UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E BIOLOGIA MOLECULAR

ESTUDO DA INTERAÇÃO ENTRE O GENE *LPHN3* E O AGRUPAMENTO GÊNICO NTAD NO TDAH EM ADULTOS

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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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Sumário

Lista de Abreviaturas

ANKK1	*Ankyrin repeat and kinase domain containing 1*
COMT	Gene *Catechol-O-methyltransferase*
CTF	Fração C-terminal
DBH	Gene *Dopamine beta-hydroxylase*
DRD2	Gene *Dopamine receptor D2*
DRD4	Gene *Dopamine receptor D4*
DRD5	Gene *Dopamine receptor D5*
DSM-5	Manual Diagnóstico e Estatístico de Transtornos Mentais 5º Ed
DSM-IV	Manual Diagnóstico e Estatístico de Transtornos Mentais 4º Ed
FLRT3	Fibronectine leucine rich transmembrane protein 3
GWAS	Estudo de associação por varredura genômica
HTR1B	Gene *5-hydroxytryptamine receptor* 1B
LPHN3/*ADGRL3*	Gene *Latrophilin 3/Adhesion G protein-coupled receptor L3*
MAOA	Gene *Monoamine oxidase A*
NCAM1	Gene *Neural cell adhesion molecule 1*
NF-kB	*Nuclear transcription factor kappa B*
NTAD	Agrupamento gênico formado por *NCAM1-TTC12-ANKK1-DRD2*
NTF	Fração N-terminal
SLC6A3/DAT1	Gene *Solute carrier family 6 member 3/Dopamine transporter 1*
SLC6A4/5-HTT	Gene *Solute carrier family 6 member 4/5-hydroxytryptamine transporter*
SNAP25	Gene *Sinaptosome associated protein 25*
SNP	Polimorfismo de nucleotídeo único
TDAH	Transtorno de Déficit de Atenção/Hiperatividade
TTC12	Gene *Tetratricopeptide repeat domain 12*

Resumo

O Transtorno de Déficit de Atenção/Hiperatividade (TDAH) é um dos transtornos psiquiátricos mais comuns em crianças e frequentemente persiste na vida adulta, sendo caracterizado por prejuízo significativo decorrente de sintomas de desatenção e hiperatividade/impulsividade. Este transtorno tem importante influência genética, sendo que as estimativas de herdabilidade aproximam-se dos 80%. Devido a esse forte componente genético, diversos estudos têm procurado elucidar os fatores genéticos específicos relacionados ao transtorno. Em sua maioria, esses estudos têm avaliado o papel de diferentes variantes genéticas sob uma ótica de efeitos aditivos, com poucos estudos avaliando o impacto de efeitos genéticos não-aditivos, como os resultantes de interações entre genes. No entanto, entre os poucos estudos que de fato consideraram efeitos de interação gênica na psiquiatria, existem evidências de que variantes do gene *LPHN3* interagem com polimorfismos ao longo do agrupamento gênico conhecido como NTAD (*NCAM1-TTC12-ANKK1-DRD2*), influenciando a susceptibilidade ao TDAH na infância. Nesse contexto, a presente dissertação apresenta estudo desenvolvido com o objetivo de analisar pela primeira vez o impacto de interações entre uma variante de *LPHN3* e polimorfismos do agrupamento NTAD na susceptibilidade e sintomatologia do TDAH em uma amostra de adultos. A análise exploratória baseada em entropia revelou uma potencial interação com aumento do ganho de informação entre um SNP de *LPHN3* e um dos SNPs de *TTC12* analisados com relação à sintomatologia do TDAH. Em concordância com tal achado, análises de covariância entre os adultos com TDAH apontaram que a interação *LPHN3*-rs6551665/*TTC12*-rs2303380 foi significativamente associada com a quantidade total de sintomas de TDAH e também com os sintomas específicos de hiperatividade/impulsividade. Além disso, foi observado que a mesma combinação de alelos da interação envolvida com aumento de sintomas de TDAH também está relacionada com a susceptibilidade ao transtorno, especialmente no que se refere ao grupo composto por indivíduos dos subtipos predominantemente hiperativo/impulsivo e combinado. Esses resultados reforçam os achados que apontam o envolvimento das interações entre o gene *LPHN3* e o agrupamento gênico NTAD na etiologia do TDAH na infância, estendendo tais efeitos também a amostras de adultos. Além disso, sugerimos que as interações gene-gene modulando os efeitos de *LPHN3* são fatores específicos que contribuem para o desenvolvimento de subtipos de TDAH com maior presença de sintomas de hiperatividade/impulsividade.

Abstract

Attention Deficit/Hyperactivity Disorder (ADHD) is one of the most common psychiatric disorders in children and often persists into adulthood as well. ADHD is characterized by a significant impairment due to inadequate levels of symptoms of inattention and hyperactivity/impulsivity. This disorder presents great genetic influence, with heritability estimates close to 80%. Given this strong genetic component, several studies have sought to elucidate the specific genetic factors related to the disorder. Most of these studies have evaluated the role of different genetic variants under additive effects, with few studies evaluating the impact of non-additive genetic effects, as the ones resulting from interactions between genes. Among the few studies that have considered genetic interaction effects in psychiatry, however, there is evidence that variants of the *LPHN3* gene interact with polymorphisms spanning the gene cluster known as NTAD (*NCAM1-TTC12-ANKK1-DRD2*), influencing the susceptibility to ADHD during childhood. In this context, the study developed in this dissertation is the first study to analyze the impact of interactions between a variant of *LPHN3* and polymorphisms of the NTAD cluster with respect to the susceptibility and symptomatology of ADHD in adult samples. Entropy-based exploratory analysis revealed a potential interaction with increased gained information between a *LPHN3* SNP and one of the *TTC12* SNPs analyzed when analyzing ADHD symptoms. In agreement with such findings, analysis of covariance within the adults with ADHD pointed that the *LPHN3*-rs6551665/*TTC12*-rs2303380 interaction was significantly associated with the total number of ADHD symptoms and also with the hyperactivity/impulsivity symptoms domain. Furthermore, it was observed that the same combination of interacting alleles involved in increasing ADHD symptom counts is also related to susceptibility to the disorder, particularly in the group composed of individuals of predominantly hyperactive/impulsive and combined subtypes. These results support other studies showing that the interaction between the *LPHN3* gene and the NTAD gene cluster are involved in the etiology of ADHD, expanding these effects in an adult sample. Moreover, we suggest that the gene-gene interactions modulating *LPHN3* effects are specific factors contributing to the development of ADHD subtypes with greater presence of hyperactivity/impulsivity.

O Transtorno de Déficit de Atenção/Hiperatividade (TDAH)

O TDAH é um dos transtornos mais comuns diagnosticados na prática psiquiátrica, tendo uma prevalência mundial estimada entre 3-5% em crianças e adolescentes (Polanczyk et al., 2007) e cerca de 2-4% em adultos (Kessler et al., 2006; Simon et al., 2009). Este transtorno é caracterizado por prejuízos clinicamente significativos causados por sintomas persistentes de desatenção e/ou hiperatividade e impulsividade inadequados para o nível de desenvolvimento do indivíduo.

Apesar de ser tradicionalmente considerado como um transtorno de neurodesenvolvimento, com manifestação precoce dos sintomas, sabe-se que uma parcela das crianças afetadas mantém o diagnóstico na idade adulta. Além disso, recentemente, três estudos de coortes populacionais indicaram que não há uma sobreposição completa dos grupos com diagnóstico de TDAH na infância com aqueles que o apresentam na vida adulta. De acordo com esses estudos, existe um grupo de indivíduos que apresenta o início dos sintomas de TDAH apenas na idade adulta, havendo ainda a possibilidade de que o início precoce ou tardio esteja na verdade refletindo a presença de dois tipos de transtornos distintos (Moffitt et al., 2015; Caye et al., 2016; Agnew-Blais et al., 2016). A manifestação do TDAH em indivíduos adultos está relacionada com importantes prejuízos nos âmbitos acadêmico e profissional além de afetar relacionamentos afetivos e sociais (Wilens and Spencer, 2010).

O diagnóstico do TDAH é essencialmente clínico e comumente segue os critérios propostos pelo Manual Diagnóstico e Estatístico dos Transtornos Mentais (DSM-5; APA 2013). Para adultos, tais critérios requerem a presença de ao menos cinco de nove sintomas em uma ou ambas as áreas (desatenção e hiperatividade/impulsividade), manifestação sintomatológica antes dos doze anos de idade e prejuízo decorrente do transtorno em ao menos dois contextos da vida (escola/trabalho e relações interpessoais, por exemplo). O transtorno apresenta três subtipos ou apresentações, que estão diretamente relacionados à presença de sintomas de desatenção e/ou hiperatividade/impulsividade, sendo eles: predominantemente desatento, predominantemente hiperativo/impulsivo e combinado.

Evidências apontam para uma complexa base neurobiológica do TDAH, com variações estruturais e funcionais em múltiplos sistemas de neurotransmissores

envolvidos na gênese do transtorno (Purper-Ouakil et al., 2011; Tripp and Wickens, 2009). Dentre estes, evidências de estudos psicofarmacológicos e modelos animais sugerem o envolvimento dos sistemas dopaminérgico, adrenérgico, serotoninérgico e colinérgico (Kooij et al., 2008; Wilens, 2008). Ainda, suportando o atual conjunto de evidências sobre a neurobiologia do TDAH está também o mecanismo de ação da principal terapia farmacológica empregada, o uso de medicamentos da classe dos estimulantes do sistema nervoso central. O medicamento de primeira escolha no tratamento do TDAH é o metilfenidato (conhecido comercialmente como Ritalina), considerado seguro e eficaz na redução dos sintomas tanto em crianças como em adultos (Kooij et al., 2008; Wigal, 2009; Kaplan and Newcorn, 2011; Castells et al., 2011). O metilfenidato atua bloqueando os transportadores de dopamina e noradrenalina, e, consequentemente, aumentando a concentração extracelular dessas catecolaminas em áreas do córtex pré-frontal e estriado (Sulzer et al., 2005).

Heterogeneidade Clínica

Assim como em outros transtornos psiquiátricos, a apresentação clínica do TDAH é heterogênea, sendo influenciada pela presença diferencial de sintomas (de desatenção, hiperatividade/impulsividade e/ou ambos), presença de outras comorbidades psiquiátricas e ainda variação na idade de início dos sintomas. Essas características clínicas contribuem para a alta heterogeneidade fenotípica presente no transtorno, e potencialmente modulam efeitos de gravidade e resposta à intervenção farmacológica, por exemplo.

Estima-se que 80% dos adultos com TDAH apresente simultaneamente alguma comorbidade psiquiátrica (Kessler et al., 2006). Em geral, os transtornos psiquiátricos são divididos em duas categorias distintas de acordo com a manifestação sintomatológica, os Transtornos Internalizantes e os Transtornos Externalizantes (Kessler et al., 2011). Dentre as comorbidades do eixo internalizante mais comuns estão o Transtorno Depressivo Maior, o Transtorno de Ansiedade Generalizada e Transtornos do Pânico e Bipolar; já dentre os Transtornos Externalizantes mais frequentes encontram-se o Transtorno da Personalidade Antissocial, Transtorno da Conduta e Transtorno por Uso de Substâncias (Kessler et al., 2006).

O TDAH é classicamente considerado um Transtorno Externalizante, porém pode apresentar características dos dois domínios (externalizantes e internalizantes) (Jacob et al., 2014). A faceta externalizante do TDAH é atribuída principalmente aos sintomas de hiperatividade e impulsividade (Jacob et al. 2014), sendo que a presença desses sintomas em adultos está relacionada com um maior risco de presença de outras comorbidades (Cadman et al., 2016), especialmente o Transtorno por Uso de Substâncias (Liebrenz et al., 2015). Além disso, indivíduos que apresentam maior sintomatologia no domínio de hiperatividade/impulsividade (subtipos combinado e hiperativo/impulsivo) têm um perfil neuropsicológico (Dobson-Patterson et al., 2016), e de personalidade (Salgado et al., 2009; Gomez et al., 2012) característicos, contribuindo para a diferenciação das apresentações clínicas do TDAH.

Genética do TDAH

O TDAH tem etiologia multifatorial, sendo causado pela confluência de fatores genéticos e ambientais, bem como de suas interações (Stergiakouli and Thapar, 2010). Dentre os transtornos psiquiátricos, o TDAH desponta como um dos que apresenta maior componente genético, com herdabilidade estimada, tanto em crianças como em adultos, em aproximadamente 80% (Faraone et al., 2005; Chang et al., 2013).

Devido ao grande componente genético implicado na etiologia do TDAH, muitos estudos têm tentado identificar genes associados. A maioria dos estudos, principalmente os mais antigos, utilizou a abordagem clássica de estudar genes candidatos, definidos a *priori* por hipóteses neurobiológicas.

Nesse contexto, os genes codificadores dos componentes dos sistemas de neurotransmissão têm sido o foco principal, destacando-se os sistemas dopaminérgico e serotonérgico (Franke et al., 2012). Entre os principais genes candidatos amplamente investigados estão os genes codificadores dos receptores de dopamina D2, D4 e D5 (*DRD2*, *DRD4* e *DRD5*, respectivamente), do transportador de dopamina (*SLC6A3*, também conhecido como *DAT1*) e de serotonina (*SLC6A4*, também conhecido como *5-HTT*), do receptor 1B de serotonina (*HTR1B*), da proteína associada ao sinaptossoma de 25 kDa (*SNAP25*) e das enzimas dopamina-beta-hidroxilase (*DBH*), monoamino oxidase (*MAOA*) e catecol-O-metiltransferase (*COMT*) (Gizer et al., 2009). Meta-análises têm sustentado associações individuais em amostras de crianças, entretanto

há significativa heterogeneidade entre os achados de diferentes estudos (Gizer et al., 2009). Em relação a adultos com TDAH, os resultados ainda são considerados contraditórios ou inconclusivos (Franke et al., 2011).

Até o momento, os estudos de associação por varredura genômica (GWAS, do inglês *Genome-Wide Association Studies*) realizados em amostras de TDAH não foram capazes de apontar associações significativas a nível genômico (Hinney et al., 2011; Neale et al., 2008; Lasky-Su et al., 2008b, 2008a; Stergiakouli et al., 2012; Neale et al., 2010b; Zayats et al., 2015; Mick et al., 2010; Neale et al., 2010a). Assim, mesmo em era genômica, a identificação de fatores genéticos específicos relacionados ao desenvolvimento do transtorno permanece uma questão em aberto, motivando os estudos com genes-candidatos com hipóteses baseadas em mecanismos fisiopatológicos envolvidos no transtorno e estudos com modelos animais.

Além disso, a maioria dos estudos desenvolvidos até o momento tem focado na influência de efeitos genéticos aditivos, negligenciando um possível papel de efeitos não-aditivos, como, por exemplo, os resultantes de interações gene-gene. No entanto, perspectivas mais recentes têm valorizado o potencial desta abordagem na elucidação de fenômenos biológicos importantes, e aliado a isso, uma importante e recente meta-análise avaliou as estimativas de herdabilidade do TDAH encontrada entre os estudos com gêmeos. Os resultaram indicaram que o transtorno possui um excesso de similaridade entre gêmeos monozigóticos (rMZ) do que seria esperado quando comparado com gêmeos dizigóticos (rDZ), ou seja, rMZ > 2rDZ (Polderman et al., 2015). Esse achado sugere que o componente genético do TDAH não poderia ser explicado apenas por variação genética aditiva, corroborando a hipótese de que fatores genéticos não-aditivos também desempenham um papel na susceptibilidade ao transtorno. Desta forma, se faz necessário buscar abordagens alternativas para a identificação de fatores genéticos que contribuam para a alta herdabilidade do TDAH.

