Universidade Federal do Rio Grande do Sul

Polimorfismos nos genes DAT1 e DRD4 e transtorno de déficit de atenção e hiperatividade (TDAH) em adultos.

Francine Zanchetta Coelho Marques

Dissertação submetida ao Programa de Pós-Graduação em Genética e Biologia Molecular da UFRGS como requisito parcial para a obtenção do grau de Mestre.

Orientador: Prof. Dr. Claiton Henrique Dotto Bau

Porto Alegre

Março de 2006

Este trabalho foi desenvolvido no Laboratório de Genética Humana Molecular do Departamento de Genética do Instituto de Biociências da Universidade Federal do Rio Grande do Sul.

Instituições financiadoras:

CNPq
FINEP
FAPERGS
CAPES
PRONEX

AGRADECIMENTOS

Ao professor Claiton Henrique Dotto Bau, meu orientador, pelo apoio, confiança e incentivo desde a graduação. Sempre serei grata por toda a paciência e pelo o que aprendi nos últimos anos!

Aos professores Mara Hutz, Tatiana Roman e Homero Vallada, por aceitarem o convite para participar da banca.

Aos meus colegas e amigos de mestrado, por tornarem essa fase divertida e cheia de boas lembranças.

Aos amigos dos laboratórios 114 e 116, principalmente à Silvana Almeida e à Marilu Fiegenbaum, que me ensinaram grande parte das coisas que sei.

Aos amigos das salas 107 e 109, pelos anos de convivência e bons momentos.

Aos demais amigos do departamento de Genética, principalmente à Taiana Haag, à Verônica Contini e à Valéria Muschner, pela amizade e carinho em todos os momentos.

Ao Elmo e à Ellen, secretários do PPGBM, sempre bem-humorados e dispostos a ajudar.

À professora Marion Schiengold, que sempre me apoiou nos meus sonhos e fez com que meu estágio didádico fosse maravilhoso.

À professora Margarete Mattevi, minha primeira orientadora no departamento, por ter me recebido carinhosamente e depois ter me indicado para meu atual orientador.

À professora Sídia Callegari-Jacques, pela ajuda nas análises estatísticas.

À minha família, em especial ao meu pai, minha mãe e minha irmã, que sempre contribuiu para o meu trabalho, me apoiando, lendo, dando sugestões ou simplesmente respeitando minha privacidade. Obrigada por todo amor e carinho!

À família Vidal, minha segunda família, pelo apoio constante nos últimos anos, e em especial ao meu cunhado Rogério, pelo empréstimo do notebook.

À Tina, ao Wilson e à Vília, meus hamsters e minha cadela, pela companhia nas noites de trabalho.

Ao Guilherme Vidal, por todo apoio e paciência desde os primeiros anos de iniciação científica, e por todo amor e carinho nesses últimos seis anos. Apesar de não estar presente nesse momento, sei que torce para que tudo dê certo!

SUMÁRIO

LISTA DE ABREVIATURAS

DAT: proteína transportadora de dopamina

DAT1: gene da proteína transportadora de dopamina

DRD4: gene do receptor D4 de dopamina

DSM-IV: 4ª. edição do Manual Estatístico e Diagnóstico de Transtornos Mentais

FBAT: *family-based association test* (teste de associação baseado em famílias)

HRR: *Haplotype Relative Risk* (Risco Relativo de Haplótipo)

N: tamanho amostral

pb (ou *bp*): pares de base (ou *base pair*)

SNP: *single nucleotide polymorphism* (polimorfismo de nucleotídeo único)

TCI: *Temperament and Character Inventory* (Inventário de Caráter e Temperamento)

TDAH (*ADHD*): transtorno de déficit de atenção e hiperatividade (*attention-deficit/hyperactivity disorder*)

TDT: *Transmission Desequilibrium Test* (Teste de Desequilíbrio de Transmissão)

UTR: *untranslated region* (região não-traduzida)

VNTR: *variable number of tandem repeats* (número de variações repetidas em tandem)

