UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E BIOLOGIA MOLECULAR

REFINANDO ASSOCIAÇÕES DO *MEF2C* COM A MICROESTUTURA DA SUBSTÂNCIA BRANCA E TRANSTORNOS PSIQUIÁTRICOS

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Dissertação 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 Mestre em Genética e Biologia Molecular.

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Porto Alegre

Abril de 2021

Instituições e fontes financiadoras

Este trabalho foi desenvolvido no Laboratório de Genética Humana Molecular do Departamento de Genética do Instituto de Biociências da Universidade Federal do Rio Grande do Sul. A aluna recebeu bolsa de estudos concedida pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Instituições Financiadoras:

CNPq - Conselho Nacional de Pesquisa. Auxílios 466722/2014-1 e 424041/2016-2

CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior. Código 001

FIPE-HCPA – Fundo de Incentivo à Pesquisa e Eventos. Projeto 160600

FAPERGS - Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul.

Agradecimentos

Agradeço imensamente a todos participantes da pesquisa que gentilmente se dispuseram a ceder seu tempo para as avaliações psiquiátricas, coleta de material biológico e imagens do cérebro. Agradeço também a todos os membros do Programa de Déficit de Atenção e Hiperatividade do Hospital de Clínicas que trabalham incansavelmente no planejamento, organização e execução de um dos melhores ambulatórios de TDAH da América Latina.

Agradeço especialmente os ensinamentos e a ajuda das minhas colegas Renata e Cibele que me estimularam na elaboração e realização do presente estudo. Da mesma forma que meu orientador Claiton e coorientadora Bruna, sempre muito solícitos e prestativos.

Agradeço às minhas amigas, Anna, Clara, Gabriela, Julia, Juliana, Luísa, Marianna e Tatiana pelo suporte e confiança em todos os momentos. E à minha família que me incentivou a perseguir meu sonho de ser cientista e me deu todo apoio para realizá-lo. Sou eternamente grata a vocês!

SUMÁRIO

Lista de abreviaturas

CDH13 – Cadherin 13

DRD4 – Dopamine receptor D4

DRD5 – Dopamine receptor D5

DUSP6 – Dual specificity phosphatase 6

DTI – Diffusion Tensor Imaging

DSM - Manual Diagnóstico e Estatístico de Transtornos Mentais

DMN – Default Mode Network

EMN – Extrinsic Mode Network

ENIGMA – Enhancing Neuro Imaging Genetics through Meta-Analysis

FOXP2 – Forkhead box P2

GWAS – Genome Wide Association Study

GSMA – Genome Scan Meta-Analysis

HDACs – Histonas desacetilases

HTR1B - 5-hydroxytryptamine receptor 1B

IMpACT - International Multi-centre persistent ADHD CollaboraTion

LINC00461 – Long intergenic non-protein coding RNA 461

MEF2C – Miocyte enhancer factor 2C

PGC – Psychiatric Genomics Consortium

SLC6A3 – Solute carrier family 6 member 3

SLC6A4 – Solute carrier family 6 member 4

SLC6A5 – Solute carrier family 6 member 5

SNAP25 – Synaptosome associated protein 25

TDAH - Transtorno de Déficit de Atenção/Hiperatividade

TDM – Transtorno Depressivo Maior

TUS – Transtorno por Uso de Substâncias

Resumo

O Transtorno de Déficit de Atenção e Hiperatividade (TDAH) possui altas taxas de herdabilidade e prevalência, apresentando diversas comorbidades. A genômica aliada a neuroimagem representa uma perspectiva promissora para desvendar o papel de genes específicos na relação entre estruturas cerebrais e o comportamento. A substância branca é responsável pela conectividade cerebral e medidas como a Anisotropia Fracionada (FA), permitem inferir sobre a sua integridade. O *MEF2C* (Myocyte enhancer factor 2C) é um regulador central do neurodesenvolvimento, sendo amplamente associado com fenótipos psiquiátricos e de substância branca através de estudos de GWAS. O objetivo desse trabalho foi compreender como o gene *MEF2C* está associado ao TDAH, explorando a heterogeneidade do transtorno e aspectos estruturais do cérebro. O presente estudo incluiu 407 casos com TDAH e 463 controles, dos quais 85 casos e 64 controles passaram pela aquisição de imagens cerebrais. Duas metodologias foram utilizadas: (1) *gene-wide* incluindo 97 variantes dentro da região do *MEF2C* avaliadas em relação à FA média cerebral e de 11 tratos de matéria branca (2) *set-based* incluindo blocos de desequilíbrio de ligação dentro do *MEF2C* em relação à: regiões apontadas na metodologia *gene-wide*; diagnóstico e escores dimensionais de TDAH, além de comorbidades do transtorno previamente associadas ao *MEF2C*. Os resultados apontam para uma associação de regiões específicas do *MEF2C* com componentes da circuitaria fronto-temporal, relacionados com processos cognitivos e de regulação emocional, sendo esses frequentemente comprometidos nos transtornos psiquiátricos. Não foram encontradas associações significativas entre o *MEF2C* e o TDAH ou suas apresentações. Ao confirmar as relações do *MEF2C* com a microestrutura da substância branca, a estratégia empregada se mostrou eficaz na tentativa de melhor caracterizar os mecanismos biológicos por trás do papel do *MEF2C* na neurobiologia e comportamento.

Abstract

Attention Deficit Hyperactivity Disorder (ADHD) has high rates of prevalence and heritability, presenting several comorbidities. Imaging genomics represents a promising perspective to unravel the role of specific genes in the relation between cerebral structures and behavior. The white matter is responsible for brain connectivity and measures such as Fractional Anisotropy (FA) enable inferences about its integrity. The *MEF2C* (Myocyte enhancer factor 2C) regulates neurodevelopment being largely associated with psychiatric traits and white matter microstructure through GWAS studies. The objective of this work was to comprehend how *MEF2C* is associated with ADHD, exploring the disorder heterogeneity and structural aspects of the brain. The present study included 407 ADHD cases and 463 healthy controls, of which 85 cases and 64 controls underwent a magnetic resonance imaging scan. Two methodologies were used: (1) a *gene-wide* analysis including 97 variants inside *MEF2C* region evaluated in relation to the average whole brain FA and 11 white matter tracts; (2) a *set-based* analysis including the linkage disequilibrium blocks inside *MEF2C* in relation to: regions pointed in the *gene-wide* analysis; ADHD diagnosis and dimensional scores, and ADHD comorbidities previously related to *MEF2C*. The results point towards an association of *MEF2C* specific regions with components of the fronto-temporal circuitry, related to cognitive processes and emotional regulation, which are frequently impaired in psychiatric disorders. Significative associations between *MEF2C* and or its presentations were not found. When confirming the relations between *MEF2C* and the white matter microstructure, the strategy employed showed effectiveness in better characterize the underlying *MEF2C* biological mechanisms in neurobiology and behavior.

Capítulo I

Introdução geral

1. INTRODUÇÃO

O Transtorno de Déficit de Atenção e Hiperatividade/Impulsividade (TDAH) é um transtorno neuropsiquiátrico com alta prevalência em crianças e adultos (5% e 2,5-3,4%, respectivamente) (Polanczyk et al., 2014; Simon et al. 2009). Seus sintomas incluem níveis extremos de atividade motora, desatenção e/ou impulsividade, com três apresentações possíveis: desatenta, hiperativa ou combinada. Estes sintomas são crônicos e causam prejuízo para o indivíduo afetado em múltiplas áreas de sua vida, como o trabalho, estudo, lazer e relações afetivas (American Psychiatric Association, 2014).

O TDAH foi considerado por muito tempo um transtorno restrito à infância e adolescência, até que diversas evidências demonstraram a sua persistência na vida adulta (Faraone and Biederman 2016; Franke et al. 2018). As estimativas de persistência do transtorno até essa fase variam entre os estudos realizados (Faraone et al. 2006), chegando em até 80% (Cheung et al. 2015). Essa variação pode estar ligada a mudanças que ocorrem na sintomatologia do transtorno ao longo do desenvolvimento (Faraone 2019), como a tendência no sentido de um declínio nos sintomas de hiperatividade e persistência nos de desatenção ao longo da vida (Willcutt 2012). Além disso, a parcela de indivíduos do sexo masculino diagnosticados na infância/adolescência é de aproximadamente 80%, enquanto em adultos essa faixa aproxima-se dos 50% (Kooij et al. 2010). Os meninos costumam apresentar mais sintomas de hiperatividade, e as meninas, de desatenção, porém a proporção dos sintomas entre os sexos na vida adulta tende a ser mais similar (Larsson et al. 2011).

A persistência do transtorno da infância para a vida adulta é mais frequente em indivíduos que apresentam comorbidades, como Transtorno por Uso de Substâncias (TUS) (Capusan et al. 2016), Transtorno Depressivo Maior (TDM) (Anttila et al. 2018), obesidade (Cortese and Tessari 2017), Transtorno do Espectro Autista (Hawi et al. 2015), Transtorno Bipolar (TB),

Esquizofrenia e comportamento antissocial (Larsson et al. 2013). A sobreposição vista entre os sintomas de um ou mais transtornos aponta para uma vulnerabilidade genética comum, possivelmente associada a fatores ambientais e mecanismos neurobiológicos coincidentes (Hinshaw 2018; Grimm et al. 2018). Este padrão de sobreposição com outros transtornos e a ocorrência de comorbidades evidencia a complexidade e heterogeneidade do TDAH. Dessa forma, aspectos clínicos e neurobiológicos do transtorno precisam ser mais explorados para que se possa entender sua etiologia e manifestações.