De fato, apesar de escassos, estudos que avaliaram interações gene-gene já identificaram efeitos epistáticos específicos influenciando na etiologia do TDAH. Dentre estes, o estudo com o tamanho de efeito mais robusto, e com papel apoiado por meta-análise, envolve a interação entre o gene *LPHN3 (ADGRL3)* e o agrupamento gênico NTAD (*NCAM1-TTC12-ANKK1-DRD2*) (Jain et al., 2012; Acosta et al., 2011; Bruxel et al., 2015).

LPHN3 (ADGRL3)

O gene *LPHN3* (do inglês, *Latrophilin 3*), sendo também conhecido como *ADGRL3* (do inglês, *Adhesion G protein-coupled receptor L3*) está localizado em Chr: 4q13. Esse gene codifica um receptor de adesão transmembrana acoplado a proteína G, o qual é expresso predominantemente no cérebro e presente, principalmente, em neurônios glutamatérgicos (Arcos-Burgos et al., 2010). Nas áreas cerebrais onde é expresso, o receptor desempenha um papel na regulação da liberação de neurotransmissores e desenvolvimento de sinapses (O'Sullivan et al., 2014, 2012), afetando o metabolismo de circuitos neurais potencialmente envolvidos no TDAH, especialmente na via mesocortical (Arcos-Burgos et al., 2010). Esse gene foi inicialmente implicado no TDAH através de um estudo de ligação realizado em um isolado populacional da Colômbia (Arcos-Burgos et al., 2004, 2010) e, desde então, vem sendo considerado como um dos genes mais promissores para estudos da susceptibilidade ao TDAH. A associação de variantes e haplótipos do gene *LPHN3* com esse transtorno foi replicada em várias amostras de outras populações, a maioria envolvendo crianças (Arcos-Burgos et al., 2010; Ribasés et al., 2011; Choudhry et al., 2012; Bruxel et al., 2015; Hwang et al., 2015; Gomez-Sanchez et al., 2016) e parece fornecer novos *insights* sobre a genética, neurobiologia e o tratamento do TDAH.

Combinado com os resultados da meta-análise, e estudos posteriores em outras populações que avaliaram a influência do *LPHN3* no TDAH, alguns SNPs (polimorfismo de nucletídeo único, do inglês *Single Nucleotide Polimorphism*) despontam como de especial interesse para a etiologia do transtorno. Por exemplo, uma mesma variante (rs6551665) foi associada com a susceptibilidade ao TDAH (Arcos-Burgos et al., 2010; Choudhry et al., 2012; Hwang et al., 2015) em diversas amostras e também com a resposta terapêutica por uso de estimulantes (Arcos-Burgos et al., 2010; Bruxel et al., 2015; Labbe et al., 2012). Um dado interessante sobre esse SNP está relacionado a uma avaliação de risco atribuível à população. Essa análise demonstrou matematicamente que se o efeito do rs6551665 fosse controlado haveria uma redução de aproximadamente 9% nos casos de TDAH em uma amostra proveniente da Noruega (Arcos-Burgos et al., 2010). Essa variante está localizada na região intrônica do gene e ainda não tem caracterização funcional descrita, mas devido a sua consistência nos

estudos de associação de TDAH é possível especular que esteja envolvida diretamente ou refletindo efeitos funcionais de outros polimorfismos próximos implicados na genética do transtorno.

Diversos estudos com modelos animais têm procurado elucidar o papel funcional do gene *LPHN3* no TDAH. A caracterização comportamental de animais submetidos a processos de *knockout* ou *knockdown* de ortólogos do gene *LPHN3* demonstrou que esses apresentam um aumento da atividade locomotora, o que é comparável à hiperatividade observada em individuos com TDAH (Wallis et al., 2012; Lange et al., 2012; van der Voet et al., 2015; Reuter et al., 2016; Orsini et al., 2016). Além disso, esses modelos animais têm também sugerido um papel central da *LPHN3* na regulação do desenvolvimento do sistema dopaminérgico. Diferentes estudos observaram uma sinalização dopaminérgica anormal (van der Voet et al., 2015), neurônios dopaminérgicos desorganizados e em número reduzido (Lange et al., 2012), aumento dos níveis de dopamina no corpo estriado e aumento da expressão de receptores dopaminérgicos e outros genes relacionados (Wallis et al., 2012).

A partir destas observações, estudos *in vitro* procuraram entender melhor a função do *LPHN3* na regulação do desenvolvimento do sistema dopaminérgico. A descoberta dos ligantes endógenos da proteína trouxe informações relevantes para a sua funcionalidade, favorecendo a ideia da LPHN3 como responsável pela ação combinada de interações entre células e transdução de sinal (Martinez et al., 2011). LPHN3 é um receptor de adesão acoplado à proteína G, e, como tal, contêm duas subunidades, a fração C-terminal (CTF), localizado intracelularmente e responsável pela sinalização da proteína G, e a fração N-terminal (NTF), que se projeta a partir da membrana plasmática (Silva and Ushkaryov, 2010). A subunidade NTF contém vários domínios que são potencias locais de ligação para outras proteínas. Essa subunidade de LPHN3 interage com a proteína FLRT3 (*Fibronectin leucine rich transmembrane protein 3*), implicada na orientação de axônios, formando um complexo trans-sináptico (O'Sullivan et al., 2012, 2014). Esta ligação é necessária para suportar o desenvolvimento de sinapses glutamatérgicas e, portanto, está implicada no desenvolvimento do sistema nervoso.

O agrupamento gênico NTAD

O agrupamento gênico NTAD, ou *cluster* NTAD, é composto por quatro genes – *NCAM1, TTC12, ANKK1* e *DRD2* – localizado em uma região de cerca de 520kb no braço longo do cromossomo 11 (Chr: 11q22-23). Evidências apontam que o *cluster* pode ser considerado como uma unidade funcional conservada que vem mantendo sua sintenia pela influência dos genes nos processos relacionados com neurogênese e neurotransmissão (Mota et al., 2012). Além disso, evidências apontam para uma co-regulação entre os genes do cluster onde variações genéticas em um dos genes poderiam indiretamente alterar o nível de expressão ou a funcionalidade de outro gene do mesmo conjunto (Huang et al., 2009).

O gene *NCAM1* (do inglês, *Neural cell adhesion molecule 1*) codifica a proteína NCAM que é amplamente expressa no sistema nervoso, onde atua como uma proteína de membrana importante para a adesão neuronal, desenvolvimento, transdução de sinal, manutenção da arquitetura e plasticidade do sistema nervoso (Walmod et al., 2004). O SNP rs646558 já foi associado com Transtorno Bipolar (Atz et al., 2007; Arai et al., 2004) e também como modulador da heterogeneidade clínica do TDAH, afetando a susceptibilidade ao Transtorno Depressivo Maior em pacientes com TDAH (Mota et al., 2015).

As funções no cérebro da proteína codificada pelo gene *TTC12* (*Tetratricopeptide repeat 12*) ainda são pouco conhecidas, porém algumas características da proteína sugerem que ela possa estar relacionada com as funções dos genes *DRD2* e *NCAM1* na transmissão dopaminérgica e no neurodesenvolvimento (Yang et al., 2007). O polimorfismo rs2303380 tem sido consistentemente associado a comportamentos aditivos, com resultados implicando a variante na Dependência de Nicotina, Heroína e Transtorno de Jogo (Gelernter et al., 2006; Yang et al., 2008a; Nelson et al., 2013; Lobo et al., 2010; Bidwell et al., 2015).

O gene *ANKK1* (*Ankyrin repeat and kinase domains contaning 1*) dá origem a uma proteína envolvida no circuito de transdução de sinal neuronal e na ativação do fator de transcrição NF-kB (*Nuclear transcription factor kappa B*) (Huang et al., 2009). Uma variante nesse gene, o rs2734849, pode alterar a expressão de outro gene do complexo (*DRD2*) por meio de modificações no padrão de transcrição (Huang et al.,

2009). Esse polimorfismo provoca uma substituição de um aminoácido na proteína (p.Arg490Hist) na região de superfície de interações proteína-proteína, modificando a maquinaria de transdução do sinal e funcionalidade do NF-kB (Huang et al., 2009). Esse mesmo estudo demonstrou ainda que essa variante está associada com a Dependência de Nicotina.

Considerando os genes presentes neste *cluster*, o gene codificador do receptor de dopamina do tipo D2 (*DRD2)* é o mais avaliado em transtornos psiquiátricos. Esse é o receptor de dopamina mais amplamente expresso no cérebro (Alcantara et al., 2003), e alguns de seus marcadores, particularmente o rs1800497 – uma variante localizada no gene *ANKK1*, mas que está em forte desequilíbrio de ligação com polimorfismos do *DRD2* e foi inicialmente considerada como um marcador desse gene – foram amplamente estudados no TDAH. De acordo com meta-análises existem evidências de associação de diferentes polimorfismos do gene *DRD2* com o TDAH, porém com altos índices de heterogeneidade entre os estudos (Gizer et al., 2009). Nos últimos anos, outros polimorfismos localizados no gene, especialmente aqueles com efeitos funcionais já descritos, também têm sido alvo de investigações. Entre estes, o rs2283265 influencia no mecanismo de *splicing* alternativo e afeta a formação das duas isoformas do receptor, D2S e D2L. Esse SNP já foi associado com o TDAH em uma amostra de crianças com Transtorno do Espetro Autista (Gadow et al., 2014), além de estar envolvido na modulação do Transtorno Depressivo Maior em indivíduos adultos com TDAH independentemente do efeito de *NCAM1* previamente citado (Mota et al., 2015).

Recentemente, porém, o foco dos estudos genéticos tem se voltando para a análise mais ampla das variações em todo o *cluster* NTAD. Estudos apontam que os genes que compõem o *cluster* estão envolvidos, independentemente e/ou em conjunto, na etiologia do Transtorno por Uso de Substâncias (Gelernter et al., 2006; Yang et al., 2008b, 2007; Ducci et al., 2011; Nelson et al., 2013; Bidwell et al., 2015) e mais recentemente, como já descrito acima, um trabalho do nosso grupo demonstrou que variações do NTAD estão associadas também à heterogeneidade clínica do TDAH (Mota et al., 2015). Os quatro genes estão localizados dentro de um grande bloco de desequilíbrio de ligação, indicando que haplótipos funcionais dessa região não são

necessariamente restritos aos limites de genes específicos, reforçando a importância do estudo do NTAD como unidade funcional.

Interação *LPHN3* e cluster NTAD

Além do forte sinal na região da *LPHN3,* os estudos iniciais de ligação da genética do TDAH na comunidade do isolado populacional Paisa da Colômbia, reportaram outros sinais de ligação, como, por exemplo, no cromossomo 11 (Arcos-Burgos et al., 2004). A ocorrência de mais de um sinal cromossômico de ligação nessa população levantou a hipótese de que possíveis interações entre genes de diferentes cromossomos estariam afetando a susceptibilidade ao transtorno. De fato, um estudo subsequente relatou um efeito de interação entre uma variante do gene *LPHN3* (rs6551665) e um haplótipo localizado na região Chr: 11q22 englobando dois polimorfismos nos genes *NCAM1* e *TTC12* do complexo NTAD. A verificação inicial na comunidade Paisa reportou que a presença dos alelos de risco nas duas regiões (*LPHN3* e NTAD) mais que quadruplicava o risco de TDAH (Jain et al., 2012). Uma meta-análise envolvendo outras quatro populações da Europa e Estados Unidos da América corroborou esse resultado e sugere que a interação entre *LPHN3* e o NTAD duplica o risco de susceptibilidade ao TDAH, além de influenciar o perfil de resposta ao tratamento com estimulantes (Jain et al., 2012). O efeito dessa interação na susceptibilidade ao TDAH e na modulação do transtorno é um dos achados com o maior tamanho de efeito da genética psiquiátrica.

Subsequentemente, outros dois estudos apoiaram efeitos de interação entre o gene *LPHN3* e o complexo NTAD, expandindo tal associação para outros polimorfismos. Em uma análise exaustiva de interações entre pares de SNPs, cinco interações entre diferentes variantes dos genes da *LPHN3* e do cluster NTAD mostraram estar associados com a sintomatologia e a gravidade do transtorno (Acosta et al., 2011). Ainda, um estudo independente com uma amostra de TDAH em crianças reportou a associação de uma interação entre um haplótipo contendo três SNPs da *LPHN3* e uma variante do gene *NCAM1* com a susceptibilidade ao TDAH (Bruxel et al., 2015).

Em conjunto, esses dados sugerem um papel importante de interações entre diferentes variantes genéticas dos genes da *LPHN3* e dos componentes do complexo

NTAD na susceptibilidade ao TDAH, na modulação da gravidade do transtorno e também na resposta à terapia farmacológica de primeira escolha.

Justificativa

Indivíduos com transtornos psiquiátricos muitas vezes sofrem com o estigma, a discriminação, a violação de seus direitos e dificuldades financeiras relacionadas ao alto custo do tratamento e, frequentemente, à impossibilidade de continuar ativamente no mercado de trabalho. Os prejuízos causados por esses transtornos excedem o âmbito individual e comprometem também a família e a sociedade em geral.

Apesar da alta herdabilidade dos transtornos psiquiátricos, a identificação dos genes e variantes genéticas específicas associadas ao desenvolvimento dos transtornos é de grande dificuldade. No caso do TDAH, por exemplo, a alta complexidade fenotípica e genotípica dificulta ainda mais tal tarefa, e o pequeno número de estudos em adultos abre um nicho para pesquisas acerca de outros fatores genéticos envolvidos no TDAH nesta faixa etária. Além disso, o conjunto de evidências quanto ao papel de fatores genéticos não-aditivos na herdabilidade do TDAH, somado a ampla rede de interações moleculares no ambiente celular, sugerem uma perspectiva promissora para estudos de interações envolvendo genes implicados na etiologia do TDAH.

Objetivos

Objetivo geral

Avaliar o efeito de um polimorfismo no gene *LPHN3* previamente implicado no TDAH, bem como de sua interação com variantes do *cluster* gênico NTAD na susceptibilidade e heterogeneidade fenotípica do TDAH.

Objetivos específicos

1. Avaliar o papel da variante rs6551665 do gene *LPHN3* na susceptibilidade e apresentação dos sintomas do TDAH.
2. Avaliar o papel da interação entre variantes do gene *LPHN3* e do *cluster* gênico NTAD no diagnóstico e sintomatologia do TDAH.
3. Explorar os efeitos do gene *LPHN3*, tanto individualmente quanto em interação com o complexo NTAD, em relação aos domínios de sintomas clássicos e subtipos correspondentes de TDAH.