RESUMO

O transtorno de déficit de atenção e hiperatividade (TDAH) é um dos problemas psiquiátricos mais comuns na infância, o qual apresenta uma herdabilidade significativa e se caracteriza pela presença de desatenção, hiperatividade e impulsividade. Cerca da metade das crianças com TDAH permanecem com o transtorno durante a vida adulta. Embora os estudos em amostras de crianças tenham explorado diversos genes de suscetibilidade ao TDAH, os resultados são inconclusivos e há poucos estudos em adultos. A amostra aqui estudada é composta por 308 adultos portadores de TDAH e 233 doadores de sangue do Hemocentro do Rio Grande do Sul. Ambos os grupos são compostos por brasileiros descendentes de europeus. Os diagnósticos psiquiátricos foram realizados por uma equipe treinada, seguindo os critérios do DSM-IV. Este trabalho objetivou testar possíveis associações entre polimorfismos nos genes do receptor D4 de dopamina (DRD4) e da proteína transportadora de dopamina (DAT1) com o TDAH, tendo em conta os subtipos, gravidade, comorbidades e idade de início dos sintomas de TDAH.

Não foi encontrada associação entre os dois polimorfismos estudados no gene DRD4 (duplicação de 120 pares de base da região 5' e VNTR de 48 pares de base do éxon III) e o TDAH. Porém, o alelo de 7-repetições do éxon III mostrou-se associado a uma menor idade de início dos sintomas e com o transtorno bipolar do humor associado ao TDAH. Genótipos contendo o alelo de 9-repetições do VNTR de 40pb na região 3' não traduzida do gene DAT1 predisporiam ao subtipo desatento de TDAH entre os pacientes do sexo masculino e ao TDAH em geral entre as mulheres. Pacientes com o genótipo homozigoto para o alelo de 10-repetições apresentaram um maior número de sintomas de hiperatividade. Os resultados aqui obtidos apontam para a existência de diferenças substanciais no perfil genético entre as amostras de TDAH em crianças e adultos. As características amostrais relacionadas com o sexo, idade, subtipo e comorbidades mais prevalentes podem modificar a associação entre os polimorfismos estudados e o transtorno.

ABSTRACT

Attention-deficit/hyperactivity disorder (ADHD) is common psychiatry disorder, present a significant heritability and is characterized by the presence of inattention, hyperactivity and impulsivity. About half of ADHD children have persistent symptoms into adulthood. Several candidate gene association studies were performed in children samples, with generally inconclusive results. Few investigations were performed in adult samples. The present study group is composed by 308 adult ADHD patients and 233 blood donor controls, all of them Brazilians of European descent. The diagnoses were made by an extensively trained team of psychiatrists following DSM-IV criteria. This investigation tested possible associations between polymorphisms in the dopamine D4 receptor (DRD4) and dopamine transporter (DAT1) genes and ADHD, considering the subtypes, severity, comorbidities and age of onset of ADHD symptoms.

No association was found between the DRD4 polymorphisms studied (120-base pair repeat in the 5' region and exon III VNTR) and ADHD. However, the 7-repeats allele (7R) in the exon III VNTR was associated with an earlier age of onset of ADHD symptoms and the presence of comorbid bipolar disorder. Genotypes containing the 9R allele of the DAT1 3'UTR VNTR are more common among male patients of the inattentive subtype and female patients of both subtypes. Subjects with the 10/10 genotype presented more hyperactivity symptoms than those with other genotypes. Our results indicate the existence of substantial differences in the genetic profiles between children and adult ADHD. The sample's characteristics related to gender, age, subtype and more prevalent comorbidities could modify the association between the polymorphisms studied and the disorder.

