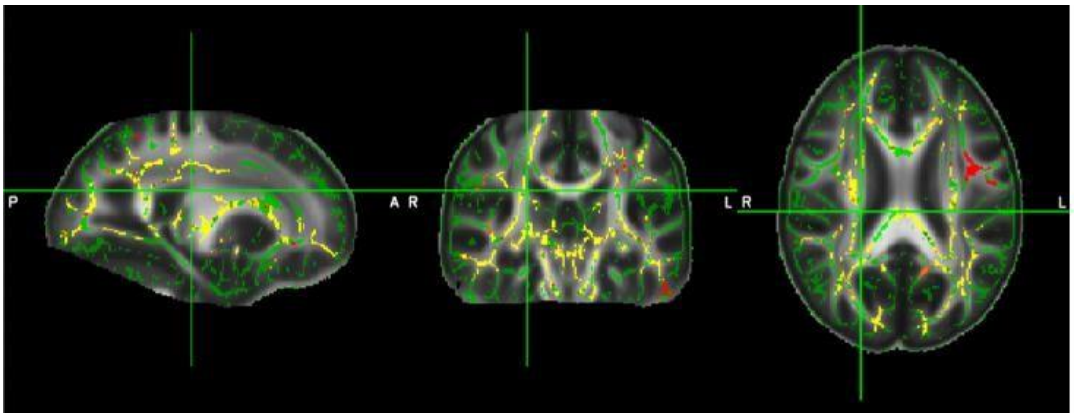
9.2.3 Resultados

Foi observado uma associação dos usuários de cocaína com valores de FA (**Figura 6**), com um efeito disseminado pelo cérebro. Foi observado também um efeito do sexo, independente do grupo (i.e., usuários ou controles), mas não foi observada interação entre sexo e grupo.

9.2.4 Conclusão preliminar e perspectivas

Esses achados apontam que o uso de cocaína tenha uma grande influência na integridade estrutural da substância branca, com alterações difusas em muitas regiões do cérebro. Análises posteriores relacionadas a tempo de uso, gravidade e performance cognitiva podem auxiliar na compreensão desse amplo efeito. Além disso, através de parcerias uma perspectiva do grupo da PUCRS é avaliar aspectos moleculares possivelmente relacionados, como comprimento de telômeros e medidas epigenômicas.

Figura 6. Associação entre FA e transtorno por uso de cocaína (P > 0.01 representado em amarelo e laranja).



9.3 Conclusão geral do projeto até o momento

Os efeitos observados do Transtorno por Uso de Crack parecem ser muito maiores e mais difusos em relação aos observados no TDAH, detectáveis mesmo em uma amostra menor, indicando um maior tamanho de efeito. Diferente do TDAH, parece não haver um efeito de interação entre o Transtorno por Uso de Crack e sexo, apesar do perfil clínico diferente entre homens e mulheres em que homens parecem desenvolver uma dependência antes, enquanto mulheres demoram mais, mas apresentam maior gravidade e um pior prognóstico (Sanvicente-Vieira et al. 2019) A maior prevalência de TUS em homens com TDAH, em relação a mulheres (Solberg et al. 2018), reforça a necessidade de se avaliar os resultados anteriores considerando o perfil de comorbidades.

Capítulo VIII

Discussão geral

Transtornos psiquiátricos estão relacionados a altas taxas de morbimortalidade no mundo inteiro, com altos índices de suicídio e comportamentos de risco (Chesney et al. 2014), por exemplo pacientes com TDAH apresentam um aumento da mortalidade em 220% (Dalsgaard et al. 2015). Em muitos casos, essa alta mortalidade é decorrente da recusa em aceitar o transtorno e a realizar o tratamento pelo estigma por trás dos transtornos psiquiátricos. Esse estigma acompanha os transtornos há séculos e fez com que muitas pessoas ao invés de serem tratados fossem considerados abomináveis ou criminosas, sob punição divina ou possuídos por entidades demoníacas (Rössler 2016). A compreensão dos transtornos como algo mais concreto e palpável, através da compreensão de bases biológicas envolvidas ("uma doença do cérebro") são os primeiros passos no sentido de romper esses estigmas, sendo dessa forma uma perspectiva promissora para que possamos superar esse obstáculo na saúde pública. Além disso, a maior compreensão da neurobiologia pode levar a avanços no diagnóstico e tratamento do transtorno.

O TDAH é muito questionado em relação a sua existência, superdiagnóstico e tratamento com estimulantes em crianças. Particularmente em adultos, só no final do século XX o transtorno foi descrito com bases científicas modernas (Wood et al. 1976). Nos últimos anos, o TDAH vem apresentando grandes mudanças conceituais, como por exemplo a inclusão formal do transtorno em adultos somente na última versão do DSM, e sendo alvo de inúmeros debates. Nesse sentido, estudos longitudinais, tanto clínicos quanto populacionais, tem se mostrado de suma importância. A observação da remissão dos sintomas de TDAH na vida adulta necessita ser melhor explorada, a exemplo do estudo, ainda em andamento, abordado no Capítulo VI. A compreensão da neurobiologia envolvida nessas trajetórias ao longo da vida pode contribuir substancialmente para estudos visando novas estratégias terapêuticas.

Apesar de que descrições fenotípicas compatíveis com TDAH datam de muito tempo atrás, inclusive da Grécia antiga (Victor et al. 2018), só nas últimas décadas, com avanços científicos e tecnológicos, está sendo possível explorar e conhecer mais sobre a etiologia do TDAH. O século XXI começa abrindo perspectivas promissoras no entendimento da etiologia de transtornos psiquiátricos, como o TDAH, através de estudos de genética e neuroimagem.

Na área da genética, grandes avanços foram alcançados nos últimos anos, culminando na identificação de diversos *loci* envolvidos no TDAH (Demontis et al. 2019). Embora ainda falte muito da herdabilidade do transtorno a ser explicada no nível molecular, e ainda seja necessária uma maior compreensão do papel desses fatores genéticos, os progressos já obtidos representam um grande avanço no entendimento da arquitetura genética do TDAH. Na área da neuroimagem é mais difícil estabelecer um marco específico no seu avanço. Novas técnicas e abordagens, desde a aquisição até análise dos dados, vêm sendo implementadas gradualmente e têm ajudado a obter diferentes informações. As técnicas de difusão (como utilizadas nos Capítulos IV e VII), relativamente recentes, possibilitaram observações antes só possíveis em estudos *post mortem*. Além disso a evolução de métodos analíticos tem permitido a detecção de novos fatores, antes muitas vezes negligenciados, como exemplificado no Capítulo III onde o uso de uma metodologia amplamente utilizada para RMN funcional se mostra útil também em RMN estrutural.

Esses avanços nos dão ideia da complexidade envolvida no TDAH e outros transtornos psiquiátricos. Por um lado, temos a genética com suas milhões de variantes interagindo entre si e com fatores ambientais. De outro, a neuroimagem com seus milhões de neurônios e células não-neuronais. Considerando esse alto grau de complexidade, a presente Tese é um exercício preliminar de avaliar bases genéticas e neurobiológicas do transtorno. Ainda, essa

Tese marca o início dos trabalhos do nosso grupo na área de neuroimagem e genética.

Através de estudos de genética e/ou de neuroimagem, a presente Tese apresenta resultados que contribuem para o entendimento da neurobiologia do TDAH, demonstrando aspectos estruturais no cérebro associados ao transtorno em duas amostras independentes, com idades diferentes e provenientes de dois diferentes continentes, o que aumenta a confiabilidade e robustez dos achados (Capítulo III). Além disso, ambos os resultados em relação a achados estruturais do cérebro no TDAH (Capítulo I e VI) por diferentes abordagens (*data-driven* e auto-segmentação) corroboram a implicação do estriado no TDAH. Além disso, ambos os estudos trazem novos achados sobre a fisiopatologia do TDAH. No Capítulo I, utilizando uma abordagem *data-driven*, onde são identificados componentes independentes demonstramos que não só a substância cinzenta, mas também a substância branca parece estar envolvida no TDAH. A maioria dos estudos estruturais de neuroimagem avaliam apenas a substância cinzenta, de forma que os achados dessa tese convergem para a importância do cérebro como estrutura mais ampla e complexa. Nas duas maiores meta-análises publicadas com achados estruturais no TDAH (Hoogman et al. 2017; Hoogman et al. 2019), os autores destacam um efeito exclusivamente, ou majoritariamente em crianças. Os resultados do Capítulo VI mostrando diferenças entre casos e controles para a maior parte de áreas corticais e volumes corticais estendem esses achados para adultos. Vale destacar no entanto que tanto a maioria dos estudos internacionais como também o nosso ainda não dispõem de análises de neuroimagem realizadas sequencialmente nos mesmos pacientes com uma perspectiva longitudinal. Tais informações seriam muito relevantes para explorar mecanismos envolvidos na persistência ou remissão dos sintomas.