Capítulo III – **Artigo Científico**

Artigo em preparação:

Interaction effects between *LPHN3* gene and NTAD gene cluster on ADHD susceptibility, symptoms, and presentation stability in adults

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Abstract

Attention-Deficit/Hyperactivity Disorder (ADHD) is a common and highly heritable neuropsychiatric disorder. Even with high heritability, there is considerable genetic complexity hampering the unraveling of specific genetic factors related to ADHD. Recent evidence suggests that gene-gene interactions can explain part of this genetic complexity. Here, we have examined the impact of interaction effects between a *LPHN3* variant, previously associated with ADHD (rs6551665), and seven SNPs spanning the NTAD gene cluster (*NCAM1-TTC12-ANKK1-DRD2*) on ADHD susceptibility and symptomatology for the first time in adults. The sample comprised 548 adults with ADHD and 643 controls. Entropy-based analysis indicated a potential interaction between the *LPHN3*-rs6551665 and *TTC12*-rs2303380 SNPs influencing ADHD symptom counts. Further analysis revealed significant interaction effects on ADHD total symptoms (p=0.002), and with hyperactivity/impulsivity symptoms counts (p=0.005). We observed that the presence of *LPHN3*-rs6551665 G allele was associated to increased ADHD risk only in individuals carrying the *TTC12*-rs2303380 AA genotype (p=0.026) in the group composed by predominantly hyperactive/impulsive and combined presentations. Moreover, the same allelic constellation is involved in maintenance of ADHD in a predominantly hyperactive/impulsive or combined presentation after a 7-year follow-up (p=0.008). These observations reinforce and replicate previous evidence suggesting that an interaction effect between the *LPHN3* gene and the NTAD gene cluster may have a role in the genetic substrate associated to the complex etiology of ADHD in adults. Moreover, we suggest that the gene-gene interactions between *LPHN3* and NTAD are specific factors contributing to the development of a refined phenotype with higher presence of hyperactive/impulsive symptoms that is maintained throughout adulthood.

Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a common and highly heritable neuropsychiatric disorder, characterized by an impairing pattern of behavior resulting in issues in social, educational or work-related settings[1]. ADHD seems to result from the combination of effects from several genes, the interactions between them, and the interplay with the environment[2]. Genome-wide association studies (GWAS) so far have shown that common genetic variability is responsible for approximately 25% of the variance in ADHD case-control status[3], but they are still far away from explaining the approximate 80% heritability of the disorder[4,5]. Moreover, the high genetic heterogeneity of psychiatric disorders challenges the identification of the specific genetic factors related to their etiology and at the present moment few genome-wide significant hits were reported for ADHD[6–8]. Contributing to the discrepancy between estimated heritability and explained heritability for the disorder is the presence of interactions between genetic factors, which have been neglected in most studies[9,10]. Recently, data from a large meta-analysis of heritability estimates suggested that ADHD can be depicted as an exception to the purely additive heritability model, and considered as a psychiatric disorder for which non-additive genetic factors are suggested[11]. Such role of non-additive genetic factors on ADHD is in agreement with previous important findings involving gene-gene interactions on the etiology of the disorder[12,13].

One notable example of non-additive genetic effects on ADHD susceptibility refers to the interaction between the *LPHN3* gene and a haplotype spanning the *NCAM1*, *TTC12*, *ANKK1* and *DRD2* genes, a region known as the NTAD gene cluster[13]. The presence of both *LPHN3* risk allele and NTAD risk haplotype increased in more than two-fold the risk of ADHD and influenced the response to treatment with methylphenidate[13]. Afterwards, it was also shown that interactions involving *LPHN3* and the NTAD cluster were involved in predicting symptom severity in patients with the disorder[12], and its effect on ADHD susceptibility was replicated in an independent sample[14].

Interestingly, both *loci* have outstanding support for their involvement in psychiatry, either from linkage studies implicating *LPHN3* and NTAD in ADHD[15],

candidate gene association studies repeatedly implicating the *LPHN3* gene in ADHD[14,16–21] or relating the NTAD cluster to Substance Use Disorder [22–29], and even GWAS, where the *DRD2* (a member of the NTAD cluster), one of the most traditional candidates in psychiatric genetics, corresponds to one of the significant hits in the latest Schizophrenia GWAS meta-analysis[30].

Thus, considering that previous studies showing an interaction effect of *LPHN3* and NTAD SNPs on ADHD susceptibility[12–14] were conducted in either a sample from a genetically isolated population or samples of children, the goal of our study was to further elucidate the extent of *LPHN3*-NTAD interaction effects on ADHD by trying to replicate such interaction in a sample of adults. For this, we examined ADHD susceptibility and symptomatology, attempting to explore if such interaction effects are specific for or stronger on a particular ADHD symptom domain, as well as if these effects are stable throughout adulthood.

Materials and Methods

Study participants

The ADHD sample comprises 548 adults recruited from the Adult Division of the ADHD Outpatient Program at the Hospital de Clínicas de Porto Alegre (HCPA). Diagnostic procedures followed DSM-IV criteria for ADHD[31] and were carried out by trained psychiatrists through application of clinical and semi-structured interviews, as the Portuguese version of the Schedule for Affective Disorders and Schizophrenia for School-Age Children–Epidemiologic version (K-SADS-E adapted to the adult life context)[32]. Individuals included in this study were 18 years or older and fulfilled ADHD diagnosis both currently and during childhood (retrospectively). Regarding ethnicity, all individuals are white Brazilians of primarily European descent. Individuals were excluded if they presented clinically significant history of neurological disease (e.g., delirium, dementia, epilepsy, head trauma, multiple sclerosis), past or present symptoms of psychosis and/or an estimated IQ <70. Detailed sample characteristics are presented in Table 1.

A subset of the ADHD sample was reassessed in a 7-year clinical follow-up of ADHD in adulthood, and a full description of demographic and clinical variables of this

study is published elsewhere[33]. Clinical ADHD presentation for those with persistent ADHD after 7-year follow-up was available for 148 subjects.

A sample of 643 adults with negative screening scores for ADHD in the Adult ADHD Self-Rated Scale[34] (ASRS) composed our control group. They were recruited at the blood donation center of the same hospital where cases were ascertained. The inclusion and exclusion criteria were similar to the ADHD group, with the exception of lack of DSM-IV ADHD diagnosis (Table 1).

This work was carried out in accordance with the Declaration of Helsinki and all participants signed an informed consent form approved by the Ethics Committee of HCPA (Institutional Review Board number 0000921).

Genotyping and SNP selection

We selected polymorphisms that were previously implicated in ADHD susceptibility, symptoms severity, clinical heterogeneity and/or had evidence of functionality, and that had a minor allele frequency >15% and commercially validated TaqMan assays. The studied genetic polymorphisms were rs6551665, considered one of the most exquisite SNPs at the *LPHN3* gene based on previous meta-analyses[17], and seven SNPs spanning the NTAD gene cluster, as described elsewhere[35]: rs646558 (*NCAM1*), rs723077 and rs2303380 (*TTC12*), rs2734849 and rs1800497 (*ANKK1*), rs6277 and rs2283265 (*DRD2*). All SNPs were genotyped by TaqMan allelic discrimination assays according to manufacturer's specifications (Applied Biosystems). Genotypes were grouped in dominant models according to minor allele frequencies in order to minimize the number of cells with insufficient sample size.

Statistical analyses

Firstly, we adopted a model-free approach using entropy-based analysis implemented in MDR v3.0.2[36] to select potential interactions contributing to the examined phenotypes from all the possible pairwise combinations between SNPs from the *LPHN3* gene and the NTAD gene cluster. From these analyses, potential pairwise connections can be selected and further explored with appropriate adjustments for covariates (defined as described below). We examined the contribution of potential

pairwise connections to ADHD susceptibility and also undertook a dimensional approach by examining their effects on ADHD symptom counts within ADHD cases.

Based on the overall results from the entropy-based analyses described above, we then performed Analysis of Covariance (ANCOVA) to evaluate the effect of the appointed potential interactions on total ADHD symptom counts as well as on inattention and hyperactivity/impulsivity domains separately. Variables such as sex, age and common comorbid psychiatric disorders (i.e. Major Depressive Disorder, Generalized Anxiety Disorder, Bipolar Disorder, Oppositional Defiant Disorder and Substance Use Disorder) were considered as possible covariates and included in the model when associated at a level of $p<0.2$ with both the outcome and at least one of the tested SNPs. As a third step, logistic regression analyses were performed to assess the effect of significant SNP-SNP interactions on ADHD susceptibility subdividing the cases sample in individuals with high (i.e. those of predominantly hyperactive/impulsive and combined presentations) and with low (i.e. those of predominantly inattentive presentation) hyperactivity/impulsivity symptom scores.

Lastly, considering recent evidence implicating variants in the *LPHN3* gene in a refined phenotype with stability of ADHD presentation from childhood to adolescence[16], we evaluated the effects of significant interactions regarding ADHD presentation on a 7-year follow-up with Fisher's Exact Test.

Although not in the original scope of this study, main effects for the *LPHN3*-rs6551665 regarding ADHD susceptibility in this sample have been tested in order to enable the inclusion of these results in future meta-analyses. Main effects regarding ADHD symptomatology can be found in Table 2, and further detailed results are presented in Supplementary Table 1. The effects of NTAD cluster SNPs in this sample have been previously reported[35].

Results

Entropy-based *LPHN3*-NTAD interaction analyses: ADHD symptoms and diagnosis in adults

We conducted a model-free entropy-based analysis in order to assess the effects of previously reported *LPHN3*-NTAD interactions on ADHD susceptibility in adults. The

Kamada-Kawai graph did not clearly suggest specific potential interactions to be taken further in-depth when evaluating case-control status in our sample (Supplementary Figure 1-A). Analysis of ADHD symptom counts within cases, however, indicated a potential interaction between the evaluated *LPHN3* SNP and one of the *TTC12* SNPs analyzed, with an increased gain of information when evaluating both polymorphisms jointly (Supplementary Figure 1-B). Independently, *LPHN3*-rs6551665 and *TTC12*-rs2303380 would account for, respectively, 0.19% and 0.08% of the observed outcome variance, while the two SNPs cooperatively explain 1.09% of the ADHD total symptom count variance. Other interactions did not show any greater information gain.

***LPHN3*-rs6551665/*TTC12*-rs2303380 interaction**

Following the above interaction analyses, and in order to further understand the effects of the *LPHN3*-rs6551665/*TTC12*-rs2303380 interaction, ANCOVA was used to evaluate its effects for the total ADHD symptom count, as well as for inattention and hyperactivity/impulsivity symptoms separately. Confounders for these analyses were presence of current Oppositional Defiant Disorder and Bipolar Disorder. The *LPHN3*-rs6551665/*TTC12*-rs2303380 interaction was significantly associated with ADHD total symptom count (p=0.002), and with hyperactivity/impulsivity symptoms (p=0.005), while the same was not observed with the inattention symptoms measure (p=0.257) (Table 2). The post-hoc analysis showed that, when compared to the reference allelic constellation (*LPHN3*-rs6551665 AA/*TTC12*-rs2303380 AA), the presence of *LPHN3*-rs6551665 G allele was associated to increased ADHD total symptoms in individuals with the *TTC12*-rs2303380 AA genotype (p=0.004) but not in individuals carrying the *TTC12*-rs2303380 G allele (p=0.587) (Figure1-A). In order to further validate this finding as an interaction effect we compared ADHD total symptoms scores between the two groups of *LPHN3*-rs6551665 G allele carriers (i.e. *TTC12*-rs2303380 AA and *TTC12*-rs2303380 G carriers; represented by red dots in Figure 1-A), and found a significant difference between these two sets (p=0.002, not shown). These results suggest that the *LPHN3*-rs6551665 G carriers/*TTC12*-rs2303380 AA represents a distinct group from those formed by either the alternative *LPHN3* genotype (reference allelic constellation) or the alternative *TTC12* genotype (*LPHN3*-rs6551665 G carrier/*TTC12*-rs2303380 G carriers) groups regarding ADHD total symptoms. A similar effect was observed when

only hyperactive/impulsive symptoms were considered (Figure 1-B); while no interaction effects were found when exclusively inattention symptoms were evaluated (Figure 1-C).

ADHD susceptibility in specific presentations

Since the ANCOVA analyses showed a significant effect on ADHD symptomatology, which seems to be mostly related to the hyperactivity/impulsivity symptoms domain, we performed logistic regression in order to test the interaction on ADHD susceptibility in adults separately in those with combined or hyperactive/impulsive ADHD presentation and in those with inattentive ADHD presentation. Case-control analyses performed in the predominantly hyperactive/impulsive and combined group showed that there was an interaction effect, where the presence of *LPHN3*-rs6551665 G allele was associated to increased ADHD risk in individuals carrying the *TTC12*-rs2303380 AA genotype (OR ($CI_{95\%}$)= 1.664 (1.062-2.606); p=0.026), while there was no effect in individuals caring the *TTC12*-rs2303380 G allele (OR ($CI_{95\%}$)= 1.169 (0.755-1.810); p=0.484) (Figure 1-D). Confirming the interaction effect between the two groups of *LPHN3*-rs6551665 G allele carriers (*TTC12*-rs2303380 AA and *TTC12*-rs2303380 G carriers), there was a significant difference between these two sets (p=0.045, not shown). The observed *LPHN3* effect shows a similar pattern to the one observed for the total ADHD and hyperactivity/impulsivity symptom measures. Such *LPHN3*/*TTC12* interaction effect was not observed in the analysis regarding predominantly inattentive individuals (Figure 1-E), which is also in agreement with the lack of effect observed for the inattention symptoms measure.

ADHD presentation in a clinical follow-up

We performed Fisher's Exact Test in order to assess if the *LPHN3*/*TTC12* interaction was also associated to stability of ADHD presentation during adulthood in a 7-year clinical follow-up. Given the reduced sample size with available follow-up data we tested the influence of the established risk allelic constellation (*LPHN3*-rs6551665 G+/*TTC12*-rs2303380 AA) against the other allelic combinations. Cross-tabulation demonstrated that presence of the risk allelic constellation (*LPHN3*-rs6551665

G+/*TTC12*-rs2303380 AA) was significantly associated to stability of ADHD presentation (p=0.008). There was an excess of expected subjects retaining diagnosis as combined or predominantly hyperactive/impulsive ADHD after follow-up, while subjects retaining diagnosis as inattentive presentation were reduced (Table 3). Taking into account that ADHD subjects with a combined presentation report more symptoms, and this is one of the predicting factors of ADHD persistence[33,37], we also performed this analyses adjusting for a possible confounding effect of total symptom counts. The inclusion of such covariate did not result in major changes in the results.

Discussion

This study replicates previous evidence on the presence of interaction effects between *LPHN3* and NTAD markers on ADHD susceptibility, expanding this finding for the first time to a sample of adults. Furthermore, the present study shows that such effect is restricted to hyperactivity/impulsivity symptoms within ADHD cases and, consequently, to ADHD susceptibility in hyperactive/impulsive or combined presentations only. We also provide additional evidence that this gene-gene interaction is involved in the persistence of ADHD hyperactive/impulsive or combined presentation throughout adulthood. Overall, these findings suggest that the *LPHN3*/NTAD may be involved in the underpinnings of ADHD susceptibility.

In this context, we observed that regarding hyperactivity/impulsivity symptoms and ADHD susceptibility in presentations with clear presence of hyperactivity/impulsivity a *LPHN3*/*TTC12* interaction is able to modulate the *LPHN3* variant effects, where the risk effect of *LPHN3*-rs6551665 G allele is only observable in individuals with *TTC12*-rs2303380 AA genotype, being abolished in the presence of *TTC12*-rs2303380 G allele. Meanwhile, no significant interaction effect was observed on inattention symptom counts in ADHD cases nor on ADHD susceptibility when individuals with predominantly inattentive presentation were analyzed separately.