I. INTRODUÇÃO

1. DIAGNÓSTICO E EPIDEMIOLOGIA DO TDAH

Os sintomas do transtorno de déficit de atenção e hiperatividade (TDAH) reconhecidos pela quarta edição do Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM-IV; *American Psychiatric Association*, 1994) se dividem em dois grupos: desatenção e hiperatividade-impulsividade (Tabela 1). De acordo com o DSM-IV, são critérios diagnósticos para esse transtorno seis ou mais sintomas em um ou ambos os grupos, por pelo menos seis meses; caracterização de prejuízo em função destes sintomas em no mínimo dois ambientes (casa, escola e/ou trabalho); e início do prejuízo antes dos sete anos de idade. Através disso, três subtipos podem ser estabelecidos: predominantemente desatento, predominantemente hiperativo-impulsivo e combinado (presença de ao menos seis sintomas em ambos os grupos).

Os principais sintomas do TDAH em crianças são a desatenção, a hiperatividade e a impulsividade (*American Psychiatric Association*, 1994); porém, alguns dos sintomas nucleares se modificam com o passar da idade, enquanto outros permanecem estáveis. Sintomas relatados na infância podem desaparecer, enquanto o impacto de outros se torna ainda mais evidente na vida adulta. A impulsividade, por exemplo, freqüentemente muda de significado e passa a ser observada nas tomadas de decisão do indivíduo, em diferentes contextos de sua vida diária. Já a hiperatividade diminui significativamente, e pode ser canalizada em várias atividades simultâneas ou então dar lugar a uma sensação subjetiva de inquietude. Os déficits atentivos passam a se associar com comprometimento da memória e freqüente prejuízo funcional, uma vez que as demandas da vida adulta são maiores e mais complexas (Mattos e cols., 2003).

O TDAH é um transtorno psiquiátrico comum na infância. Em Porto Alegre, a prevalência situa-se entre 3 e 6% das crianças (Rohde e cols., 1999). Diferentemente do que se pensava até algumas décadas atrás, o problema pode permanecer durante a vida adulta. De acordo com a *American Academy of Child and Adolescent Psychiatry* (AACAP, 1997), a prevalência do transtorno seria estimada em 2-7% em adultos, dado apoiado pelo estudo longitudinal de Wender e cols. (2001). A persistência, contudo, varia de acordo com a sua definição e os critérios diagnósticos, com uma amplitude entre 15 e 65% (Faraone e cols., 2006).

Tabela 1. Sintomas do TDAH de acordo com o DSM-IV (American Psychiatric Association, 1994).

Desatenção	**Hiperatividade**	**Impulsividade**
• Dificuldade em organizar tarefas e atividades; • Dificuldade em seguir instruções e finalizar tarefas; • Dificuldade em manter a atenção durante atividades ou brincadeiras; • Evita se engajar em tarefas que exijam esforço mental sustentado; • Perda freqüente de coisas necessárias a tarefas; • Parece não estar ouvindo; • Fácil distração por estímulos externos; • Esquecimento em atividades diárias; • Não dá atenção a detalhes.	• É inquieto com as mãos e os pés quando sentado; • Parece estar sempre com o motor ligado; • Corre pelo ambiente e "escala" tudo, em momentos inapropriados; • Dificuldade em brincar ou se engajar em atividades de lazer quieto; • Dificuldade em ficar sentado, em sala de aula e outras situações; • Fala excessivamente.	• Dá respostas impulsivas, sem esperar o final da pergunta; • Dificuldade em esperar pela sua vez; • Interrompe os outros facilmente.

Diferentemente dos estudos clínicos de crianças, onde a proporção de meninos é maior que a de meninas (6:1 até 9:1) (Gittelman e cols., 1985; Weiss e cols., 1985; Arnold, 1996; Faraone e cols., 1998), em amostras de adultos com o transtorno, a razão entre homens e mulheres é próxima a 1:1 (Wender e cols, 1981, 1985; Biederman e cols., 1993;

Murphy e Barkley, 1996). Segundo Cantwell (1996) e Biederman e cols. (1999), as meninas seriam subdiagnosticadas porque possuiriam poucos sintomas de agressividade/impulsividade, baixas taxas de transtorno de conduta e freqüente comorbidade com transtorno de humor e ansiedade. Desse modo, a idade diagnóstica tende a ser mais avançada em relação aos meninos. Outra explicação provável é dada por Biederman e cols. (2002), que sugerem que as diferenças entre os sexos podem ser operantes na expressão fenotípica do TDAH. Por exemplo, o sexo feminino estaria mais associado ao subtipo predominantemente desatento do que o sexo masculino, e os meninos apresentariam mais distúrbios de aprendizagem e outros problemas de comportamento escolar em relação às meninas.