O gene *SYT1* já foi previamente implicado em diversos fenótipos psiquiátricos, incluindo o TDAH, através de diferentes abordagens (Sokolov et al. 2000; Lasky-Su et al. 2008; Ge et al. 2013; Cupertino et al. 2016; Lee et al. 2018; Nagel et al. 2018a; Nagel et al. 2018b). Resultados apresentados no Capítulo IV mostram a influência do gene *SYT1* em aspectos cerebrais relacionados a conectividade estrutural. Além dos diversos fenótipos já implicados, o efeito pleiotrópico do gene *SYT1* é evidenciado na Tese pela sua associação, em abordagens *single-SNP* à susceptibilidade ao transtorno por uso de cocaína/crack (Capítulo V) e também em anexos envolvendo desempenho cognitivo (item 10.1.4), TDAH e fenótipos relacionados (item 10.1.2) e resposta ao tratamento (item 10.2.5). Avaliar genes com efeito pleiotrópico é uma perspectiva de extrema relevância por auxiliar no desenvolvimento de novos estudos voltados para fenótipos mais próximos da base biológica dos transtornos. Essa perspectiva é preconizada pelo *National Institute of Mental Health* (NIMH) no quadro de investigação RDoC (*Research Domain Criteria Iniciative*) (Shankman and Gorka 2015).

Tendo em vista a alta heterogeneidade e o pequeno tamanho de efeito envolvido, grandes consórcios e colaborações têm sido fundamentais para o avanço tanto na neuroimagem quanto na genética do TDAH. Nesse sentido, essa Tese envolve trabalhos desenvolvidos em colaboração com outros centros, como a Universidade Radboud na Holanda, envolvendo principalmente TDAH, mas também a dependência de crack, buscando compreender mais sobre a etiologia compartilhada entre os transtornos.

Esse conjunto de estudos responde a algumas perguntas, mas, principalmente, gera hipóteses e perspectivas de pesquisa. Tendo em vista o efeito amplo e complexo no cérebro, estudos multi-modalidades, incluindo aspectos de difusão, morfológicos e funcionais, podem ajudar a esclarecer as bases neurobiológicas do TDAH. Além disso, a respeito da *SYT1,* estudos moleculares,

com modelos animais e culturas celulares podem auxiliar na compreensão de mecanismos subjacentes ao seu efeito em fenótipos psiquiátricos e na integridade da substância branca. Considerando os dados longitudinais, outra perspectiva desse trabalho é avaliar o papel da *SYT1* nos desfechos ou trajetórias de TDAH, incluindo não só variantes genéticas, mas também variação epigenética, considerando padrões de metilação do gene ao longo do tempo.

Assim, está claro que o horizonte repleto de perguntas sobre a genética e neuroimagem no TDAH é muito mais extenso do que o caminho de respostas já percorrido. No entanto, se levarmos em conta a trajetória de mais de 2000 anos de indagações sobre esse intrigante fenótipo, é possível afirmar que a velocidade com que os avanços vêm sendo obtidos na última década é algo nunca antes experimentado e extremamente promissor.

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10. Produções científicas adicionais e Premiações

10.1 Produção científica relacionadas a tese

10.1.1 SNARE complex in developmental psychiatry: neurotransmitter exocytosis and beyond

SNARE complex in developmental psychiatry: neurotransmitter exocytosis and beyond

Renata Basso Cupertino[1] · Djenifer B. Kappel[1] · Cibele Edom Bandeira[1] · Jaqueline Bohrer Schuch[1] · Bruna Santos da Silva[1] · Diana Müller[1] · Claiton Henrique Dotto Bau[1] · Nina Roth Mota[1]

Received: 23 September 2015 / Accepted: 20 January 2016

Abstract Multiple biological processes throughout development require intracellular vesicular trafficking, where the SNARE (soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein (SNAP) receptors) complex plays a major role. The core proteins forming the SNARE complex are SNAP-25 (synaptosomal-associated protein 25), VAMP (vesicle-associated membrane protein) and Syntaxins, besides its regulatory proteins, such as Synaptotagmin. Genes encoding these proteins (*SNAP25*, *VAMP1*, *VAMP2*, *STX1A*, *SYT1* and *SYT2*) have been studied in relation to psychiatric disorders susceptibility. Here, we review physiological aspects of SNARE complex and genetic association results reported for attention deficit hyperactivity disorder, both in children and adults, autism spectrum disorders, major depressive disorder, bipolar disorder and schizophrenia. Moreover, we included findings from expression, pharmacogenetics and animal model studies regarding these clinical phenotypes. The overall scenario depicted here suggests that the SNARE complex may exert distinct roles throughout development, with age-specific effects of genetic variants in psychiatric disorders. Such perspective should be considered in future studies regarding SNARE complex genes.

Keywords SNARE · Development · Psychiatry disorders · ADHD · DEVELOPM.PSYCH

Introduction

SNARE (soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein receptors) complex is a large family of proteins that plays a major role in intracellular vesicular trafficking in eukaryotic cells. Such process is essential in different biological events, such as cell division, maintenance of subcellular compartments, protein and hormone secretion and neurotransmitter release (Zylbersztejn and Galli 2011). The SNARE complex is formed by members of the SNAP-25 (Synaptosomal-Associated Protein 25), VAMP (Vesicle-Associated Membrane Protein) and Syntaxins families. These proteins interact creating a four-helix bundle, formed by two helices of SNAP-25, one vesicle-transmembrane VAMP and one presynaptic plasma membrane Syntaxin that approximates the vesicle and plasmatic membranes (Sutton et al. 1998; Brunger 2000) (Fig. 1). Other proteins interact with the SNARE complex and regulate it, such as Munc-18, Complexin, Synaptophysin and the better studied Syt (Synaptotagmin) (Südhof

10.1.2 Replicated association of Synaptotagmin (SYT1) with ADHD and its broader influence in externalizing behaviors

Replicated association of Synaptotagmin (*SYT1*) with ADHD and its broader influence in externalizing behaviors

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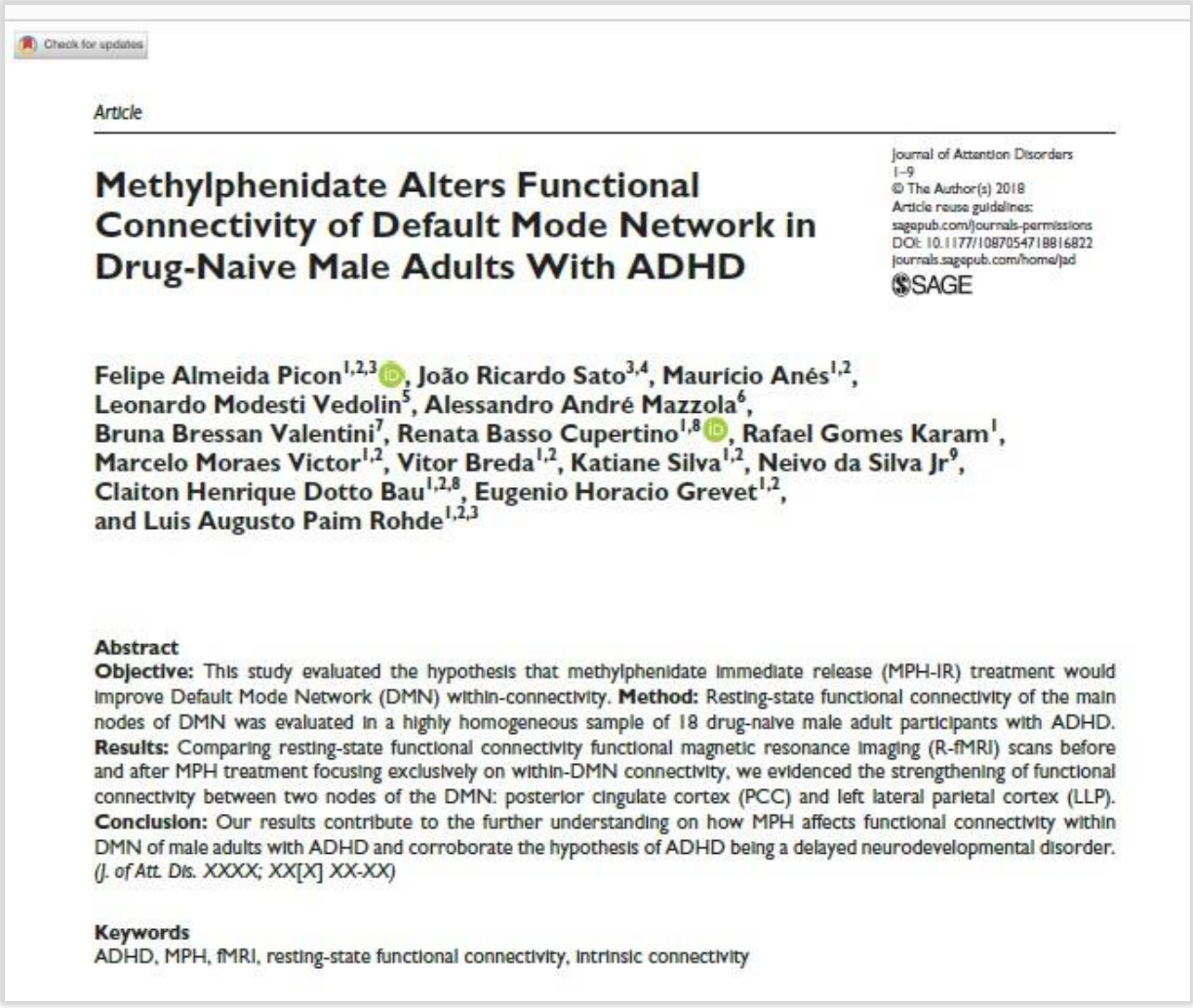
Received 13 July 2016; received in revised form 30 October 2016; accepted 5 January 2017