1.1 Genética do TDAH e comorbidades

Múltiplas variantes genéticas de pequeno efeito e altamente polimórficas na população estão envolvidas na etiologia do TDAH (Faraone et al., 2019; B. Franke et al., 2012; Grimm, Kittel-Schneider, Reif, 2019; Hawi et al., 2015). Com uma herdabilidade estimada em ~76% (Faraone et al 2005), o TDAH é um dos transtornos psiquiátricos com maior componente genético envolvido. A busca por variantes capazes de explicar esse efeito iniciou com estudos de ligação e gene-candidato e, mais recentemente, estudos de associação por varredura genômica (GWAS).

Embora os estudos de ligação permitissem uma abordagem de varredura genômica, nunca conseguiram resultados replicáveis, provavelmente porque o seu delineamento é otimizado para a detecção de variantes com grande efeito (Grimm; Kittel-Schneider; Reif, 2019). Posteriormente, os estudos de associação foram realizados com a abordagem gene-candidato, baseando-se principalmente nos genes componentes dos sistemas de neurotransmissão dopaminérgicos (*SLC6A3, DRD4, DRD5)* e serotoninérgicos *(HTR1B, SNAP25, SLC6A4 e SLC6A5)* (Gizer et al. 2009; Franke et al. 2012; Faraone et al. 2019). Esses estudos também tiveram poucos resultados replicados (Gizer et al. 2009), possivelmente porque não incluíam a maioria dos loci que posteriormente se mostraram relevantes.

Já os estudos de GWAS, assim como os de ligação, não possuem hipótese prévia, e avaliam sítios polimórficos em todo o genoma na busca de variantes comuns associadas ao desfecho de interesse. Esses estudos começaram no início do milênio e por muitos anos não foram capazes de indicar variantes associadas com o TDAH que sobrevivessem à correção para múltiplos testes. Essa dificuldade estava relacionada com o grande número de variantes avaliadas (100.000 – 1.000.000), que para atingirem a significância ($p<10^{-8}$) precisavam de um enorme poder amostral (Franke et al. 2009; Sullivan et al. 2012).

Consórcios internacionais tais como o PGC (*Psychiatric Genomic Consortium*) concentram dados genômicos de amostras coletadas por grupos ao redor de todo o mundo. Devido a esses esforços, foi possível alcançar grandes tamanhos amostrais. O IMpACT (*International Multi-centre persistent ADHD CollaboraTion* - "https://www.impactadhdgenomics.com/"*),* por sua vez, reúne os grupos envolvidos com TDAH na vida adulta. O maior GWAS de TDAH já publicado envolveu uma amostra de 20.183 casos e 35.191 controles pertencentes a 9 coortes compostas por crianças e 3 por adultos, onde se identificaram 12 loci significativamente associados ao TDAH (Demontis et al. 2019). Entre os genes presentes nessas regiões destacam-se *FOXP2, DUSP6, LINC00461, MEF2C;* por serem expressos em regiões cerebrais, estarem envolvidos em vias biológicas de alguma forma relacionadas ao TDAH, ou ainda terem sido associados a outros transtornos psiquiátricos (Faraone et al. 2019). O último estudo de GWAS, realizado com 22.406 adultos (6.532 casos e 15.874 controles) e 27.154 crianças (10.617 casos, 16.537 controles) demonstrou nove novos loci associados ao transtorno (Rovira et al. 2019), porém não replicou as variantes reportadas por Demontis e colaboradores (2019) que apresentaram uma associação nominal, com a mesma direção de efeito. Este estudo também demonstrou um *background* genético compartilhado entre adultos e crianças, entretanto, quando avaliados separadamente, os grupos demonstraram associações a loci distintos.

Estudos de GWAS conduzidos por outros grupos dentro do PGC também demonstraram que a arquitetura genética complexa evidenciada para o TDAH é compartilhada por outros transtornos psiquiátricos (Smoller et al. 2019). Atualmente, estudos analisando conjuntamente dados de GWAS obtidos para transtorno distintos tentam elucidar qual a parcela de sobreposição dos transtornos, identificando variantes pleiotrópicas associadas ao risco de transtornos relacionados. Os achados até agora apontam que tanto variantes aliadas ao risco específico de um determinado transtorno, quanto aquelas compartilhadas por mais de um transtorno são altamente expressas em regiões cerebrais, além de estarem envolvidas no neurodesenvolvimento, interferindo na regulação da plasticidade neuronal, neurotransmissão e sinapse (Lee et al. 2019).

1.2 Regiões cerebrais associadas ao TDAH e comorbidades

Devido à dificuldade inerente ao diagnóstico de transtornos psiquiátricos, existe uma grande busca por marcadores biológicos capazes de acessar de forma mais direta e objetiva os sintomas, possibilitando um melhor diagnóstico e por conseguinte um tratamento mais eficaz. Exames de neuroimagem são uma grande aposta na identificação de traços compartilhados por indivíduos afetados. Os estudos realizados em pacientes utilizam, entre outras técnicas, a ressonância magnética para avaliar características estruturais, funcionais e de conectividade/integridade das estruturas corticais e subcorticais (Klein et al. 2017; Albajara Sáenz et al. 2019). Essas técnicas tem revelado mecanismos neurais comuns e sobrepostos entre transtornos psiquiátricos incluindo TDM, Esquizofrenia, TB, TDAH, entre outros (Mukherji 2020). O sistema límbico, responsável pela regulação das emoções, parece ser um ponto comum subjacente aos distúrbios, além das regiões corticais frontais envolvidas nos processos cognitivos (Mukherji 2020).

1.2.1 Avaliações estruturais

Na mega-análise de Hoogman et al. (2017), os autores avaliaram alterações estruturais no TDAH em sete regiões subcorticais, e identificaram quatro regiões cerebrais (hipocampo, amígdala, núcleo caudado e putamen) com menor volume em casos quando comparados a controles. Além disso, também foi observada diferença significativa no volume intracraniano total. Um estudo do mesmo grupo (Hoogman et al., 2019) também apontou uma menor espessura cortical em pacientes com TDAH. Os dois estudos relataram diferenças maiores ou presentes exclusivamente em crianças, talvez porque a amostra de pacientes maiores de 25 anos incluída em ambos era pequena. No entanto, um estudo recente do nosso grupo mostrou associações estruturais em regiões frontais e estriatais tanto em crianças e adolescentes quanto em adultos (Cupertino et al. 2020).

Uma redução no volume total do cérebro e no volume de substância cinzenta de pacientes com TDAH também foi identificada, especialmente nas regiões fronto estriatais, córtex pré-frontal e lobos parietais e occipitais (Valera et al. 2007; Nakao et al. 2011; Norman et al. 2016; Silk et al. 2016; Ambrosino et al. 2017; Albajara Sáenz et al. 2019). Além disso, reduções na área de superfície cortical também foram encontradas em regiões corticais orbitais, mediais e superiores, além de regiões temporais (Hoogman et al. 2019).

Outros estudos apontaram que regiões cerebrais componentes de redes identificadas no TDAH também estavam associadas a outros transtornos psiquiátricos. Os achados incluíam a relevância do córtex medial pré-frontal para o TDM (Zhao et al. 2014), Esquizofrenia (Ren et al. 2013) e o córtex medial orbitofrontal para o TUS (Mackey et al. 2016). Regiões subcorticais como o hipocampo, tálamo e amígdala (Mukherji 2020), além de reduções específicas na espessura e área de superfície corticais (Thompson et al. 2020) também aparecem relacionadas aos mesmos transtornos e ao TB (Frazier et al. 2005) .

1.2.2 Avaliações de substância branca e conectividade

As regiões corticais e subcorticais conectam-se através dos tratos de matéria branca, que são os prolongamentos axonais provenientes dos corpos nucleares que formam o córtex (Le Bihan et al. 2001a). Imagens de ressonância magnética processadas pela técnica de DTI (*Diffusion-Tensor Imaging*) são capazes de inferir sobre a integridade da matéria branca, baseando-se na difusão das moléculas de água através do tecido cerebral (Le Bihan et al. 2001a). A anisotropia fracionada é uma das medidas mais utilizadas no contexto da difusão, e caracteriza propriedades relacionadas com a mielinização, densidade axonal e a integridade e organização das fibras (Basser, 1995).

Embora ainda não existam dados suficientes para uma mega-análise e resultados conflitantes tenham sido publicados em relação ao TDAH, uma conexão atípica inter-hemisférica através do corpo caloso tem sido verificada em vários estudos (Aoki et al. 2018; Albajara Sáenz et al. 2019). A tractografia é outro método que utiliza medidas de DTI para reconstruir tratos de matéria branca, e estudos com essa metodologia têm mostrado alterações em crianças com TDAH em conexões pré-frontal-estriatais (Hong et al. 2014) e frontal-accumbens (Cha et al. 2015).

Uma menor integridade dos circuitos fronto-temporais também foi indentificada na Esquizofrenia (Kelly et al. 2018), enquanto que regiões inter-hemisferiais envolvidas na regulação emocional como o corpo caloso também foram associadas ao TDM (van Velzen et al. 2020), ao TB (Lu et al. 2012) e ao TUS (Ogretmen 2019). Além disso o fascículo uncinado, que conecta a amígdala ao córtex orbitofrontal, parece estar relacionado à Esquizofrenia (Mandl et al. 2013) e a traços de ansiedade, chegando a ser considerado um possível endofenótipo para o comportamento ansioso (Linke 2019; Lee and Lee 2020).