Já foi sugerido que a heterogeneidade pode obscurecer um achado que seria positivo em estudos sobre a etiologia de uma doença, e deve ser considerada como um fator de confusão (Alsobrook e Pauls, 1998). Pacientes adultos com TDAH têm uma prevalência ao menos duas vezes maior de transtornos psiquiátricos que adultos sem o TDAH (Weiss e cols., 1985; Mannuzza e cols., 1991; Biederman e cols., 1993). Estas comorbidades incluem a dependência de álcool, abuso de substâncias, personalidade anti-social, depressão maior, transtorno de ansiedade e transtorno bipolar do humor (Murphy e Barkley, 1996; Biederman, 2004).

2. BASE NEUROBIOLÓGICA

A variação nas manifestações clínicas do TDAH reflete a complexidade dos processos biológicos implicados na origem de seus sintomas, supondo-se que alterações em diferentes sistemas de neurotransmissores devam estar envolvidas. Os circuitos neuronais associados com o transtorno incluem o córtex pré-frontal, gânglios da base e cerebelo. Um

fraco controle inibitório da região cortical-frontal sobre as funções límbicas seria a origem dos sintomas desse transtorno (Satterfield e Dawson, 1971). Dados de estudos neuropsicológicos mostraram que crianças com TDAH têm um desempenho prejudicado em tarefas que demandam funções cognitivas tais como atenção, percepção, planejamento e organização, além de falhas na inibição comportamental, processos esses relacionados com o lobo frontal e com áreas subcorticais (Swanson e cols., 1998a; Tannock, 1998).

As primeiras teorias bioquímicas propostas para explicar o TDAH foram baseadas nas catecolaminas, visto que regiões implicadas na sua fisiopatologia são primariamente inervadas por esses neurotransmissores (Faraone e Biederman, 1998; Swanson e cols., 1998a). Evidências apoiam a disfunção dopaminérgica no TDAH em três áreas de pesquisa: a neurofarmacologia de medicações estimulantes (Zametkin e Rapoport, 1987; Amara e Kuhar, 1993), modelos animais bioquímicos e de comportamento (Giros e cols., 1996; Gainetdinov e cols., 1999; Jaber e cols., 1999; Russell e cols., 2000; Russell e cols., 2005), e estudos de neuroimagem em adultos com TDAH (Dougherty e cols., 1999; Krause e cols., 2000). O principal suporte para a hipofunção dopaminérgica surgiu da observação de que o metilfenidato, principal medicamento usado para tratamento do transtorno, aumenta a disponibilidade de dopamina na fenda sináptica em regiões específicas (Vaidya e cols., 1998). Além disso, os efeitos pré e pós-sinápticos dos estimulantes parecem ser diferentes conforme a região dopaminérgica considerada (Swanson e cols., 1998a).

É bastante provável um papel da noradrenalina no TDAH, havendo uma série de estudos que apontam para a sua participação na modulação da função cognitiva no lobo pré-frontal (ver revisão em Arnsten, 2000). Os circuitos fronto-subcorticais, possivelmente implicados no TDAH, são ricos tanto em dopamina como em noradrenalina (Faraone e Biederman, 1998). Além disso, a dopamina é sintetizada no mesmo processo que a

noradrenalina. Algumas regiões cerebrais primariamente moduladas por redes noradrenérgicas, como o *locus coeruleus* e a região parietal, parecem estar envolvidas em processos de atenção seletiva (Arnsten e cols., 1996; Pliszka e cols., 1996). Outros fármacos psicoativos eficazes no tratamento do TDAH têm comprovada ação noradrenérgica, como antidepressivos tricíclicos e agonistas de noradrenalina (Biederman e Spencer, 1999).