Multiple studies have shown main effects of *LPHN3* variants on ADHD susceptibility, most of them regarding childhood ADHD[14,17–21], providing new insights into the genetics, neurobiology and treatment of ADHD. Similar to what we observed here with the *LPHN3*-*TTC12* interaction, some of these studies have also revealed that

the genetic association between *LPHN3* and ADHD is either driven by, or present only in samples of ADHD with higher presence of hyperactivity/impulsivity[19,21].

Also, a recent longitudinal study was able to show that a *LPHN3* polymorphism is associated with a specific ADHD phenotype characterized by maintenance of ADHD diagnosis with a combined presentation from childhood into adolescence[16]. Similarly, here we report that the presence of the risk allelic constellation (*LPHN3*-rs6551665 G+/*TTC12*-rs2303380 AA) was associated to maintenance of ADHD presentation in the hyperactive/impulsive or combined form throughout adulthood in a 7-year follow-up. Interestingly, most children with ADHD have clinical characteristics of high hyperactivity/impulsivity symptomatology, but the persistence of these symptoms into adulthood seems to be less common than of the inattention symptoms[38]. Nevertheless, adults with ADHD that show increased symptomatology on the hyperactivity/impulsivity domain refer higher presence of other psychiatric comorbidities[39], especially increased risk of Substance Use Disorder[40]. These results implicate the *LPHN3/TTC12* interaction as being involved in the development and persistence of a more externalizing form of ADHD.

The observed effect of *LPHN3* and its interactions with the NTAD cluster in regulating hyperactivity/impulsivity is further supported by studies with different animal models. Animals submitted to either knockout or knockdown of *LPHN3* orthologues were behaviorally characterized as having increased locomotor activity, which is comparable to the hyperactivity observed in individuals with ADHD[41–45]. The behavioral hyperactivity in Drosophila was considered to be derived from abnormal dopamine-mediated signaling[44]. In zebrafish dopaminergic neurons were disorganized and in smaller number[41,43]. Additionally, *Lphn3* null mice presented increased levels of dopamine in the striatum and increased expression of dopaminergic receptors (including *Drd2*), and other related genes, such as *Ncam,* another member of the NTAD cluster[45]. Together, these findings suggest that the *LPHN3* gene is implicated in regulating the development of the dopaminergic system.

Considering the central role of the dopaminergic system in the neurobiology of ADHD[46–49], particularly in the subcortical mechanisms related to motor responses, and the effects of LPHN3 in the development and regulation of dopaminergic signaling, it seems likely that the influence of the interaction between *LPHN3* and *TTC12* reported

here can be related to changes in the subcortical dopaminergic pathways, as in the mesolimbic system. This hypothesis is interesting because it also enhances the neurodevelopmental aspects of ADHD, demonstrating that the effects of genetic variants involved in modifying the development of the central nervous system can also be detected and have persistent effects in adult subjects with the disorder.

A screening of potential mutations in the *LPHN3* gene in patients with ADHD revealed that its effect is most likely caused by partial reduction of gene activity, and not by a complete loss of function[50]. Therefore, we could hypothesize that the *LPHN3* variation studied herein - rs6551665 -, which has been associated to ADHD in several samples[17,18,20], figures as a potential causal variant when it comes to *LPHN3* role in ADHD. Alternatively, it is also possible that we are, in fact, capturing the by-proxy effect of another functional variant in linkage disequilibrium.

Overall, the preponderance of the evidence supports the association between *LPHN3* and ADHD; however, the *TTC12* effects are much less clear. Jain et al.[13] demonstrated that an 11q haplotype, spanning the NTAD cluster, despite showing a significant interaction effect with *LPHN3*, does not exhibit main effects on ADHD susceptibility by itself. Similarly, a previous study performed on our sample did not reveal main effects of NTAD cluster SNPs on ADHD susceptibility, including the *TTC12* SNPs evaluated here[35]. In spite of the fact that *TTC12* gene function remains unclear, its properties and location within the NTAD gene cluster suggest it to be involved in cell adhesion, neurogenesis and modulation of dopaminergic transmission[28,35,51]. Thus, it is possible that the *TTC12*-rs2303380 variant is involved in *TTC12*-driven modulations of the dopaminergic system, that when interacting with *LPHN3*-rs6551665 can either counter-act or enhance the effects of the later.

Our findings should be understood in the context of some limitations. First, although our sample size of adults with ADHD is moderate, the expected effect size for these interactions is small. Thus, it might be still premature to completely rule out the possibility of false-negative findings regarding other possible *LPHN3*-NTAD interactions. On the other hand, this is the first replication study in an adult sample but considering the investigations in other independent samples also providing evidence in the same direction, decreases the chances of false-positive findings. Additionally, one strength of our approach is that we departed from a model-free entropy-based

method to screen for the potential interactions, and narrowed down the area of association derived from the original 11q haplotype[13]. Finally, the exact biological process that is depicted by this interaction is still unknown.

In conclusion, our results further corroborate to understand *LPHN3* role in ADHD and help to elucidate its interactions with the NTAD gene cluster as a genetic substrate to ADHD, extending previous findings in children to adults. Moreover, we suggest that the gene-gene interactions modulating *LPHN3* effects are specific factors contributing to the development of a more refined ADHD phenotype, with increased and persistent hyperactivity/impulsivity. Our study sheds light on the biological underpinnings of ADHD and also shows that our current understanding on the functional roles of *LPHN3* and *TTC12*, and their interaction on ADHD susceptibility is still not complete.

Conflict of Interest

The author(s) declare the following potential conflict of interest with respect to the research, authorship and/or publication of this article: Dr. Grevet was on the speaker's bureau for Novartis and Shire for the last 3 years. He also received travel awards (air tickets and hotel accommodations) for participating in two psychiatric meetings from Shire and Novartis. Dr. Rohde has received Honoraria, has been on the speakers' bureau/advisory board and/or has acted as a consultant for Eli-Lilly, Janssen-Cilag, Novartis and Shire in the last three years. He receives authorship royalties from Oxford Press and ArtMed. He also received travel awards for taking part of 2014 APA and 2015 WFADHD meetings from Shire. The ADHD and Juvenile Bipolar Disorder Outpatient Programs chaired by him received unrestricted educational and research support from the following pharmaceutical companies in the last three years: Eli-Lilly, Janssen-Cilag, Novartis, and Shire. All other authors report no biomedical financial interests or potential conflicts of interest.

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References

1 APA. Diagnostic and statistical manual of mental disorders (DSM 5). Am. Psychiatr. Assoc. 2013.

2 Stergiakouli E, Thapar A. Fitting the pieces together: current research on the genetic basis of attention-deficit/hyperactivity disorder (ADHD). *Neuropsychiatr Dis Treat* 2010; **6**: 551–560.

3 Cross-Disorder Group of the Psychiatric Genetics Consortium. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. 2013; **381**: 1371–1379.

4 Faraone S V, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA *et al.* Molecular genetics of attention-deficit/hyperactivity disorder. *Biol Psychiatry* 2005; **57**: 1313–1323.

5 Chang Z, Lichtenstein P, Asherson PJ, Larsson H. Developmental twin study of attention problems: high heritabilities throughout development. *JAMA Psychiatry* 2013; **70**: 311–318.

6 Neale BM, Medland SE, Ripke S, Asherson P, Franke B, Lesch K-P *et al.* Meta-analysis of genome-wide association studies of attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 2010; **49**: 884–97.

7 Zayats T, Athanasiu L, Sonderby I, Djurovic S, Westlye LT, Tamnes CK *et al.* Genome-Wide Analysis of Attention Deficit Hyperactivity Disorder in Norway. *PLoS One* 2015; **10**: e0122501.

8 Zai G, Alberry B, Arloth J, Bánlaki Z, Bares C, Boot E *et al.* Rapporteur summaries of plenary, symposia, and oral sessions from the XXIIIrd World Congress of Psychiatric Genetics Meeting in Toronto, Canada, 16-20 October 2015. *Psychiatr Genet* 2016. doi:10.1097/YPG.0000000000000148.

9 Rovaris DL, Mota NR, Callegari-Jacques SM, Bau CHD. Approaching 'phantom heritability' in psychiatry by hypothesis-driven gene-gene interactions. *Front Hum Neurosci* 2013; **7**: 210.

10 Zuk O, Hechter E, Sunyaev SR, Lander ES. The mystery of missing heritability: Genetic interactions create phantom heritability. *Proc Natl Acad Sci U S A* 2012; **109**: 1193–8.

11 Polderman TJC, Benyamin B, de Leeuw CA, Sullivan PF, van Bochoven A, Visscher PM *et al.* Meta-analysis of the heritability of human traits based on fifty years of twin studies. *Nat Genet* 2015; **47**: 702–9.

12 Acosta MT, Vélez JI, Bustamante ML, Balog JZ, Arcos-Burgos M, Muenke M. A two-locus genetic interaction between LPHN3 and 11q predicts ADHD severity and long-term outcome. *Transl Psychiatry* 2011; **1**: e17.

13 Jain M, Velez J, Acosta MT, Palacio LG, Vélez JI, Balog J *et al.* A cooperative interaction between LPHN3 and 11q doubles the risk for ADHD. *Mol Psychiatry* 2012; **17**: 741–747.

14 Bruxel EM, Salatino-Oliveira A, Akutagava-Martins GC, Tovo-Rodrigues L, Genro JP, Zeni CP *et al.* LPHN3 and attention-deficit/hyperactivity disorder: A susceptibility and pharmacogenetic study. *Genes, Brain Behav* 2015; **14**: 419–427.

15 Arcos-Burgos M, Castellanos FX, Pineda D, Lopera F, Palacio JD, Palacio LG *et al.* Attention-deficit/hyperactivity disorder in a population isolate: linkage to loci at 4q13.2, 5q33.3, 11q22, and 17p11. *Am J Hum Genet* 2004; **75**: 998–1014.

16 Acosta MT, Swanson J, Stehli A, Molina BSG, Martinez AF, Arcos-Burgos M *et al. ADGRL3 (LPHN3)* variants are associated with a refined phenotype of ADHD in the MTA study. *Mol Genet Genomic Med* 2016; **3**: 1–8.

17 Arcos-Burgos M, Jain M, Acosta MT, Shively S, Stanescu H, Wallis D *et al.* A common variant of the latrophilin 3 gene, LPHN3, confers susceptibility to ADHD and predicts effectiveness of stimulant medication. *Mol Psychiatry* 2010; **15**: 1053–1066.

18 Choudhry Z, Sengupta SM, Grizenko N, Fortier ME, Thakur GA, Bellingham J *et al.* LPHN3 and attention-deficit/hyperactivity disorder: Interaction with

maternal stress during pregnancy. *J Child Psychol Psychiatry Allied Discip* 2012; **53**: 892–902.

19 Gomez-Sanchez CI, Riveiro-Alvarez R, Soto-Insuga V, Rodrigo M, Tirado-Requero P, Mahillo-Fernandez I *et al.* Attention deficit hyperactivity disorder: genetic association study in a cohort of Spanish children. *Behav Brain Funct* 2016; **12**: 2.

20 Hwang IW, Lim MH, Kwon HJ, Jin HJ. Association of LPHN3 rs6551665 A/G polymorphism with attention deficit and hyperactivity disorder in Korean children. *Gene* 2015; **566**: 68–73.

21 Ribasés M, Ramos-Quiroga JA, Sánchez-Mora C, Bosch R, Richarte V, Palomar G *et al.* Contribution of LPHN3 to the genetic susceptibility to ADHD in adulthood: A replication study. *Genes, Brain Behav* 2011; **10**: 149–157.

22 Bidwell LC, McGeary JE, Gray JC, Palmer RHC, Knopik VS, MacKillop J. An initial investigation of associations between dopamine-linked genetic variation and smoking motives in African Americans. *Pharmacol Biochem Behav* 2015; **138**: 104–10.

23 David SP, Mezuk B, Zandi PP, Strong D, Anthony JC, Niaura R *et al.* Sex differences in TTC12/ANKK1 haplotype associations with daily tobacco smoking in Black and White Americans. *Nicotine Tob Res* 2010; **12**: 251–62.

24 Ducci F, Kaakinen M, Pouta A, Hartikainen A-L, Veijola J, Isohanni M *et al.* TTC12-ANKK1-DRD2 and CHRNA5-CHRNA3-CHRNB4 influence different pathways leading to smoking behavior from adolescence to mid-adulthood. *Biol Psychiatry* 2011; **69**: 650–60.

25 Gelernter J, Yu Y, Weiss R, Brady K, Panhuysen C, Yang B-Z *et al.* Haplotype spanning TTC12 and ANKK1, flanked by the DRD2 and NCAM1 loci, is strongly associated to nicotine dependence in two distinct American populations. *Hum Mol Genet* 2006; **15**: 3498–507.

26 Lobo DSS, Zawertailo L, Selby P, Kennedy JL. The role of ANKK1 and TTC12 genes on drinking behaviour in tobacco dependent subjects. *World J Biol Psychiatry*

2012; **13**: 232–8.

27 Nelson EC, Lynskey MT, Heath AC, Wray N, Agrawal A, Shand FL *et al.* ANKK1, TTC12, and NCAM1 polymorphisms and heroin dependence: importance of considering drug exposure. *JAMA psychiatry* 2013; **70**: 325–33.

28 Yang B-Z, Kranzler HR, Zhao H, Gruen JR, Luo X, Gelernter J. Association of haplotypic variants in DRD2, ANKK1, TTC12 and NCAM1 to alcohol dependence in independent case control and family samples. *Hum Mol Genet* 2007; **16**: 2844–53.

29 Yang B-Z, Kranzler HR, Zhao H, Gruen JR, Luo X, Gelernter J. Haplotypic variants in DRD2, ANKK1, TTC12, and NCAM1 are associated with comorbid alcohol and drug dependence. *Alcohol Clin Exp Res* 2008; **32**: 2117–27.

30 Schizophrenia Working Group of the Psychiatric Genomics C. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 2014; **511**: 421–427.

31 APA. Diagnostic and statistical manual of mental disorders (DSM-IV). Am. Psychiatr. Assoc. 1994.

32 Grevet EH, Bau CHD, Salgado CAI, Ficher A, Victor MM, Garcia C *et al.* Interrater reliability for diagnosis in adults of attention deficit hyperactivity disorder and oppositional defiant disorder using K-SADS-E. *Arq Neuropsiquiatr* 2005; **63**: 307–10.

33 Karam RG, Breda V, Picon FA, Rovaris DL, Victor MM, Salgado CAI *et al.* Persistence and remission of ADHD during adulthood: a 7-year clinical follow-up study. *Psychol Med* 2015; **45**: 2045–56.

34 Kessler RC, Adler L, Ames M, Demler O, Faraone S, Hiripi E *et al.* The World Health Organization Adult ADHD Self-Report Scale (ASRS): a short screening scale for use in the general population. *Psychol Med* 2005; **35**: 245–256.

35 Mota NR, Rovaris DL, Kappel DB, Picon FA, Vitola ES, Salgado CAI *et al.* NCAM1-TTC12-ANKK1-DRD2 gene cluster and the clinical and genetic heterogeneity of adults with ADHD. *Am J Med Genet Part B Neuropsychiatr Genet* 2015; **168**.

doi:10.1002/ajmg.b.32317.