1.2.3 Avaliações funcionais

As redes funcionais cerebrais são regiões relacionadas que aumentam ou diminuem sua ativação durante tarefas cognitivas (EMN - *Extrinsic Mode Network*), ou em períodos de descanso (DMN – *Default Mode Network*) (Allen et al. 2019). Essas redes são avaliadas pela ressonância magnética funcional em estudos que podem realizar tarefas neurocomportamentais concomitantes à aquisição de imagens (Roalf R. David 2016), ou aquisição de imagens sem tarefa (resting-state) (Biswal et al. 1995).

No que tange os pacientes com TDAH, a maioria dos estudos têm demonstrado uma concordância com os achados estruturais. Foram encontradas uma redução na conectividade da DMN (Sutcubasi et al. 2020) e alterações na atividade de regiões fronto-estriatal-talâmica e fronto-parietal-cerebelar (Cortese et al. 2012; McCarthy et al. 2014; Lei et al. 2015), envolvidas com déficits atencionais e funções executivas, com destaque para a memória de trabalho e inibição (Albajara Sáenz et al. 2019).

Essa mesma concordância entre os achados funcionais e estruturais também é vista em relação ao TDM, onde déficits funcionais foram encontrados nas vias de recompensa e regulação emocional (Phillips et al. 2015) e no TB, onde uma funcionalidade comprometida de regiões do córtex pré-frontal, da amígdala e do cíngulo foi identificada (Foland-Ross et al. 2012). Em contrapartida, variações funcionais nas redes fronto-estriatais e no córtex medial pré-frontal na Esquizofrenia parecem estar conectadas à idade de início de sintomas e ao tratamento farmacológico (Gong et al. 2016; Li et al. 2016). Os transtornos também apresentaram alterações na ativação funcional e conectividade da DMN (Allen et al. 2019).

1.3 Genética relacionada às regiões cerebrais

O cérebro é uma estrutura complexa e que tem o seu desenvolvimento afetado por milhares de variantes genéticas de pequeno efeito (Hibar et al. 2015a), da mesma forma que os transtornos psiquiátricos. O consórcio ENIGMA (*Enhancing Neuro Imaging Genetics through Meta-Analysis* -http://enigma.ini.usc.edu/) foi criado com o intuito de reunir amostras genômicas e de imagens cerebrais provenientes de diversos grupos de pesquisa, aumentando assim a capacidade exploratória dessas variantes associadas às características cerebrais (Argyraki et al. 2018). Após mais de dez anos avaliando a genômica subjacente ao cérebro, o consórcio identificou mais de 200 loci que contribuem significativamente para a variação encontrada em medidas cerebrais estruturais, cada um desses loci possuindo um pequeno efeito individual (0.1-1%), que, quando agregados, podem chegar a um efeito de até 20% na variância fenotípica observada (Thompson et al. 2020). Estudos recentes de GWAS utilizando medidas de difusão também identificaram mais de 200 loci associados a medidas de substância branca, muitos dos quais estavam sobrepostos a traços cognitivos e de transtornos psiquiátricos (Zhao et al. 2019).

Os circuitos cerebrais são essenciais para o funcionamento motor e cognitivo típico e suas disfunções podem levar a distúrbios neuropsiquiátricos (Argyraki et al. 2018). Muitos dos *hits* encontrados em associação com as regiões cerebrais através de GWAS (Stein et al. 2012; Hibar et al. 2015b; Adams et al. 2016; Hibar et al. 2017; Zhao et al. 2019) estavam próximos ou relacionados a genes envolvidos na apoptose e migração celular, assim como na orientação axonal, sendo estes processos celulares capazes de influenciar na morfologia e funcionamento do tecido neuronal (Argyraki et al. 2018). Portanto, compreender as vias biológicas associadas a tais processos ou suas inter-relações é uma das formas de se auxiliar a desvendar a etiologia dos transtornos psiquiátricos.

1.3.1 Papel do MEF2C

Como mencionado acima, o gene *MEF2C* (Myocyte enhancer factor 2C), está implicado na etiologia do TDAH e fenótipos relacionados (Demontis et al., 2017). Mais especificamente, o gene também foi associado através de GWAS com sucesso escolar (Okbay et al. 2016), TDM (Howard et al. 2018), Esquizofrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014) e nominalmente associado com transtornos neurológicos e psiquiátricos como, Alzheimer (Davies et al. 2015), TUS (Wang et al. 2013) e TB (Xie et al. 2017).

Esse gene, localizado em 5q14.3, codifica a proteína MEF polipeptídeo C que possui uma ação trans-ativadora e de ligação ao DNA (Leifer et al. 1993; Hobson et al. 1995). A proteína MEF2-C faz parte da família MEF2 (*MADS box transcription enhancer factor 2*), que atua na diferenciação de tecidos durante o desenvolvimento e vida adulta, sendo fundamental na expressão gênica dependente de ativação sináptica (Leifer et al. 1993; Assali et al. 2019). Essa família regula a migração e diferenciação neuronal; influencia na sobrevivência de neurônios recém-diferenciados; na orientação e "*pruning*" axonal e formação e remodelação dos dendritos (Mao et al. 1999; Chen et al. 2012; Pulimood et al. 2017).

Proteínas da família MEF2 podem atuar como repressoras ou ativadoras da transcrição juntamente com Histonas Desacetilases (HDACs), e são reguladas por vias enzimáticas dependentes de cálcio, sendo portanto fatores fundamentais na epigenética neuronal (Potthoff and Olson 2007). Devido a sua ampla influência na expressão de variados genes, muitos envolvidos na diferenciação e desenvolvimento normal do cérebro de mamíferos, genes codificantes de proteínas dessa família são frequentemente considerados um fator de risco para transtornos do neurodesenvolvimento como o TDAH, Transtorno do Espectro Autista e deficiência intelectual (Harrington et al. 2016; Assali et al. 2019).

Estudos com modelos animais apontaram papéis do *MEF2C* na plasticidade neuronal em resposta à estímulos ambientais. Harrington et al. (2016) observaram que sua deleção no cérebro de camundongos provocou déficits de memorização e aprendizagem, além de hiperatividade motora. Entretanto a deleção pós-natal do *Mef2c* no prosencéfalo não causou os mesmos desfechos (Adachi et al. 2016), indicando uma distinção das influências de sua expressão ao longo do desenvolvimento. Já a exposição crônica de nicotina durante o início do desenvolvimento em camundongos aumentou os níveis corticais de *Mef2c*, que por sua vez alteraram a transmissão sináptica cortical, levando a dificuldades de aprendizagem (Jung et al. 2016).

Análises de expressão gênica também mostram genes regulados por essa família atuando no desenvolvimento e função sináptica, assim como na excitabilidade neuronal (Harrington et al. 2016). Os quatro genes dessa família (*Mef2a-d*) codificam fatores de transcrição que são expressos em padrões distintos e sobrepostos durante o desenvolvimento e vida adulta dos vertebrados (Potthoff and Olson 2007; Assali et al. 2019) A expressão dessas proteínas em diversos tipos celulares, incluindo neurônios, ocorre ao mesmo tempo que a ativação dos seus programas de diferenciação e o balanço entre as funções ativadoras da transcrição da família MEF2 e as funções repressoras das HDACs classe II A irão conduzir o desenvolvimento do tecido. Já em tecidos adultos a família MEF2 é crucial para o remodelamento de programas celulares em resposta ao ambiente. Em ambas as funções os genes ativados pela MEF2 irão depender de mudanças pós-translacionais específicas que a família sofre durante a interação com os seus co-fatores (Potthoff and Olson 2007).

A identificação de microdeleções e mutações de ponto na região 5q13.4 em humanos permitiu caracterizar uma síndrome genética causada pela haploinsuficiência do MEF2C (Le Meur et al. 2010). Os fenótipos relatados são deficiência intelectual grave, epilepsia, hipotonia

muscular e anomalias cerebrais (Bienvenu et al. 2013; Rocha et al. 2016). Existem nove casos dessa síndrome descritos até hoje e entre as alterações cerebrais mais frequentes estão o atraso na mielinização e alterações em medidas da substância branca (Rocha et al. 2016).

O *MEF2C* também faz parte dos genes encontrados como significativamente associados à microestrutura da substância branca em um grande estudo de GWAS com 42.919 indivíduos neurotípicos avaliados (Zhao et al. 2020). Outros estudos também demonstraram associações nominais do *MEF2C* com o hipocampo (Hibar et al. 2015a), lóbulo frontal, ventrículos laterais e volume cerebral total (Seshadri et al. 2007), sugerindo um papel crucial desse gene no desenvolvimento e plasticidade do tecido neuronal e, por conseguinte, nas possíveis alterações cerebrais relacionadas a transtornos psiquiátricos.

Capítulo II

Justificativa e objetivos

2. JUSTIFICATIVA

Sendo o Transtorno de Déficit de Atenção e Hiperatividade um traço altamente herdável, com uma prevalência significativa na população (Simon et al. 2009; Polanczyk et al. 2014) e que traz uma série de prejuízos individuais e sociais para os acometidos (American Psychiatric Association, 2014) é imprescindível que existam esforços para melhor compreendê-lo nos diversos campos relacionados às suas manifestações e assim buscar auxiliar no diagnóstico e tratamento da sua sintomatologia.