O sistema serotoninérgico parece também ter importância na fisiopatologia do TDAH. Um estudo em cobaias demonstrou que, nos animais sem o gene da proteína transportadora de dopamina (DAT1), a intensa hiperatividade motora foi revertida tanto com a administração de psicoestimulantes como de agentes serotoninérgicos, sem alteração nos níveis extracelulares de dopamina, diferentemente do que ocorreu com a linhagem com DAT1. Esses achados sugereriram que o psicoestimulante pode ter outros sítios de ação no controle dos sintomas do TDAH, além da proteína transportadora de dopamina, e que a hiperatividade seria mediada pela serotonina em algumas formas do transtorno (Gainetdinov e cols., 1999). Outros estudos sugerem uma interação entre o sistema dopaminérgico e serotoninérgico, no qual a serotonina regularia a liberação da dopamina em algumas áreas, influenciando assim os comportamentos mediados por esse neurotransmissor (ver revisão em Quist e Kennedy, 2001).

3. ETIOLOGIA

O TDAH é um transtorno psiquiátrico heterogêneo, multifatorial, envolvendo a interação entre diversos fatores neurobiológicos, como os genéticos os e fatores ambientais.

3.1. Fatores ambientais

Transtornos do desenvolvimento neurológico podem resultar da exposição do feto ou da criança a contaminantes ambientais; porém, evidências apóiam a hipótese que contaminantes ambientais podem contribuir para apenas uma porção pequena da incidência de TDAH (Woodruff e cols., 2004). A maioria das crianças com TDAH não tiveram exposição a eles, e muitas das que tiveram não desenvolveram o transtorno (Faraone e Biederman, 1998).

Entre os fatores ambientais, agentes psicossociais não específicos, que atuam no funcionamento adaptativo e saúde emocional geral da criança, parecem ter participação no surgimento e manutenção do transtorno, pelo menos em alguns casos. Tais agentes seriam, por exemplo, desentendimentos familiares, presença de transtornos mentais nos pais e criminalidade paterna, condições que parecem ser mais prevalentes nas famílias das crianças afetadas (Faraone e Biederman, 1998). Certas adversidades presentes durante a gravidez, como o uso de álcool, nicotina, cocaína e maconha pela mãe, também parecem agir como fatores de risco para o TDAH (Linnet e cols., 2003; Lautch e Schmidt, 2004; Button e cols., 2005; Noland e cols., 2005). É importante ressaltar que a maioria dos estudos sobre possíveis agentes ambientais apenas evidenciaram uma associação destes fatores com o TDAH, não sendo possível estabelecer uma relação clara de causa e efeito entre eles (Faraone e Biederman, 1998).

3.2. Fatores genéticos

O estudo da genética do TDAH, assim como em qualquer outro transtorno psiquiátrico, envolve dois tipos diferentes de investigações: os estudos genéticos clássicos e os estudos moleculares. Os estudos clássicos compreendem as pesquisas com famílias,

comparação entre gêmeos mono e dizigóticos e crianças adotados. Por meio desses estudos, pode ser confirmada a existência de um componente genético determinando ou influenciando o transtorno em questão. Para se definir quais genes estão envolvidos, utilizam-se os estudos moleculares voltados para os chamados "genes candidatos" (genes possivelmente relevantes para a neurobiologia da característica) (Tannock, 1998).

3.2.1. Estudos genéticos clássicos

Diversos estudos têm mostrado uma prevalência elevada de TDAH entre familiares de indivíduos afetados. Os primeiros estudos observaram que o risco de ter TDAH entre pais e irmãos de crianças afetadas estaria aumentado em 2 a 8 vezes (Faraone e Doyle, 2000). Já que outras diferenças ambientais também poderiam aumentar o risco, estudos caso-controle duplo-cegos examinaram especificamente o risco para irmãos de crianças com TDAH quando os fatores ambientais são controlados (Biederman e cols., 1990, 1992; Faraone e cols., 1992). Após fazer o controle pelo sexo, pela estabilidade da família e pelo nível socioeconômico, esses estudos confirmaram a familiaridade do TDAH. Outros estudos de famílias com esse transtorno foram realizados, os quais mostraram consistentemente uma recorrência familial (Tannock, 1998).