KEYWORDS
SNARE;
Attention-Deficit/Hyperactivity Disorder;
Synaptotagmin;
ASPD;
Antisocial Personality Disorder;
Meta-analysis

Abstract
Attention-Deficit/Hyperactivity Disorder (ADHD) is a common psychiatric disorder, affecting both children and adults. The Soluble N-ethylmaleimide sensitive factor Attachment REceptors (SNARE) complex has been implicated in ADHD pathophysiology since it is a key component of neurotransmitter release events and neurodevelopment processes, and SNPs in this complex have been associated with ADHD. Here we aim to analyze the effects of SNARE complex variants on ADHD susceptibility and its clinical heterogeneity in affected adults. We tested the association between ADHD and polymorphisms on the SNARE genes *STX1A* (rs2228607), *SYT1* (rs1880867 and rs2251214), *VAMP2* (26bp Ins/Del) and *SNAP25* (rs6108461 and rs8636) on a sample comprised of 548 adults with ADHD and 644 non-affected controls. Regarding clinical heterogeneity, we further investigated the effects of associated SNPs on age at onset of

10.1.3 Methylphenidate Alters Functional Connectivity of Default Mode Network in Drug-Naïve Male Adults with ADHD



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Methylphenidate Alters Functional Connectivity of Default Mode Network in Drug-Naive Male Adults With ADHD

Journal of Attention Disorders
1–9

DOI: 10.1177/1087054718816822
journals.sagepub.com/home/jad
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Abstract

Objective: This study evaluated the hypothesis that methylphenidate immediate release (MPH-IR) treatment would improve Default Mode Network (DMN) within-connectivity. **Method:** Resting-state functional connectivity of the main nodes of DMN was evaluated in a highly homogeneous sample of 18 drug-naive male adult participants with ADHD. **Results:** Comparing resting-state functional connectivity functional magnetic resonance imaging (R-fMRI) scans before and after MPH treatment focusing exclusively on within-DMN connectivity, we evidenced the strengthening of functional connectivity between two nodes of the DMN: posterior cingulate cortex (PCC) and left lateral parietal cortex (LLP). **Conclusion:** Our results contribute to the further understanding on how MPH affects functional connectivity within DMN of male adults with ADHD and corroborate the hypothesis of ADHD being a delayed neurodevelopmental disorder. *(J. of Att. Dis. XXXX; XX[X] XX-XX)*

Keywords

ADHD, MPH, fMRI, resting-state functional connectivity, intrinsic connectivity

10.1.4 Association between cognitive performance and *SYT1*-rs2251214 among women with cocaine use disorder

Journal of Neural Transmission
https://doi.org/10.1007/s00702-019-02086-w

PSYCHIATRY AND PRECLINICAL PSYCHIATRIC STUDIES - SHORT COMMUNICATION

Association between cognitive performance and *SYT1*-rs2251214 among women with cocaine use disorder

Thiago Wendt Viola[1] · Jaqueline Bohrer Schuch[2] · Diego Luiz Rovaris[3,4] · Rafael Genovese[1] · Lucca Tondo[1] · Breno Sanvicente-Vieira[5] · Aline Zaparte[1] · Renata Basso Cupertino[4,6] · Bruna Santos da Silva[4,6] · Claiton Henrique Dotto Bau[4,6] · Rodrigo Grassi-Oliveira[1]

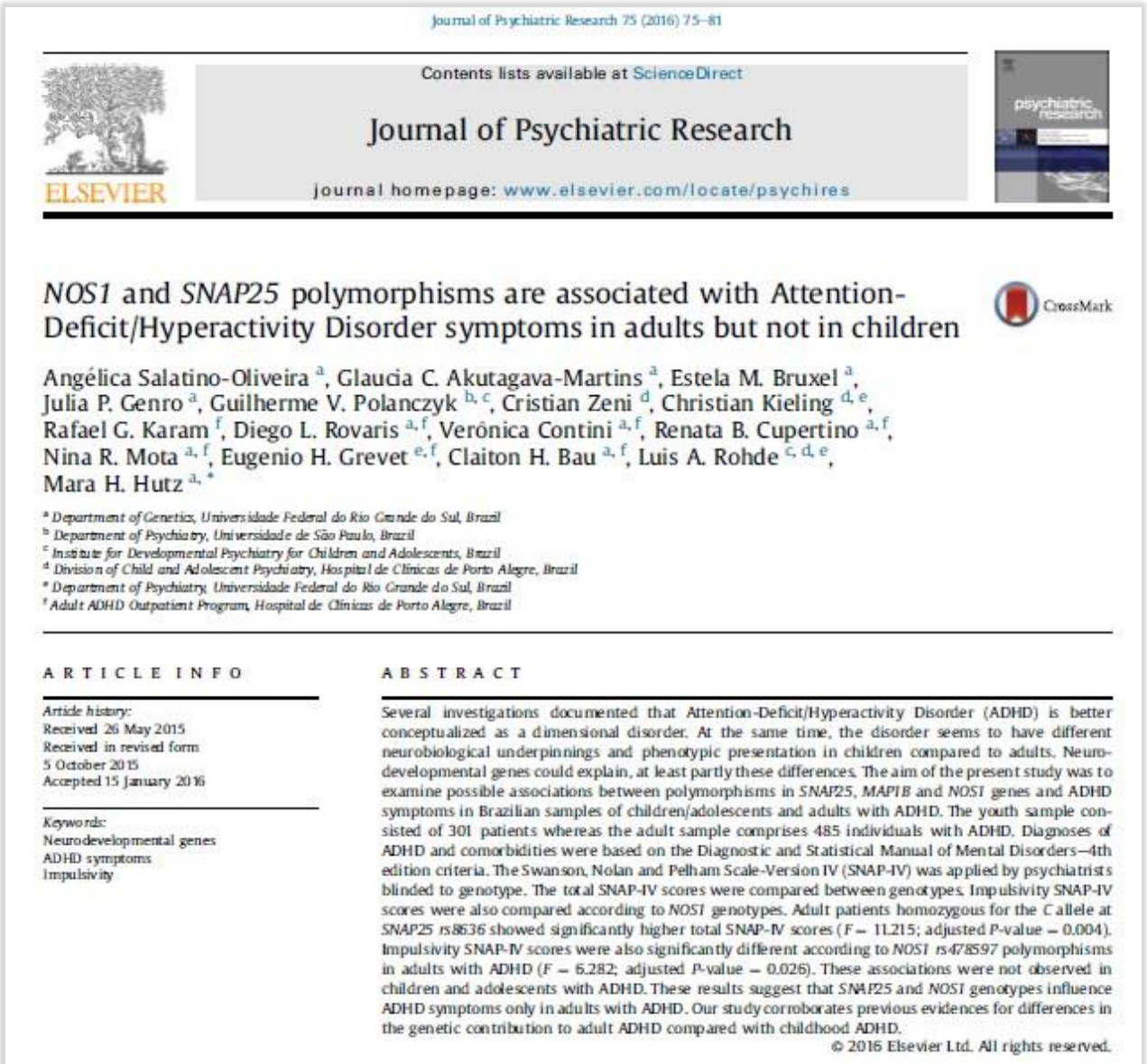
Received: 25 June 2019 / Accepted: 20 September 2019

Abstract
The SNP rs2251214 of the SYT1 gene was recently associated with externalizing phenotypes, including ADHD and cocaine use disorder (CUD). Here, we investigated whether *SYT1*-rs2251214 could also be implicated with cognitive performance variations among women with CUD. Results showed that G homozygous ($n = 146$) have lower cognitive performance in the Stroop, Trail Making and Matrix Reasoning tests compared with A-allele carriers ($n = 64$), suggesting that rs2251214 may influence the severity of cognitive impairments in CUD.

Keywords SNARE complex · Cocaine addiction · Substance use disorders · Stimulants · Cognition

10.2 Produção científica não relacionadas ao tema da tese

10.2.1 NOS1 and SNAP25 polymorphisms are associated with Attention-Deficit/Hyperactivity Disorder symptoms in adults but not in children.



Journal of Psychiatric Research 75 (2016) 75–81

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

NOS1 and *SNAP25* polymorphisms are associated with Attention-Deficit/Hyperactivity Disorder symptoms in adults but not in children

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ARTICLE INFO

Article history:
Received 26 May 2015
Received in revised form
5 October 2015
Accepted 15 January 2016

Keywords:
Neurodevelopmental genes
ADHD symptoms
Impulsivity

ABSTRACT

Several investigations documented that Attention-Deficit/Hyperactivity Disorder (ADHD) is better conceptualized as a dimensional disorder. At the same time, the disorder seems to have different neurobiological underpinnings and phenotypic presentation in children compared to adults. Neurodevelopmental genes could explain, at least partly these differences. The aim of the present study was to examine possible associations between polymorphisms in *SNAP25*, *MAPIB* and *NOS1* genes and ADHD symptoms in Brazilian samples of children/adolescents and adults with ADHD. The youth sample consisted of 301 patients whereas the adult sample comprises 485 individuals with ADHD. Diagnoses of ADHD and comorbidities were based on the Diagnostic and Statistical Manual of Mental Disorders—4th edition criteria. The Swanson, Nolan and Pelham Scale-Version IV (SNAP-IV) was applied by psychiatrists blinded to genotype. The total SNAP-IV scores were compared between genotypes. Impulsivity SNAP-IV scores were also compared according to *NOS1* genotypes. Adult patients homozygous for the C allele at *SNAP25 rs8636* showed significantly higher total SNAP-IV scores ($F = 11.215$; adjusted P-value = 0.004). Impulsivity SNAP-IV scores were also significantly different according to *NOS1 rs478597* polymorphisms in adults with ADHD ($F = 6.282$; adjusted P-value = 0.026). These associations were not observed in children and adolescents with ADHD. These results suggest that *SNAP25* and *NOS1* genotypes influence ADHD symptoms only in adults with ADHD. Our study corroborates previous evidences for differences in the genetic contribution to adult ADHD compared with childhood ADHD.