A mudança de paradigma dos estudos de gene-candidato para os de varredura genômica que ocorreu na última década abriu espaço para a investigação de novas variantes genéticas associadas ao TDAH (Faraone 2019). Variantes no *MEF2C* estão significativamente associadas ao transtorno e à matéria branca através do GWAS (Demontis et al. 2017; Zhao et al. 2019) e também são relacionadas com o neurodesenvolvimento e a plasticidade neuronal a partir de estudos com modelos animais (Assali et al. 2019), tornando-se uma provável fonte de descoberta tanto de vias biológicas quanto de mecanismos moleculares envolvidos no transtorno.

Exames de ressonância magnética têm sido amplamente utilizados como uma forma de se auxiliar a compreender a etiologia dos transtornos psiquiátricos (Thompson et al. 2020), e a análise conjunta de dados de medidas de difusão provenientes de exames de neuroimagem e de varredura genômica tornou-se um campo vasto com múltiplas possibilidades de ser explorado e que, ao mesmo tempo, atua de forma promissora na elucidação da etiologia dos transtornos. Dessa forma, reunir e analisar dados genômicos e de microestrutura da substância branca, traçando conexões entre eles, é relevante para o melhor entendimento de como a genética, as regiões cerebrais e suas conexões contribuem para a manifestação desses transtornos.

Como o *MEF2C* possui uma extensa participação na regulação do desenvolvimento cerebral e de outros tecidos (Harrington et al. 2016) é provável que as vias biológicas direta ou indiretamente relacionadas com este gene sejam relevantes para o desenvolvimento típico do sistema neuronal. Portanto, explorar a associação do *MEF2C* ao TDAH e à estrutura de suas regiões cerebrais envolvidas é uma perspectiva promissora para desvendar mecanismos subjacentes ao transtorno.

3. OBJETIVOS

3.1 Objetivos Gerais

Compreender como o gene *MEF2C* está associado ao TDAH, explorando a heterogeneidade do transtorno e aspectos estruturais do cérebro.

3.2 Objetivos Específicos

- Identificar variantes do *MEF2C* associadas ao TDAH e a microestrutura de matéria branca;
- Identificar possíveis relações do *MEF2C* com características clínicas associadas ao transtorno;
- Avaliar possíveis rotas biológicas associadas ao *MEF2C* e sua relação com a microestrutura da matéria branca;

Capítulo III

Artigo Científico

4. Refining patterns of MEF2C associations with white matter microstructure and psychiatric features

Artigo em preparação, a ser submetido na revista *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*

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Keywords: *MEF2C*, white matter, fractional anisotropy, psychiatry genetics, neuroimaging, MRI

Short/running title: Refining *MEF2C* associations to white matter and psychiatric features

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Abstract

Background: The Myocyte Enhancer Factor 2 C has a central role in neurodevelopment, tissue differentiation, axonal orientation and pruning, as well as dendritic formation and remodulation. Its encoding gene (*MEF2C*) has been associated with white matter microstructure and several psychiatric disorders. Considering the broad pattern of associations, we aim to refine the *MEF2C* effect in an integrated analysis of both white matter and psychiatric phenotypes in an extensively characterized sample.

Methods: This study included 407 subjects with Attention-Deficit/Hyperactivity Disorder (ADHD) and 463 controls assessed through a psychiatric interview, with a followed-up subsample of 139 subjects (85 ADHD cases, 54 controls) who underwent a Magnetic Resonance Imaging scan. We evaluated variants in the *MEF2C* region with two approaches: 1) a *gene-wide analysis* which uses the sum of polymorphism p-values, and 2) *set-based* analysis restricted to 5 linkage disequilibrium (LD) blocks within the gene. The outcomes included fractional anisotropy (FA brain-derived metric to estimate white matter microstructure), ADHD and comorbidities.

Results: While our gene-wide analyses pointed to nominal associations between *MEF2C* and the whole brain average FA, as well as 3 out of 11 tracts, *MEF2C* LD blocks were significantly associated with the temporal portion of the superior longitudinal fasciculus and uncinate fasciculus, and nominally to ADHD comorbidities.

Conclusion: Our findings showing specific *MEF2C* regions associated with temporo-frontal circuitry components might raise hypothesis of how the *MEF2C* gene underlies a broad range of psychiatric phenotypes, since these regions are relevant to executive and cognitive functions.

Introduction

The white matter (WM) comprises the connective segment of the brain, relating cortical and subcortical region (Le Bihan et al. 2001b), having an estimated average SNP heritability of 48.7% across tracts (Zhao et al. 2019). Microstructural characteristics of WM can be inferred by Diffusion Tensor Imaging (DTI) data through water diffusivity properties in different neural tissues (Basser, 2019). One of the most common DTI measures is Fractional Anisotropy (FA), a global value regarding the water direction in WM fibers, varying from zero (fully isotropic) to one (fully anisotropic), allowing inferences on WM orientation and integrity (Mori et al. 2007). FA measures are altered in a series of psychiatric disorders, such as Generalized Anxiety Disorder, Major Depression Disorder (MDD) and Attention-Deficit/Hyperactivity (ADHD) (Mufford et al. 2017).

The Myocyte Enhancer Factor 2C (*MEF2C)* gene, located in the 5q14.3 region, was associated to WM integrity in a recent GWAS (Zhao et al. 2020). In addition, *MEF2C* has also been associated through GWAS studies to ADHD (Demontis et al. 2019), Schizophrenia (SCZ)(Kelly et al. 2018), MDD (Howard et al. 2018) and Educational Attainment (Okbay et al. 2016), outcomes with high rates of shared heritability (Lee et al. 2019).

MEF2C is part of a gene-family of enhancers, the MEF2s, and part of a bigger transcription factors family, the MADS-BOX genes. The MEF2s have a broad role in development and tissue differentiation, acting in muscular and neural crest cells, endothelium, chondrocytes, neurons and lymphocyte development (Potthoff and Olson 2007). Moreover, *MEF2C* interacts with a great variety of development-related proteins, and 16% of these interactions are with proteins relevant to neuronal development (Dong et al. 2017).

Altogether, these several independent GWAS pointing *MEF2C* associations with white matter microstructure and psychiatric features pose this gene as an important factor for a broad

range of phenotypes. The aim of this study was to narrow down the *MEF2C* association patterns in an integrated analysis of both white matter and psychiatric phenotypes in an extensively characterized sample of subjects with ADHD and healthy controls.

Methods and Materials

Sample

The sample consists of 407 adults (mean age: 33.6 years; males: 53.1%) recruited in the ADHD Outpatient Program, adult division (ProDAH-A) from Hospital de Clínicas de Porto Alegre (HCPA). The diagnosis of ADHD followed DSM-IV (American Psychiatric Association, 1996) criteria, using the Portuguese version of the Kiddie Schedule for Affective Disorders and Schizophrenia (KSADS - E; Mercadante et al., 1995) adapted for adults (Grevet et al. 2005). Comorbidities were evaluated through the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First et al. 1998). Exclusion criteria were evidence of clinically significant neurological disease (e.g., delirium, dementia, epilepsy, head trauma) and intelligence quotient (IQ) ≤ 70.

The control sample comprised 463 subjects (mean age: 29.37 years; males: 47.9%) with negative screening for ADHD, assessed by the 6-item Adult ADHD Self-Rated Scale Screener (ASRS) (Kessler 2005). Psychiatric disorders were evaluated through the screening module of SCID-IV (First et al. 1998) covering anxiety, mood, psychosis, substance use, and eating disorders. Both samples are characterized in Table 1. All participants signed an informed consent form approved by the hospital ethical committee (IRB 0000921).

Magnetic Resonance Imaging (MRI) scan

After approximately 13 years of the first evaluation in ProDAH-A, a subsample including subjects with ADHD (n=85, mean age: 34.3 years; males: 43.5%) and controls (n=54,

mean age: 29.3 years, males: 61.1%) was scanned in a 3T Siemens Spectra MRI scanner with a 16-channel head coil. The neuroimaging subsample is characterized in Table 2. The diffusion-weighted imaging acquisition protocol applied a single-shot echo planar imaging sequence (62 contiguous axial slices, TE=110ms, TR=11000ms, voxel size=2x2x2mm, slice thickness=2.0mm, FOV=240mm, one b0 image and 64 diffusion-weighted images with gradient directions b=1400s/mm^2). An adapted protocol with reduced acquisition time (with differences on the number of diffusion-weighted images=32, TE=106ms, voxel size=2.4x2.4x2.4mm and slice thickness=2.4mm) was applied for the restless or claustrophobic individuals (n=25).

The preprocessing and correction for motion and eddy currents were done using FMRIB Software Library (FSL) tools (https://fsl.fmrib.ox.ac.uk/fsl/; Woolrich et al., 2009), with posterior visual quality control. Through *dtifit* we generate a whole-brain FA map per each individual. All their maps were registered and created a skeleton using tract based spatial statistics (TBSS) according to the John Hopkins University white matter tractography atlas (Mori et al., 2007; Wakana et al., 2007, https://identifiers.org/neurovault.image:1403). 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).