Já que, na falta de dados genéticos moleculares, os estudos de famílias não podem ser totalmente separados das fontes ambientais de transmissão, foram realizados estudos de gêmeos e de adoção para determinar se haveria um componente genético na transmissão do transtorno. Dois estudos concluíram que parentes biológicos de crianças hiperativas têm maior probabilidade de apresentar hiperatividade do que parentes adotivos (Cantwell, 1975; Morrison e Stewart, 1973). Além disso, pesquisas com pais de adotados encontraram uma freqüência significativamente maior de TDAH entre os pais biológicos de crianças afetadas

do que entre os pais adotivos (Thapar e cols., 1999; Sprich e cols., 2000). A média de herdabilidade do TDAH em 20 estudos de gêmeos dos Estados Unidos, da Austrália, da Escandinávia e do Reino Unido foi estimada em 76%, mostrando que o TDAH está entre os transtornos psiquiátricos mais herdáveis (ver revisão em Faraone e cols., 2005).

3.2.2. Estudos moleculares

Grande parte dos estudos sobre componentes genéticos do TDAH emprega a abordagem de associação alélica. Neste tipo de análise, investiga-se uma possível associação entre formas distintas de um gene (alelos) e o fenótipo de interesse. A metodologia é útil para a identificação de alelos relacionados a doenças, particularmente de genes que conferem suscetibilidade a doenças complexas (Pericak-Vance, 1998). A associação pode ter várias causas, entre as quais estão a ação biológica direta do polimorfismo e o desequilíbrio de ligação com um outro polimorfismo que confere a suscetibilidade diretamente. Dessa forma, o estudo de haplótipos tem sido freqüentemente empregado nos estudos de genes candidatos.

Os estudos moleculares podem ser realizados através da abordagem caso-controle, nos quais se compara as freqüências alélicas de um determinado gene candidato entre um grupo de casos e um grupo-controle obtido da população em geral. O problema dos estudos com controles populacionais é o fato de, teoricamente, estarem sujeitos a efeitos de estratificação populacional, pois as freqüências alélicas da maioria dos marcadores genéticos são muito variáveis entre diferentes populações e etnias (Cavalli-Sforza e cols., 1994). A maior parte das investigações realizadas com o TDAH, contudo, utilizam os métodos com base na família, ou seja, o método de risco relativo de haplótipos (HRR) e o

teste de desequilíbrio de transmissão (TDT). Esses métodos de famílias não são muito utilizados em amostras de adultos, pois é mais difícil obter material biológico dos pais.

3.2.2.1. Estudos com genes candidatos no TDAH

Foram realizadas diversas varreduras genômicas em amostras de TDAH, descritas na Tabela 2. Esses estudos sugerem a ausência de um gene principal, com muitos genes de efeito pequeno ou moderado. De modo geral, as regiões cromossômicas mais freqüentemente observadas foram 5p, 7p, 16p e 17p, sugerindo que genes nessas regiões possam ter um papel significativo na etiologia da doença. Em nenhum dos estudos, porém, as regiões dos genes da proteína transportadora de dopamina (DAT1) e receptor D4 de dopamina (DRD4), os mais estudados até agora no TDAH, são significantes, indicando que se os mesmos realmente apresentarem um efeito, esse deve ser pequeno. De todos esses estudos, somente Arcos-Burgos e cols. (2004a) analisaram alguns pacientes adultos juntamente com as crianças.

Tabela 2. Estudos de varredura genômica envolvendo o TDAH.

