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EFEITOS DO TDAH E DE VARIANTES GENÉTICAS DO RECEPTOR DE GLICOCORTICOIDE SOBRE VOLUMES CEREBRAIS

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SUMÁRIO

Lista de abreviaturas

ACTH – Adrenocorticotrofina

BAIAP2 – Gene B*rain-specific Angiogenesis Inhibitor 1-Associated Protein*

CRH - Hormônio Liberador de Corticotrofina

CRHR1 – Receptor do Hormônio Liberador de Corticotrofina tipo 1

DSM - Manual Diagnóstico e Estatístico de Transtornos Mentais

FDR – Taxa de Falsa Descoberta

FKBP5 – Gene *FK506 binding protein 5*

GWAS – Estudo de associação por varredura genômica

GR – Receptor de glicocorticoide

HPA – Hipotálamo-Pituitária-Adrenal

IMpACT - *International Multi-centre persistent ADHD CollaboraTion*

MR - Receptor de mineralocorticoide

NR3C1 – Gene *Nuclear Receptor Subfamily 3 Group C Member 1*

PGC – Consórcio de Genômica Psiquiátrica

POMC - Pró-opiomelanocortina

TC – Transtorno de Conduta

TDAH - Transtorno de Déficit de Atenção/Hiperatividade

TOD - Transtorno de Oposição Desafiante

SNAP-IV - Escala Swanson, Nolan e Pelham-IV

Resumo

Vários estudos relacionaram o TDAH à uma desregulação do eixo hipotálamo-pituitária-adrenal (HPA), envolvido na resposta ao estresse, e a alterações neuroanatômicas, como a redução do volume de certas regiões cerebrais. O receptor de glicocorticoide (GR), codificado pelo gene *NR3C1*, desempenha um papel fundamental na resposta ao estresse. A ativação do fator de transcrição GR regula a expressão de um grande número de genes e tem efeitos rápidos na excitabilidade neuronal. Considerando as evidências que ligam a variação genética do eixo HPA em transtornos psiquiátricos e volumes cerebrais, nós hipotetizamos que a variação no *NR3C1* poderia moderar a associação relatada entre o TDAH e volume subcortical cerebral. Para isso, avaliamos os volumes do *accumbens*, amígdala, caudado, hipocampo, putâmen e volume intracraniano em 100 adultos com TDAH e 60 controles avaliados no Hospital de Clínicas de Porto Alegre. O diagnóstico de TDAH seguiu os critérios do DSM-5 e a aquisição das imagens foi conduzida em um scanner Siemens Magnetom Spectra 3T. A genotipagem foi realizada pela plataforma Infinium PsychArray-24 BeadChip, com posterior seleção de polimorfismos baseados em filtros genômicos e agrupamento por desequilíbrio de ligação, resultando em 47 variantes independentes incluídas na análise final. Interações entre as variantes e o diagnóstico de TDAH nas seis regiões cerebrais investigadas foram avaliadas usando modelo linear geral seguido de correção para múltiplos testes. Análises *in silico* foram realizadas para avaliar potenciais efeitos funcionais dos SNPs significativamente associados nas análises de interação. Os volumes intracraniano e do hipocampo se mostraram menores em casos comparado aos controles. Dos polimorfismos incluídos nas análises finais, vários apresentaram efeitos opostos de acordo com o diagnóstico de TDAH, principalmente no *accumbens* e na amígdala. Os SNPs rs10052957 e rs41423247, com papéis funcionais definidos, encontraram-se entre aqueles com efeitos significativos. Análises *in silico* revelaram que os SNPs que sobreviveram à correção de múltiplos testes e que apresentaram potenciais efeitos funcionais estavam em desequilíbrio de ligação com o rs6198, um SNP conhecido por estabilizar a isoforma GRβ, aumentando sua expressão. Nossos resultados indicam que as diferenças de volume de regiões cerebrais em indivíduos com TDAH e controles podem ser influenciadas por variantes no gene codificador de GR.

Abstract

Several studies have suggested that ADHD is associated with dysregulation of the Hypothalamic–Pituitary–Adrenal (HPA) axis, involved in stress-response, and with neuroanatomic alterations, such as reduced volume in certain brain regions. The glucocorticoid receptor (GR), encoded by the *NR3C1* gene, plays a pivotal role in the stress response. The activated GR transcription factor regulates the expression of a large number of genes, and it has rapid effects on neuronal excitability. Considering the evidence linking HPA axis genetic variation in psychiatric disorders and brain volumes, we hypothesize that variation in *NR3C1* could moderate the reported association between ADHD and brain subcortical volume. For this purpose, we evaluated the volumes of accumbens, amygdala, caudate, hippocampus, putamen and intracranial volume in 100 adults with ADHD and 60 controls, assessed at Hospital de Clínicas de Porto Alegre. The diagnosis of ADHD followed DSM-5 criteria and the images acquisition were conducted in a Siemens Magnetom Spectra 3T scanner. Genotyping was performed on the Infinium PsychArray-24 BeadChip platform, with a posterior selection of polymorphisms based on genomic filters and pruning, resulting in 47 independent variants included in the final analysis. Interactions between variants and ADHD diagnosis on the six brain regions investigated were evaluated using general linear model followed by correction for multiple tests. In silico analyses were performed to assess the potential functionality effects for the SNPs significantly associated in the interaction analyses. The hippocampus and intracranial volumes were decreased in cases when compared to controls. Of the polymorphisms included in the final analyses, several presented opposite directions of effects according to ADHD status, mostly on accumbens and amygdala. The rs10052957 and rs41423247, with functional role described, were among the significant SNPs. In silico analyses revealed that the SNPs that survived multiple test correction and had potential functional effects were in strong Linkage Disequilibrium with rs6198, a SNP known for stabilize the isoform GRβ, increasing its expression. Our findings indicate that volume differences of specific brain regions in subjects with ADHD and controls might be influenced by variants in the GR encoding gene.

Capítulo I

Introdução geral

1 INTRODUÇÃO

1.1 Transtorno de Déficit de Atenção/Hiperatividade

O Transtorno de Déficit de Atenção/Hiperatividade (TDAH) é uma condição neuropsiquiátrica altamente comum, com prevalência estimada em 2,8% em adultos (Fayyad et al. 2017). Os critérios para o diagnóstico de TDAH em adultos são a presença de cinco ou mais sintomas de desatenção e/ou de hiperatividade/impulsividade, presentes antes dos 12 anos e que causem prejuízo em ao menos dois diferentes contextos de vida, tais como ambiente familiar e âmbito profissional (Manual Diagnóstico e Estatístico de Transtornos Mentais, 5ª versão - DSM-5, APA 2013). É possível distinguir três apresentações do transtorno: predominantemente desatento, predominantemente hiperativo/impulsivo ou combinado.

O TDAH possui etiologia multifatorial, na qual variantes genéticas comuns de pequeno efeito são implicadas na sua susceptibilidade, bem como a interação de tais variantes com componentes ambientais (APA 2013). Em adultos, o TDAH está associado à presença de comorbidades em aproximadamente 80% dos casos (Torgersen et al. 2006; Sobanski et al. 2007). Entre as principais comorbidades apresentadas, estão o Transtorno de Personalidade Antissocial, os Transtornos de Humor e Ansiedade e os Transtornos por Uso de Substâncias (Magnin and Maurs 2017; Reale et al. 2017). A complexidade etiológica, somada a diferentes perfis de comorbidades, confere uma significativa heterogeneidade clínica ao TDAH (Sobanski 2006).

Em relação à neurobiologia do TDAH, o envolvimento dos sistemas dopaminérgico, adrenérgico, serotoninérgico e colinérgico foi demonstrado por evidências de estudos psicofarmacológicos (Wilens 2008). O envolvimento dos sistemas dopaminérgico e adrenérgico na fisiopatologia do transtorno foi corroborado por modelos animais (van der Kooij and Glennon 2007). A complexidade da neurobiologia do TDAH parece ser consequência da interação entre vários sistemas neurofisiológicos disfuncionais.

1.2 Fatores etiológicos

1.2.1 Genéticos

Estudos com gêmeos estimam uma herdabilidade de 70 a 80% para o TDAH, tanto para crianças e adolescentes quanto para adultos (Faraone et al. 2005; Brikell et al. 2015). Com isso, é crescente a busca por variantes genéticas que possam estar implicadas nesse transtorno. As principais abordagens utilizadas ao longo do tempo foram os estudos de gene candidato (Bonvicini et al. 2016; Cupertino et al. 2017; Kappel et al. 2017) e estudos de associação por varredura genômica (*Genome-Wide Association Studies* – GWAS) (Neale et al. 2010b; Zayats et al. 2015; Demontis et al. 2019).

Os estudos com genes candidatos são conduzidos com hipóteses prévias formuladas a partir do conhecimento da neurobiologia dos transtornos. Visto que o TDAH parece envolver modificação dos sistemas dopaminérgico e noradrenérgico (Ernst et al. 1998; Ernst et al. 1999; Faraone et al. 2014) e que o principal fármaco utilizado no seu tratamento (metilfenidato) atua nos transportadores de dopamina e noradrenalina (Sulzer et al. 2005), os genes candidatos mais explorados foram os componentes dessas vias (Gizer et al. 2009; Franke et al. 2012; Faraone et al. 2014; Badgaiyan et al. 2015). Entretanto, o único gene que manteve um resultado positivo na mais ampla meta-análise realizada em amostras de adultos foi o *BAIAP2* (*brain-specific angiogenesis inhibitor 1-associated protein*) (Bonvicini et al. 2016). Em função das dificuldades de replicação e consistência dos resultados, nos últimos anos os esforços se deslocaram em direção às abordagens genômicas, incluindo GWAS.

As varreduras genômicas apresentam a vantagem de analisar de centenas a milhões de polimorfismos simultaneamente, sem a necessidade de uma hipótese prévia. Assim, os resultados de GWAS podem sugerir novas rotas ou novos genes a ser investigados. Essa abordagem, entretanto, deve contar com um tamanho amostral muito maior do que o necessário para estudos com gene candidato. Isso se dá porque a correção para múltiplos testes aumenta o rigor do limiar de significância que deve ser atingido ($p < 5x10^{-8}$). Nesse sentido, as primeiras tentativas de GWAS para o TDAH - com tamanhos amostrais limitados - obtiveram sucesso aquém do esperado (Lasky-Su et al. 2008a; Lasky-Su et al. 2008b; Neale et al. 2008; Neale et al. 2010a; Mick et al. 2010; Neale et al. 2010b; Stergiakouli et al. 2012; Fliers et al. 2012; Yang et al. 2013; Ebejer et al. 2013).