36 Moore JH, Andrews PC. Epistasis analysis using multifactor dimensionality reduction. *Methods Mol Biol* 2015; **1253**: 301–14.

37 Victor MM, Rovaris DL, Salgado CAI, Silva KL, Karam RG, Vitola ES *et al.* Severity But Not Comorbidities Predicts Response to Methylphenidate in Adults With Attention-Deficit/Hyperactivity Disorder. *J Clin Psychopharmacol* 2014; **34**: 212–217.

38 Biederman J, Mick E, Faraone S V. Age-dependent decline of symptoms of attention deficit hyperactivity disorder: impact of remission definition and symptom type. *Am J Psychiatry* 2000; **157**: 816–8.

39 Cadman T, Findon J, Eklund H, Hayward H, Howley D, Cheung C *et al.* Six-year follow-up study of combined type ADHD from childhood to young adulthood: Predictors of functional impairment and comorbid symptoms. *Eur Psychiatry* 2016; **35**: 47–54.

40 Liebrenz M, Gamma A, Ivanov I, Buadze A, Eich D. Adult attention-deficit/hyperactivity disorder: Associations between subtype and lifetime substance use – a clinical study. *F1000Research* 2015; : 1–9.

41 Lange M, Norton W, Coolen M, Chaminade M, Merker S, Proft F *et al.* The ADHD-susceptibility gene lphn3.1 modulates dopaminergic neuron formation and locomotor activity during zebrafish development. *Mol Psychiatry* 2012; **17**: 946–954.

42 Orsini CA, Setlow B, DeJesus M, Galaviz S, Loesch K, Ioerger T *et al.* Behavioral and transcriptomic profiling of mice null for Lphn3, a gene implicated in ADHD and addiction. *Mol Genet genomic Med* 2016; **4**: 322–43.

43 Reuter I, Knaup S, Romanos M, Lesch K-P, Drepper C, Lillesaar C. Developmental exposure to acetaminophen does not induce hyperactivity in zebrafish larvae. *J Neural Transm* 2016. doi:10.1007/s00702-016-1556-z.

44 van der Voet M, Harich B, Franke B, Schenck A. ADHD-associated dopamine

transporter, latrophilin and neurofibromin share a dopamine-related locomotor signature in Drosophila. *Mol Psychiatry* 2016; **21**: 565–73.

45 Wallis D, Hill DS, Mendez IA, Abbott LC, Finnell RH, Wellman PJ *et al.* Initial characterization of mice null for Lphn3, a gene implicated in ADHD and addiction. *Brain Res* 2012; **1463**: 85–92.

46 Faraone S V., Asherson P, Banaschewski T, Biederman J, Buitelaar JK, Ramos-Quiroga JA *et al.* Attention-deficit/hyperactivity disorder. *Nat Rev Dis Prim* 2015; **1**: 15020.

47 Arnsten AFT. Toward a New Understanding of Attention-Deficit Hyperactivity Disorder Pathophysiology An Important Role for Prefrontal Cortex Dysfunction. 2009; : 33–41.

48 Genro JP, Kieling C, Rohde LA, Hutz MH. Attention-deficit/hyperactivity disorder and the dopaminergic hypotheses. *Expert Rev Neurother* 2010; **10**: 587–601.

49 Purper-Ouakil D, Ramoz N, Lepagnol-Bestel, Aude-Marie Gorwood P, Simonneau M. Neurobiology of attention deficit/hyperactivity disorder. *Pediatr Res* 2011; **69**: 69–76.

50 Domené S, Stanescu H, Wallis D, Tinloy B, Pineda DE, Kleta R *et al.* Screening of human LPHN3 for variants with a potential impact on ADHD susceptibility. *Am J Med Genet Part B Neuropsychiatr Genet* 2011; **156**: 11–18.

51 Katoh M, Katoh M. Identification and characterization of TPARM gene in silico. *Int J Oncol* 2003; **23**: 1213–7.

Table 1: Participants demographic and psychiatric characteristics

	ADHD (n = 548)	Controls (n = 643)
	Mean (SD)	Mean (SD)
Age (years)	33.63 (10.80)	29.15 (8.66)
Total ADHD symptoms[a]	12.94 (2.99)	-
Hyperactivity/impulsivity symptoms[a]	5.44 (2.65)	-
Inattention symptoms[a]	7.50 (1.36)	-
	n (%)	n (%)
Gender (male)	303 (55.3)	316 (49.1)
Predominantly inattentive presentation	238 (43.4)	-
Combined presentation	280 (51.1)	-
Predominantly hyperactive/impulsive presentation	30 (5.5)	-

ADHD: Attention Deficit/Hyperactivity Disorder; SD: standard deviation
[a] ADHD symptoms evaluated through K-SADS-E questionnaire

Table 2: Analyses of Covariance regarding *LPHN3*-rs6551665/*TTC12*-rs2303380 effects and interaction on ADHD symptom counts

	LPHN3-rs6551665[a]		*TTC12*-rs2303380[b]		*LPHN3*TTC12*[c]	
	$F_{(1,545)}$	p-value	$F_{(1, 541)}$	p-value	$F_{(1, 538)}$	p-value
ADHD total symptoms	1.324	0.250	2.807	0.094	9.490	**0.002**
ADHD hyperactivity/impulsivity symptoms	1.873	0.172	1.854	0.174	7.969	**0.005**
ADHD inattention symptoms	0.017	0.895	0.897	0.344	1.288	0.257

[a] adjusted for presence of Bipolar Disorder as covariate
[b] adjusted for presence of Oppositional Defiant Disorder as covariate
[c] adjusted for presence of Oppositional Defiant Disorder and Bipolar Disorder as covariates

Table 3: ADHD clinical presentation and stability of diagnosis at baseline and 7-year follow-up

	Combined to Combined[a] n=60	Inattentive to Inattentive[b] n=49	Combined to Inattentive[c] n=27	Inattentive to Combined[d] n=12	Fisher's Exact Test p-value
LPHN3 G+/*TTC12* AA	22 (56.4)	5 (12.8)	9 (23.1)	3 (7.7)	0.008
Else	38 (34.9)	44 (40.4)	18 (16.5)	9 (8.3)	
Residual p-value	0.016	0.002	0.368	0.920	

Data are expressed as n and (%)

[a] Retained combined or predominantly hyperactive/impulsive presentation

[b] Retained predominantly inattentive presentation

[c] Switched to predominantly inattentive presentation

[d] Switched to combined or predominantly hyperactive/impulsive presentation

Multinomial regression analyses adjusted for ADHD symptom counts retrieved the same results.

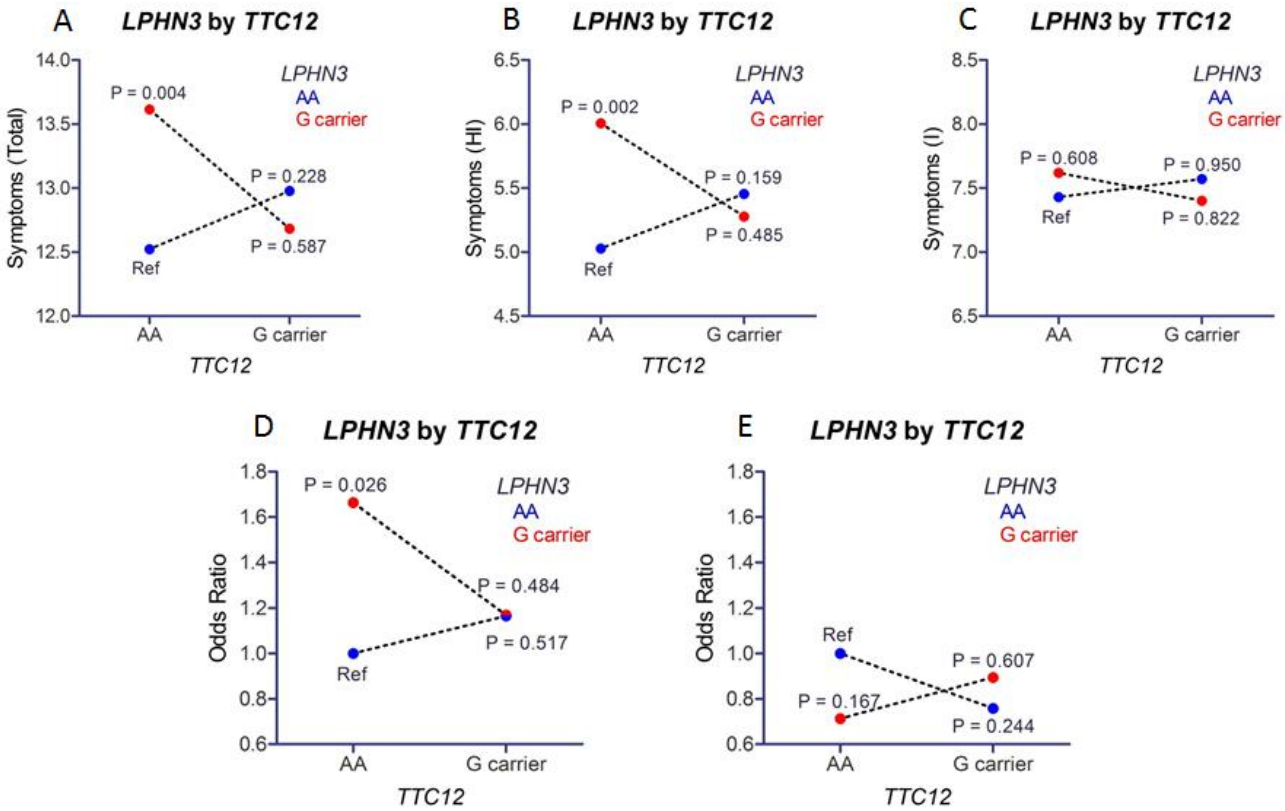


Figure 1: Interaction effects for ADHD symptom counts and diagnosis
A – Mean total symptom scores according to *LPHN3*-rs6551665 and *TTC12*-rs2303380 genotypes in adults with ADHD.
B – Mean symptom scores for the hyperactivity/impulsivity domain (HI) according to the *LPHN3*-rs6551665 and *TTC12*-rs2303380 genotypes.
C – Mean symptom scores for the inattention domain (I) according to the *LPHN3*-rs6551665 and *TTC12*-rs2303380 genotypes.
D – Influence of *LPHN3*-rs6551665 and *TTC12*-rs2303380 genotypes on the susceptibility to ADHD in individuals with high hyperactivity/impulsivity symptom measures (i.e. predominantly hyperactive/impulsive and combined presentations).
E – Influence of *LPHN3*-rs6551665 and *TTC12*-rs2303380 genotypes on the susceptibility to ADHD in individuals with low hyperactivity/impulsivity symptom measures (i.e. inattentive presentation).

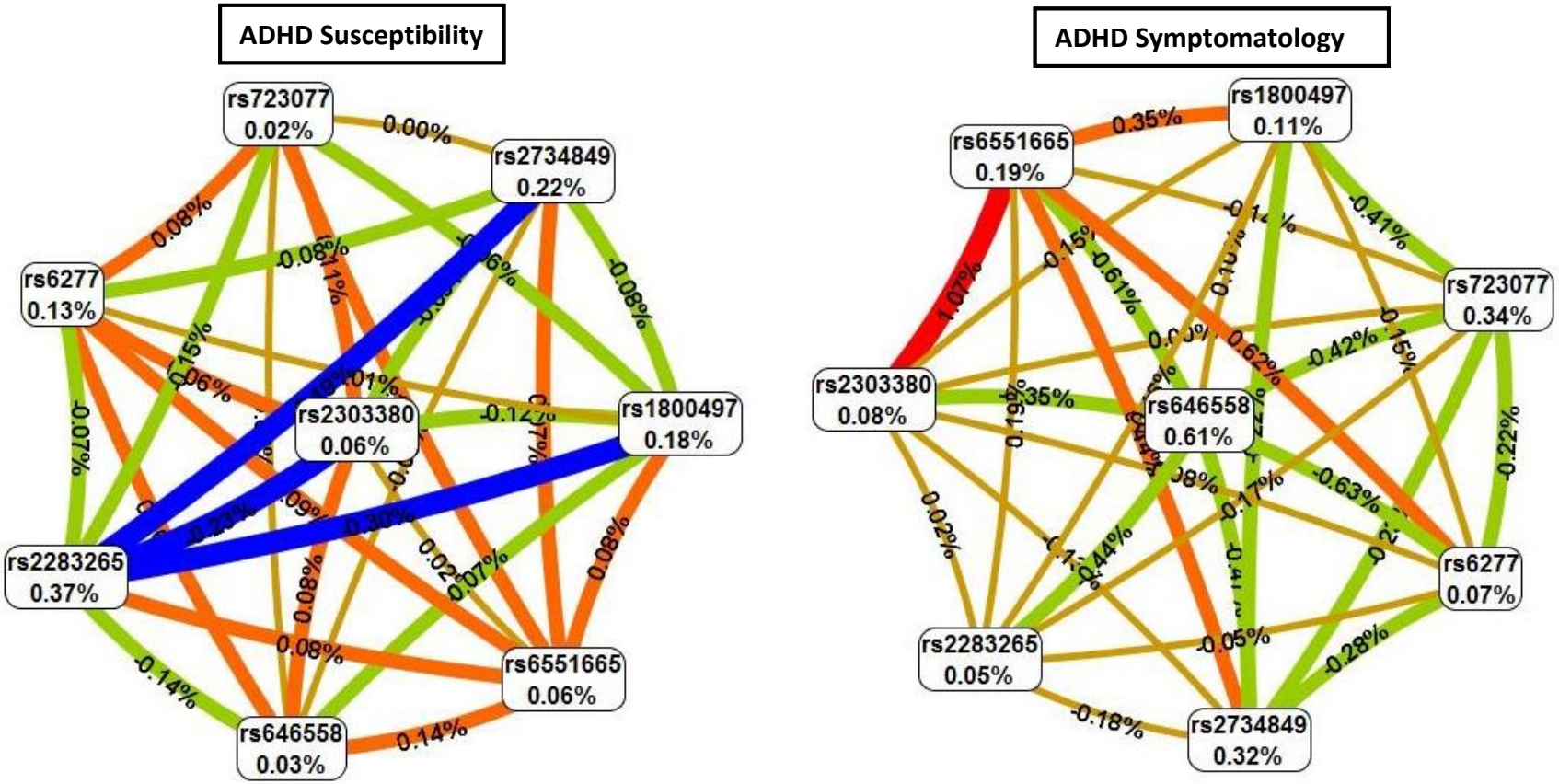
Supplementary Table 1. Logistic regression for *LPHN3*-rs6551665 main effects regarding ADHD susceptibility in adults

Gene - Polymorphism	Genotype groups (n (%))			
	Reference	Test	OR (CI $_{95\%}$)	p-value
LPHN3 – rs6551665	AA	AG + GG		
Cases (N=548)	210 (38.3)	338 (61.7)	1.168 (0.92-1.49)	0.206
Controls (N=643)	263 (40.9)	380 (59.1)		

ADHD: Attention-Deficit/Hyperactivity Disorder

OR: Odds Ratio; CI: Confidence Interval

Adjusted for presence of comorbid Bipolar Disorder.