Genotyping

Peripheral blood of all subjects was collected, and DNA extracted using *salting out* method (Lahiri and Nurnberger 1991). The variants were genotyped through the Illumina Infinium PsychArray-24 v1.1. Pre-imputation quality control at individual and SNP levels, as

well as principal component analyses for ancestry genetic outlier detection were performed through the *Rapid Imputation and COmputational PIpeLIne* (Ricopili). Phasing of genotype data, and imputation were performed using SHAPEIT2 and IMPUTE2 algorithms, respectively, 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

We compared the FA measures obtained in whole brain and each tract between ADHD cases and controls using linear regression, adjusted for sex, age and headmotion. In addition, we tested if the ADHD dimensional scores were associated with the FA measures. These analyses were conducted in SPSS v26 (George et al. 2020). Analysis to evaluate *MEF2C* were conducted using two approaches: 1) a *gene-wide analysis* and 2) analysis restricted to 5 linkage disequilibrium (LD) blocks, as described in the following sessions.

Gene-wide analysis

We extracted the *MEF2C* gene region plus a window of 10kb upstream and 10kb downstream (GRCh37 genomic positions 5:88,004,058 to 5:88,209,922), in PLINK v1.9 (Chang et al. 2015), comprising 97 variants. We first analyzed these variants in relation to FA of eleven WM tracts and the whole brain in a set-based analysis using the additive model, with ten thousand permutations. This analysis provides an empirical p-value relative to the association of the whole gene to the outcome, calculated as the mean of a single SNP statistics.

Set-based analyses

Hereafter, we assessed the LD blocks inside *MEF2C* (Figure 2) using the *--blocks* command in PLINK (Chang et al., 2015), to account for the heterogeneity across the gene region. WM tracts with suggestive evidence for association with *MEF2C* in the gene-wide

analysis were selected for LD block set-based analyses, which were adjusted for MDD and ADHD diagnosis, age, sex, headmotion, and the first ten principal components.

In addition, we evaluated the LD blocks in relation to the ADHD diagnosis, dimensional scores and related comorbidities (n = 407 ADHD cases). This analysis was adjusted for sex, age, and the ten first principal components. All analyses were performed in PLINK 1.9 software (Chang et al. 2015). Covariates were included according to their association ($p \leq 0.2$) with predictor and outcome (Maldonado and Greenland 1993), or its clinical relevance. False discovery rate (FDR) was applied to correct for multiple tests. We also identified the index SNPs for each LD block associated in the set-based analysis to compare possible overlapping SNPs and the magnitude of association with the outcomes evaluated.

In-silico

The *MEF2C* independent variants were also evaluated through in silico analyses, to explore possible regulatory functions. We utilized the following tools: GWASatlas (https://atlas.ctglab.nl/; Watanabe et al., 2019) to evaluate associations to neurological and psychiatric features, Variant Effect Prediction (VEP), to predict functional effects of the variants (www.ensemble.org; Yates et al., 2016); UCSC Genome Browser, to visualize genomic data; HaploReg v4.1, to examine noncoding genome annotations of disease-associated loci by GWAS (pubs.broadinstitute.org;); RegulomeDB, to annotate the SNPs with known and predicted regulatory elements in the intergenic regions of the genome (www.regulomedb.org; Boyle et al., 2012); SNPinfo web server, to predict functional characteristics of coding and noncoding variants (snpinfo.niehs.nih.gov; Xu & Taylor, 2009).

Results

FA measures of the whole brain and the eleven white matter tracts evaluated did not differ between ADHD cases and controls and were not associated with ADHD dimensional scores (Supplementary Tables 1 and 2). In this sense, we performed *gene-wide* and *set-based* analyses between *MEF2C* and the white matter tracts pooling cases and controls together (n=139), controlling for ADHD.

Gene-wide analysis

The results pointed to nominal associations between *MEF2C* and FA measures in four out of eleven WM tracts tested (Temporal Portion of the Superior Longitudinal Fasciculus (SLFTEMP), Uncinate Fasciculus (UF), Forceps Minor), and the whole brain average FA (Table 2).

LD blocks analysis

Five LD blocks were identified within the *MEF2C* (Figure 1) and were investigated in relation to whole brain average FA and the three tracts mentioned above presenting nominal associations with the gene (i.e., SLFTEMP, UF and Forceps Minor). We found distinct patterns of associations across the gene regions and the WM tracts (Table 3). The associations with SLFTEMP and UF remained significant after FDR correction (Considering all 20 tests - i.e., 5 blocks on 4 WM tracts). The index SNPs of each block associated to SLFTEMP are shown in Table 4 and Supplementary Figures 1-4, all of them were associated with lower SLFTEMP FA. The fourth LD block was the only associated with more than one WM tract, exhibiting index SNPs in opposite directions of association with SLFTEMP and with UF (Table 5).

In the analysis including the clinical sample (n = 407) we did not find any significant association with ADHD diagnosis, or dimensional scores. Nevertheless, we found that three of

the five LD blocks tested were associated with at least one of the included psychiatric comorbidities (Supplementary Table 3). None of the associations remained significant after the FDR correction (Considering all 20 tests 5 LD blocks, and 4 comorbidities). In silico analysis are detailed in Supplementary Table 4.

Discussion

This is the first study to simultaneously address associations relating *MEF2C*, DTI measurements and psychiatric features. Specific *MEF2C* regions were associated to temporo-frontal circuitry components relevant to executive and cognitive functions. Our results involving *MEF2C* and WM microstructure extend previous findings relating *MEF2C* and neurological outcomes, and support *MEF2C* genome-wide associations to DTI measures (Zhao et al. 2020).

Our finding of association between *MEF2C* and uncinate fasciculus supports previous GWAS association of this gene with FA in the external capsule (Zhao et al. 2020). The external capsule is a structure that has relevant roles in executive functions, emotional regulation, and cognitive control (Korgaonkar et al. 2014), and is composed by the Uncinate and Inferior Fronto Occipital Fasciculi. Furthermore, our findings include the temporal portion of the SLF, a bidirectional association fiber tract connecting the parietal, occipital and temporal lobes with frontal cortices (Schmahmann et al. 2007), involved in processes related to human cognition, such as attention, language, memory and emotions (Kamali et al. 2014). The SLF has been related with psychiatric features associated to *MEF2C*, such as MDD (van Velzen et al. 2020), Substance Use Disorder (Ogretmen 2019), Bipolar Disease (Ching et al. 2020), and SCZ (Kelly et al., 2018), while UF has also been associated to Bipolar Disease (Ching et al. 2020), and extensively related to General Anxiety Disorder (Wang et al. 2016; Linke 2019).

The MEF2C transcripts have three domains with binding sites to different proteins, DNA dimerization and epigenetic marks (Shalizi and Bonni 2005). All 4 LD blocks found associated with SLFTEMP have variants associated to promoter or enhancer features in epigenetic marks present in brain tissues, supporting its role in the central nervous system. Exploring the index SNPs of these associations we observed that the rs619584 polymorphism disrupts binding sites to important transcript factors (TFs) involved in embryogenesis, cell development and differentiation, while rs80043958 binds to the TATA Binding Protein, a central transcription regulator. All blocks have polymorphisms that bind central proteins involved in transcription, gene expression, tissue development and differentiation.

The fourth LD block is the only block that has distinct variants associated with the WM tracts: rs80043958 G carriers' status is associated with lower FA of SLFTEMP, while rs57055575 A carriers present higher FA of UF. The rs80043958 G carriers have more affinity to ZNF219 gene transcript, which acts in inactivation of chromatin. On the other hand, rs57055575 A carriers have more affinity to the Ascl2 transcription factor, involved in the determination of central nervous system neuronal precursors. These functional annotations could help to explain why rs80043958 relates to a lower WM microstructure integrity, and rs57055575 to a higher one.

The nominal findings regarding the *MEF2C* LD blocks and ADHD comorbidities are plausible as every associated block has SNPs linked to SCZ (Pardiñas et al. 2018), MDD (Howard et al. 2019), or both disorders, in addition to be expressed in brain tissues. Furthermore, MEF2C integrates the MAPK cascade, which in turn regulates the Neurotrophin signaling pathway, that coordinates axonal and dendritic growth and guidance, synaptic structure and connections, neurotransmitter release, long-term potentiation (LTP) and synaptic plasticity (Park and Poo 2013). This route has also been recently suggested as a potential shared

pathway between multimorbid psychiatric disorders (Golovina et al. 2020). So, we hypothesized that the association suggested between *MEF2C* and WM microstructure, and its possible effect in psychiatric disorders could be related to the regulation of this molecular pathway.

The lack of difference between FA measures in subjects with ADHD and controls reported here is in line with another recent DTI study with a larger sample of 654 subjects, being 258 ADHD cases (Damatac et al. 2020). We also did not see any alterations in FA measures concerning the ADHD dimensional scores. In fact, previous meta-analysis pointed to inconsistent results observed so far relating DTI measures and ADHD (Albajara Sáenz et al. 2019).

Despite ADHD GWAS findings pointing to the *MEF2C* region as a significant hit, we did not find any association between *MEF2C* and the symptoms of ADHD, or its diagnosis. However, it is noteworthy that the *MEF2C* significant GWAS variant found by Demontis and colleagues (2019) did not pass the credible set analysis, and another subsequent ADHD GWAS (Rovira et al. 2020), did not replicate the association with *MEF2C.* These differences can be addressed to the highly heterogeneous presentation of the disorder, with underlying distinct functional deficits that converge in the diagnosis, in addition to the high rates of psychiatric comorbidities (Mueller et al. 2017). As *MEF2C* was also a significant hit in GWAS of other psychiatric disorders comorbid with ADHD, as MDD (Howard et al. 2018), and SCZ (Ripke et al. 2014), the specific association with ADHD could represent a shared underlying trait among these disorders.