A fim de modificar o cenário inicialmente negativo, grandes consórcios e parcerias foram formados, aumentando os tamanhos amostrais em estudos de varredura genômica. Nosso grupo faz parte do IMpACT (*International Multi-centre persistent ADHD CollaboraTion*), um consórcio que reúne esforços da Holanda, Alemanha, Espanha, Noruega, Reino Unido, Suécia, EUA e Brasil. Atualmente, o IMpACT conta com uma amostra de mais de 4.000 casos e mais de 8.000 controles (https://www.impactadhdgenomics.com/). Essa

amostra faz parte de um consórcio maior, o PGC (*Psychiatric Genomics Consortium*), formado por diversos grupos de trabalho. O grupo com foco no TDAH conta com 106 investigadores de 14 países e 29 instituições (http://www.med.unc.edu/pgc/). O PGC disponibiliza para download os resultados de varreduras genômicas, permitindo o uso desses dados em outras abordagens e em estudos de replicação.

A formação desse tipo de colaboração internacional possibilitou recentemente a realização de uma meta-análise de GWAS, que identificou 12 loci associados ao TDAH em uma amostra de 20.183 casos e 35.191 controles, incluindo crianças e adultos (Demontis et al. 2019). Torna-se evidente que os estudos de varredura genômica precisam de grandes tamanhos amostrais para atingir poder necessário para detectar variantes com pequenos tamanhos de efeito. Vale destacar que de maneira geral os resultados de GWAS contribuem no entendimento da herdabilidade molecular explicada pela variância genética aditiva, que responde por apenas 25% da herdabilidade total. Assim, é importante considerar outros fatores não aditivos tais como interações gene-gene e gene-ambiente.

1.2.2 Ambientais

Diversos fatores ambientais já foram associados ao TDAH, dentre eles aspectos nutricionais e psicossociais (Thapar and Cooper 2016; Weissenberger et al. 2017). Alguns estudos constataram que exposição passiva e/ou ativa a tabaco pela mãe durante a gravidez aumenta o risco de desenvolvimento de TDAH nos filhos (Zhu et al. 2014; Han et al. 2015; Holbrook 2016; Gustavson et al. 2017). Adicionalmente, o consumo de álcool (Han et al. 2015; Furtado and Roriz 2016; Eichler et al. 2017; Eilertsen et al. 2017), drogas prescritas (ex: paracetamol) e substâncias ilícitas (Liew et al. 2014; Thompson et al. 2014; Nygaard et al. 2016) pela mãe no período gestacional também foi relacionado a uma maior prevalência de TDAH nos filhos, quando comparados a indivíduos não expostos. Fatores perinatais como baixo peso ao nascer e prematuridade também parecem contribuir para o aumento do risco (Bhutta et al. 2002).

Quanto a fatores psicossociais, níveis socioeconômicos mais baixos e famílias com a presença de um único genitor foram associados a uma maior incidência de TDAH (Klein et al. 2012; Russell et al. 2016). Além desses, eventos traumáticos na infância, tais como negligência e abuso, foram descritos mais frequentemente em crianças (Ford et al. 2000; Ouyang et al. 2008; Jimenez et al. 2017; Brown et al. 2017) e em adultos (Rucklidge et al.

2006; Fuller-Thomson and Lewis 2015; Capusan et al. 2016; Konstenius et al. 2017) com TDAH do que em controles.

O conjunto de variantes genéticas e fatores ambientais associados ao TDAH descritos na literatura explicam apenas parte da susceptibilidade ao transtorno. Como mencionado anteriormente, uma maior fatia do espectro também pode, em princípio, ser explicada por interações, que ocorrem quando, de maneira não aditiva, fatores ambientais ou genéticos modificam o efeito de variantes genéticas.

Nesse sentido, a exposição ao estresse e a variabilidade nos genes relacionados à resposta ao estresse podem estar envolvidos em interações gene-ambiente. Vários estudos sugeriram que o TDAH está associado à desregulação do sistema de estresse - eixo Hipotálamo-Pituitária-Adrenal (HPA) (Corominas et al. 2012; van der Meer et al. 2017; Schloß et al. 2018). A maioria das associações apontam para uma hipoatividade do eixo HPA. Especificamente, uma meta-análise com biomarcadores associados ao TDAH encontrou uma associação significativa, embora pequena, entre baixa secreção de cortisol salivar basal e TDAH (Scassellati et al. 2012). Uma outra meta-análise sobre os parâmetros de secreção de cortisol e sintomas externalizantes (incluindo sintomas de transtornos de oposição desafiante e de conduta - TOD e TC, respectivamente - além de sintomas de TDAH) forneceu achados semelhantes (Alink et al. 2008). Crianças com TDAH têm uma resposta a estressores psicossociais alterada, bem como menores níveis diurnos de cortisol (Scerbo and Kolko 1994; King et al. 1998; Randazzo et al. 2008; van West et al. 2009; Ma et al. 2011; Schloß et al. 2018).

1.2.3 *NR3C1*

O gene *NR3C1* (*Nuclear Receptor Subfamily 3 Group C Member 1*) está localizado na região Chr5q31.3. Esse gene codifica o receptor de glicocorticoide (GR), que desempenha um papel essencial na resposta ao estresse ao se ligar ao cortisol (Buckingham 2006; Rovaris et al. 2016) (**Figura 1**) e inibir sua liberação através de um *feedback* negativo (Mizoguchi et al. 2003). Após sua ativação, o GR regula a expressão de centenas de genes (Buckingham 2006; Revollo et al. 2013; Vockley et al. 2016), bem como o desenvolvimento cerebral e a plasticidade e excitabilidade neuronal (Buckingham 2006; Groeneweg et al. 2011).

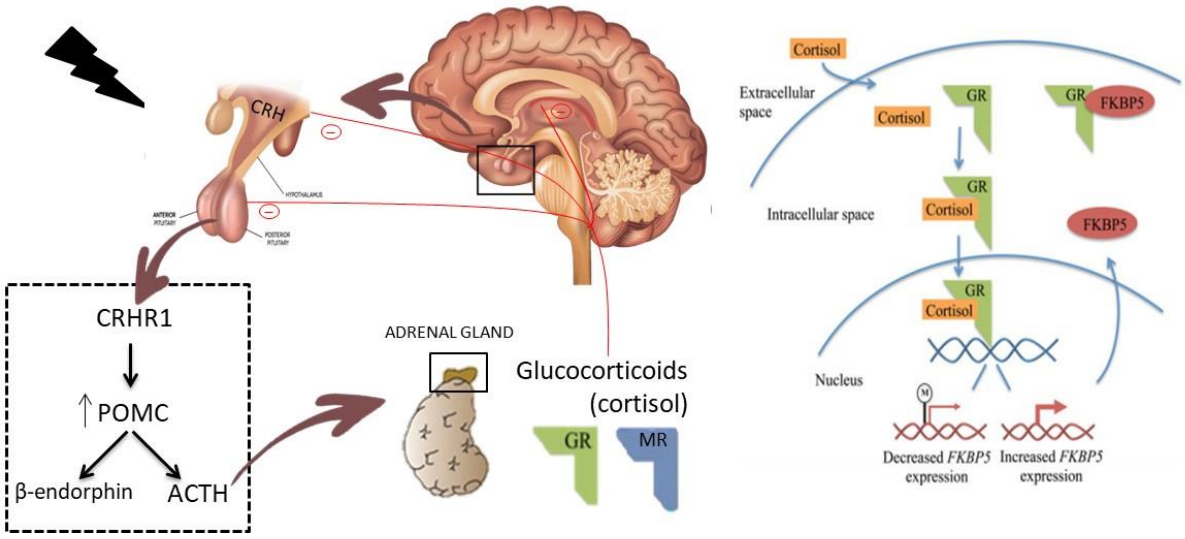

Figura 1. Funcionamento do eixo HPA em humanos. Quando o corpo é exposto a um estressor, há uma cascata de eventos que culminam na liberação do Hormônio Liberador de Corticotrofina (*Corticotropin Releasing Hormone*, CRH). Na pituitária anterior, o CRH ativa seu receptor do tipo 1 (*Corticotropin Releasing Hormone Receptor 1*, CRHR1), resultando em um aumento da expressão de Pró-opiomelanocortina (*Pró-opiomelanocortin*, POMC). A POMC é clivada, gerando β-endorfina e adrenocorticotrofina (*Adrenocorticotropic hormone*, ACTH). A ACTH atua como um hormônio endócrino, estimulando a produção de glicocorticoides (cortisol) na glândula adrenal. O cortisol ativa os receptores de glicocorticoide (GR) e de mineralocorticoide (MR). A ativação do GR resulta em uma rápida indução da expressão do *FKBP5* (*FK506 binding protein 5*), que diminui a sensibilidade do GR ao cortisol, exercendo um *feedback* negativo à atividade do cortisol. O aumento nos níveis de cortisol também resulta em um *feedback* negativo pelo GR no hipotálamo, pituitária anterior e no hipocampo.

O *NR3C1* é transcrito em muitas regiões cerebrais (Morimoto et al. 1996; Erickson et al. 2003), e sua atividade diferencial, juntamente com o cortisol, tem sido associados com funções relacionadas ao TDAH, como atenção, excitabilidade, percepção, memória e regulação emocional (Erickson et al. 2003). Com isso, embora as meta-análises de GWAS disponíveis ainda não situem o *NR3C1* como um hit (Demontis et al. 2019), muitos estudos de genes candidatos avaliam tal gene, além de considerar o seu potencial envolvimento em interações e consequente contribuição na variância não-aditiva.

Fortier et al. (2013) relataram uma associação entre um haplótipo contendo quatro polimorfismos funcionais do *NR3C1* (rs6189-rs6195-rs41423247-rs6198) e comportamentos relacionados ao TDAH (problemas atencionais e de função executiva, comportamento agressivo, comorbidade com TOD, e resposta ao tratamento em crianças com TDAH) em um estudo de associação baseado em família. Esse haplótipo influencia a atividade do GR (Claes 2009). Adicionalmente, Schote et al. (2016) encontraram uma associação nominal entre outro haplótipo do *NR3C1* (rs10052957-rs10482605-rs6189/rs6190) e TDAH, bem como entre um SNP específico (rs56149945) e a probabilidade de crianças com TDAH terem TC como comorbidade. Outro SNP do *NR3C1* também foi relacionado ao TDAH sem comorbidades em crianças, e com desatenção (mas não com hiperatividade/impulsividade na escala Swanson, Nolan and Pelham-IV Questionnaire - SNAP-IV) (Chen et al. 2019).

Como um fator de transcrição, a proteína Nr3c1 parece inibir a atividade promotora e a transcrição de microRNAs específicos (miR-138-1, 34c*, 296, and 494), que são negativamente regulados em um modelo animal de TDAH (*spontaneously hypertensive rats*). Esse estudo mostrou que a desregulação da rota envolvendo Nr3c1 e o alvo desses microRNAs estava envolvida na susceptibilidade ao TDAH (Wu et al. 2017).