10.2.2 Pleiotropic effects of Chr15q25 nicotinic gene cluster and the relationship between smoking, cognition and ADHD.

Journal of Psychiatric Research 80 (2016) 73–78

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

Pleiotropic effects of Chr15q25 nicotinic gene cluster and the relationship between smoking, cognition and ADHD

Jaqueline B. Schuch [a, 1], Evelise R. Polina [a, 1], Diego L. Rovaris [a], Djenifer B. Kappel [a], Nina R. Mota [a], Renata B. Cupertino [a], Katiane L. Silva [b], Paula O. Guimarães-da-Silva [b], Rafael G. Karam [b], Carlos A.I. Salgado [b], Melanie J. White [d], Luis A. Rohde [b, c], Eugenio H. Grevet [b, c], Claiton H.D. Bau [a, b, *]

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ARTICLE INFO

Article history:
Received 28 December 2015
Received in revised form
1 June 2016
Accepted 2 June 2016

Keywords:
Nicotinic receptor
Nicotine
CHRNA5-CHRNA3-CHRNB4
WAIS-R
IQ

ABSTRACT

Polymorphisms in the *CHRNA5-CHRNA3-CHRNB4* gene cluster (Chr15q25) have been robustly associated with nicotine dependence, including genome-wide studies, as well as with cognitive and neuropsychological measures. In addition, cognitive processes can be influenced by nicotine use through nicotinic acetylcholine receptors (nAChRs). Here, we evaluated the effect of polymorphisms in *CHRNA5-CHRNA3-CHRNB4* gene cluster and their interaction with tobacco smoking status on cognition in patients with Attention Deficit/Hyperactivity Disorder (ADHD). Eight SNPs from the *CHRNA5-CHRNA3-CHRNB4* gene cluster were evaluated on a clinical sample of 403 adults with ADHD. Cognitive performance was assessed using the Wechsler Adult Intelligence Scale-Revised (WAIS-R). Analyses of covariance were used to assess the influence of single markers and their interaction with smoking status in the Vocabulary and Block Design subtests of WAIS-R. Correction for multiple comparisons was applied. Lifetime smoking was associated to Vocabulary subtest. The TT genotypes of CHRNA5 SNPs rs588765 and rs514743 showed a trend towards association with, respectively, higher and lower scores on the Vocabulary subtest. There was a significant interaction between intergenic SNP rs8023462 and smoking on Vocabulary scores. Our results are consistent with an influence of variants in the *CHRNA5–CHRNA3–CHRNB4* gene cluster on cognitive measures. The overall scenario suggests a pleiotropic role of Chr15q25 nicotinic gene cluster with complex influences in ADHD, tobacco smoking and cognitive performance, characteristics that can be partially interdependent and may share underlying genetic factors.

10.2.3 Effects of corticotropin-releasing hormone receptor 1 SNPs on major depressive disorder are influenced by sex and smoking status

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research paper

Effects of corticotropin-releasing hormone receptor 1 SNPs on major depressive disorder are influenced by sex and smoking status

Bruna S. da Silva [a], Diego L. Rovaris [a], Jaqueline B. Schuch [a], Nina R. Mota [a], Renata B. Cupertino [a], Angelita P. Aroche [a], Guilherme P. Bertuzzi [a], Rafael G. Karam [b], Eduardo S. Vitola [b], Luciana Tovo-Rodrigues [c], Eugenio H. Grevet [b,d], Claiton H.D. Bau [a,b,*]

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ARTICLE INFO

Article history:
Received 26 January 2016
Received in revised form
10 July 2016
Accepted 11 August 2016
Available online 13 August 2016

Keywords:
Depression
Smoking
Stress
Gender
Genetics
CRHR1

ABSTRACT

Background: The corticotropin-releasing hormone receptor 1 (*CRHR1*) gene has been repeatedly implicated in Major Depressive Disorder (MDD) in humans and animal models; however, the findings are not absolutely convergent. Since recent evidence from genome-wide association studies suggests that narrowing the phenotypic heterogeneity may be crucial in genetic studies of MDD, the aim of this study was to evaluate the effects of *CRHR1* polymorphisms on MDD while addressing the influence of sex and smoking status.

Methods: The association of the *CRHR1* SNPs rs12944712, rs110402, and rs878886 with MDD was evaluated in 629 Brazilian adults of European descent recruited from the general population [180 (28.6%) with lifetime MDD]. The sample was subdivided according to sex and smoking status

Results: Among nonsmokers, there were nominal associations between MDD and all tested SNPs (rs12944712, P=0.042; rs110402, P=0.031, and rs878886, P=0.040), regardless of sex. In addition, there were significant effects of rs110402 in women (P_{corr}=0.034) and rs878886 in men (P_{corr}=0.013). Among lifetime smokers, there were no significant associations between *CRHR1* SNPs and MDD

Limitations: The lack of a depression rating scale; scarcity of information on the functionality of the *CRHR1* SNPs; and relatively small sample sizes in some subgroups.

Conclusions: Our results strengthen the evidence for the role of *CRHR1* SNPs in MDD susceptibility and suggest that their effects may be modulated by sex and smoking status. These findings suggest the perspective that reducing phenotypic heterogeneity is warranted in genetic studies of MDD.

10.2.4 Childhood Maltreatment Linked with a Deterioration of Psychosocial Outcomes in Adult Life for Southern Brazilian Transgender Women.

J Immigrant Minority Health
DOI 10.1007/s10903-016-0528-6

ORIGINAL PAPER

Childhood Maltreatment Linked with a Deterioration of Psychosocial Outcomes in Adult Life for Southern Brazilian Transgender Women

Anna Martha Vaitses Fontanari[1,5] · Diego Luiz Rovaris[2] · Angelo Brandelli Costa[3,5] · Andrew Pasley[4] · Renata Basso Cupertino[2] · Bianca Machado Borba Soll[1,5] · Karine Schwarz[1,5] · Dhiordan Cardoso da Silva[1,5] · André Oliveira Borba[5] · Andressa Mueller[1] · Clailton Henrique Dotto Bau[2] · Maria Inês Rodrigues Lobato[1,5]

Abstract A history of childhood maltreatment (HCM) has been associated with detrimental psychiatric outcomes. This is particularly true for transgender, for whom there is initial evidence that HCM may be associated with psychiatric morbidity. Our study aimed to further characterize the relationship between HCM and the development of mental disorder in adult life, based on a sample of Brazilian transgender women. Cross-sectional data were collected from a consecutive sample of 289 transgender women who attended the Hospital Clínicas clinic for gender dysphoria, in Porto Alegre, between 1998 and 2014. Our results demonstrated a greater risk of deteriorating mental health amongst participants who had experienced HCM. Given the disproportionally high rate of HCM in transgender persons, we advocate for greater assistance for transgender persons.

Keywords Gender identity · Sex work · Minority stress · Southern Brazil · Childhood maltreatment