Autores (ano)	***n***	***Regiões cromossômicas***
Fisher e cols. (2002)	126	5p12, 10q26, 12q23, 16p13
Smalley e cols. (2002)	203	16p13
Bakker e cols. (2003)	164	15q15, 7p12, 9q33
Arcos-Burgos e cols. (2004a)	16	8q12, 11q23, 4q23, 17p11, 12q23, 8p23
Ogdie e cols. (2003 e 2004)	308	5p13, 6q12, 16p13, 17p11
Hebebrand e cols. (2006)	155	5p17, 6q, 7p, 9q, 11q, 12q, 17p

Serão aqui revisados os efeitos de genes para os quais há indícios prévios de estudos de associação sugerindo que possam estar envolvidos no TDAH. O sistema mais estudado

na predisposição ao TDAH tem sido o dopaminérgico. Fazem parte desse sistema os dois genes mais estudados em amostras de crianças, e incluídos no presente trabalho com adultos (DRD4 e DAT1). Esses genes serão detalhadamente abordados posteriormente. O polimorfismo mais estudado no gene da proteína receptora D5 de dopamina (DRD5) tem sido uma repetição dinucleotídica que fica aproximadamente 18,5 kb da região 5' do sítio de início de transcrição (Hawi e cols., 2003). As meta-análises envolvendo o alelo de 148 pares de base (pb) desse polimorfismo no TDAH e estudos baseados em famílias têm se mostrado positivas (Maher e cols., 2002; Lowe e cols., 2004a; Faraone e cols., 2005). Já as meta-análises envolvendo os genes DRD2, DRD3, tirosina hidroxilase (TH) e catecol-O-metiltransferase (COMT) foram negativas (Faraone e cols., 2005). As investigações com a maioria desses marcadores, assim como dos demais sistemas, ainda são bastante reduzidas, impedindo conclusões definitivas.

No sistema noradrenérgico, os estudos concentram-se principalmente no gene que codifica a enzima dopamina-beta-hidroxilase (DBH), que transforma dopamina em noradrenalina. Nesse gene, tem-se investigado preferencialmente um sítio de restrição *TaqI* localizado no íntron 5 do gene (Roman e cols., 2002a), o qual teve resultado significativo em uma recente meta-análise (Farone e cols., 2005). Outro polimorfismo bastante promissor é um sítio de restrição *HhaI* (-1021C/T) localizado no promotor do gene, com efeito funcional (Zabetian e cols., 2001), pouco estudado até o momento. Alguns genes de receptores adrenérgicos também foram analisados, porém, somente um polimorfismo no promotor do gene do receptor α2A de noradrenalina (ADRA2A, C/G na posição -1291) mostrou associação entre o alelo G e sintomas de desatenção (Comings e cols., 1999; Roman e cols., 2003, 2006; Wang e cols., 2006). Além disso, o mesmo alelo mostrou-se associado com transtorno de leitura em pacientes com TDAH (Stevenson e cols., 2005). As

análises dos demais receptores adrenérgicos, ADRA2C e ADRA1C, em amostras de pacientes com TDAH foram todas negativas (Barr e cols., 2001a; De Luca e cols., 2004a). O gene da proteína transportadora de noradrenalina (NET1, SLC6A2) também tem sido alvo de estudos, visto que algumas drogas que bloqueiam essa proteína são eficazes no tratamento da doença (Biederman e Spencer, 1999). Primeiramente, foi demonstrada associação entre sintomas de TDAH e um polimorfismo de nucleotídeo único (SNP, *single nucleotide polymorphism*) em NET1 em pacientes com Síndrome de Tourette (Comings e cols., 2000). Após isso, diversos estudos analisaram este e outros polimorfismos no gene (Barr e cols., 2002; McEvoy e cols., 2002; De Luca e cols., 2004b; Bobb e cols., 2005), mas somente Bobb e cols. (2005) observaram tal associação com o TDAH.