A proteína codificada pelo *NR3C1* pode se apresentar em várias isoformas, geradas a partir de *splicing* alternativo e da iniciação alternativa da tradução (Lu and Cidlowski 2004; Lu and Cidlowski 2006). As isoformas do GR têm distribuições tecido-específicas e perfis diferentes de regulação da transcrição. Dos 9 éxons presentes no gene, o primeiro e o último são alvos de *splicing* alternativo e produzem as duas isoformas mais estudadas (GRα and β). A partir do mRNA dessas duas isoformas, outras são criadas por iniciação alternativa da tradução. O GRα é expresso em todo o corpo e se torna funcional quando ativado por glicocorticoides (cortisol, por ex.). O GRβ, entretanto, é incapaz de se ligar aos glicocorticoides devido a um domínio de ligação alterado, possuindo uma distribuição tecido-dependente (Lu and Cidlowski 2004). A proteína GRβ é expressa abundantemente nas células epiteliais que revestem os pulmões, no timo e no ducto biliar no fígado (Lu and Cidlowski 2006). Embora o tratamento com glicocorticoides aumente a ligação de DNA apenas pelo GRα (e não pelo β) (Oakley et al. 1999), a última é considerada uma isoforma dominante negativa, atuando como repressora da isoforma α e da ação dos glicocorticoides (Fortier et al. 2013). A consideração das isoformas do GR, no entanto, não será alvo de análises no presente estudo.

1.3 Alterações neuroanatômicas relacionadas ao TDAH e à exposição ao estresse

Estudos com neuroimagem apontam a existência de alterações estruturais e funcionais em regiões cerebrais de pacientes com TDAH (Hoogman et al. 2017; Klein et al. 2017b; Klein et al. 2017a). Duas meta-análises demonstraram que pacientes com TDAH apresentavam uma redução significativa no volume dos núcleos da base no hemisfério direito (putâmen, globo pálido e caudado; Nakao et al. 2011; Frodl and Skokauskas 2012). Uma mega-análise recente detectou que indivíduos com TDAH possuem volumes menores do núcleo *accumbens*, da amígdala, do caudado, do hipocampo e do putâmen, quando comparados com controles (Hoogman et al. 2017), sendo esse efeito mais pronunciado em crianças (**Figura 2**). A análise desses volumes em adultos com TDAH, principalmente a partir da meia idade, foi até hoje pouco desenvolvida, mesmo em grandes estudos colaborativos.

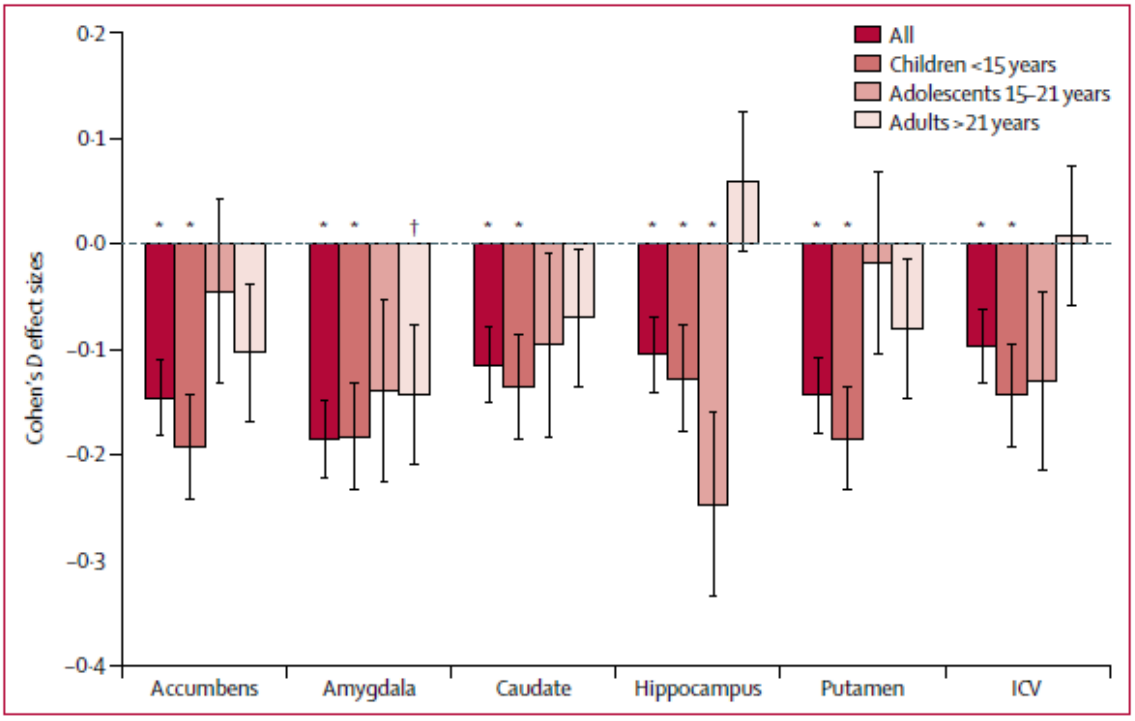

Figura 2. Tamanho de efeito (d de Cohen) para as diferenças entre indivíduos com TDAH e controles para volumes subcorticais e volume intracranial (ICV). Os tamanhos de efeito foram estimados para todos os indivíduos, para crianças (<15 anos), adolescentes (15–21 anos) e adultos (>21 anos). Barras de erros correspondem ao erro padrão. *Significante após correção por *False Discovery Rate* (FDR). †Nominalmente significante (p<0,05). Fonte: Hoogman et al. 2017

Um dos fatores que poderiam explicar tais características cerebrais relacionadas ao TDAH seria aqueles relacionados com trauma e estresse. A presença de eventos traumáticos leva à desregulação do sistema de resposta ao estresse, que pode ter como maiores consequências reduções no tamanho do corpo caloso, do neocórtex esquerdo, do hipocampo e da amígdala, bem como desregulações na atividade frontotemporal. Essas alterações, por sua vez, aumentam o risco de desenvolvimento dos sintomas de TDAH e de outros transtornos psiquiátricos (Teicher et al. 2002; McEwen et al. 2015).

Estudos conduzidos em modelos animais de estresse relataram menores volumes do *accumbens* e da amigdala (Aleksić et al. 2016) em ratos expostos ao estresse quando comparados com controles, bem como encurtamento dos ramos neuronais da amígdala e do comprimento dendrítico neuronal do *accumbens* (Wang et al. 2012). Adicionalmente, uma meta-análise constatou que indivíduos com Transtorno de Estresse Pós-Traumático possuíam, em média, volumes menores de hipocampo e amígdala (Logue et al. 2018).

Além do estresse, o receptor de glicocorticoide, especificamente, também têm sido explorado em relação a aspectos cerebrais, visto sua ampla expressão no cérebro e suas funções de regulação do desenvolvimento cerebral, e plasticidade e excitabilidade neuronal (Buckingham 2006; Groeneweg et al. 2011), já mencionadas previamente. Em relação à genética, Zhao et al. (2018) relataram as herdabilidades de inúmeras regiões cerebrais, corroborando a existência de fatores genéticos associados ao volume cerebral.

Alguns estudos têm empregado técnicas de neuroimagem para investigar a relação entre *NR3C1* e aspectos cerebrais. Devido à relação entre estresse e emoção e memória (Finsterwald and Alberini 2014), tais estudos exploraram principalmente o hipocampo, a amígdala e o córtex medial pré-frontal (Dedovic et al. 2009). Um estudo com genes candidatos, incluindo *NR3C1*, sugeriu que exposição a estresse e "risco genético" estariam vinculados a uma conectividade mais fraca entre a amígdala e os giros frontais inferior e médio, o caudado e o giro parahipocampal em crianças. Adicionalmente, a conectividade na amígdala mostrou-se associada a sintomas de ansiedade e habilidades de regulação emocional (Pagliaccio et al. 2015). Além disso, eventos de vida estressantes estavam associados a um menor volume da amígdala esquerda em indivíduos com um perfil genético considerado como de risco (Pagliaccio et al. 2014). Esses resultados são consistentes com outros achados de volumes menores da amígdala em depressão (Keller et al. 2008; Sacher et al. 2012) e após administração de cortisol (Brown et al. 2008).

Considerando os múltiplos efeitos que parecem estar associados a variações genéticas no GR, pode-se inferir que o *NR3C1* tem efeitos pleitrópicos, o que é plausível tendo em conta a sua atuação como fator de transcrição e seu importante papel e na excitabilidade e plasticidade neuronal. Assim, uma longa série de estudos será necessária para elucidar os mecanismos subjacentes às relações entre *NR3C1*, aspectos cerebrais e TDAH.

Capítulo II

Justificativa e objetivos

2. JUSTIFICATIVA

A alta prevalência de transtornos psiquiátricos na população mundial é responsável por prejuízos tanto nos indivíduos afetados quanto nos seus familiares e pessoas próximas. Apesar de possuir uma alta herdabilidade (Faraone et al. 2005), poucas variantes genéticas têm sido identificadas como relevantes na etiologia do TDAH. Mesmo as abordagens em larga escala desenvolvidas na última década não permitiram explicar mais do que 25% da herdabilidade. Além disso, as variantes identificadas apresentam um pequeno tamanho de efeito na susceptibilidade ao transtorno.

Uma estratégia na busca de melhor compreender a etiologia do TDAH, envolve o uso de possíveis endofenótipos. Nesse sentido, aspectos cerebrais com significativa herdabilidade, principalmente anatômicos, têm sido associados ao TDAH em grandes meta-analises. O presente trabalho procura compreender melhor a relação entre aspectos estruturais de neuroimagem e TDAH, considerando o papel de variantes genéticas.

Nesse sentido, variantes no gene *NR3C1* apresentam-se como excelentes candidatas, visto seu papel como fator de transcrição e codificador do receptor de glicocorticoide, uma proteína essencial para o funcionamento do eixo hipotálamo-pituitária-adrenal (HPA). Diferentes respostas do eixo HPA foram previamente associadas a transtornos psiquiátricos como TDAH, e a alterações de volumes cerebrais. Dessa forma, polimorfismos no *NR3C1* poderiam moderar a associação entre TDAH e volumes cerebrais.

3. OBJETIVOS

3.1 Objetivos Gerais

Avaliar o possível papel de variações no gene *NR3C1* nas relações entre TDAH e volume de estruturas cerebrais.

3.2 Objetivos Específicos

- Seleção de variantes independentes no *NR3C1* com base em desequilíbrio de ligação;
- Análise de associações entre o TDAH e o volume de estruturas cerebrais;
- Avaliar efeitos de interação entre variantes do *NR3C1* e diagnóstico de TDAH sobre volume de estruturas cerebrais;
- Explorar a potencial funcionalidade das variantes que apresentarem interação significativa com TDAH sobre volumes cerebrais.