10.2.5 Exocytosis-related genes and response to methylphenidate treatment in adults with ADHD.

Molecular Psychiatry (2017) 00, 1–7

www.nature.com/mp

ORIGINAL ARTICLE

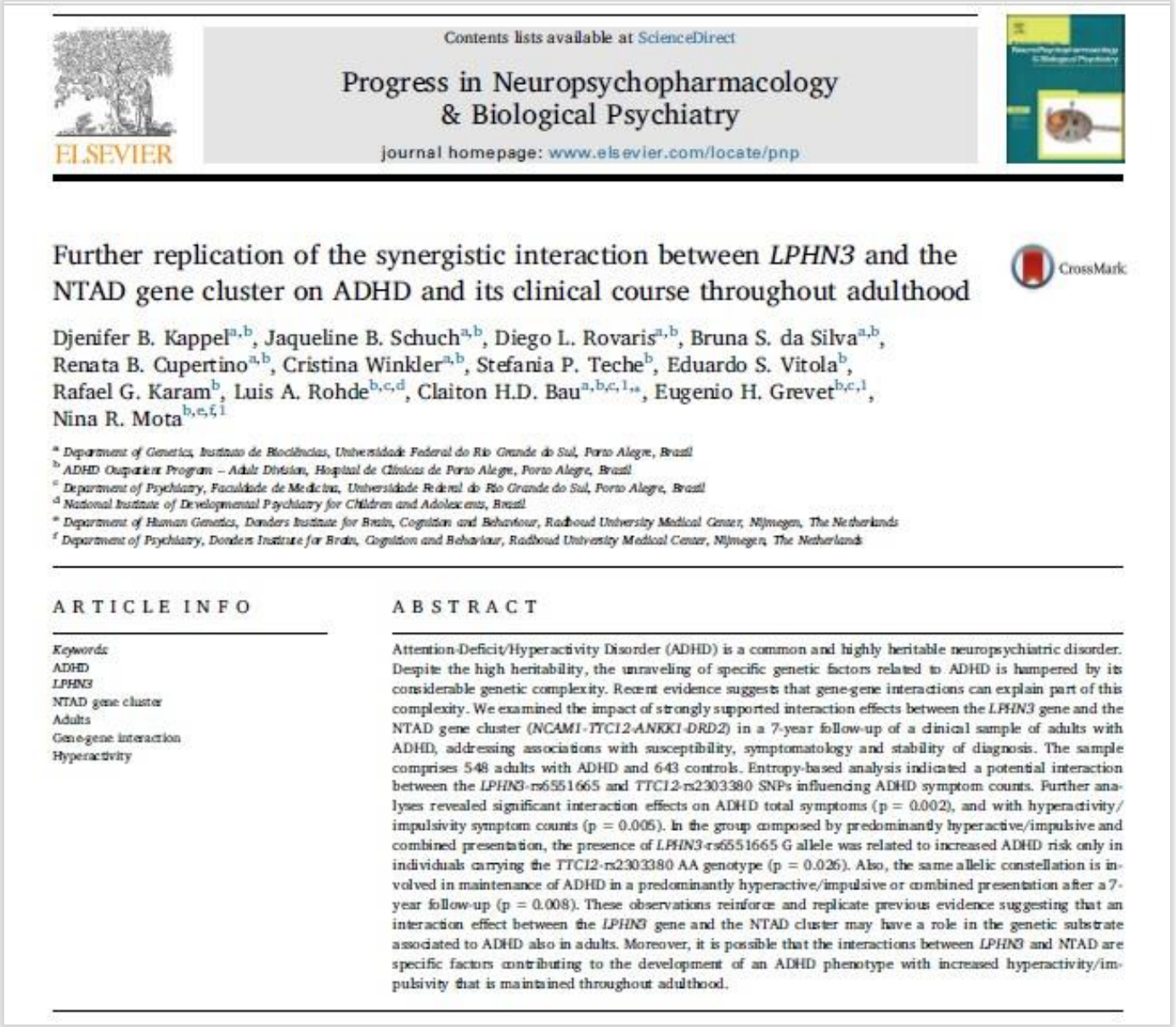
Exocytosis-related genes and response to methylphenidate treatment in adults with ADHD

BS da Silva[1], RB Cupertino[1], DL Rovaris[1], JB Schuch[1], DB Kappel[1], D Müller[1], CE Bandeira[1], MM Victor[2], RG Karam[2], NR Mota[1,3], LA Rohde[2,4], V Contini[5], EH Grevet[2,4] and CHD Bau[1,2]

Experimental studies have demonstrated that methylphenidate (MPH) modulates the synaptic vesicle trafficking and synaptotagmin-1 (Sytl) mRNA levels. Sytl is a regulatory protein of the SNARE complex, a neurotransmitter exocytosis mediator. Despite this evidence, most SNARE complex-related genes have never been evaluated in attention-deficit/hyperactivity disorder (ADHD) pharmacogenetics. This study evaluates, for we believe the first time, polymorphisms on the SNARE complex-related genes *STX1A* (rs2228607), *VAMP2* (26bp Ins/Del) and *SYT1* (rs1880867 and rs2251214) on the response to immediate-release methylphenidate (IR-MPH) in a naturalistic sample of adults with ADHD. The sample comprised 433 subjects, of which 272 (62.8%) have completed the short-term IR-MPH treatment (at least 30 days). The main outcome measure was the categorical variable of short-term response to IR-MPH based on the Swanson, Nolan and Pelham Rating Scale version 4 (SNAP-IV), and on the clinical global impression-improvement scale. Additional analyses evaluated the percentage of SNAP-IV symptom reduction for each dimension as well as short- and long- (7 years) term treatment persistence. *SYT1*-rs2251214 was associated with the categorical short-term response to IR-MPH ($P=0.006$, $P_{FDR}=0.028$), and with the percentage of inattention and oppositional defiant disorder symptoms reduction ($P=0.007$, $P_{FDR}=0.028$ and $P=0.017$, $P_{FDR}=0.048$, respectively). *SYT1*-rs2251214 was also associated with short-term treatment persistence ($P=0.018$, $P_{FDR}=0.048$), and with months of treatment ($P=0.002$, $P_{FDR}=0.016$) in the long-term protocol. Our findings suggest that *SYT1*-rs2251214 presents a broad influence in IR-MPH response variability in adults with ADHD, being involved with both symptom response and treatment persistence. If such findings are replicated, Sytl could represent a key element in MPH pharmacodynamics in adults with ADHD.

Molecular Psychiatry advance online publication, 2 May 2017; doi:10.1038/mp.2017.90

10.2.6 Further replication of the synergistic interaction between LPHN3 and the NTAD gene cluster on ADHD and its clinical course throughout adulthood.



Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Further replication of the synergistic interaction between *LPHN3* and the NTAD gene cluster on ADHD and its clinical course throughout adulthood

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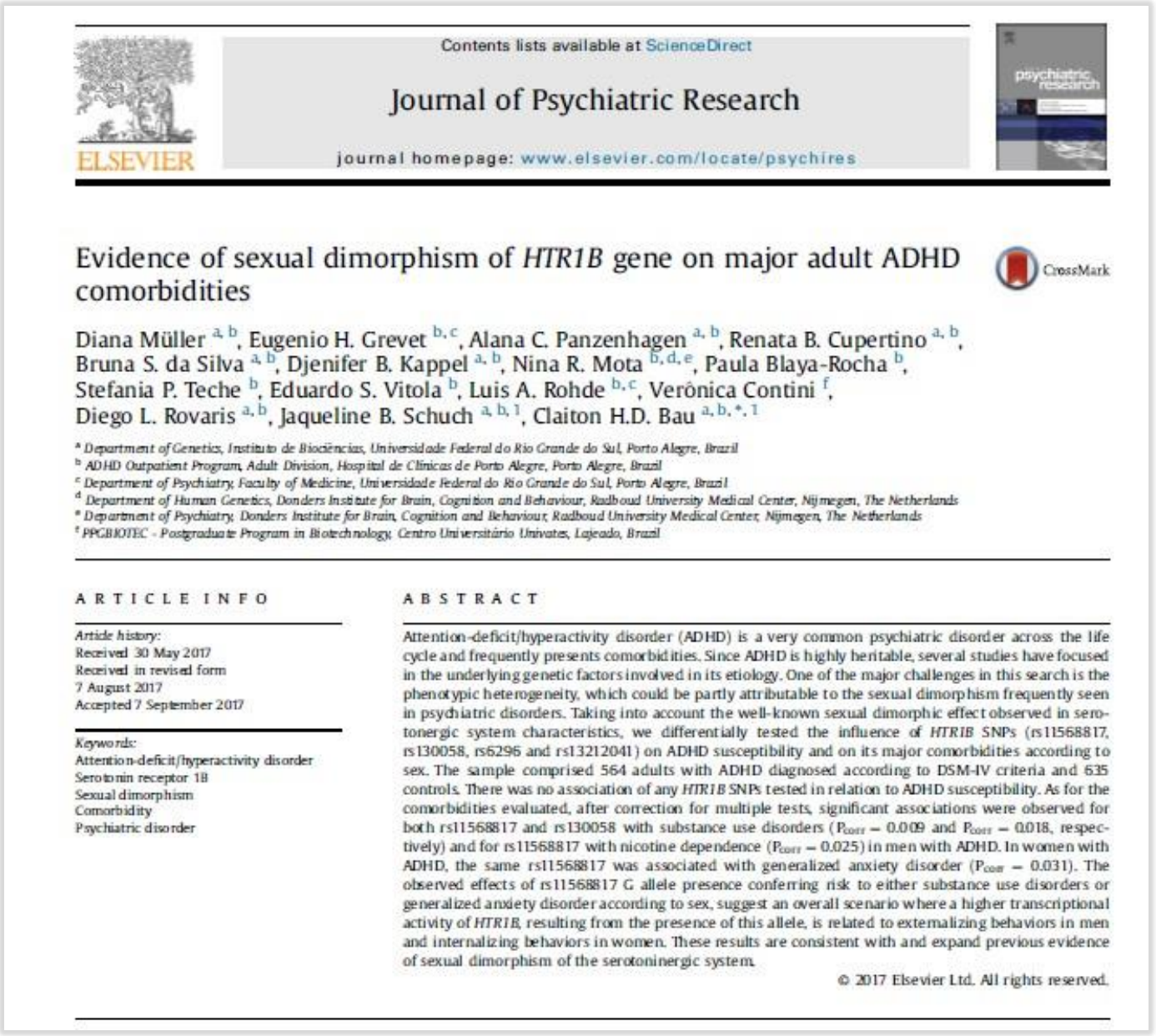
ARTICLE INFO

Keywords:
ADHD
LPHN3
NTAD gene cluster
Adults
Gene-gene interaction
Hyperactivity

ABSTRACT

Attention-Deficit/Hyperactivity Disorder (ADHD) is a common and highly heritable neuropsychiatric disorder. Despite the high heritability, the unraveling of specific genetic factors related to ADHD is hampered by its considerable genetic complexity. Recent evidence suggests that gene-gene interactions can explain part of this complexity. We examined the impact of strongly supported interaction effects between the *LPHN3* gene and the NTAD gene cluster (*NCAM1-TTC12-ANKK1-DRD2*) in a 7-year follow-up of a clinical sample of adults with ADHD, addressing associations with susceptibility, symptomatology and stability of diagnosis. The sample comprises 548 adults with ADHD and 643 controls. Entropy-based analysis indicated a potential interaction between the *LPHN3*-rs6551665 and *TTC12*-rs2303380 SNPs influencing ADHD symptom counts. Further analyses revealed significant interaction effects on ADHD total symptoms ($p = 0.002$), and with hyperactivity/impulsivity symptom counts ($p = 0.005$). In the group composed by predominantly hyperactive/impulsive and combined presentation, the presence of *LPHN3*-rs6551665 G allele was related to increased ADHD risk only in individuals carrying the *TTC12*-rs2303380 AA genotype ($p = 0.026$). Also, the same allelic constellation is involved in maintenance of ADHD in a predominantly hyperactive/impulsive or combined presentation after a 7-year follow-up ($p = 0.008$). These observations reinforce and replicate previous evidence suggesting that an interaction effect between the *LPHN3* gene and the NTAD cluster may have a role in the genetic substrate associated to ADHD also in adults. Moreover, it is possible that the interactions between *LPHN3* and NTAD are specific factors contributing to the development of an ADHD phenotype with increased hyperactivity/impulsivity that is maintained throughout adulthood.