Supplementary Figure 1: Kamada-Kawai interaction graph based on entropy measurements, comprised of nodes representing information gain of individual SNPs (main effect) with connected lines representing all pairwise connections between them (interaction effects) to the outcome. Positive entropy (plotted in red) indicates interaction effects and negative entropy (plotted in green or blue) indicates redundancy or correlation (e.g. linkage disequilibrium). Independence is represented by the gold color.

LPHN3= rs6551665; *NCAM1*= rs646558; *TTC12*= rs723077, rs2303380; *ANKK1*= rs2734849, rs1800497 and *DRD2*= rs6277, rs2283265.

Capítulo IV – **Discussão Geral**

Os resultados apresentados nesta dissertação fornecem evidências relevantes para a busca dos fatores genéticos específicos relacionados ao desenvolvimento do TDAH em adultos. O manuscrito incluído oferece uma discussão centrada nos principais resultados observados, neste capítulo serão discutidos aspectos gerais relevantes a um contexto mais abrangente da genética do TDAH em adultos.

Considerando a alta herdabilidade do TDAH, tanto em crianças como em adultos (Faraone et al., 2005; Chang et al., 2013), muitos grupos têm empreendido esforços na tentativa de elucidar os fatores genéticos associados a este transtorno. Embora mais recentemente tais esforços estejam se voltando para as varreduras genômicas, o volume crescente de resultados em genes candidatos permanece resultando em revisões anuais sobre o atual estado da arte nesses estudos em TDAH (Akutagava-Martins et al., 2013; Franke et al., 2012; Elia et al., 2012). Até recentemente, essas revisões eram centradas em genes candidatos clássicos apoiados por meta-análises, sendo a principal destas o célebre estudo de Gizer et al. (2009). Em uma perspectiva mais atual, as grandes revisões sobre a genética do TDAH tem incluído também o gene *LPHN3*, apontando este como mais um dos potenciais fatores centrais na etiologia do transtorno (Hawi et al., 2015; Li et al., 2014).

O atual conjunto de evidências aponta o gene *LPHN3* como importante moderador do desenvolvimento do sistema nervoso central, especialmente nos sistemas dopaminérgico e glutamatérgico. Além dos estudos *in vitro* e *in vivo* que ajudaram a elucidar o papel funcional do gene, uma ampla gama de estudos de associação tem evidenciado sua influência nas bases genéticas do TDAH (Arcos-Burgos et al., 2010; Ribasés et al., 2011; Choudhry et al., 2012; Bruxel et al., 2015; Hwang et al., 2015; Gomez-Sanchez et al., 2016; Acosta et al., 2016). No entanto, apesar de abranger vários estudos, com replicações em amostras de diferentes grupos e etnias, a maioria destes foi realizada em amostras de crianças com TDAH.

Os resultados apresentados no manuscrito corroboram achados prévios de influência do gene *LPHN3* e das interações deste gene com o *cluster* NTAD na etiologia do TDAH. De fato esse é o primeiro estudo a avaliar o papel da interação entre *LPHN3* e

NTAD em uma amostra composta de adultos. Além disso, tais resultados demonstram um efeito específico em fenótipos onde predomina a hiperatividade/impulsividade. Esses resultados devem ser interpretados no contexto de que, apesar de no presente estudo estarmos reportando uma evidência de interação entre *LPHN3* e NTAD na susceptibilidade ao TDAH apenas em indivíduos com maior presença de hiperatividade/impulsividade (subtipos combinado e hiperativo/impulsivo), este não é o primeiro estudo a reportar que os efeitos da *LPHN3* no TDAH podem estar sendo levados por amostras com essas características (Ribasés et al., 2011; Gomez-Sanchez et al., 2016; Acosta et al., 2016).

A primeira tentativa de replicação da associação de *LPHN3* no TDAH em adultos demonstrou que um haplótipo contendo três polimorfismos do gene estava fortemente relacionado com a apresentação do TDAH na forma combinada (Ribasés et al., 2011). Além disso, os subtipos combinado e hiperativo/impulsivo são mais frequentes em crianças do que em adultos e o efeito de *LPHN3* nessa apresentação mais externalizante do transtorno em crianças foi relatado por Gomez-Sanchez et al. (2016). Ainda, em um recente estudo com uma amostra longitudinal, foi demonstrado que uma variante de *LPHN3* parece estar relacionada com a manutenção da apresentação do TDAH como subtipo combinado desde a infância até o início da vida adulta (Acosta et al., 2016).

A manifestação do TDAH com presença de hiperatividade/impulsividade representa a faceta mais externalizante do transtorno e está relacionada a um fenótipo de graves prejuízos clínicos. Por exemplo, casos com predomínio de hiperatividade tendem a ser diagnosticados mais precocemente do que aqueles com predomínio de desatenção (Applegate et al., 1997) e apresentam maior risco de presença de transtornos psiquiátricos externalizantes ao decorrer da vida (Smith et al., 2016). Nesse mesmo contexto, indivíduos adultos com TDAH dos subtipos com sintomatologia de hiperatividade/impulsividade apresentam mais comorbidades psiquiátricas (Cadman et al., 2016), com um perfil externalizante de alta prevalência de TUS (Liebrenz et al., 2015).

Outra perspectiva interessante vem da ideia de que os transtornos psiquiátricos representam o extremo de fenótipos que naturalmente ocorrem na população em geral. Por exemplo, já foi demonstrado que escores de risco poligênico para o TDAH estavam

associados com traços caraterísticos do transtorno, como os sintomas de desatenção e hiperatividade/impulsividade, em amostras populacionais (Martin et al., 2014). Nesse contexto, é possível especular que os efeitos de interação das variantes genéticas reportados neste estudo estejam também influenciando a manifestação de sintomas de hiperatividade/impulsividade na população geral, sugerindo, assim, a necessidade de mais estudos que envolvam não somente amostras clínicas de TDAH.

Além disso, estudos que buscam entender o papel de interações gênicas denotam novos modelos interessantes no contexto de elucidar os mecanismos biológicos envolvidos no desenvolvimento do TDAH. Por exemplo, considerando o papel central do sistema dopaminérgico na neurobiologia do TDAH e os efeitos da LPHN3 no desenvolvimento e regulação da sinalização de dopamina, parece provável que a influência da interação entre *LPHN3* e *TTC12* reportada aqui está relacionada com alterações no desenvolvimento do sistema dopaminérgico. Essa hipótese também é interessante porque reforça os aspectos neurodesenvolvimentais do TDAH, demonstrando que os efeitos de variantes genéticas envolvidas no desenvolvimento do sistema nervoso central podem ser detectados também em indivíduos adultos com o transtorno.

Ainda, é importante ressaltar que o estudo de interações pode contribuir muito para a compreensão da arquitetura genética de doenças (Rovaris et al., 2013; Mäki-Tanila and Hill, 2014). Até o presente momento as varreduras genômicas realizadas no TDAH têm tido pouco sucesso em apontar associações significativas a nível genômico (Hinney et al., 2011; Neale et al., 2008; Lasky-Su et al., 2008b, 2008a; Stergiakouli et al., 2012; Neale et al., 2010b; Zayats et al., 2015; Mick et al., 2010; Neale et al., 2010a). É possível especular que, além dos atuais tamanhos amostrais reduzidos e limitações de cunho estatístico, outro fator contribuindo para essa caraterística é o alto número de interações entre genes relacionados ao sistema nervoso central, o que dificultaria a identificação dos genes e variantes genéticas implicados na etiologia do TDAH.

Em conjunto, esses dados sugerem que a melhor caracterização fenotípica dos indivíduos com TDAH é uma abordagem promissora aos estudos de associação, principalmente levando em consideração as replicações entre estudos originalmente

realizados em crianças e posteriormente em adultos. De fato, além das diferenças entre prevalência dos subtipos em crianças e adultos, em amostras clínicas de crianças com TDAH a proporção entre o gênero masculino e feminino é de aproximadamente 4:1, enquanto que em amostras de adultos a proporção é próxima de 1:1 (APA, 2013). Além disso, o perfil de transtornos psiquiátricos presentes em comorbidade ao TDAH em crianças e adultos é distinto, sendo classicamente externalizante nas crianças com aumento gradual de um perfil de internalização nos adultos.

Em conclusão, esse estudo contribui para o atual contexto de busca pelos fatores genéticos associados à complexa arquitetura etiológica do TDAH, demonstrando que abordagens mais amplas incluindo também os efeitos de interações entre genes, além de uma melhor caracterização fenotípica do transtorno são fatores-chave para o êxito em estudos de associação na genética psiquiátrica.

Referências

Acosta, MT, Swanson, J, Stehli, A, Molina, BSG, Martinez, AF, Arcos-Burgos, M, Muenke, M. 2016. *ADGRL3 (LPHN3)* variants are associated with a refined phenotype of ADHD in the MTA study. Mol. Genet. Genomic Med. 3: 1–8.

Acosta, MT, Vélez, JI, Bustamante, ML, Balog, JZ, Arcos-Burgos, M, Muenke, M. 2011. A two-locus genetic interaction between LPHN3 and 11q predicts ADHD severity and long-term outcome. Transl. Psychiatry 1: e17.

Agnew-Blais, JC, Polanczyk, G V, Danese, A, Wertz, J, Moffitt, TE, Arseneault, L. 2016. Evaluation of the Persistence, Remission, and Emergence of Attention-Deficit/Hyperactivity Disorder in Young Adulthood. JAMA psychiatry 73: 713–20.

Akutagava-Martins, GC, Salatino-Oliveira, A, Kieling, CC, Rohde, LA, Hutz, MH. 2013. Genetics of attention-deficit/hyperactivity disorder: current findings and future directions. Expert Rev Neurother 13: 435–445.

Alcantara, AA, Chen, V, Herring, BE, Mendenhall, JM, Berlanga, ML. 2003. Localization of dopamine D2 receptors on cholinergic interneurons of the dorsal striatum and nucleus accumbens of the rat. Brain Res. 986: 22–29.

APA. 2013. Diagnostic and statistical manual of mental disorders (DSM 5). Am. Psychiatr. Assoc.

Applegate, B, Lahey, BB, Hart, EL, Biederman, J, Hynd, GW, Barkley, RA, Ollendick, T, Frick, PJ, Greenhill, L, McBurnett, K, Newcorn, JH, Kerdyk, L, Garfinkel, B, Waldman, I, Shaffer, D. 1997. Validity of the age-of-onset criterion for ADHD: a report from the DSM-IV field trials. J. Am. Acad. Child Adolesc. Psychiatry 36: 1211–21.

Arai, M, Itokawa, M, Yamada, K, Toyota, T, Arai, M, Haga, S, Ujike, H, Sora, I, Ikeda, K, Yoshikawa, T. 2004. Association of neural cell adhesion molecule 1 gene polymorphisms with bipolar affective disorder in Japanese individuals. Biol. Psychiatry 55: 804–10.

Arcos-Burgos, M, Castellanos, FX, Pineda, D, Lopera, F, Palacio, JD, Palacio, LG, Rapoport, JL, Berg, K, Bailey-Wilson, JE, Muenke, M. 2004. Attention-deficit/hyperactivity

disorder in a population isolate: linkage to loci at 4q13.2, 5q33.3, 11q22, and 17p11. Am. J. Hum. Genet. 75: 998–1014.

Arcos-Burgos, M, Jain, M, Acosta, MT, Shively, S, Stanescu, H, Wallis, D, Domené, S, Vélez, JI, Karkera, JD, Balog, J, Berg, K, Kleta, R, Gahl, W a, Roessler, E, Long, R, Lie, J, Pineda, D, Londoño, a C, Palacio, JD, Arbelaez, a, Lopera, F, Elia, J, Hakonarson, H, Johansson, S, Knappskog, PM, Haavik, J, Ribases, M, Cormand, B, Bayes, M, Casas, M, Ramos-Quiroga, J a, Hervas, a, Maher, BS, Faraone, S V, Seitz, C, Freitag, CM, Palmason, H, Meyer, J, Romanos, M, Walitza, S, Hemminger, U, Warnke, a, Romanos, J, Renner, T, Jacob, C, Lesch, K-P, Swanson, J, Vortmeyer, a, Bailey-Wilson, JE, Castellanos, FX, Muenke, M. 2010. A common variant of the latrophilin 3 gene, LPHN3, confers susceptibility to ADHD and predicts effectiveness of stimulant medication. Mol. Psychiatry 15: 1053–1066.

Atz, ME, Rollins, B, Vawter, MP. 2007. NCAM1 association study of bipolar disorder and schizophrenia: polymorphisms and alternatively spliced isoforms lead to similarities and differences. Psychiatr. Genet. 17: 55–67.

Bidwell, LC, McGeary, JE, Gray, JC, Palmer, RHC, Knopik, VS, MacKillop, J. 2015. An initial investigation of associations between dopamine-linked genetic variation and smoking motives in African Americans. Pharmacol. Biochem. Behav. 138: 104–10.

Bruxel, EM, Salatino-Oliveira, A, Akutagava-Martins, GC, Tovo-Rodrigues, L, Genro, JP, Zeni, CP, Polanczyk, G V., Chazan, R, Schmitz, M, Arcos-Burgos, M, Rohde, LA, Hutz, MH. 2015. LPHN3 and attention-deficit/hyperactivity disorder: A susceptibility and pharmacogenetic study. Genes, Brain Behav. 14: 419–427.

Cadman, T, Findon, J, Eklund, H, Hayward, H, Howley, D, Cheung, C, Kuntsi, J, Glaser, K, Murphy, D, Asherson, P. 2016. Six-year follow-up study of combined type ADHD from childhood to young adulthood: Predictors of functional impairment and comorbid symptoms. Eur. Psychiatry 35: 47–54.

Castells, X, Ramos-Quiroga, JA, Rigau, D, Bosch, R, Nogueira, M, Vidal, X, Casas, M. 2011. Efficacy of methylphenidate for adults with attention-deficit hyperactivity disorder: a meta-regression analysis. CNS Drugs 25: 157–69.

Caye, A, Rocha, TB-M, Anselmi, L, Murray, J, Menezes, AMB, Barros, FC, Gonçalves, H, Wehrmeister, F, Jensen, CM, Steinhausen, H-C, Swanson, JM, Kieling, C, Rohde, LA. 2016. Attention-Deficit/Hyperactivity Disorder Trajectories From Childhood to Young Adulthood: Evidence From a Birth Cohort Supporting a Late-Onset Syndrome. JAMA psychiatry 73: 705–12.

Chang, Z, Lichtenstein, P, Asherson, PJ, Larsson, H. 2013. Developmental twin study of attention problems: high heritabilities throughout development. JAMA Psychiatry 70: 311.

Choudhry, Z, Sengupta, SM, Grizenko, N, Fortier, ME, Thakur, GA, Bellingham, J, Joober, R. 2012. LPHN3 and attention-deficit/hyperactivity disorder: Interaction with maternal stress during pregnancy. J. Child Psychol. Psychiatry Allied Discip. 53: 892–902.

Dobson-Patterson, R, O'Gorman, JG, Chan, RCK, Shum, DHK. 2016. ADHD subtypes and neuropsychological performance in an adult sample. Res. Dev. Disabil. 55: 55–63.

Ducci, F, Kaakinen, M, Pouta, A, Hartikainen, A-L, Veijola, J, Isohanni, M, Charoen, P, Coin, L, Hoggart, C, Ekelund, J, Peltonen, L, Freimer, N, Elliott, P, Schumann, G, Järvelin, M-R. 2011. TTC12-ANKK1-DRD2 and CHRNA5-CHRNA3-CHRNB4 influence different pathways leading to smoking behavior from adolescence to mid-adulthood. Biol. Psychiatry 69: 650–60.