Capítulo III

Artigo científico

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Glucocorticoid receptor polymorphisms present differential effects on brain volumes according to ADHD status

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ABSTRACT

ADHD has been associated with dysregulation of the Hypothalamic–Pituitary–Adrenal (HPA) axis, involved in stress-response, and with neuroanatomic alterations, such as reduced volume in certain brain regions. The glucocorticoid receptor, encoded by the *NR3C1* gene, plays a pivotal role in the stress response and has effects on neuronal excitability. Considering the evidence linking HPA axis genetic variation in psychiatric disorders and brain volumes, we hypothesize that *NR3C1* variation could moderate the reported association between ADHD and brain subcortical volume. For this purpose, we evaluated the volumes of accumbens, amygdala, caudate, hippocampus, putamen and intracranial volume in 100 adults with ADHD and 60 controls. The diagnosis of ADHD followed DSM-5 criteria and the images acquisition were conducted in a Siemens Magnetom Spectra 3T scanner. Genotyping was performed on the Infinium PsychArray-24 BeadChip platform, followed by application of genomic filters and pruning, which resulted in 47 independent variants analyzed. The hippocampus and intracranial volumes were decreased in cases when compared to controls. Several of the polymorphisms included in the final analyses presented opposite direction of effects according to ADHD status, mostly on accumbens and amygdala. The functional and well-studied rs10052957 and rs41423247 SNPs were among the significant ones. In silico analyses revealed that the significant SNPs were in strong Linkage Disequilibrium with rs6198, another variant known for their functional effect. Our findings indicate that volume differences of specific brain regions in subjects with ADHD and controls might be influenced by *NR3C1* variants.

Keywords: ADHD; *NR3C1*; gene-disorder interaction; brain volumes

1. Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a highly prevalent disorder, occurring in 2.8% of adults (Fayyad et al. 2017). Several studies have suggested that ADHD is associated with dysregulation of the Hypothalamic–Pituitary–Adrenal (HPA) axis (Corominas et al. 2012; van der Meer et al. 2017; Schloß et al. 2018), which is involved in stress-response, and in multiple other functions, from maintenance of homeostatic systems in the body to involvement in metabolic, cardiovascular, immune and reductive systems (Del Rey et al. 2008).

Most of the associations between HPA axis and ADHD indicate a hypoactivity of this stress-system. Meta-analyses on ADHD-associated biomarkers found associations of low basal salivary cortisol secretion with ADHD (Scassellati et al. 2012) and with externalizing symptoms (Alink et al. 2008). Additionally, children with ADHD present a blunted cortisol response to psychosocial stressors, and decreased daytime cortisol levels (Scerbo and Kolko 1994; King et al. 1998; Randazzo et al. 2008; van West et al. 2009; Ma et al. 2011; Schloß et al. 2018).

The glucocorticoid receptor (GR) participates directly in stress response, providing a negative feedback of cortisol activity (Mizoguchi et al. 2003). Once GR is activated by cortisol, it moves for nucleus and acts as transcription factor, regulating several genes (Buckingham 2006; Rovaris et al. 2016). It also participates in neuronal excitability (Groeneweg et al. 2011), and its differential activity has been associated with functions related to ADHD such as attention, arousal, perception, memory and emotional processing (Erickson et al. 2003).

Despite genetic variations in the GR coding gene (*NR3C1*) do not reach genome-wide significance for ADHD, the gene presented a nominal association in a gene-based approach (Demontis et al. 2019). Individuals carrying a putative ADHD risk haplotype of *NR3C1* (Fortier et al. 2013) showed significantly more positive relation between stress exposure and ADHD severity (van der Meer et al. 2016). Such *NR3C1* haplotype also moderated the association between stress and gray matter volume in the cerebellum and parahippocampal cortex.

Due to its effects on neuronal plasticity and regulation of brain development (Buckingham 2006), *NR3C1* also has been investigated in brain aspects. The relationship described between stress and emotion and memory lead to exploration of, mainly, hippocampus, amygdala and medial prefrontal cortex (Dedovic et al. 2009).

An interaction between stress exposure and "genetic risk" (considering *NR3C1* and other candidate genes) was associated with weakened functional connectivity between the amygdala and other structures in children at resting state (Pagliaccio et al. 2015). Additionally, connectivity in the amygdala was associated with anxiety symptoms and emotional regulation abilities (Pagliaccio et al. 2015) and stressful life events were associated with lower left amygdala volume in individuals with a high genetic risk profile (Pagliaccio et al. 2014). These findings are consistent with research indicating decreased amygdala volumes in depression (eg, Keller et al. 2008; Sacher et al. 2012) and with cortisol administration (Brown et al. 2008).

Considering the evidence linking HPA axis genetic variation in psychiatric disorders and brain volumes, and the evidence for a significant role of non-additive genetic variance in ADHD (Polderman et al. 2015), we hypothesize that variation in *NR3C1* (a major HPA regulator with pleiotropic effects) could moderate the reported association between ADHD and brain subcortical volume.

2. Methods

2.1 Participants

This study comprised 100 patients recruited at the ADHD Outpatient Program – Adult Division of the Hospital de Clínicas de Porto Alegre (HCPA), and 60 controls negatively screened for ADHD assessed in the blood donation center at the same hospital. The inclusion criteria were (i) being Brazilian of European descent, (ii) aged at least 18 years, (iii) have been previously interviewed at least once by the group. Exclusion criteria were the presence of (i) clinically significant neurological disease (e.g., delirium, dementia, epilepsy, head trauma), (ii) current or past history of psychosis, (iii) estimated intelligence quotient (IQ) $\leq$70, and (iv) ineligibility for magnetic resonance imaging (e.g presence of metals in the head, symptoms of claustrophobia).

The diagnosis of ADHD followed DSM-5 criteria using the Portuguese version of the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS-E) in which questions about symptoms originally used for children were adapted for adults (Grevet et al. 2005). The study was approved by the institutional review board of HCPA (IRB number 0000921). Participants were fully informed of study procedures and provided signed consent.

2.2 Genotyping

DNA was extracted from peripheral blood by salting out method (Lahiri et al. 1991). Genotyping was performed on the Infinium PsychArray-24 BeadChip platform (Illumina, San Diego, CA, USA) at the Broad Institute of Harvard and MIT (Cambridge, MA, USA). Quality control (QC) and genotype imputation procedures were implemented using the default values standard on the Psychiatric Genomics Consortium pipeline (RICOPILI) (https://sites.google.com/a/broadinstitute.org/ricopili/home) with the European population of the 1,000 Genomes Project Phase 1 built 19hg as reference panel.

2.3 MRI acquisition and data processing

All individuals underwent to Magnetic resonance imaging (MRI) scan in a whole-body Siemens Magnetom Spectra 3T scanner equipped with a 12-channel phased-array head coil at the Radiology Clinic Serdil, Porto Alegre. High-resolution structural MRI sequences were acquired for each individual using a T1-weighted 3D magnetization prepared rapid acquisition with gradient echoes (MPRAGE) sequence with 192 slices, flip angle=7°, TE=2.55ms, TR=2530ms, TI=1100ms, matrix size=256x256, slice thickness 1mm, lasting for 06:02 min. All subjects were instructed on how MRI works and sounds previously to the scanning day, in order to improve the subjects' engagement to the procedure.

For the images processing, we used fully automated and validated neuroimaging segmentation algorithms based on FreeSurfer version 5.3 (http://surfer.nmr.mgh.harvard.edu/; Fischl 2012). The quality control of images was conducted using the Enhancing Neuro Imaging Genetics through Meta-Analysis (ENIGMA) protocol (http://enigma.ini.usc.edu/). All images were individually inspected before and after the application of the ENIGMA quality control protocol to ensure the absence of preprocessing errors. In order to narrow the number of tests performed, we evaluated *NR3C1* effects only in the brain regions that were previously associated with ADHD in a larger mega-analysis (Hoogman et al. 2017): accumbens, amygdala, caudate, hippocampus, putamen and intracranial volume.

2.4 Polymorphisms selection

NR3C1 gene start and end positions (plus a window of 2 kb upstream and 1 kb downstream) were derived from NCBI genome browser gateway (NCBI37/hg19). All polymorphisms genotyped and imputed in our dataset were extracted using the Plink v1.07

software (http://zzz.bwh.harvard.edu/plink/anal.shtml; Purcell et al. 2007). A total of 1.060 variants was kept in a new database. A QC was applied to the extracted database to remove individuals with high rate of genotype missingness (≥ 95%) and SNPs with low call rate (≤ 95%), low minor allele frequency (≤ 10%) or failing Hardy-Weinberg equilibrium test (≥ $5x10^{-5}$). After the QC procedure, we pruned our data-set using the "-- indep" command following the default parameters suggested in the PLINK manual. Polymorphisms within a 50 SNP window that had $r^2 > 0.5$ (corresponding to a variance inflation factor [VIF] of two) with all other variants in the window were removed. At the end, 47 variants survived the quality checks and pruning (**Figure 1**).

2.5 Interaction analyses

Interactions between SNPs and ADHD diagnosis on the six brain regions investigated were evaluated using general linear model in the Statistical Package for the Social Sciences version 18.0 (SPSS, Chicago, IL, USA). False discovery rate (FDR) was applied to account for the multiple tests performed and false discovery rate significance threshold was set to 5% (Benjamini and Hochberg 1995).

2.6 In silico analysis

HaploReg v4.1, RegulomeDB, and the SNP Function Prediction of the SNPinfo were used to evaluate the possible role of variants that survive multiple tests correction in regulatory mechanisms. HaploReg is a tool that examines annotations of the noncoding genome at disease-associated loci by GWAS (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php; Ward and Kellis 2012). 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).

3. Results

The main characteristics of the sample are given in **Table 1**. There were no significant differences regarding sex and age between cases and controls at $p < 0.05$. The accumbens,

caudate, hippocampus, and intracranial volumes were decreased in cases when compared to controls. Since age and sex reached the threshold criteria for their use as covariates ($p < 0.2$), the tests were conducted including these variables in the model, resulting in differences between cases and controls only for hippocampus and intracranial volumes.

3.1 Interaction analyses

Of the 47 variants included in the final analyses, 25 presented opposite direction of effects on accumbens in cases and controls (**Supplementary Table 1**). Among these, 12 significantly interacted with ADHD status and 5 survived the multiple test correction. The functional and well-studied rs10052957 and rs41423247 SNPs were among the nominal and significant SNPs (respectively) that interacted with ADHD on accumbens mean volume. In amygdala, 33 polymorphisms presented opposite direction of effects in cases and controls (**Supplementary Table 2**), for which 9 significantly interacted with ADHD and 6 survived the multiple test correction. Once again, rs10052957 and rs41423247 SNPs presented nominal and significant associations. Regarding caudate, hippocampus, putamen, and intracranial volume, 27, 24, 26, and 27 variants presented opposite direction of effects in cases and controls, respectively (**Supplementary Tables 3 to 6**). Among these, 5, 8, 4 and 2 variants significantly interacted with ADHD, respectively. There was no SNP by ADHD status interaction that survived to the multiple test correction for these brain areas.