This study must be seen in light of some limitations. First, the size of our neuroimaging subsample was relatively limited considering the demands of the gene-wide analysis. However, our LD block analysis is a valid approach to optimize statistical power. Second, we analyzed in

this study complex and multifactorial features that are all deeply related to each other, hindering the establishment of causal effects. As multifactorial features, the WM and psychiatric disorders have large polygenicity, with most common variants presenting small effects, and therefore larger samples are needed to replicate the associations suggested here.

The overall pattern of our findings supports the view that *MEF2C* gene could affect WM microstructure and this relation could partially explain the several associations of *MEF2C* in GWAS. Further studies are needed to better understand how *MEF2C*, psychiatric disorders and white matter microstructure are interconnected.

Acknowledgments

We would like to acknowledge the patients assessed in ProDAH-A and all its members. This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES, Finance Code 001) and FIPE-HCPA 160600, GPPG-HCPA 01-321. In addition to the financial support received from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Grants 466722/2014-1, 424041/2016-2, 426905/2016-2, 431472/2018-1, 140853/2019-7), and the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (Grants PqG-19/2551-0001731-6; PqG-19/2551-0001668-9). The funding agencies mentioned were not involved in study design, in the collection, analysis and interpretation of data, in the writing of the report or in the decision to submit the article for publication.

Disclosures

The authors declare the following potential conflict of interest: Dr. Grevet was on the speaker's bureau for Novartis and Shire for three years, and received travel awards (air tickets and hotel accommodations) for participating in two psychiatric meetings from Shire

and Novartis. All other authors declare that they have no conflict of interest.

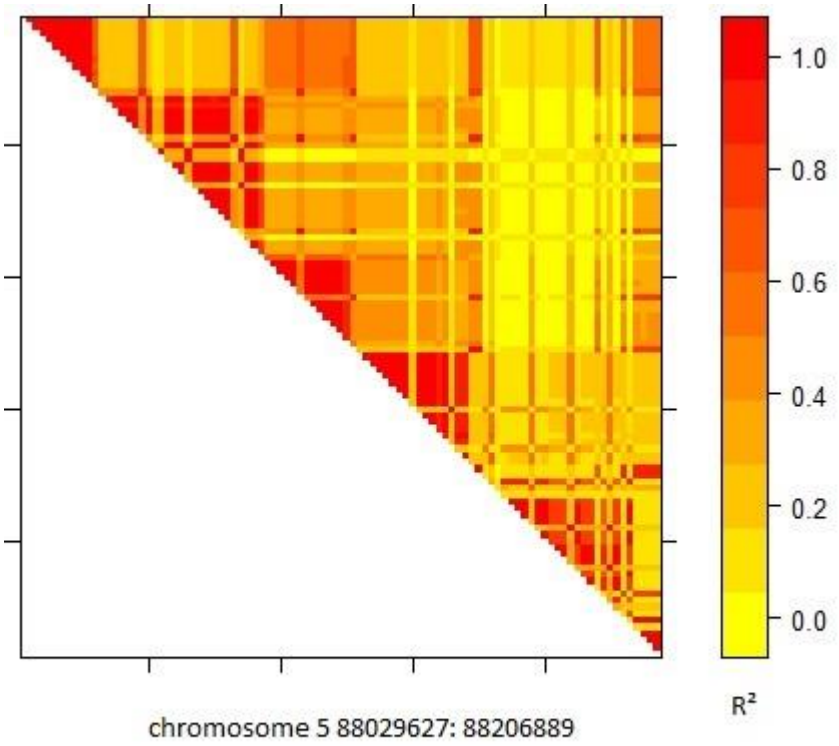


Figure 1. LD blocks pattern inside *MEF2C* gene region
R^2 denotes the LD between the gene variants

Table 1. Overview of the Neuroimaging and clinical sample

Neuroimaging sample	*ADHD cases (n=85)*		*Controls (n=54)*	
	n	*%*	*n*	*%*
Gender (male)	37	43,5	33	61,1
Age (years)[a]	29,3	8,9	34,3	9,8
Comorbidities lifetime				
Generalized Anxiety Disorder	63	74,1	7	13
Bipolar Disorder	31	36,5	-	
Major Depressive Disorder	37	43,5	22	40,7
Substance Use Disorder	29	34,1	5	9,2

Clinical sample	*ADHD cases (n=407)*		*Controls (n=463)*	
	n	*%*	*n*	*%*
Gender (male)	216	53	222	47,9
Age (years)[a]	29,4	8,7	33,6	10,8
Comorbidities lifetime				
Generalized Anxiety Disorder	154	37,8	63	13,6
Bipolar Disorder	94	23,1	16	3,4
Major Depressive Disorder	166	40,8	141	30,4
Substance Use Disorder	98	24	22	4,7

n is the number of subjects and % the percentage considering the whole sample
[a] n is the mean years and % the standard deviation

Table 2. Gene-wide results including *MEF2C* 97 variants and the 11 WM tracts and the whole brain FA measures.

	N_{SNP}	N_{SIG}	P_{-emp}
Inferior Fronto-occipital Fasciculus	97	16	0.204
Inferior Longitudinal Fasciculus	97	8	0.069
Superior Longitudinal Fasciculus	97	43	0.109
Uncinate Fasciculus	97	39	**0.020***
Temporal Portion of SLF	97	31	**0.002***
Anterior Thalamic Radiation	97	16	0.279
Corticospinal Tract	97	1	0.364
Dorsal Cingulate Gyrus	97	42	0.103
Ventral Cingulate Gyrus	97	3	0.299
Forceps Major	97	28	0.124
Forceps Minor	97	21	**0.049***
Average Fractional Anisotropy	97	59	**0.046***

N SNPs are the number of SNPs in the MEF2C gene region
N_{SIG} are the number of significative SNPs associated with the outcome
P-emp stands for the P-empirical value corrected for 10 thousand permutations
*None of the associations remained significant after FDR correction
Analysis adjusted for: ADHD and MDD diagnosis, sex, age, headmotion and the ten first principal components.

Table 3. Set-based results including *MEF2C* 5 LD blocks and the WM regions associated in Table 2

	Temporal Portion of SLF (SLFTEMP)		Uncinate Fasciculus		Forceps Minor		Whole Brain	
5 LD blocks - N SNPs	N_{SIG}	p_{-EMP}	N_{SIG}	p_{-EMP}	N_{SIG}	p_{-EMP}	N_{SIG}	p_{-EMP}
block 1 - 11	-	1	-	1	-	1	1	0.032
block 2 - 26	21	**0.004**	2	0.150	-	1	22	0.054
block 3 - 8	1	**0.003**	-	1	-	1	8	0.023
block 4 - 25	3	**0.008**	17	**0.002**	15	0.022	19	0.030
block 5 - 27	6	**0.005**	20	0.027	6	0.043	9	0.084

N SNPs are the number of SNPs in the *MEF2C* gene region
N_{SIG} are the number of significative SNPs associated with the outcome
P-emp stands for the P-empirical value corrected for 10 thousand permutations
Associations in bold remained significant after FDR correction
Analysis adjusted for: ADHD and MDD diagnosis, sex, age, headmotion and the ten first principal components.

Table 4. Associations between the index SNP representing each LD block with SLFTEMP FA measures

			SLFTEMP		
	index SNPs	A1	beta (ci 95%)	SE	p
block 2	rs619584	G	-0.018 (-0.027 -0.008)	0.004	**<0.001**
block 3	rs159950	T	-0.019 (-0.031 -0.007)	0.006	**0.002**
block 4	rs80043958	G	-0.019 (-0.031 -0.006)	0.005	**0.003**
block 5	rs4351182	T	-0.020 (-0.032 -0.008)	0.006	**0.002**

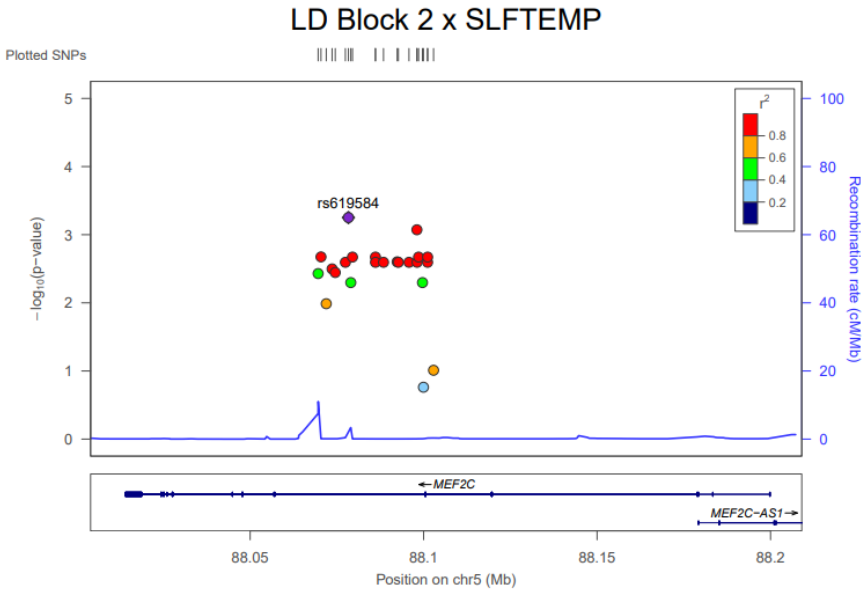
A1 is the reference allele
SE denotes standard error
Analysis adjusted for: ADHD and MDD diagnosis, sex, age, headmotion and the ten first principal components.