Elia, J, Sackett, J, Turner, T, Schardt, M, Tang, S-C, Kurtz, N, Dunfey, M, McFarlane, NA, Susi, A, Danish, D, Li, A, Nissley-Tsiopinis, J, Borgmann-Winter, K. 2012. Attention-deficit/hyperactivity disorder genomics: update for clinicians. Curr. Psychiatry Rep. 14: 579–89.

Faraone, S V, Perlis, RH, Doyle, AE, Smoller, JW, Goralnick, JJ, Holmgren, MA, Sklar, P. 2005. Molecular genetics of attention-deficit/hyperactivity disorder. Biol Psychiatry 57: 1313–1323.

Franke, B, Faraone, S V, Asherson, P, Buitelaar, J, Bau, CHD, Ramos-Quiroga, JA, Mick, E, Grevet, EH, Johansson, S, Haavik, J, Lesch, KP, Cormand, B, Reif, A, IMpACT, on behalf of the IM persistent AC. 2011. The genetics of attention deficit/hyperactivity disorder in adults, a review. Mol. Psychiatry 17: 960–987.

Franke, B, Faraone, S V, Asherson, P, Buitelaar, J, Bau, CHD, Ramos-Quiroga, JA, Mick, E, Grevet, EH, Johansson, S, Haavik, J, Lesch, K-P, Cormand, B, Reif, A, International Multicentre persistent ADHD CollaboraTion. 2012. The genetics of attention deficit/hyperactivity disorder in adults, a review. Mol. Psychiatry 17: 960–87.

Gadow, KD, Pinsonneault, JK, Perlman, G, Sadee, W. 2014. Association of dopamine gene variants, emotion dysregulation and ADHD in autism spectrum disorder. Res. Dev. Disabil. 35: 1658–1665.

Gelernter, J, Yu, Y, Weiss, R, Brady, K, Panhuysen, C, Yang, B-Z, Kranzler, HR, Farrer, L. 2006. Haplotype spanning TTC12 and ANKK1, flanked by the DRD2 and NCAM1 loci, is strongly associated to nicotine dependence in two distinct American populations. Hum. Mol. Genet. 15: 3498–507.

Gizer, IR, Ficks, C, Waldman, ID. 2009. Candidate gene studies of ADHD: A meta-analytic review. Hum. Genet. 126: 51–90.

Gomez, R, Woodworth, R, Waugh, M, Corr, PJ. 2012. Attention-Deficit/Hyperactivity Disorder symptoms in an adult sample: Associations with Cloninger's temperament and character dimensions. Pers. Individ. Dif. 52: 290–294.

Gomez-Sanchez, CI, Riveiro-Alvarez, R, Soto-Insuga, V, Rodrigo, M, Tirado-Requero, P, Mahillo-Fernandez, I, Abad-Santos, F, Carballo, JJ, Dal-Ré, R, Ayuso, C. 2016. Attention deficit hyperactivity disorder: genetic association study in a cohort of Spanish children. Behav Brain Funct 12: 2.

Hawi, Z, Cummins, TDR, Tong, J, Johnson, B, Lau, R, Samarrai, W, Bellgrove, MA. 2015. The molecular genetic architecture of attention deficit hyperactivity disorder. Mol. Psychiatry 20: 289–97.

Hinney, A, Scherag, A, Jarick, I, Albayrak, Ö, Pütter, C, Pechlivanis, S, Dauvermann, MR, Beck, S, Weber, H, Scherag, S, Nguyen, TT, Volckmar, A-L, Knoll, N, Faraone, S V, Neale, BM, Franke, B, Cichon, S, Hoffmann, P, Nöthen, MM, Schreiber, S, Jöckel, K-H, Wichmann, H-E, Freitag, C, Lempp, T, Meyer, J, Gilsbach, S, Herpertz-Dahlmann, B, Sinzig, J, Lehmkuhl, G, Renner, TJ, Warnke, A, Romanos, M, Lesch, K-P, Reif, A, Schimmelmann, BG, Hebebrand, J. 2011. Genome-wide association study in German

patients with attention deficit/hyperactivity disorder. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 156B: 888–97.

Huang, W, Payne, TJ, Ma, JZ, Beuten, J, Dupont, RT, Inohara, N, Li, MD. 2009. Significant association of ANKK1 and detection of a functional polymorphism with nicotine dependence in an African-American sample. Neuropsychopharmacology 34: 319–30.

Hwang, IW, Lim, MH, Kwon, HJ, Jin, HJ. 2015. Association of LPHN3 rs6551665 A/G polymorphism with attention deficit and hyperactivity disorder in Korean children. Gene 566: 68–73.

Jacob, C, Gross-Lesch, S, Jans, T, Geissler, J, Reif, A, Dempfle, A, Lesch, KP. 2014. Internalizing and externalizing behavior in adult ADHD. Atten. Defic. Hyperact. Disord. 6: 101–110.

Jain, M, Velez, J, Acosta, MT, Palacio, LG, Vélez, JI, Balog, J, Roessler, E, Pineda, D, Londoño, a C, Palacio, JD, Arbelaez, a, Lopera, F, Elia, J, Hakonarson, H, Seitz, C, Freitag, CM, Palmason, H, Meyer, J, Romanos, M, Walitza, S, Hemminger, U, Warnke, a, Romanos, J, Renner, T, Jacob, C, Lesch, K-P, Swanson, J, Castellanos, FX, Bailey-Wilson, JE, Arcos-Burgos, M, Muenke, M. 2012. A cooperative interaction between LPHN3 and 11q doubles the risk for ADHD. Mol. Psychiatry 17: 741–747.

Kaplan, G, Newcorn, JH. 2011. Pharmacotherapy for Child and Adolescent Attention-deficit Hyperactivity Disorder. Pediatr. Clin. North Am. 58: 99–120.

Kessler, PDR, Adler, MDL, Barkley, PDR, Biederman, MDJ, Conners, PDC, Demler, MAMPHO, Faraone, PDS, Greenhill, MDL, Howes, PDM, Secnik, PDK, Spencer, MDT, Ustun, MDT, Walters, MSE, Zaslavsky, PDA. 2006. The Prevalence and Correlates of Adult ADHD in the United States: Results From the National Comorbidity Survey Replication. Am. J. Psychiatry 163: 716–723.

Kessler, RC, Adler, L, Ames, M, Demler, O, Faraone, S, Hiripi, E, Howes, MJ, Jin, R, Secnik, K, Spencer, T, Ustun, TB, Walters, EE. 2005. The World Health Organization Adult ADHD Self-Report Scale (ASRS): a short screening scale for use in the general population. Psychol. Med. 35: 245–256.

Kessler, RC, Petukhova, M, Zaslavsky, AM. 2011. The role of latent internalizing and

externalizing predispositions in accounting for the development of comorbidity among common mental disorders. Curr. Opin. Psychiatry 24: 307.

Kooij, JS, Boonstra, AM, Vermeulen, SH, Heister, AG, Burger, H, Buitelaar, JK, Franke, B. 2008. Response to methylphenidate in adults with ADHD is associated with a polymorphism in SLC6A3 (DAT1). Am. J. Med. Genet. B. Neuropsychiatr. Genet. 147B: 201–208.

Labbe, A, Liu, A, Atherton, J, Gizenko, N, Fortier, M ??ve, Sengupta, SM, Ridha, J. 2012. Refining psychiatric phenotypes for response to treatment: Contribution of LPHN3 in ADHD. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. 159 B: 776–785.

Lange, M, Norton, W, Coolen, M, Chaminade, M, Merker, S, Proft, F, Schmitt, A, Vernier, P, Lesch, K-P, Bally-Cuif, L. 2012. The ADHD-susceptibility gene lphn3.1 modulates dopaminergic neuron formation and locomotor activity during zebrafish development. Mol. Psychiatry 17: 946–954.

Lasky-Su, J, Anney, RJL, Neale, BM, Franke, B, Zhou, K, Maller, JB, Vasquez, AA, Chen, W, Asherson, P, Buitelaar, J, Banaschewski, T, Ebstein, R, Gill, M, Miranda, A, Mulas, F, Oades, RD, Roeyers, H, Rothenberger, A, Sergeant, J, Sonuga-Barke, E, Steinhausen, HC, Taylor, E, Daly, M, Laird, N, Lange, C, Faraone, S V. 2008a. Genome-wide association scan of the time to onset of attention deficit hyperactivity disorder. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. 147: 1355–1358.

Lasky-Su, J, Neale, BM, Franke, B, Anney, RJL, Zhou, K, Maller, JB, Vasquez, AA, Chen, W, Asherson, P, Buitelaar, J, Banaschewski, T, Ebstein, R, Gill, M, Miranda, A, Mulas, F, Oades, RD, Roeyers, H, Rothenberger, A, Sergeant, J, Sonuga-Barke, E, Steinhausen, HC, Taylor, E, Daly, M, Laird, N, Lange, C, Faraone, S V. 2008b. Genome-wide association scan of quantitative traits for attention deficit hyperactivity disorder identifies novel associations and confirms candidate gene associations. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. 147: 1345–1354.

Li, Z, Chang, S hua, Zhang, L yan, Gao, L, Wang, J. 2014. Molecular genetic studies of ADHD and its candidate genes: A review. Psychiatry Res. 219: 10–24.

Liebrenz, M, Gamma, A, Ivanov, I, Buadze, A, Eich, D. 2015. Adult attention-

deficit/hyperactivity disorder: Associations between subtype and lifetime substance use – a clinical study. F1000Research: 1–9.

Liu, J. 2004. Childhood externalizing behavior: theory and implications. J. Child Adolesc. Psychiatr. Nurs. 17: 93–103.

Lobo, DSS, Souza, RP, Tong, RP, Casey, DM, Hodgins, DC, Smith, GJ, Williams, RJ, Schopflocher, DP, Wood, RT, El-Guebaly, N, Kennedy, JL. 2010. Association of functional variants in the dopamine D2-like receptors with risk for gambling behaviour in healthy Caucasian subjects. Biol. Psychol. 85: 33–7.

Mäki-Tanila, A, Hill, WG. 2014. Influence of gene interaction on complex trait variation with multilocus models. Genetics 198: 355–367.

Martin, J, Hamshere, ML, Stergiakouli, E, O'Donovan, MC, Thapar, A. 2014. Genetic risk for attention-deficit/hyperactivity disorder contributes to neurodevelopmental traits in the general population. Biol. Psychiatry 76: 664–671.

Martinez, AF, Muenke, M, Arcos-Burgos, M. 2011. From the black widow spider to human behavior: Latrophilins, a relatively unknown class of G protein-coupled receptors, are implicated in psychiatric disorders. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. 156: 1–10.

Mick, E, Todorov, A, Smalley, S, Hu, X, Loo, S, Todd, RD, Biederman, J, Byrne, D. 2010. Family Based Genome-Wide Association Scan of Attention -Deficit/Hyperactivity Disorder. J Am Acad Child Adolesc Psychiatry 49: 898–905.

Moffitt, TE, Houts, R, Asherson, P, Belsky, DW, Corcoran, DL, Hammerle, M, Harrington, H, Hogan, S, Meier, MH, Polanczyk, G V, Poulton, R, Ramrakha, S, Sugden, K, Williams, B, Rohde, LA, Caspi, A. 2015. Is Adult ADHD a Childhood-Onset Neurodevelopmental Disorder? Evidence From a Four-Decade Longitudinal Cohort Study. Am. J. Psychiatry 172: 967–77.

Mota, NR, Araujo-Jnr, EV, Paixão-Côrtes, VR, Bortolini, MC, Bau, CHD. 2012. Linking dopamine neurotransmission and neurogenesis: The evolutionary history of the NTAD (NCAM1-TTC12-ANKK1-DRD2) gene cluster. Genet. Mol. Biol. 35: 912–8.

Mota, NR, Rovaris, DL, Kappel, DB, Picon, FA, Vitola, ES, Salgado, CAI, Karam, RG, Rohde, LA, Grevet, EH, Bau, CHD. 2015. NCAM1-TTC12-ANKK1-DRD2 gene cluster and the clinical and genetic heterogeneity of adults with ADHD. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. 168.

Neale, BM, Lasky-Su, J, Anney, R, Franke, B, Zhou, K, Maller, JB, Vasquez, AA, Asherson, P, Chen, W, Banaschewski, T, Buitelaar, J, Ebstein, R, Gill, M, Miranda, A, Oades, RD, Roeyers, H, Rothenberger, A, Sergeant, J, Steinhausen, HC, Sonuga-Barke, E, Mulas, F, Taylor, E, Laird, N, Lange, C, Daly, M, Faraone, S V. 2008. Genome-wide association scan of attention deficit hyperactivity disorder. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. 147B: 1337–1344.

Neale, BM, Medland, SE, Ripke, S, Asherson, P, Franke, B, Lesch, K-P, Faraone, S V, Nguyen, TT, Schäfer, H, Holmans, P, Daly, M, Steinhausen, H-C, Freitag, C, Reif, A, Renner, TJ, Romanos, M, Romanos, J, Walitza, S, Warnke, A, Meyer, J, Palmason, H, Buitelaar, J, Vasquez, AA, Lambregts-Rommelse, N, Gill, M, Anney, RJL, Langely, K, O'Donovan, M, Williams, N, Owen, M, Thapar, A, Kent, L, Sergeant, J, Roeyers, H, Mick, E, Biederman, J, Doyle, A, Smalley, S, Loo, S, Hakonarson, H, Elia, J, Todorov, A, Miranda, A, Mulas, F, Ebstein, RP, Rothenberger, A, Banaschewski, T, Oades, RD, Sonuga-Barke, E, McGough, J, Nisenbaum, L, Middleton, F, Hu, X, Nelson, S, Psychiatric GWAS Consortium: ADHD Subgroup. 2010a. Meta-analysis of genome-wide association studies of attention-deficit/hyperactivity disorder. J. Am. Acad. Child Adolesc. Psychiatry 49: 884–97.

Neale, BM, Su, J, Anney, R, Franke, B, Maller, JB, Vasquez, AA, Asherson, P, Chen, W, Buitelaar, J, Ebstein, R, Gill, M, Miranda, A, Oades, D, Roeyers, H, Rothenberger, A, Sergeant, J, Steinhausen, C, Sonuga-barke, E, Mulas, F, Taylor, E. 2010b. Genome-wide Association Scan of Attention Deficit Hyperactivity Disorder. 3255: 1337–1344.

Nelson, EC, Lynskey, MT, Heath, AC, Wray, N, Agrawal, A, Shand, FL, Henders, AK, Wallace, L, Todorov, AA, Schrage, AJ, Saccone, NL, Madden, PAF, Degenhardt, L, Martin, NG, Montgomery, GW. 2013. ANKK1, TTC12, and NCAM1 polymorphisms and heroin dependence: importance of considering drug exposure. JAMA psychiatry

70: 325–33.

O'Sullivan, M, Wit, J De, Savas, J. 2012. Postsynaptic FLRT proteins are endogenous ligands for the black widow spider venom receptor Latrophilin and regulate excitatory synapse development. Neuron 73: 903–910.

O'Sullivan, ML, Martini, F, von Daake, S, Comoletti, D, Ghosh, A. 2014. LPHN3, a presynaptic adhesion-GPCR implicated in ADHD, regulates the strength of neocortical layer 2/3 synaptic input to layer 5. Neural Dev. 9: 1–11.