This first step of multiple test correction was based on the number of polymorphisms tested in each brain area investigated. This approach, combined with the evaluation of the effect directions in cases and controls (Supplementary Tables 1 to 6) was used in order to capture the effect pattern of *NR3C1* gene on the brain regions investigated. However, we also carried out the multiple test correction considering all the interaction tests performed (47 variants multiplied by six brain areas = 282). This step was used to select the most relevant interaction effects, and results in 5 top interactions between SNPs and ADHD status, 3 of them related to accumbens. The presence of minor alleles of rs17209237 (C), rs2398631 (C), and rs41423247 (C) was associated with decreased accumbens volumes in cases, while the opposite effect was observed for controls (**Figure 2A-C**). Regarding amygdala, rs11740747 (A) and rs10482689 (T) minor alleles were associated with decreased volumes in controls with no significant differences in cases (**Figure 2D-E**).

3.2 In silico analysis

In silico analyses were performed to predict the potential functionality of the *NR3C1* variants that survived to the strict multiple test correction. Considering that rs41423247 is a well-studied SNP in *NR3C1* gene (extensively reviewed in Rovaris et al. 2017), we only evaluated the potential functionality of rs2398631, rs17209237, rs11740747, and rs10482689. Regulome DB had available data for rs17209237, rs11740747, and rs10482689 showing minimal evidence of transcription factors binding on these SNP positions (scores of 4, 5, and 6, respectively). The SNP Function Prediction of SNPinfo web server did not reveal any involvement of the four SNPs tested in splicing regulatory mechanisms.

However, HaploReg suggests that rs2398631 is potentially involved in gene expression regulatory mechanisms. This SNP presents different histone marks in several brain tissues (H3K4me1, H3K27ac, and H3K9ac). eQTL analysis showed that rs17209237 has been correlated to *NR3C1* expression. HaploReg also showed that rs11740747 and rs10482689 are in strong Linkage Disequilibrium with rs6198 in the CEU population. This SNP have functional effects already described in the literature.

4. Discussion

Our findings indicate that volume differences of specific brain regions in subjects with ADHD and controls might be moderated by variants in the GR encoding gene (*NR3C1*). These findings are in line with the reported mediating effects of transcription factor variants such as GR on polygenic disease risk (Reshef et al. 2018).

Two SNPs evaluated in our study had functional effects described: rs41423247 and rs10052957. These SNPs were associated with differences in the expression of GR isoforms in the dorsolateral pre-frontal cortex in humans (Sinclair et al. 2012) and with diurnal and evening cortisol levels (Rosmond et al. 2000b; Rosmond et al. 2000a; Schatzberg et al. 2014). The rs41423247 SNP is located between *NR3C1* exons 2 and 3 and confers a guanine-to-cytosine substitution. We found that the C-minor allele was associated with smaller accumbens and amygdala volume in cases and greater volume in controls. Previous studies related the C allele with increased glucocorticoid sensitivity (van Rossum and Lamberts 2004; Souza et al. 2014), higher cortisol response to a stress test (Li-Tempel et al. 2016) and higher *NR3C1* expression levels (Schote et al. 2018). Since rs41423247 is an intronic polymorphism, it is possible that its effect on gene expression may occur by actuation on repressor or

enhancer sites. Regarding, rs10052957, it is characterized by a guanine-to-adenine change in the intron between untranslated exons $1A_{1-3}$ and 1D (van Rossum and Lamberts 2004; Zobel et al. 2008). The A allele, associated in this study with larger volume in ADHD cases and smaller in controls, was previously associated with increased BDNF levels in crack cocaine addicted patients (Rovaris et al. 2017).

Additionally, some of the other significant SNPs here were in LD with the extensively studied rs6198. This SNP consists in an adenine-to-guanine substitution at the 3669-nucleotide position that occurs on the first adenine of an ATTTA sequence. The ATTTA motif is responsible for the GRβ isoform mRNA destabilization, favoring the GRα expression. Therefore, the presence of the guanine allele of rs6198 results in an increased GRβ expression due to a better mRNA stability (Derijk et al. 2001; Rovaris et al. 2015). Previous studies linked the G-allele with an altered cortisol response (Kumsta et al. 2008) and with major depressive disorder (Szczepankiewicz et al. 2011).

The interpretation of our findings could lead to at least two possibilities for the directionality of causal relationships. The first (*NR3C1* SNPs – brain volumes – ADHD) proposes that *NR3C1* variation would interact with brain volumes, leading to greater or lower risk to ADHD. This perspective is in agreement with the greater heritability of brain volumes over ADHD itself (Zhao et al. 2018). In this regard, brain regions such as accumbens and amygdala have been implicated in differences in stress susceptibility or resilience (Fekete et al. 2009; Narayanan et al. 2011; Chaudhury et al. 2013; Anacker et al. 2016; Logue et al. 2018), which can lead to different susceptibilities to ADHD. The alternative perspective (*NR3C1* SNPs – ADHD - brain volumes) would be that *NR3C1* variants interact with the context of ADHD or associated psychopathology in early childhood to differentially affect brain volume. In favor of this perspective is the fact that a *NR3C1* haplotype modified the relationship between stress exposure and ADHD severity and moderated the association between stress and gray matter volume in the cerebellum and parahippocampal cortex (van der Meer et al. 2016). Pagliaccio et al. (2014) reported that stressful life events predicted decreased amygdala volume in subjects with lower, but not higher, genetic profile scores for disorders (in which *NR3C1* was included). Further studies with longitudinal approaches may help to better elucidate which of the perspectives proposed is more solid.

Concerning limitations, a larger sample size would increase the statistical power and therefore the reliability of the results presented here. Besides, anatomical alterations of the brain do not necessarily imply that functional alterations are also present. Further evaluation of functional MRI and spectroscopy should be conducted to address this issue.

Considering that only about a quarter of SNP heritability in ADHD is explained by additive genetic variance (Demontis et al. 2019), in-depth analyses of genes with potential for non-additive effects are warranted, especially due to the role of non-additive variance in ADHD (Polderman et al. 2015). The pattern of results reported in the current study represents a plausible, albeit partial, scenario for the complex relationship between genetics, stress, brain structures and ADHD.

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Table 1. Sample characteristics

	Cases (n = 98)	Controls (n = 60)	P-value	P-value[a]
	Mean (SD)	Mean (SD)		
Age (years)	34.2 (10.1)	31.2 (9.7)	0.065	
Sex (% male)	43.9%	55.0%	0.176	
Accumbens volume[b]	-0.10 (0.93)	0.22 (1.07)	**0.051**	0.309
Amygdala volume[b]	-0.10 (0.98)	0.18 (1.03)	0.088	0.521
Caudate volume[b]	-0.14 (0.95)	0.25 (1.03)	**0.017**	0.097
Hippocampus volume[b]	-0.17 (0.90)	0.29 (1.10)	**0.004**	**0.043**
Putamen volume[b]	-0.06 (1.02)	0.12 (0.96)	0.280	0.944
Intracranial volume[b]	-0.15 (1.00)	0.27 (0.95)	**0.010**	**0.042**

[a] Adjusted for sex and age.
[b] Volumes were transformed in z-scores.