Table 5. Associations between the index SNP representing the fourth LD block with SLFTEMP and UF

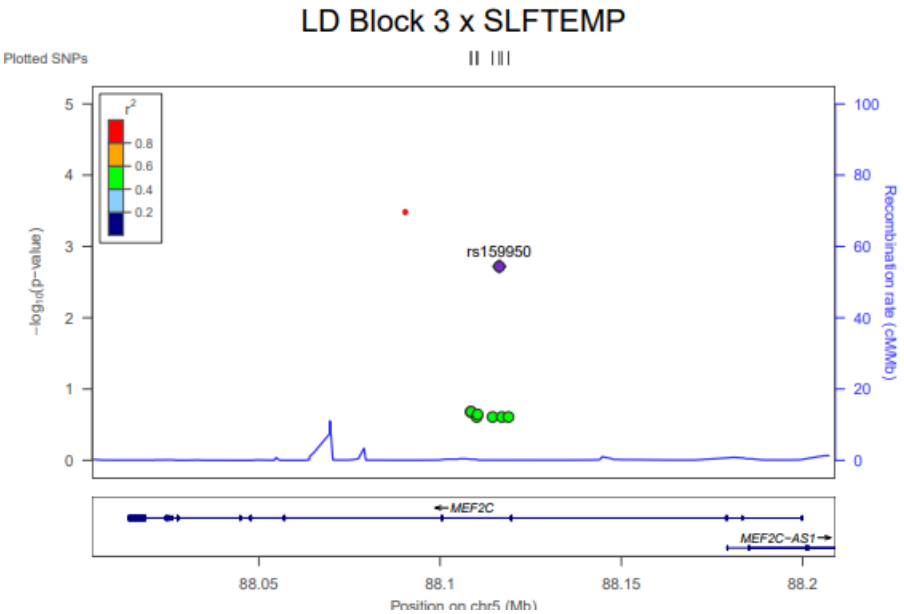
			SLFTEMP			UF		
	index SNPs	A1	beta (ci 95%)	SE	p	beta (ci 95%)	SE	p
block 4	rs57055575	A	0.009 (-0.001 0.019)	0.005	0.083	0.015 (0.0075 - 0.022)	0.004	**<0.001**
	rs80043958	G	-0.019 (-0.031 -0.006)	0.005	**0.003**	-0.008 (-0.018 - 0.001)	0.005	0.101

A1 is the reference allele
SE denotes standard error
Analysis adjusted for: ADHD and MDD diagnosis, sex, age, headmotion and the ten first principal components.

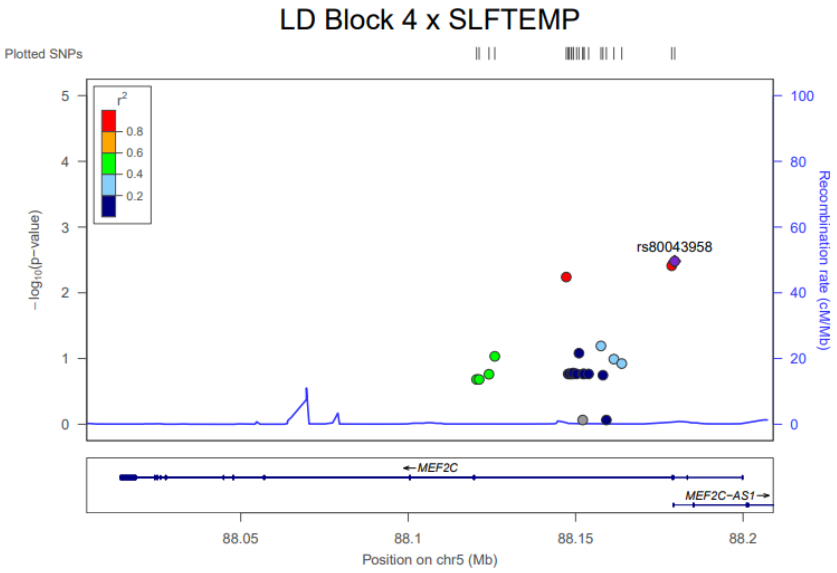
Supplementary Figure 1.



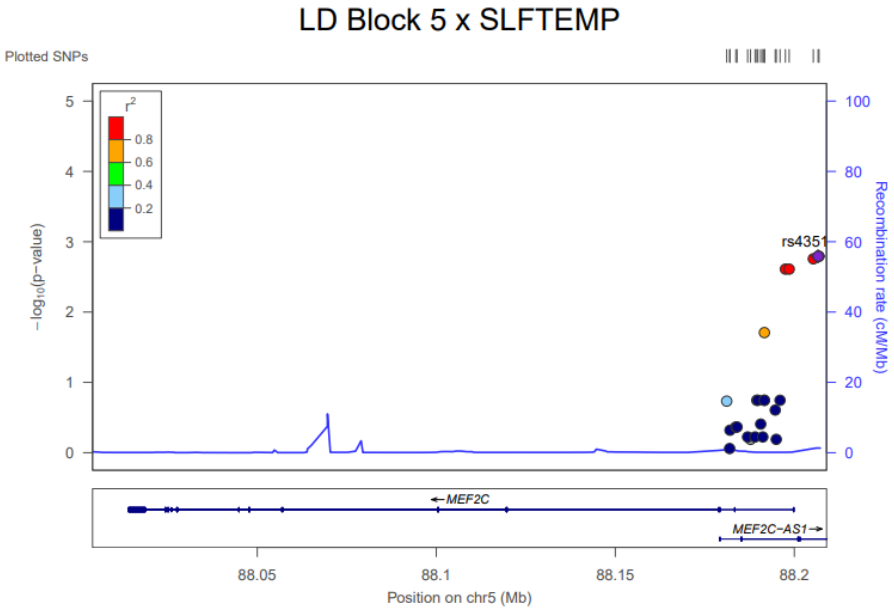
Supplementary Figure 2.



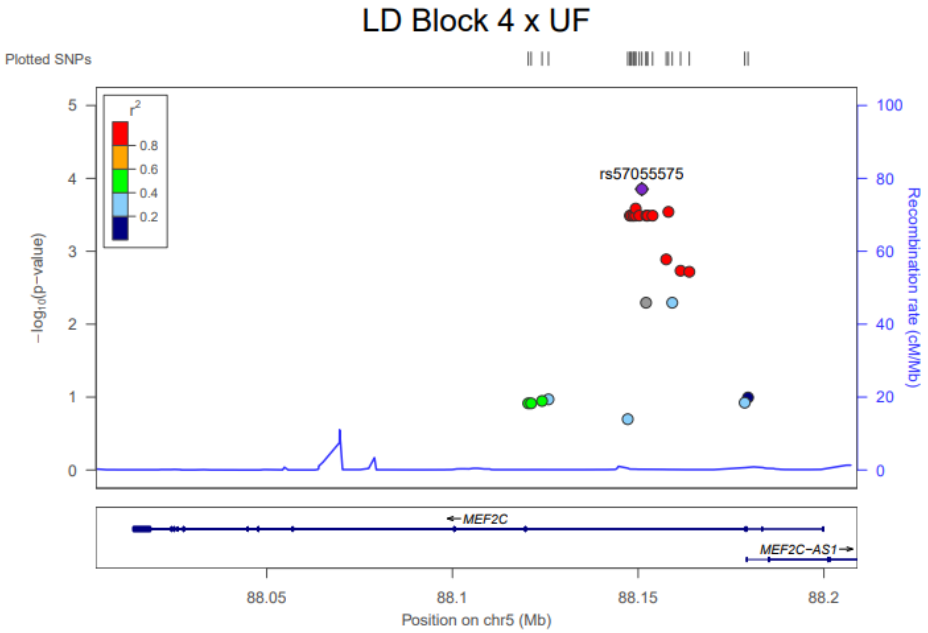
Supplementary Figure 3.



Supplementary Figure 4.



Supplementary Figure 5.



Supplementary Figures 1-5. Index SNP plot between each associated LD block and its respective WM tract.

Supplementary Table 1. Cohen's D size effect of ADHD diagnosis in the 11 WM tracts and the whole brain FA measures

	Effect Size and 95% C.I.					
	Cohen's D	SE	Lower limit	Upper limit	Z-value	p
Inferior Fronto-occipital Fasciculus	-0.101	0.174	-0.445	0.245	1,231	0.566
Inferior Longitudinal Fasciculus	0.045	0.175	-0.301	0.390	0,505	0.799
Superior Longitudinal Fasciculus	-0.030	0.175	-0.375	0.315	0,089	0.863
Uncinate Fasciculus	-0.055	0.174	-0.289	0.246	0,498	0.751
Temporal Portion of SLF	-0.099	0.174	-0.443	0.443	2,635	0.573
Anterior Thalamic Radiation	-0.013	0.175	-0.359	0.332	0,661	0.939
Corticospinal Tract	-0.161	0.174	-0.505	0.183	0,000	0.357
Dorsal Cingulate Gyrus	0.108	0.174	-0.236	0.453	0,357	0.535
Ventral Cingulate Gyrus	-0.172	0.174	-0.516	0.172	0,694	0.325
Forceps Major	0.084	0.174	-0.261	0.429	0,004	0.632
Forceps Minor	0.004	0.175	-0.341	0.350	0,007	0.980
Whole Brain Fractional Anisotropy	-0.074	0.174	-0.419	0.271	1,304	0.673

SE denotes Standard Error
Lower and Upper Limit regarding the 95% Confidence Interval
Analysis adjusted for: sex, age, and headmotion.