10.2.7 Evidence of sexual dimorphism of HTR1B gene on major adult ADHD comorbidities.



Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

Evidence of sexual dimorphism of *HTR1B* gene on major adult ADHD comorbidities

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ARTICLE INFO

Article history:
Received 30 May 2017
Received in revised form
7 August 2017
Accepted 7 September 2017

Keywords:
Attention-deficit/hyperactivity disorder
Serotonin receptor 1B
Sexual dimorphism
Comorbidity
Psychiatric disorder

ABSTRACT

Attention-deficit/hyperactivity disorder (ADHD) is a very common psychiatric disorder across the life cycle and frequently presents comorbidities. Since ADHD is highly heritable, several studies have focused in the underlying genetic factors involved in its etiology. One of the major challenges in this search is the phenotypic heterogeneity, which could be partly attributable to the sexual dimorphism frequently seen in psychiatric disorders. Taking into account the well-known sexual dimorphic effect observed in serotonergic system characteristics, we differentially tested the influence of *HTR1B* SNPs (rs11568817, rs130058, rs6296 and rs13212041) on ADHD susceptibility and on its major comorbidities according to sex. The sample comprised 564 adults with ADHD diagnosed according to DSM-IV criteria and 635 controls. There was no association of any *HTR1B* SNPs tested in relation to ADHD susceptibility. As for the comorbidities evaluated, after correction for multiple tests, significant associations were observed for both rs11568817 and rs130058 with substance use disorders ($P_{corr} = 0.009$ and $P_{corr} = 0.018$, respectively) and for rs11568817 with nicotine dependence ($P_{corr} = 0.025$) in men with ADHD. In women with ADHD, the same rs11568817 was associated with generalized anxiety disorder ($P_{corr} = 0.031$). The observed effects of rs11568817 G allele presence conferring risk to either substance use disorders or generalized anxiety disorder according to sex, suggest an overall scenario where a higher transcriptional activity of *HTR1B*, resulting from the presence of this allele, is related to externalizing behaviors in men and internalizing behaviors in women. These results are consistent with and expand previous evidence of sexual dimorphism of the serotoninergic system.

10.2.8 Effects of DRD2 splicing-regulatory polymorphism and DRD4 48 bp VNTR on crack cocaine addiction.

Journal of Neural Transmission
https://doi.org/10.1007/s00702-018-1946-5

PSYCHIATRY AND PRECLINICAL PSYCHIATRIC STUDIES - ORIGINAL ARTICLE

Effects of *DRD2* splicing-regulatory polymorphism and *DRD4* 48 bp VNTR on crack cocaine addiction

Anderson R. Stolf[1] · Renata B. Cupertino[2] · Diana Müller[2] · Breno Sanvicente-Vieira[3] · Tatiana Roman[2] · Eduardo S. Vitola[4] · Eugenio H. Grevet[4] · Lisia von Diemen[1] · Felix H. P. Kessler[1] · Rodrigo Grassi-Oliveira[3] · Claiton H. D. Bau[1,4] · Diego L. Rovaris[2] · Flavio Pechansky[1] · Jaqueline B. Schuch[2,5]

Received: 20 April 2018 / Accepted: 17 October 2018

Abstract
There is evidence that dopamine receptors D2 (*DRD2*) and D4 (*DRD4*) polymorphisms may influence substance use disorders (SUD) susceptibility both individually and through their influence in the formation of DRD2–DRD4 heteromers. The dopaminergic role on the vulnerability to addiction appears to be influenced by sex. A cross-sectional study with 307 crack cocaine addicts and 770 controls was conducted. The influence of *DRD2* rs2283265 and *DRD4* 48 bp VNTR in exon 3 variants, as well as their interaction on crack cocaine addiction susceptibility and severity were evaluated in women and men separately. An association between the *DRD2* T allele and crack cocaine addiction was found in women. In this same group, interaction analysis demonstrated that the presence of *DRD2*-T allele and concomitant absence of *DRD4*-7R allele were associated with risk for crack cocaine addiction. No influence of *DRD2* and *DRD4* variants was observed in men regarding addiction severity. This study reinforces the role of dopaminergic genes in externalizing behaviors, especially the influence of DRD2–DRD4 interaction on SUD. This is the fourth sample that independently associated the DRD2–DRD4 interaction with SUD itself or related disorders. In addition, our findings point out to a potential difference of dopaminergic neurotransmission across sex influencing addiction susceptibility.

Keywords Cocaine · Crack · Dependence · Dopamine receptor D2 · Dopamine receptor D4 · Substance use disorder

10.2.9 The Role of Gene Encoding Variation of *DRD4* in the Relationship between Inattention and Seasonal Daylight

Submetido a revista Translational Psychiatry, disponível no BioRxiv - doi: https://doi.org/10.1101/825083

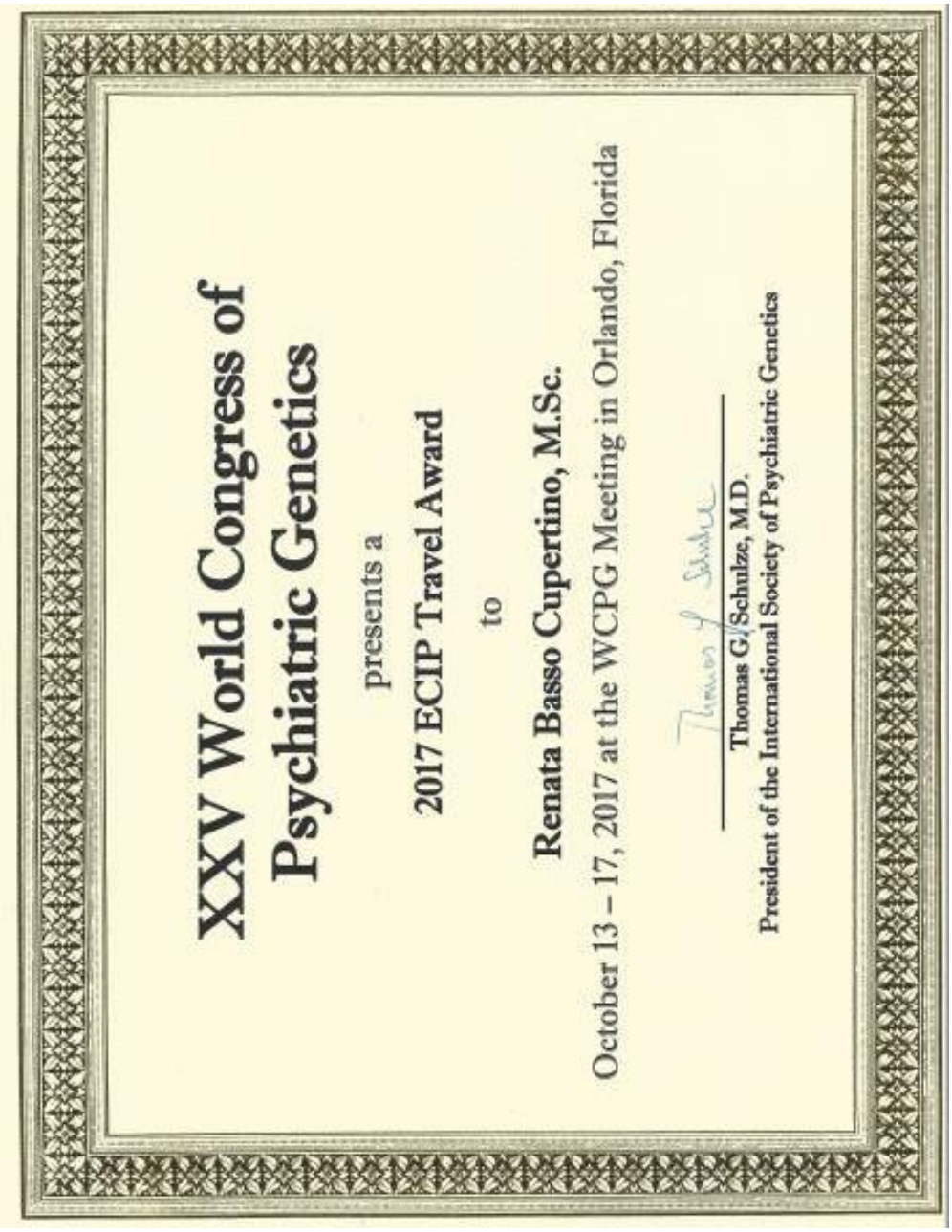
The Role of Gene Encoding Variation of *DRD4* in the Relationship between Inattention and Seasonal Daylight