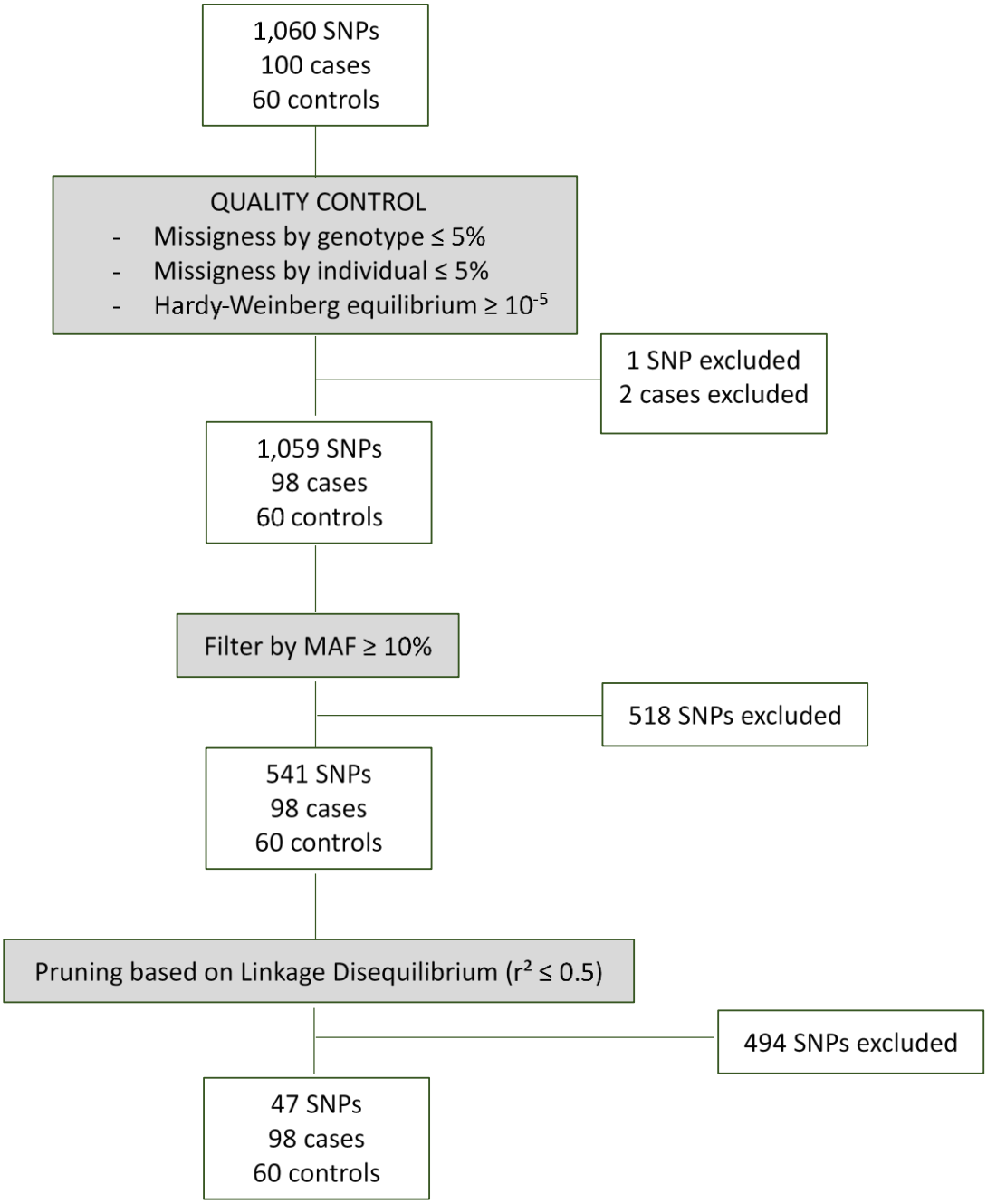

Figure 1. Quality controls and pruning procedures of the genome-wide dataset

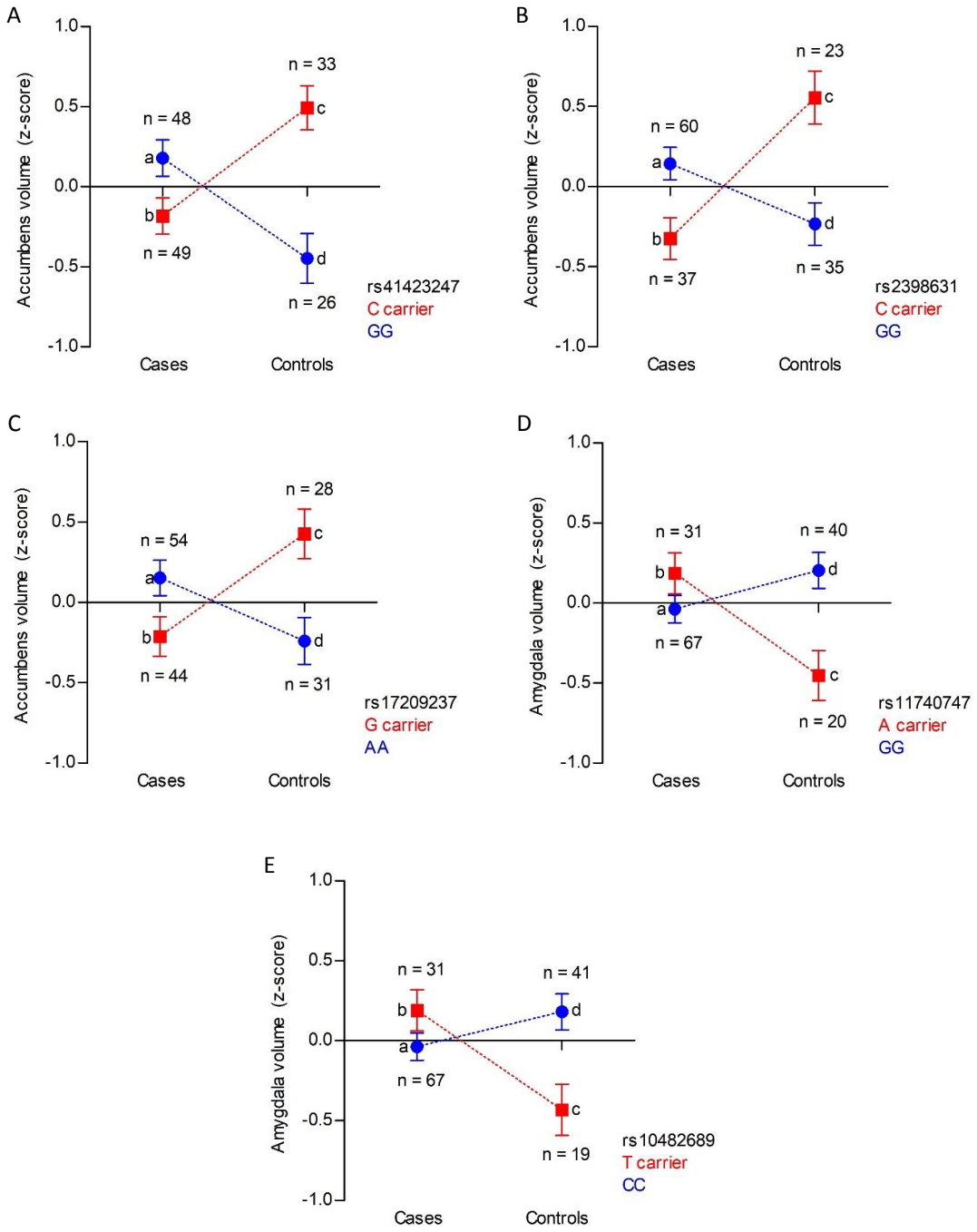

Figure 2. Significant SNP by ADHD status interactions

A) $P_{interaction}$ = 0.000002; $P_{interaction}$ corrected = 0.000564. Post hoc: a>b; a=d; c>b,d.
B) $P_{interaction}$ = 0.000006; $P_{interaction}$ corrected = 0.000846. Post hoc: a>b; a=d; c>b,d.
C) $P_{interaction}$ = 0.0002; $P_{interaction}$ corrected = 0.0188. Post hoc: a>b; a=d; c>b,d.
D) $P_{interaction}$ = 0.0004; $P_{interaction}$ corrected = 0.0282. Post hoc: a=b; a<d; b = c; c<d.
E) $P_{interaction}$ = 0.0008; $P_{interaction}$ corrected = 0.0451. Post hoc: a=b; a<d; b = c; c<d.

Supplementary Table 1. Polymorphisms by ADHD interactions on accumbens volume

Marker	Effect Allele	Effect in Cases	Effect in controls	Statistics (t-test)	P-value (uncorrected)	P-value (corrected)
chr5_143113237_D	D	-	-	0.682	0.4102	0.6659
chr5_143114500_D	D	+	-	0.283	0.5957	0.7291
rs10052957	A	+	-	6.200	**0.0139**	0.0742
rs10057473	G	+	-	0.509	0.4767	0.6659
rs10482682	T	-	+	1.040	0.3095	0.6612
rs10482689	T	+	-	10.241	**0.0017**	**0.0160**
rs11167813	G	-	-	0.555	0.4574	0.6659
rs11738754	T	-	+	0.417	0.5196	0.6784
rs11740747	A	+	-	10.264	**0.0017**	**0.0160**
rs11749182	G	-	+	0.510	0.4763	0.6659
rs11750064	A	+	+	0.760	0.3847	0.6659
rs12153243	T	+	-	5.528	**0.0200**	0.0855
rs12518265	T	-	-	0.307	0.5801	0.7291
rs12521436	A	-	+	5.132	**0.0249**	0.0975
rs12657648	A	-	+	1.621	0.2050	0.5165
rs13168199	T	-	-	0.247	0.6196	0.7291
rs13172698	G	+	-	0.010	0.9205	0.9205
rs13354365	C	-	-	0.013	0.9109	0.9205
rs1348701	A	-	-	0.472	0.4933	0.6659
rs17100289	T	+	+	0.149	0.7004	0.7838
rs17209237	G	-	+	14.728	**0.0002**	**0.0031**
rs17404863	C	-	-	0.163	0.6867	0.7838
rs190488	G	+	-	6.151	**0.0142**	0.0742
rs2398631	C	-	+	21.945	**0.000006**	**0.000141**
rs258747	G	+	-	2.801	0.0963	0.3233
rs258750	G	+	-	5.769	**0.0175**	0.0823
rs258814	T	+	-	6.151	**0.0142**	0.0742
rs34205186	T	+	+	1.594	0.2088	0.5165
rs34226461	T	+	-	6.880	**0.0096**	0.0742
rs41423247	C	-	+	24.973	**0.000002**	**0.000094**
rs4428446	A	+	+	2.620	0.1077	0.3375
rs4501370	G	+	+	1.339	0.2490	0.5714
rs4912924	C	-	-	0.048	0.8268	0.9037
rs56704867	G	-	-	0.740	0.3912	0.6659
rs6191	A	+	-	2.801	0.0963	0.3233
rs6196	G	-	+	0.537	0.4648	0.6659
rs62374564	T	-	+	0.017	0.8960	0.9205
rs62375975	G	+	+	0.466	0.4959	0.6659
rs6865777	T	+	+	0.019	0.8918	0.9205
rs6872499	C	-	-	1.738	0.1893	0.5165
rs6878628	A	-	-	0.618	0.4330	0.6659
rs72806724	C	+	+	0.721	0.3973	0.6659
rs7713008	T	-	-	1.304	0.2553	0.5714
rs7716360	T	+	-	2.382	0.1249	0.3669
rs7732001	G	-	-	0.745	0.3895	0.6659
rs888993	A	+	+	0.733	0.3931	0.6659
rs9324927	G	+	-	0.246	0.6205	0.7291

All analyses were adjusted for age, sex and intracranial volume.

Supplementary Table 2. Polymorphisms by ADHD interactions on amygdala volume

Marker	Effect Allele	Effect in Cases	Effect in controls	Statistics (t-test)	P-value (uncorrected)	P-value (corrected)
chr5_143113237_D	D	-	+	4.354	**0.0386**	0.2016
chr5_143114500_D	D	-	-	0.518	0.4727	0.6348
rs10052957	A	+	-	8.920	**0.0033**	**0.0290**
rs10057473	G	+	-	1.597	0.2083	0.4275
rs10482682	T	+	-	1.244	0.2665	0.4811
rs10482689	T	+	-	11.722	**0.0008**	**0.0188**
rs11167813	G	+	-	1.903	0.1698	0.4275
rs11738754	T	-	+	0.946	0.3324	0.5040
rs11740747	A	+	-	13.275	**0.0004**	**0.0188**
rs11749182	G	+	-	1.661	0.1994	0.4275
rs11750064	A	-	-	1.528	0.2183	0.4275
rs12153243	T	+	-	1.625	0.2044	0.4275
rs12518265	T	-	-	0.250	0.6178	0.7075
rs12521436	A	-	-	0.163	0.6874	0.7343
rs12657648	A	+	+	2.030	0.1563	0.4275
rs13168199	T	-	+	1.095	0.2971	0.4815
rs13172698	G	-	+	0.559	0.4558	0.6348
rs13354365	C	-	-	0.343	0.5587	0.6910
rs1348701	A	-	+	1.582	0.2105	0.4275
rs17100289	T	-	+	0.085	0.7714	0.7882
rs17209237	G	-	+	0.170	0.6804	0.7343
rs17404863	C	-	+	1.194	0.2764	0.4811
rs190488	G	+	-	8.672	**0.0037**	**0.0290**
rs2398631	C	-	+	3.249	0.0735	0.3140
rs258747	G	+	+	0.230	0.6322	0.7075
rs258750	G	+	-	8.894	**0.0033**	**0.0290**
rs258814	T	+	-	8.672	**0.0037**	**0.0290**
rs34205186	T	-	+	2.390	0.1242	0.3892
rs34226461	T	-	-	3.590	0.0600	0.2820
rs41423247	C	-	+	5.930	**0.0161**	0.1081
rs4428446	A	-	+	1.772	0.1852	0.4275
rs4501370	G	-	+	0.431	0.5127	0.6694
rs4912924	C	-	+	1.568	0.2124	0.4275
rs56704867	G	-	+	2.836	0.0942	0.3406
rs6191	A	+	+	0.230	0.6322	0.7075
rs6196	G	+	+	0.003	0.9595	0.9595
rs62374564	T	+	-	0.961	0.3285	0.5040
rs62375975	G	-	+	3.001	0.0852	0.3337
rs6865777	T	+	-	0.115	0.7353	0.7680
rs6872499	C	+	+	2.477	0.1176	0.3892
rs6878628	A	-	-	0.631	0.4281	0.6288
rs72806724	C	+	-	1.329	0.2509	0.4717
rs7713008	T	-	-	0.534	0.4662	0.6348
rs7716360	T	+	-	0.363	0.5477	0.6910
rs7732001	G	-	-	0.263	0.6089	0.7075
rs888993	A	-	+	4.373	**0.0382**	0.2016
rs9324927	G	-	+	1.120	0.2916	0.4815

All analyses were adjusted for age, sex and intracranial volume.