Supplementary Table 2. ADHD dimensional scores regression coefficients with the 11 WM tracts and the whole brain FA measures

	Innatention		Hyperactivity		ADHD score	
	beta	p_{lin}	beta	p_{lin}	beta	p_{lin}
Inferior Fronto-occipital Fasciculus	-0,100	0,263	0,047	0,580	-0,033	0,714
Inferior Longitudinal Fasciculus	-0,039	0,658	0,149	0,077	0,059	0,501
Superior Longitudinal Fasciculus	-0,109	0,237	-0,004	0,966	-0,066	0,471
Uncinate Fasciculus	-0,076	0,416	-0,022	0,804	-0,057	0,540
Temporal Portion of SLF	-0,107	0,253	-0,006	0,950	-0,065	0,479
Anterior Thalamic Radiation	-0,099	0,256	0,071	0,396	-0,019	0,826
Corticospinal Tract	-0,164	0,064	-0,008	0,929	-0,100	0,255
Dorsal Cingulate Gyrus	0,021	0,816	-0,017	0,844	0,003	0,974
Ventral Cingulate Gyrus	-0,119	0,179	0,072	0,394	-0,030	0,735
Forceps Major	-0,019	0,834	0,034	0,688	0,008	0,929
Forceps Minor	-0,043	0,622	0,064	0,442	0,010	0,907
Whole Brain Fractional Anisotropy	-0,092	0,297	0,026	0,758	-0,039	0,652

beta denotes the linear coefficient regression
p-lin denotes the association p-value
Analysis adjusted for: sex, age, and headmotion.

Supplementary Table 3. Set-based results including *MEF2C* 5 LD blocks and ADHD comorbidities

	GAD		MDD		BD		SUD	
5 LD blocks - N SNPs	N_{SIG}	p_{-EMP}	N_{SIG}	p_{-EMP}	N_{SIG}	p_{-EMP}	N_{SIG}	p_{-EMP}
block 1 - 11	-	1	-	1	-	1	-	1
block 2 - 26	-	1	18	**0.022***	5	0.054	12	**0.039***
block 3 - 8	-	1	7	**0.024***	4	0.083	7	**0.015***
block 4 - 25	-	1	5	0.127	12	0.196	20	**0.007***
block 5 - 27	-	1	-	1	-	1	5	0.074

N SNPs are the number of SNPs of each LD block
N_{SIG} are the number of significative SNPs associated with the outcome
P-emp stands for the P-empirical value corrected for 10 thousand permutations
GAD (Generalized Anxiety Disorder) MDD (Major Depression Disorder) BP (Bipolar Disease) SUD (Substance Use Disorder)
*None of the associations remained significant after FDR correction
Analysis adjusted for: sex, age and the ten first principal components.

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Capítulo IV

Discussão geral

5. DISCUSSÃO

A busca por um melhor entendimento dos mecanismos fisiopatológicos dos transtornos psiquiátricos tem reunido diferentes áreas do conhecimento em enormes esforços colaborativos ao redor do mundo. Tratam-se de consórcios para coleta e análise de material biológico e traços fenotípicos, incluindo diagnósticos clínicos, dados genômicos e de neuroimagem (Smoller et al. 2019; Thompson et al. 2020). Os resultados desses estudos confirmam os modelos multifatoriais clássicos, indicando que os transtornos parecem fazer parte de um contínuo, com um compartilhamento do risco genético e de redes funcionais cerebrais, além de certos distúrbios estarem mais relacionados dentro da sua fisiopatologia, sendo que essas relações nem sempre são as mesmas observadas clinicamente (Allen et al. 2019; Smoller et al. 2020).

A relação entre a genômica e o funcionamento cerebral é extremamente complexa, por ser o cérebro um órgão com extensa plasticidade anatômica e funcional, moldado por uma imbricada rede de fatores biológicos e ambientais interagindo entre si (Elliott et al. 2018). De que forma a interação desses fatores acaba por resultar numa vulnerabilidade maior para o aparecimento de transtornos psiquiátricos é um dos pontos centrais de estudo da psiquiatria (Sullivan and Geschwind 2019).

Vias de sinalização envolvidas no desenvolvimento do tecido neuronal foram extensamente relacionadas a transtornos psiquiátricos e podem ser parcialmente responsáveis por déficits funcionais do sistema nervoso central que acabam acarretando a expressão desses transtornos (Golovina et al. 2020). Compreender quais variantes genéticas implicadas nessas vias estão associadas ao funcionamento cerebral e de que forma elas influenciam os distúrbios mentais é de extrema importância (Smoller et al. 2019).

O *MEF2C* foi amplamente associado à traços psiquiátricos (Ripke et al. 2014; Demontis et al. 2019; Howard et al. 2019) e neurológicos (Lambert et al. 2013; Zhao et al. 2020) através de estudos de GWAS, além de ser um regulador central de programas de diferenciação e desenvolvimento tecidual, estando relacionado na formação típica do tecido neuronal e suas conexões (Shalizi and Bonni 2005; Li et al. 2008; Li et al. 2018), atuando na atividade sináptica (Harrington et al. 2016) e plasticidade neuronal (Barbosa et al. 2008). Os achados desse estudo demonstram regiões específicas do *MEF2C* associadas à tratos de matéria branca componentes de redes cerebrais envolvidas na cognição e regulação emocional (Schmahmann et al. 2007; Kamali et al. 2014), sendo essas redes amplamente relacionadas aos transtornos psiquiátricos (Mukherji 2020).

Vale destacar a possível conexão das associações encontradas e a funcionalidade de polimorfismos com ação regulatória na expressão gênica e no neurodesenvolvimento. Isto está em acordo com outro estudo recente que relacionou o *MEF2C* ao risco genético e epigenético da esquizofrenia e possivelmente outros transtornos, através de sua influência regulatória na plasticidade e desenvolvimento cerebral (Mitchell et al. 2018). Além disso, os achados auxiliam a compreensão da associação entre o *MEF2C* e características cerebrais e indicam uma possível explicação para a influência deste gene nos transtornos psiquiátricos.

A divisão do *MEF2C* em blocos de desequilíbrio de ligação conseguiu captar especificidades de associações entre as regiões do gene e aumentar o poder estatístico limitado pela análise de *gene-wide*. Além disso, a delimitação otimizada das variantes possibilitou a exploração da funcionalidade de polimorfismos específicos nas análises *in silico* e sua relação com as medidas de anisotropia fracionada. Portanto, a estratégia empregada se mostrou eficaz na tentativa de melhor caracterizar os mecanismos biológicos por trás do papel do *MEF2C* na neurobiologia e comportamento.

De maneira geral os resultados do presente estudo confirmam o envolvimento do *MEF2C* na integridade da microestrutura da substância branca, e a sua associação com transtornos psiquiátricos. No entanto, ainda não temos claro qual o espectro total das possíveis consequências fenotípicas da variabilidade do *MEF2C*, portanto, muitos estudos deverão ser realizados explorando as implicações deste gene e das vias que ele regula, para compreender melhor sua influência nos fenótipos associados. Esse esforço vai na direção de se desvendar a arquitetura genética compartilhada pelos transtornos psiquiátricos e mecanismos biológicos por trás de sua etiologia, possibilitando formas de avaliação e tratamento mais eficazes.

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Anexos

Aprovação do Comitê de Ética em Pesquisa do HCPA

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 PICON
KATIANE LILIAN DA SILVA
EDUARDO SCHNEIDER VITOLA
DJENIFER KAPPEL
VERÔNICA CONTINI
JAQUELINE BOHRER SCHUCH
BRUNA SANTOS DA SILVA
DIEGO LUIZ ROVARIS
CLAITON HENRIQUE DOTTO BAU
RENATA BASSO CUPERTINO

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 avalição 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

NeuroImage: Clinical 28 (2020) 102403

Contents lists available at ScienceDirect

NeuroImage: Clinical

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

Reduced fronto-striatal volume in attention-deficit/hyperactivity disorder in two cohorts across the lifespan

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ARTICLE INFO

Keywords:
ADHD
White matter
Fronto-striatal
Tensor-based morphometry
Independent component analysis

ABSTRACT

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 data-driven multivariate approach to investigate neuroanatomical features associated with ADHD in two independent cohorts: the Dutch NeuroIMAGE cohort (n = 890, 17.2 years) and the Brazilian IMpACT cohort (n = 180, 44.2 years). Using independent component analysis of whole-brain morphometry images, 375 neuroanatomical components were assessed for association with ADHD. In both discovery (corrected-p = 0.0085) and replication (p = 0.032) cohorts, ADHD was associated with reduced volume in frontal lobes, striatum, and their interconnecting white-matter. Current results provide further evidence for the role of the fronto-striatal circuit in ADHD in children, and for the first time show its relevance to ADHD in adults. The fact that the cohorts are from different continents and comprise different age ranges highlights the robustness of the findings.

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