Orsini, CA, Setlow, B, DeJesus, M, Galaviz, S, Loesch, K, Ioerger, T, Wallis, D. 2016. Behavioral and transcriptomic profiling of mice null for Lphn3, a gene implicated in ADHD and addiction. Mol. Genet. genomic Med. 4: 322–43.

Polanczyk, G, de Lima, MS, Horta, BL, Biederman, J, Rohde, LA. 2007. The worldwide prevalence of ADHD: a systematic review and metaregression analysis. Am J Psychiatry 164: 942–948.

Polderman, TJC, Benyamin, B, de Leeuw, CA, Sullivan, PF, van Bochoven, A, Visscher, PM, Posthuma, D. 2015. Meta-analysis of the heritability of human traits based on fifty years of twin studies. Nat. Genet. 47: 702–9.

Purper-Ouakil, D, Ramoz, N, Lepagnol-Bestel, Aude-Marie Gorwood, P, Simonneau, M. 2011. Neurobiology of attention deficit/hyperactivity disorder. Pediatr. Res. 69: 69–76.

Reuter, I, Knaup, S, Romanos, M, Lesch, K-P, Drepper, C, Lillesaar, C. 2016. Developmental exposure to acetaminophen does not induce hyperactivity in zebrafish larvae. J. Neural Transm.

Ribasés, M, Ramos-Quiroga, JA, Sánchez-Mora, C, Bosch, R, Richarte, V, Palomar, G, Gastaminza, X, Bielsa, A, Arcos-Burgos, M, Muenke, M, Castellanos, FX, Cormand, B, Bayés, M, Casas, M. 2011. Contribution of LPHN3 to the genetic susceptibility to ADHD in adulthood: A replication study. Genes, Brain Behav. 10: 149–157.

Rovaris, DL, Mota, NR, Callegari-Jacques, SM, Bau, CHD. 2013. Approaching "phantom heritability" in psychiatry by hypothesis-driven gene-gene interactions. Front. Hum.

Neurosci. 7: 210.

Salgado, CA, Bau, CH, Grevet, EH, Fischer, AG, Victor, MM, Kalil, KL, Sousa, NO, Garcia, CR, Belmonte-de-Abreu, P. 2009. Inattention and hyperactivity dimensions of ADHD are associated with different personality profiles. Psychopathology 42: 108–112.

Silva, JP, Ushkaryov, YA. 2010. The latrophilins, "split-personality" receptors. Adv. Exp. Med. Biol. 706: 59–75.

Simon, V, Czobor, P, Balint, S, Meszaros, A, Bitter, I. 2009. Prevalence and correlates of adult attention-deficit hyperactivity disorder: meta-analysis. Br J Psychiatry 194: 204–211.

Smith, E, Meyer, BJ, Koerting, J, Laver-Bradbury, C, Lee, L, Jefferson, H, Sayal, K, Treglown, L, Thompson, M, Sonuga-Barke, EJS. 2016. Preschool hyperactivity specifically elevates long-term mental health risks more strongly in males than females: a prospective longitudinal study through to young adulthood. Eur. Child Adolesc. Psychiatry.

Stergiakouli, E, Hamshere, M, Holmans, P, Langley, K, Zaharieva, I, Hawi, Z, Kent, L, Gill, M, Williams, N, Owen, MJ, O'Donovan, M, Thapar, A, Faraone, S V., Neale, BM, Ripke, S, Daly, M, Medland, SE, Asherson, P, Franke, B, Steinhausen, HC, Lesch, KP, Freitag, C, Reif, A, Renner, TJ, Nguyen, TT, Romanos, M, Romanos, J, Walitza, S, Schäfer, H, Warnke, A, Meyer, J, Palmason, H, Buitelaar, J, Vasquez, AA, Lambregts-Rommelse, N, Anney, RJL, Sergeant, J, Roeyers, H, Mick, E, Biederman, J, Doyle, A, Smalley, S, Loo, S, Hakonarson, H, Elia, J, Todorov, A, Miranda, A, Mulas, F, Ebstein, R, Rothenberger, A, Banaschewski, T, Oades, R, Sonuga-Barke, E, McGough, J, Nisenbaum, L, Middleton, F, Hu, X, Nelson, S. 2012. Investigating the contribution of common genetic variants to the risk and pathogenesis of ADHD. Am. J. Psychiatry 169: 186–194.

Stergiakouli, E, Thapar, A. 2010. Fitting the pieces together: current research on the genetic basis of attention-deficit/hyperactivity disorder (ADHD). Neuropsychiatr. Dis. Treat. 6: 551–560.

Sulzer, D, Sonders, MS, Poulsen, NW, Galli, A. 2005. Mechanisms of neurotransmitter

release by amphetamines: A review. Prog. Neurobiol. 75: 406–433.

Tripp, G, Wickens, JR. 2009. Neurobiology of ADHD. Neuropharmacology 57: 579–589.

van der Voet, M, Harich, B, Franke, B, Schenck, a. 2015. ADHD-associated dopamine transporter, latrophilin and neurofibromin share a dopamine-related locomotor signature in Drosophila. Mol. Psychiatry 10: 1–9.

Wallis, D, Hill, DS, Mendez, IA, Abbott, LC, Finnell, RH, Wellman, PJ, Setlow, B. 2012. Initial characterization of mice null for Lphn3, a gene implicated in ADHD and addiction. Brain Res. 1463: 85–92.

Walmod, PS, Kolkova, K, Berezin, V, Bock, E. 2004. Zippers make signals: NCAM-mediated molecular interactions and signal transduction. Neurochem. Res. 29: 2015–35.

Wigal, SB. 2009. Efficacy and safety limitations of attention-deficit hyperactivity disorder pharmacotherapy in children and adults. CNS Drugs 23: 21–31.

Wilens, TE. 2008. Effects of methylphenidate on the catecholaminergic system in attention-deficit/hyperactivity disorder. J. Clin. Psychopharmacol. 28: S46–53.

Wilens, TE, Spencer, TJ. 2010. Understanding attention-deficit/hyperactivity disorder from childhood to adulthood. Postgr. Med 122: 97–109.

Yang, BZ, Kranzler, HR, Zhao, H, Gruen, JR, Luo, X, Gelernter, J. 2008a. Haplotypic variants in DRD2, ANKK1, TTC12, and NCAM1 are associated with comorbid alcohol and drug dependence. Alcohol. Clin. Exp. Res. 32: 2117–2127.

Yang, B-Z, Kranzler, HR, Zhao, H, Gruen, JR, Luo, X, Gelernter, J. 2007. Association of haplotypic variants in DRD2, ANKK1, TTC12 and NCAM1 to alcohol dependence in independent case control and family samples. Hum. Mol. Genet. 16: 2844–53.

Yang, B-Z, Kranzler, HR, Zhao, H, Gruen, JR, Luo, X, Gelernter, J. 2008b. Haplotypic variants in DRD2, ANKK1, TTC12, and NCAM1 are associated with comorbid alcohol and drug dependence. Alcohol. Clin. Exp. Res. 32: 2117–27.

Zayats, T, Athanasiu, L, Sonderby, I, Djurovic, S, Westlye, LT, Tamnes, CK, Fladby, T, Aase, H, Zeiner, P, Reichborn-Kjennerud, T, Knappskog, PM, Knudsen, GP, Andreassen, OA, Johansson, S, Haavik, J. 2015. Genome-Wide Analysis of Attention Deficit

Hyperactivity Disorder in Norway. PLoS One 10: e0122501.

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 E COMISSÃO DE PESQUISA E ÉTICA EM SAÚDE

RESOLUÇÃO

A Comissão Científica e a Comissão de Pesquisa e Ética em Saúde, que é reconhecida pela Comissão Nacional de Ética em Pesquisa (CONEP)/MS como Comitê de Ética em Pesquisa do HCPA e pelo Office For Human Research Protections (OHRP)/USDHHS, como Institucional Review Board (IRB0000921) analisaram o projeto:

Projeto: 01-321 **Versão do Projeto:** 22/01/2002 **Versão do TCLE:** 22/01/2002

Pesquisadores:
PAULO SILVA BELMONTE DE ABREU
CLAITON H. O. BAU
EUGENIO GREVET
CARLOS ALBERTO IGLESIAS SALGADO
BETINA CHAIT

Título: ESTUDO DAS BASES MOLECULARES DO TRANSTORNO DE DÉFICIT DE ATENÇÃO/HIPERATIVIDADE EM ADULTOS

Este projeto foi Aprovado em seus aspectos éticos e metodológicos, inclusive quanto ao seu Termo de Consentimento Livre e Esclarecido, de acordo com as Diretrizes e Normas Internacionais e Nacionais, especialmente as Resoluções 196/96 e complementares do Conselho Nacional de Saúde. Os membros do CEP/HCPA não participaram do processo de avaliação dos projetos onde constam como pesquisadores. Toda e qualquer alteração do Projeto, assim como os eventos adversos graves, deverão ser comunicados imediatamente ao CEP/HCPA.

Por pertencer a uma área temática especial este projeto somente poderá ser iniciado após a sua aprovação pela Comissão Nacional de Ética em Pesquisa (CONEP).

Porto Alegre, 25 de janeiro de 2002.

Profa. Themis Reverbel da Silveira
Coordenadora do GPPG e CEP-HCPA

Produção Científica Adicional durante o Período do Mestrado

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

NCAM1-TTC12-ANKK1-DRD2 Gene Cluster and the Clinical and Genetic Heterogeneity of Adults With ADHD

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Dysfunctions of the dopaminergic system have been implicated on the etiology of Attention Deficit/Hyperactivity Disorder (ADHD). Meta-analyses addressing the association of the dopamine receptor D2 (*DRD2*) gene and ADHD were inconclusive due to excessive heterogeneity across studies. Both the great phenotypic heterogeneity of ADHD and the complexity of the genomic region where *DRD2* is located could contribute to the inconsistent findings. Most previous *DRD2* studies focused on the well-known Taq1A (rs1800497) SNP, which is actually placed in a neighbor gene (*ANKK1*). These two genes, together with *NCAM1* and *TTC12*, form the NTAD gene cluster on Chr11q22–23. In order to address the reasons for the high heterogeneity previously reported on *DRD2* effects on ADHD, this study investigates the role of NTAD variants on ADHD susceptibility in adults and on the modulation of comorbidity and personality profiles in these patients. Functional polymorphisms from NTAD were analyzed, both individually and in haplotypes, on a sample of 520 adults with ADHD and 630 non-ADHD controls. No direct association of NTAD variants with ADHD susceptibility itself was observed. However, different NTAD polymorphisms and haplotypes were associated to various phenotypes relevant to the clinical heterogeneity of ADHD, including Major Depressive Disorder, Generalized Anxiety Disorder, and Harm Avoidance and Persistence temperament scores. Therefore, these findings represent a possible explanation for the multiple conflicting findings regarding polymorphisms in this genomic region in psychiatry. The NTAD cluster may comprise a variety of independent molecular influences on various brain and behavior characteristics eventually associated with ADHD comorbidities and personality traits.

Key words: dopamine receptor; attention deficit/hyperactivity disorder; comorbidity; personality

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INTRODUCTION

Attention Deficit/Hyperactivity Disorder (ADHD) is one of the most common psychiatric disorders, affecting 5.3% of children [Polanczyck et al., 2007] and 2.5–4.4% of adults worldwide [Kessler et al., 2006; Simon et al., 2009]. It is associated with a wide range of functional impairments across life domains and with higher incidence of other psychiatric disorders [Biederman et al., 2006; Kessler et al., 2006]. High heritability estimates (around 76–80%) have been indicated for ADHD, both in children [Faraone et al., 2005] and adults [Chang et al., 2013].

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SNARE complex in developmental psychiatry: neurotransmitter exocytosis and beyond

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

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

Introduction

SNARE (soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein receptors) complex is a large family of proteins that plays a major role in intracellular vesicular trafficking in eukaryotic cells. Such process is essential in different biological events, such as cell division, maintenance of subcellular compartments, protein and hormone secretion and neurotransmitter release (Zylbersztejn and Galli 2011). The SNARE complex is formed by members of the SNAP-25 (Synaptosomal-Associated Protein 25), VAMP (Vesicle-Associated Membrane Protein) and Syntaxins families. These proteins interact creating a four-helix bundle, formed by two helices of SNAP-25, one vesicle-transmembrane VAMP and one presynaptic plasma membrane Syntaxin that approximates the vesicle and plasmatic membranes (Sutton et al. 1998; Brunger 2000) (Fig. 1). Other proteins interact with the SNARE complex and regulate it, such as Munc-18, Complexin, Synaptophysin and the better studied Syt (Synaptotagmin) (Südhof 2013).

According to cell tissue and developmental stage, distinct family members of SNARE complex present different expression profiles. SNAP-25 family members are characterized by the presence of two SNARE domains, which are the binding sites between SNAP-25 and VAMP and Syntaxin SNARE domains, in order to form the core SNARE complex. The most studied member of this protein family is SNAP-25, which is expressed in neurons and directly involved in neurotransmitter release. It is anchored to the presynaptic plasma membrane through palmitoylation of cysteine

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

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ABSTRACT

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

1. Introduction

Modern hypothesis-driven research is currently focusing on previous findings from genome-wide association studies (GWAS), which provide the main source of new research questions for specific genotype-phenotype relationships. One of the most robust GWAS findings in psychiatry involves a nicotinic acetylcholine receptors (nAChRs) gene cluster (*CHRNA5-CHRNA3-CHRNB4*), which comprise a gene cluster located on chromosome 15q25. In addition to GWAS (Bierut et al., 2006; Caporaso et al., 2009; Thorgeirsson et al., 2008), meta-analysis (Liu et al., 2010) and candidate gene studies (Bierut et al., 2008; Broms et al., 2012; Buczkowski et al., 2015; Sarginson et al., 2011; Schlaepfer et al., 2008; Sherva et al., 2010; Stephens et al., 2013; Stevens et al., 2008; Weiss et al., 2008) have consistently shown that this cluster is involved in the susceptibility to smoking behavior. However, this scenario is complex, since there is evidence that a psychiatric background, i.e. Attention Deficit/Hyperactivity Disorder (ADHD) diagnosis, may modify the relationship between nAChRs and nicotine dependence (Polina et al., 2014).

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Glucocorticoid receptor gene modulates severity of depression in women with crack cocaine addiction

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KEYWORDS
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Abstract
Crack cocaine addicted inpatients that present more severe withdrawal symptoms also exhibit higher rates of depressive symptoms. There is strong evidence that the identification of genetic variants in depression is potentialized when reducing phenotypic heterogeneity by studying selected groups. Since depression has been associated to dysregulation of the hypothalamic-pituitary-adrenal axis, this study evaluated the effects of SNPs in stress-related genes on depressive symptoms of crack cocaine addicts at early abstinence and over the detoxification treatment (4th, 11th and 18th day post admission). Also, the role of these SNPs on the re-hospitalization rates after 2.5 years of follow-up was studied. One hundred eight-two women were enrolled and eight SNPs in four genes (*NR3C2*, *NR3C1*, *FKBP5* and *CRHR1*) were genotyped. A significant main effect of *NR3C1*-rs41423247 was found, where the C minor allele increased depressive symptoms at early abstinence. This effect remained significant after 10,000 permutations to account for multiple SNPs tested (P=0.0077). There was no effect of

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