Supplementary Table 3. Polymorphisms by ADHD interactions on caudate volume

Marker	Effect Allele	Effect in Cases	Effect in controls	Statistics (t-test)	P-value (uncorrected)	P-value (corrected)
chr5_143113237_D	D	+	-	2.487	0.1169	0.4362
chr5_143114500_D	D	-	-	0.207	0.6499	0.7636
rs10052957	A	+	-	0.676	0.4123	0.6386
rs10057473	G	+	+	0.477	0.4910	0.6659
rs10482682	T	+	+	0.003	0.9576	0.9750
rs10482689	T	+	-	4.678	**0.0321**	0.3017
rs11167813	G	+	+	0.613	0.4348	0.6386
rs11738754	T	-	+	0.213	0.6453	0.7636
rs11740747	A	+	-	5.262	**0.0232**	0.2726
rs11749182	G	+	+	0.118	0.7319	0.8073
rs11750064	A	+	+	0.378	0.5395	0.6673
rs12153243	T	+	-	1.553	0.2146	0.5309
rs12518265	T	-	-	0.408	0.5242	0.6659
rs12521436	A	-	+	0.414	0.5208	0.6659
rs12657648	A	+	+	0.064	0.8013	0.8559
rs13168199	T	+	-	0.112	0.7386	0.8073
rs13172698	G	-	+	2.243	0.1363	0.4362
rs13354365	C	-	-	2.584	0.1101	0.4362
rs1348701	A	-	-	2.215	0.1388	0.4362
rs17100289	T	-	-	0.001	0.9750	0.9750
rs17209237	G	+	+	1.917	0.1682	0.4650
rs17404863	C	+	-	0.129	0.7196	0.8073
rs190488	G	+	-	0.749	0.3881	0.6386
rs2398631	C	-	+	3.416	0.0666	0.4024
rs258747	G	-	-	1.334	0.2499	0.5593
rs258750	G	+	-	0.697	0.4052	0.6386
rs258814	T	+	-	0.749	0.3881	0.6386
rs34205186	T	-	+	10.199	**0.0017**	0.0799
rs34226461	T	+	+	0.422	0.5169	0.6659
rs41423247	C	-	+	7.413	**0.0073**	0.1716
rs4428446	A	-	+	6.616	**0.0111**	0.1739
rs4501370	G	-	+	3.672	0.0572	0.4024
rs4912924	C	+	-	1.210	0.2731	0.5834
rs56704867	G	-	-	0.481	0.4891	0.6659
rs6191	A	-	-	1.334	0.2499	0.5593
rs6196	G	-	+	2.109	0.1485	0.4362
rs62374564	T	+	-	1.593	0.2089	0.5309
rs62375975	G	-	+	2.755	0.0991	0.4362
rs6865777	T	-	+	2.524	0.1143	0.4362
rs6872499	C	-	+	3.366	0.0685	0.4024
rs6878628	A	-	-	0.709	0.4012	0.6386
rs72806724	C	-	+	1.003	0.3184	0.6386
rs7713008	T	-	-	0.002	0.9661	0.9750
rs7716360	T	-	-	0.865	0.3539	0.6386
rs7732001	G	-	-	0.938	0.3345	0.6386
rs888993	A	+	-	2.200	0.1402	0.4362
rs9324927	G	-	+	0.635	0.4270	0.6386

All analyses were adjusted for age, sex and intracranial volume.

Supplementary Table 4. Polymorphisms by ADHD interactions on hippocampus volume

Marker	Effect Allele	Effect in cases	Effect in controls	Statistics (t-test)	P-value (uncorrected)	P-value (corrected)
chr5_143113237_D	D	-	+	2.497	0.1162	0.4201
chr5_143114500_D	D	-	-	0.652	0.4206	0.6377
rs10052957	A	+	-	4.306	**0.0397**	0.2332
rs10057473	G	+	-	1.540	0.2166	0.5737
rs10482682	T	+	-	2.527	0.1140	0.4201
rs10482689	T	+	-	7.716	**0.0062**	0.2092
rs11167813	G	+	+	0.891	0.3466	0.6196
rs11738754	T	-	-	0.067	0.7954	0.9346
rs11740747	A	+	-	7.033	**0.0089**	0.2092
rs11749182	G	+	-	4.442	**0.0367**	0.2332
rs11750064	A	+	-	2.572	0.1108	0.4201
rs12153243	T	+	-	1.533	0.2176	0.5737
rs12518265	T	-	+	4.647	**0.0327**	0.2332
rs12521436	A	+	-	1.974	0.1621	0.5442
rs12657648	A	+	+	0.027	0.8700	0.9786
rs13168199	T	+	-	0.829	0.3641	0.6196
rs13172698	G	-	-	0.750	0.3878	0.6285
rs13354365	C	-	-	0.984	0.3229	0.6196
rs1348701	A	-	-	0.015	0.9037	0.9786
rs17100289	T	-	-	0.005	0.9459	0.9786
rs17209237	G	+	-	0.351	0.5545	0.7897
rs17404863	C	+	-	0.811	0.3691	0.6196
rs190488	G	+	-	4.940	**0.0277**	0.2332
rs2398631	C	-	-	0.111	0.7396	0.8913
rs258747	G	-	+	1.195	0.2761	0.5899
rs258750	G	+	-	4.571	**0.0342**	0.2332
rs258814	T	+	-	4.940	**0.0277**	0.2332
rs34205186	T	-	-	0.009	0.9254	0.9786
rs34226461	T	-	-	1.519	0.2197	0.5737
rs41423247	C	-	+	0.697	0.4053	0.6350
rs4428446	A	-	-	0.018	0.8937	0.9786
rs4501370	G	+	-	0.405	0.5257	0.7721
rs4912924	C	+	+	0.271	0.6038	0.8347
rs56704867	G	-	-	0.180	0.6721	0.8538
rs6191	A	-	-	1.195	0.2761	0.5899
rs6196	G	-	+	0.152	0.6973	0.8625
rs62374564	T	+	-	2.617	0.1078	0.4201
rs62375975	G	-	-	0.910	0.3415	0.6196
rs6865777	T	-	-	0.001	0.9786	0.9786
rs6872499	C	-	-	1.855	0.1752	0.5490
rs6878628	A	-	-	1.243	0.2667	0.5899
rs72806724	C	+	+	0.002	0.9643	0.9786
rs7713008	T	-	-	0.242	0.6231	0.8367
rs7716360	T	-	-	1.436	0.2327	0.5756
rs7732001	G	-	-	1.090	0.2981	0.6092
rs888993	A	-	+	2.578	0.1105	0.4201
rs9324927	G	-	+	0.206	0.6505	0.8493

All analyses were adjusted for age, sex and intracranial volume.

Supplementary Table 5. Polymorphisms by ADHD interactions on putamen volume

Marker	Effect Allele	Effect in cases	Effect in controls	Statistics (t-test)	P-value (uncorrected)	P-value (corrected)
chr5_143113237_D	D	-	+	3.559	0.0612	0.3954
chr5_143114500_D	D	-	-	0.245	0.6212	0.8853
rs10052957	A	-	-	0.819	0.3668	0.6779
rs10057473	G	+	-	2.403	0.1233	0.5080
rs10482682	T	-	-	0.671	0.4138	0.6946
rs10482689	T	+	-	6.440	**0.0122**	0.2898
rs11167813	G	+	-	3.617	0.0591	0.3954
rs11738754	T	+	-	0.049	0.8251	0.9100
rs11740747	A	+	-	5.667	**0.0185**	0.2898
rs11749182	G	+	+	0.312	0.5773	0.8853
rs11750064	A	+	+	0.012	0.9117	0.9117
rs12153243	T	-	+	0.234	0.6292	0.8853
rs12518265	T	-	+	6.153	**0.0142**	0.2898
rs12521436	A	+	-	2.282	0.1330	0.5080
rs12657648	A	+	+	0.157	0.6923	0.8853
rs13168199	T	+	+	0.019	0.8906	0.9100
rs13172698	G	-	-	1.195	0.2761	0.6488
rs13354365	C	-	+	2.808	0.0959	0.5008
rs1348701	A	-	-	1.098	0.2965	0.6636
rs17100289	T	-	+	3.401	0.0672	0.3954
rs17209237	G	-	+	0.037	0.8484	0.9100
rs17404863	C	+	+	0.034	0.8531	0.9100
rs190488	G	-	-	0.792	0.3750	0.6779
rs2398631	C	-	-	0.225	0.6359	0.8853
rs258747	G	-	+	0.133	0.7158	0.8853
rs258750	G	-	-	0.607	0.4373	0.7087
rs258814	T	-	-	0.792	0.3750	0.6779
rs34205186	T	-	+	0.084	0.7729	0.9100
rs34226461	T	+	-	0.811	0.3692	0.6779
rs41423247	C	-	+	2.307	0.1309	0.5080
rs4428446	A	-	+	0.211	0.6469	0.8853
rs4501370	G	+	+	0.983	0.3231	0.6779
rs4912924	C	-	+	1.433	0.2332	0.5976
rs56704867	G	-	+	1.652	0.2006	0.5893
rs6191	A	-	+	0.133	0.7158	0.8853
rs6196	G	-	+	3.397	0.0673	0.3954
rs62374564	T	+	+	0.050	0.8236	0.9100
rs62375975	G	+	-	1.443	0.2316	0.5977
rs6865777	T	-	+	1.849	0.1760	0.5515
rs6872499	C	-	-	4.965	**0.0273**	0.3208
rs6878628	A	-	+	1.895	0.1707	0.5515
rs72806724	C	+	+	0.677	0.4118	0.6946
rs7713008	T	-	-	0.058	0.8099	0.9100
rs7716360	T	-	+	0.152	0.6968	0.8853
rs7732001	G	-	-	1.382	0.2416	0.5976
rs888993	A	+	-	2.195	0.1405	0.5080
rs9324927	G	+	-	0.023	0.8791	0.9100

All analyses were adjusted for age, sex and intracranial volume.