Vollebregt, M.A.[1 *], Franke, B. [2,3], Buitelaar, J.K. [4,5], Arnold, L.E. [6], Faraone, S.V[7],Grevet, E.H. [8,9], Reif, A [10], Zayats, T. [11], Bralten J.[4] , Bau, C.H.D.[9,12], Haavik, J.[11,13], Kuntsi, J.[14], Cupertino, R.B.[9,12], Loo, S.K.,[15], Lundervold, A.J.,[16], Ribasés, M. [17-19], Sánchez-Mora, C. [17-19], Ramos-Quiroga, J.A.[17-20], Asherson, P. [14], Swanson, J.M.[21], Arns, M.[1,22]

ABSTRACT

Daylight is the strongest synchronizer of human circadian rhythms. The circadian pathway hypothesis posits that synchrony between daylight and the circadian system relates to (in)attention. The dopamine neurotransmitter system is implicated in regulating the circadian system as well as in (attention)-deficit hyperactivity disorder [ADHD]. We studied the role of functional genetic variation in the gene encoding of dopamine-receptor-D4 (DRD4) in the relationship between inattention and seasonal daylight (changes). Gene-by-environment (GxE) mega-analyses were performed across eight studies including 3757 adult participants (with and without ADHD). We tested 1) the Spring-focus hypothesis, in which attention in 7R-carriers normalizes with increasing daylight levels preceding measurement, 2) the Summer-born ADHD hypothesis, in which 7R-carriers report more inattention when born in spring/summer than in autumn/winter, 3) the Winter-born ADHD hypothesis, opposing the second hypothesis. The Spring-focus hypothesis was upheld (1386 ADHD, 760 controls; d=-0.16 between periods); 7R-carriers reported even less inattention than 7R-non-carriers after winter solstice (d=0.27 between genotype-groups). Results were diagnosis-independent. Sensitivity analyses at individual study level confirmed the circannual patterns for 7R-carriers. Incorporating geographic changes into the independent measure, we also calculated changes in sunlight levels. This approach likewise showed that inattention correlated negatively with increasing light levels in 7R-carriers (r=-.135). Results emphasize peripheral effects of dopamine and the effects of (seasonal) daylight changes on cognition.

10.3 ECIP Travel Award – WCPG 2017



XXV World Congress of Psychiatric Genetics

presents a

2017 ECIP Travel Award

to

Renata Basso Cupertino, M.Sc.

October 13 – 17, 2017 at the WCPG Meeting in Orlando, Florida

Thomas G. Schulze

Thomas G. Schulze, M.D.

President of the International Society of Psychiatric Genetics

11. Anexos

11.1 Termos de consentimento aprovado – Comissão de Pesquisa e Ética em Saúde – HCPA

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

N° do projeto GPPG 16-0600 ou CAAE 60633516.2.1001.5327

Título do Projeto: **Estudo prospectivo de pacientes com transtorno de déficit de atenção/hiperatividade diagnosticados na vida adulta (Grupo de pacientes que já participaram de projeto anterior)**

Você está sendo convidado a participar de uma pesquisa cujo objetivo é comparar padrões em imagens de ressonância magnética entre indivíduos com e sem Transtorno de Déficit de Atenção/Hiperatividade (TDAH). Você está sendo convidado a participar porque já possui diagnóstico de TDAH e já participou do projeto 01-321 "Estudos das bases moleculares do Transtorno de Déficit de Atenção/Hiperatividade em Adultos" do mesmo grupo de pesquisa. Esta pesquisa está sendo realizada pelo Serviço de Psiquiatria do Hospital de Clínicas de Porto Alegre (HCPA).

Se você aceitar participar da pesquisa, os procedimentos envolvidos em sua participação são os seguintes: Você responderá a uma entrevista avaliando sintomas do TDAH e outros transtornos psiquiátricos comuns (com duração média de 1h30min), assim como a avaliação neuropsicológica (com duração média de 1h). Caso não tenha material biológico (sangue) armazenado, que foi coletado na pesquisa anterior da qual você participou, você passará por uma nova coleta de sangue (10ml, equivalente a duas colheres de chá) para extração de DNA para avaliar genes que podem estar relacionados ao diagnóstico. Além disso, você será solicitado também a coletar uma amostra fecal (de fezes) para análise de microrganismos no intestino, que podem estar relacionados aos sintomas do transtorno.

As entrevistas realizadas e/ou a coleta de amostra fecal poderão gerar desconfortos psicológicos ou constrangimento, entretanto a qualquer momento você poderá desistir de participar. A coleta de sangue é um procedimento comum, no entanto podem ocorrer pequenos sangramentos ou hematoma (mancha roxa) no local na coleta.

A participação na pesquisa não trará benefícios diretos aos participantes, porém, contribuirá para o aumento do conhecimento sobre o assunto estudado, e poderá beneficiar futuros pacientes.

Sua participação na pesquisa é totalmente voluntária, ou seja, não é obrigatória. Caso você decida não participar, ou ainda, desistir de participar e retirar seu consentimento, não haverá nenhum prejuízo ao atendimento que você recebe ou possa vir a receber na instituição.

Caso ocorra alguma intercorrência ou dano, resultante de sua participação na pesquisa, você receberá todo o atendimento necessário, sem nenhum custo pessoal.

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Os dados coletados durante a pesquisa serão sempre tratados confidencialmente. Os resultados serão apresentados de forma conjunta, sem a identificação dos participantes, ou seja, o seu nome não aparecerá na publicação dos resultados.

Caso você tenha dúvidas, poderá entrar em contato com o pesquisador responsável Prof. Dr. Eugenio Horacio Grevet, pelo telefone 051-33598094, ou com o pesquisador Prof. Dr. Claiton Henrique Dotto Bau, pelo telefone 51-33086718 ou com a pesquisadora Renata Basso Cupertino, pelo telefone 51-81837278 ou com o Comitê de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre (HCPA), pelo telefone (51) 33597640, ou no 2º andar do HCPA, sala 2227, de segunda à sexta, das 8h às 17h.

Esse Termo é assinado em duas vias, sendo uma para o participante e outra para os pesquisadores.

_____	_____
Nome do participante da pesquisa	Nome do pesquisador que aplicou o Termo
_____	_____
Assinatura	Assinatura

Local e Data: _____

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

N° do projeto GPPG 16-0600 ou CAAE 60633516.2.1001.5327

Título do Projeto: **Estudo prospectivo de pacientes com transtorno de déficit de atenção/hiperatividade diagnosticados na vida adulta**

(Grupo de controles que já participaram de projeto anterior)

Você está sendo convidado a participar de uma pesquisa cujo objetivo é estudar fatores genéticos e fenotípicos relacionados com a evolução clínica e cognitiva em pacientes com Transtorno de Déficit de Atenção/Hiperatividade (TDAH) e controles. Você está sendo convidado a participar porque não possui o diagnóstico de TDAH e já participou do projeto 01-321 "Estudos das bases moleculares do Transtorno de Déficit de Atenção/Hiperatividade em Adultos" do mesmo grupo de pesquisa. Esta pesquisa está sendo realizada pelo Serviço de Psiquiatria do Hospital de Clínicas de Porto Alegre (HCPA).

Se você aceitar participar da pesquisa, os procedimentos envolvidos em sua participação são os seguintes: Você responderá a uma entrevista avaliando sintomas presentes nos pacientes com TDAH e outros transtornos psiquiátricos comuns (com duração média de 1h30min), assim como a avaliação neuropsicológica (com duração média de 1h). Caso não tenha material biológico (sangue) armazenado, que foi coletado na pesquisa anterior da qual você participou, você passará por uma nova coleta de sangue (10ml, equivalente a duas colheres de chá) para extração de DNA para avaliar genes que podem estar relacionados ao diagnóstico. Além disso, você será solicitado também a coletar uma amostra fecal (de fezes) para análise de microrganismos no intestino, que podem estar relacionados aos sintomas do transtorno.

As entrevistas realizadas e/ou a coleta de amostra fecal poderão gerar desconfortos psicológicos ou constrangimento, entretanto a qualquer momento você poderá desistir de participar. A coleta de sangue é um procedimento comum, no entanto podem ocorrer pequenos sangramentos ou hematoma (mancha roxa) no local na coleta.

A participação na pesquisa não trará benefícios diretos aos participantes, porém, contribuirá para o aumento do conhecimento sobre o assunto estudado, e poderá beneficiar futuros pacientes.

Sua participação na pesquisa é totalmente voluntária, ou seja, não é obrigatória. Caso você decida não participar, ou ainda, desistir de participar e retirar seu consentimento, não haverá nenhum prejuízo ao atendimento que você recebe ou possa vir a receber na instituição.

Caso ocorra alguma intercorrência ou dano, resultante de sua participação na pesquisa, você receberá todo o atendimento necessário, sem nenhum custo pessoal.

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Os dados coletados durante a pesquisa serão sempre tratados confidencialmente. Os resultados serão apresentados de forma conjunta, sem a identificação dos participantes, ou seja, o seu nome não aparecerá na publicação dos resultados.