Supplementary Table 6. Polymorphisms by ADHD interactions on intracranial volume

Marker	Effect Allele	Effect in cases	Effect in controls	Statistics (t-test)	P-value (uncorrected)	P-value (corrected)
chr5_143113237_D	D	+	+	0.593	0.4423	0.7041
chr5_143114500_D	D	+	-	0.352	0.5539	0.7457
rs10052957	A	+	-	2.223	0.1381	0.6742
rs10057473	G	+	-	2.129	0.1466	0.6742
rs10482682	T	-	-	0.057	0.8115	0.8538
rs10482689	T	-	-	0.701	0.4038	0.7041
rs11167813	G	+	-	0.572	0.4506	0.7041
rs11738754	T	+	+	0.704	0.4028	0.7041
rs11740747	A	-	-	0.608	0.4368	0.7041
rs11749182	G	-	-	0.630	0.4285	0.7041
rs11750064	A	-	-	1.288	0.2582	0.6742
rs12153243	T	+	-	0.819	0.3668	0.7041
rs12518265	T	+	+	0.472	0.4933	0.7245
rs12521436	A	-	+	1.523	0.2191	0.6742
rs12657648	A	-	+	0.699	0.4044	0.7041
rs13168199	T	-	-	0.940	0.3339	0.7041
rs13172698	G	+	+	0.071	0.7906	0.8538
rs13354365	C	-	+	1.984	0.1610	0.6742
rs1348701	A	-	+	0.004	0.9486	0.9486
rs17100289	T	+	+	0.538	0.4644	0.7041
rs17209237	G	-	+	0.869	0.3528	0.7041
rs17404863	C	-	-	1.077	0.3011	0.7041
rs190488	G	+	-	2.716	0.1014	0.6742
rs2398631	C	-	+	1.346	0.2478	0.6742
rs258747	G	-	+	0.322	0.5712	0.7245
rs258750	G	+	-	3.192	0.0760	0.6742
rs258814	T	+	-	2.716	0.1014	0.6742
rs34205186	T	+	-	1.809	0.1807	0.6742
rs34226461	T	-	-	0.058	0.8093	0.8538
rs41423247	C	+	+	0.145	0.7040	0.8272
rs4428446	A	+	-	1.706	0.1935	0.6742
rs4501370	G	+	-	0.117	0.7329	0.8402
rs4912924	C	-	+	4.147	**0.0435**	0.6742
rs56704867	G	-	-	0.174	0.6768	0.8256
rs6191	A	-	+	0.322	0.5712	0.7457
rs6196	G	+	-	2.181	0.1418	0.6742
rs62374564	T	-	-	0.053	0.8175	0.8538
rs62375975	G	+	+	0.035	0.8515	0.8700
rs6865777	T	+	-	1.525	0.2188	0.6742
rs6872499	C	-	+	2.832	0.0944	0.6742
rs6878628	A	-	+	1.646	0.2015	0.6742
rs72806724	C	+	-	0.878	0.3502	0.7041
rs7713008	T	+	+	0.229	0.6328	0.8038
rs7716360	T	-	+	0.347	0.5567	0.7457
rs7732001	G	-	+	1.380	0.2420	0.6742
rs888993	A	+	+	4.289	**0.0400**	0.6742
rs9324927	G	+	+	0.165	0.6851	0.8256

All analyses were adjusted for age and sex.

Capítulo IV

Discussão geral

Pelo menos desde o século XVII, com o trabalho pioneiro de Willis (Molnár 2004), o cérebro vem sendo estudado por anatomistas e outros estudiosos e compreendido como o órgão-sede das enfermidades mentais. No entanto, a demonstração de associações entre fenótipos e volume de estruturas cerebrais foi limitada, por mais de trezentos anos, a alterações muito evidentes e traços muito graves. Somente com o surgimento de técnicas modernas de neuroimagem a ciência pode avaliar tais associações com um certo refinamento, bem como relacionar estruturas cerebrais com características comportamentais, como o TDAH. Nesse sentido, ainda há certa dificuldade em aceitar que genes e estruturas cerebrais estejam associados a determinados comportamentos. Assim podem ser compreendidas as críticas ao trabalho recente de Martine Hoogman (2017) sobre as relações entre estruturas cerebrais e o TDAH (expressas em correspondências publicadas em resposta ao artigo), muitas vezes citado nessa dissertação. Uma das críticas questiona as conclusões daquele artigo serem baseadas em dados da infância, presumindo que poderia haver uma normalização na vida adulta. Os presentes dados, entretanto, replicam parte dos resultados de Hoogman em uma amostra de adultos. A despeito das críticas, vale ressaltar o discurso de Thomas Insel (Ted Talks 2013), que diz que não há o menor risco de alguém ser determinista quando o assunto é o cérebro. A complexidade do cérebro afasta qualquer interpretação de tal forma simplória.

A escolha do gene alvo desse estudo *NR3C1* se deu em função do seu conhecido papel na resposta ao estresse. No presente estudo, nós encontramos um efeito moderador de SNPs no *NR3C1* na associação entre TDAH e volumes cerebrais. Tais resultados permitem supor que a associação entre volume e TDAH em adultos, tem como moderadora variantes genéticas subjacentes. Apesar de termos explorado apenas o efeito de um gene, podemos inferir que outras variantes também participem dessa interação. Essa inferência se torna plausível ao considerarmos que o *NR3C1* em si, como fator de transcrição, interage com outras centenas de genes e tais efeitos também são influenciados por fatores ambientais. A hipótese de que outros genes também estariam influenciando a relação TDAH-volume em adultos poderia ser testada, primeiramente, avaliando outros genes do eixo HPA com os quais o *NR3C1* interage diretamente e, posteriormente, com uma rede gênica na qual *NR3C1* estaria no centro.

Outro fator a ser considerado, porém não abordado nessa dissertação, são as isoformas do GR. De acordo com (Derijk et al. 2001), um SNP do *NR3C1* estabiliza o mRNA da variante GRβ, aumentando a expressão dessa isoforma. O efeito de outros SNPs no *splicing* alternativo das isoformas do GR, entretanto, ainda não foi avaliado. Assim, a relação entre as

principais isoformas (GRα/GRβ) e sua dinâmica em diferentes contextos (em variações do *NR3C1* e em diferentes tecidos, por ex.) permanece um grande campo a ser abordado.

Em uma visão mais ampla, estudos que explorem o efeito diferencial do *NR3C1* em psicopatologias podem ajudar a elucidar se tal efeito tem um padrão (ex: efeito diferencial apenas em psicopatologias externalizantes) ou não. Nesse sentido, Rovaris et al. (2017) também reportaram um efeito diferencial do gene em usuários de crack/cocaína. De uma forma alternativa, mas não excludente, deve ser testado se tal efeito de interação também ocorre em outras regiões cerebrais (que poderiam estar associadas ao TDAH, mas não foram relatadas por falta de inclusão de variantes genéticas na análise). Essa hipótese não é improvável, haja vista a constatação de efeitos significativos de alguns SNPs da interação no volume cerebral total.

Também se torna necessário, com os resultados da presente dissertação, averiguar qual seria a sequência de eventos mais plausível na relação volume-TDAH (se os sintomas de TDAH causariam menos desenvolvimento do cérebro, ou se um menor volume do cérebro ocasionado por outros fatores causariam desenvolvimento de TDAH). Uma possibilidade de solução dessa questão seria a realização de estudos longitudinais que avaliem a idade de surgimento dos sintomas de TDAH, bem como a evolução do tamanho cerebral. Deve-se também levar em consideração que tal questão pode não ter uma resposta definida, ou, ainda, ser uma mistura das duas hipóteses.

De maneira geral, os resultados encontrados permitem seguir inúmeras direções de estudo. Podemos explorar outras variações cerebrais não abordadas aqui, como conectividade, funcionalidade, metabolismo etc., considerando interação dos mesmos com fatores genéticos. Outras direções são abordagens bioquímicas e de biologia molecular para avaliar o efeito de fatores de transcrição em transtornos psiquiátricos, o efeito de SNPs na expressão das isoformas do GR, e se tal efeito varia de acordo com o tipo celular no qual se encontra. No âmbito genético, devemos considerar a interação de variantes genéticas entre si, bem como seus efeitos na transcrição e tradução proteica. Fatores ambientais, como exposição ao estresse, poderiam, ainda, reforçar o efeito dessas variantes ou "silenciá-las epigeneticamente". A investigação de cada um desses aspectos pode ajudar a melhor compreender a etiologia do TDAH.

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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

Produção Científica Adicional durante o Período do Mestrado

Molecular Psychiatry (2017) 00, 1–7

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ORIGINAL ARTICLE

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

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Experimental studies have demonstrated that methylphenidate (MPH) modulates the synaptic vesicle trafficking and synaptotagmin-1 (Syt1) mRNA levels. Syt1 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, Syt1 could represent a key element in MPH pharmacodynamics in adults with ADHD.

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INTRODUCTION

Several guidelines suggest that the first-line pharmacological treatment for adults with attention-deficit/hyperactivity disorder (ADHD) consists of psychostimulant medications, in particular methylphenidate (MPH),[1,2] which is the most commonly prescribed drug for the management of ADHD symptoms.[3,4] Meta-analyses and systematic reviews have shown that MPH is safe and efficacious in attenuating the core symptoms of ADHD, promoting overall clinical improvement.[5–7] Nevertheless, there is considerable interindividual variability regarding the dose required, tolerability and response rates to MPH and, patients frequently discontinue therapy over time.[8–11] In this context, there has been a growing interest in assessing clinical and genetic predictors of MPH treatment outcomes in ADHD.[12–14]

The vast majority of pharmacogenetic studies have been conducted in children.[15] So far, there are only 6 studies in adults evaluating a total of 15 genes, and they show predominantly non-significant results.[16–21] These studies have focused mainly in genes involved on MPH pharmacodynamics as potential predictors of treatment response variability.[15] Although MPH pharmacodynamics is not completely understood, a well-known mechanism is the inhibition of the reuptake of dopamine (DA) and norepinephrine into presynaptic neurons through the blockade of their transporters (DAT and NET, respectively). This process leads to increased extracellular levels of these neurotransmitters in the brain.[22,23] In view of this, the gene encoding DAT (*SLC6A3*, also known as *DAT1*) is the most widely investigated in pharmacogenetic studies, in particular the 40-pb variable number tandem repeat (VNTR) polymorphism in the 3′-untranslated region. However, recent meta-analyses did not support an association of this polymorphism with response to MPH.[24,25] Therefore, the initial intuitive hypothesis that polymorphisms in *DAT1* gene would be the major source of variations in treatment response has not been robustly supported yet, indicating that new research efforts should be devoted to alternative promising pathways on the MPH pharmacodynamics.

In this sense, there is evidence from *in vivo* pharmacological studies describing additional effects of MPH in synaptic vesicle trafficking.[26] In agreement with this hypothesis, a systematic review using systeomic-oriented approaches to the pharmacogenetics of ADHD[15] suggested a possible role of proteins related to soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) complex. The core members of the SNARE complex are the syntaxin-1A protein, the vesicle-associated membrane protein 2 (VAMP2 or synaptobrevin-2), and synaptosomal-associated protein 25 kDa (SNAP-25). The assembly of these proteins forming a four-helix bundle is essential for

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