Caso você tenha dúvidas, poderá entrar em contato com o pesquisador responsável Prof. Dr. Eugenio Horacio Grevet, pelo telefone 051-33598094, ou com o pesquisador Prof. Dr. Claiton Henrique Dotto Bau, pelo telefone 51-33086718 ou com a pesquisadora Renata Basso Cupertino, pelo telefone 51-81837278 ou com o Comitê de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre (HCPA), pelo telefone (51) 33597640, ou no 2º andar do HCPA, sala 2227, de segunda à sexta, das 8h às 17h. Ou com o Comitê de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre (HCPA), pelo telefone 51 - 33597640, ou no 2º andar do HCPA, sala 2227, de segunda à sexta, das 8h às 17h.

Esse Termo é assinado em duas vias, sendo uma para o participante e outra para os pesquisadores.

_____	_____
Nome do participante da pesquisa	Nome do pesquisador que aplicou o Termo
_____	_____
Assinatura	Assinatura

Local e Data: _____

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

N° do projeto GPPG 16-0600 ou CAAE 60633516.2.1001.5327

Título do Projeto: **Estudo prospectivo de pacientes com transtorno de déficit de atenção/hiperatividade diagnosticados na vida adulta**

(Grupo de pacientes que participarão da neuroimagem)

Você já está participando de uma pesquisa cujo objetivo é estudar fatores genéticos e fenotípicos relacionados com a evolução clínica e cognitiva em pacientes com Transtorno de Déficit de Atenção/Hiperatividade (TDAH) e controles. Apenas alguns participantes participarão desta etapa da pesquisa, você foi selecionado aleatoriamente (por sorteio) para realizar um exame de ressonância magnética.

Se você aceitar participar dessa parte da pesquisa, os procedimentos envolvidos em sua participação são os seguintes: A realização da Ressonância Magnética (RM), a ser realizada na clínica de radiologia SERDIL (Rua São Luis n° 96 - Porto Alegre, RS). Esse um exame que avalia estruturas cerebrais e sua ativação em determinadas áreas. Para isso você será colocado na posição adequada na mesa de RM e um dispositivo chamado de Bobina será colocado em volta da área de interesse do exame (é como se fosse um capacete). Esta bobina é usada para receber as imagens do seu corpo. A seguir, o operador vai colocá-lo dentro do aparelho, onde permanecerá durante todo o exame. O técnico sairá da sala, mas ficará em constante contato com você através de um aparelho de comunicação interna. Em caso de qualquer desconforto haverá uma campainha para você fazer contato com a equipe. Durante o exame você ouvirá um barulho parecido com batidas em intervalos regulares. Isto significa que as imagens estão sendo adquiridas. O seu exame terá uma duração, aproximada, de 30 minutos.

Não há riscos ou desconfortos decorrentes da realização da RM relatados na literatura. A RM não utiliza contrastes ou qualquer outro tipo de substância a ser administrada e não envolve qualquer tipo de radiação. Na presença de quaisquer efeitos colaterais ou desconforto, embora nenhum tenha sido relatado na literatura nem na nossa experiência, você terá atendimento da equipe médica envolvida no estudo.

A participação na pesquisa não trará benefícios diretos aos participantes, porém, contribuirá para o aumento do conhecimento sobre o assunto estudado, e poderá beneficiar futuros pacientes. Além disso, os laudos dos exames obtidos na pesquisa poderão ser solicitados por você. Caso seja encontrado algum resultado de exame que seja considerado relevante para a

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

sua saúde, os resultados poderão ser encaminhados para o seu médico assistente para os devidos encaminhamentos, caso você esteja de acordo.

Sua participação na pesquisa é totalmente voluntária, ou seja, não é obrigatória. Caso você decida não participar, ou ainda, desistir de participar e retirar seu consentimento, não haverá nenhum prejuízo ao atendimento que você recebe ou possa vir a receber na instituição.

Caso ocorra alguma intercorrência ou dano, resultante de sua participação na pesquisa, você receberá todo o atendimento necessário, sem nenhum custo pessoal.

Os dados coletados durante a pesquisa serão sempre tratados confidencialmente. Os resultados serão apresentados de forma conjunta, sem a identificação dos participantes, ou seja, o seu nome não aparecerá na publicação dos resultados.

Caso você tenha dúvidas, poderá entrar em contato com o pesquisador responsável Prof. Dr. Eugenio Horacio Grevet, pelo telefone 051-33598094, ou com o pesquisador Prof. Dr. Claiton Henrique Dotto Bau, pelo telefone 51-33086718 ou com a pesquisadora Renata Basso Cupertino, pelo telefone 51-81837278 ou com o Comitê de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre (HCPA), pelo telefone (51) 33597640, ou no 2º andar do HCPA, sala 2227, de segunda à sexta, das 8h às 17h.

Esse Termo é assinado em duas vias, sendo uma para o participante e outra para os pesquisadores.

_____	_____
Nome do participante da pesquisa	Nome do pesquisador que aplicou o Termo
_____	_____
Assinatura	Assinatura

Local e Data:

_____

11.2 Aprovação – Comissão de Pesquisa e Ética em Saúde – HCPA

Projeto 16-0600/CAAE 60633516.2.1001.5327

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
GRUPO DE PESQUISA E PÓS-GRADUAÇÃO

COMISSÃO CIENTÍFICA

A Comissão Científica do Hospital de Clínicas de Porto Alegre analisou o projeto:

Projeto: 160600
Data da Versão do Projeto: 17/11/2016

Pesquisadores:
EUGENIO HORACIO GREVET
FELIPE ALMEIDA ROSA
KATIANE LILIAN DA SILVA
EDUARDO SCHNEIDER VITOLA
DJENIFER KAPPEL
VERÔNICA CONTI
JAQUELINE BOHRER SCHUCH
BRUNA SANTOS DA SILVA
DIEGO LUIZ ROVARIS
CLAITON HENRIQUE DOTTO BAU
RENATA RASSO GUPERTINO

Título: Estudo prospectivo de indivíduos com e sem transtorno de déficit de atenção/hiperatividade diagnosticados na vida adulta

Este projeto foi APROVADO em seus aspectos éticos, metodológicos, logísticos e financeiros para ser realizado no Hospital de Clínicas de Porto Alegre.
Esta aprovação está baseada nos pareceres dos respectivos Comitês de Ética e do Serviço de Gestão em Pesquisa.

- Os pesquisadores vinculados ao projeto não participaram de qualquer etapa do processo de avaliação de seus projetos.

- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao Grupo de Pesquisa e Pós-Graduação (GPPG)

Porto Alegre, 03 de janeiro de 2017

Prof. José Roberto Goldim
Coordenador CEP/HCPA

11.3 Aprovação – Comissão de Ética em Pesquisa da PUCRS

Anexo IV - Aprovação Comissão de Ética em Pesquisa da PUCRS

Pontifícia Universidade Católica do Rio Grande do Sul
Faculdade de Psicologia
Programa de Pós-Graduação em Psicologia

Ofício 063/2010 – SGL

Porto Alegre, 16 de novembro de 2010.

Senhor(a) Pesquisador(a)

A Comissão Científica da Faculdade de Psicologia da PUCRS apreciou e aprovou seu protocolo intitulado **"COMPORTAMENTOS MOTIVADOS EM USUÁRIAS DE CRACK: RELAÇÃO COM NEGLIGÊNCIA NA INFÂNCIA E O CRAVING"**.

Sua investigação está autorizada a partir da presente data, sem a necessidade de passar pelo Comitê de Ética, devido à aprovação do projeto maior **"ESTILOS PARENTAIS, NEGLIGÊNCIA NA INFÂNCIA E O CRAVING EM USUÁRIAS DE CRACK: RELAÇÃO COM FUNÇÕES EXECUTIVAS, COMPORTAMENTO AGRESSIVO E MARCADORES BIOLÓGICOS"**, conforme ofício CEP nº 1229/10.

Atenciosamente,

Profa. Dra. Margareth da Silva Oliveira
Coordenadora da Comissão Científica da Faculdade de Psicologia

Ilmo(a) Sr(a)

Prof. Orientador: Rodrigo Grassi de Oliveira
Pesquisador(a): Ingrid D'Avila Francke

PUCRS
Campus Central
Av. Ipiranga, 6681 – P. 11 – 9º andar – CEP: 90619-900
Porto Alegre – RS – Brasil
Fone: (51) 3320-3500 – Fax (51) 3320 – 3633
E-mail: psicologia-pg@pucrs.br
www.pucrs.br/psipos

11.4 Certificado de Qualidade Ressonância Magnética

O Colégio Brasileiro de Radiologia e Diagnóstico por Imagem confere a:

Serdil Serviço Especializado em Radiodiagnóstico Ltda.

por se encontrar dentro dos padrões de qualidade exigidos pela instituição, como segue:
Médico responsável: Dr. Rodrigo Dias Duarte
Endereço: Rua São Luis, 96 - Santana - Porto Alegre - RS
Imagens examinadas: Obtidas pelo aparelho SIEMENS MAGNETOM SPECTRA 3T

Validade: até julho de 2019

São Paulo, 28 de julho de 2016

Dr. Antonio Carlos Matteoni d'Ajthayde
Presidente do CBR

Dr. Marco Antonio Rocha Mello
Coordenador da Comissão de Ressonância Magnética do CBR

CBR
Colégio Brasileiro de Radiologia e Diagnóstico por Imagem

(1*) Data de Emissão: 30/09/2016