Entre os genes do sistema serotoninérgico, o da proteína transportadora de serotonina (5-HTT) foi o mais estudado. Em uma meta-análise, uma inserção/deleção no promotor (5-HTTLPR) com efeito funcional foi associada ao TDAH (Faraone e cols., 2005). Genes de receptores de serotonina também já foram analisados, como HTR2A e HTR1B, associados negativa e positivamente, respectivamente, em meta-análises (Faraone e cols., 2005). Vários polimorfismos no gene HTR1B foram recentemente associados ao subtipo desatento em uma amostra de crianças com TDAH (Smoller e cols., 2005). Os resultados referentes aos polimorfismos analisados até o momento no gene da enzima triptofano hidroxilase (TPH) foram negativos (Tang e cols., 2001; Li e cols., 2003, 2006), somente associados em pacientes com transtorno de leitura (Li e cols., 2003) ou quando em haplótipo (Li e cols., 2006). Alguns polimorfismos do gene TPH2 têm sido associados ao TDAH em amostras de crianças (Sheehan e cols., 2005; Walitza e cols., 2005).

O gene da enzima monoamina oxidase A (MAO-A) fica localizado no cromossomo X e modera os níveis de noradrenalina, dopamina e serotonina no sistema nervoso central.

Foi descrito um polimorfismo funcional no promotor, um VNTR de 30 pb, o qual encontrou resultados contraditórios até o momento para o TDAH em crianças (positivo em Manor e cols., 2002a, para estudo caso-controle e baseado em família; negativo em Lawson e cols., 2003 em estudo baseado em famílias).

Diversos genes candidatos de outros sistemas têm sido estudados no TDAH. São eles os responsáveis por receptores de acetilcolina (CHRNA4 e CHRNA7; Kent e cols., 2001a, 2001b), receptores de glutamato (NMDA e GRIN2A; Turic e cols., 2004), família contendo o soluto 1 membro 3 (SLC1A3; Turic e cols., 2005), fator neurotrófico derivado do cérebro (BDNF, Kent e cols., 2005), proteína de 25 KDa associada a sinaptossoma (SNAP-25, Barr e cols, 2000a; Mill e cols., 2004, Feng e cols., 2005a) e *calcyon* (DRD1IP; Laurin e cols., 2005). Todos esses genes foram relativamente pouco estudados até o momento, sendo que somente o SNAP-25 foi submetido a uma meta-análise, com resultado positivo em crianças (Faraone e cols., 2005).

O TDAH na vida adulta só passou a ser reconhecido na psiquiatria há pouco tempo. Dessa forma, a maioria dos estudos de genes candidatos tem se concentrado em amostras de crianças com o transtorno. Os estudos de genes candidatos em amostras de adultos com TDAH estão descritos na Tabela 3.

Tabela 3. Estudos envolvendo genes candidatos em amostras de TDAH em adultos.

Primeiro e último autor, local e ano	***Gene e polimorfismo***	***Tipo de estudo***	***Número de casos/famílias***	***Achados***
SV Faraone; J Sakai (Boston, EUA) 1999a	DRD4, VNTR no éxon III	TDT	27 trios, filho com TDAH e ao menos um dos pais com TDAH	TDT: p=0,03
P Muglia; JL Kennedy (Toronto, Canadá) 2000	DRD4, VNTR no éxon III (7R vs não-7R)	Caso-controle TDT	66 casos, 66 controles 44 famílias (29 trios, 14 pares) 110 casos	Caso-controle: χ^2 = 5,65, p= 0,01 TDT: χ^2 = 2,00, p= 0,15
P Muglia, JL Kennedy (Toronto, Canadá) 2002a	DRD3, Ser9Gly (alelos)	TDT	39 famílias (25 trios, 14 pares)	TDT: χ^2 = 0,36, p= 0,54
P Muglia, JL Kennedy (Toronto, Canadá) 2002b	DAT13'UTR VNTR (10R/10R vs 10R/9R vs 9R/9R)	ANOVA TDT qualitativo e quantitativo	152 casos 102 famílias	ANOVA: Escala de TDA Brown, 5 subescalas e escore total: valor de p = NS TDT qualitativo e quantitativo: p= NS
M Johann, N Wodarz (Regensburg, Alemanha) 2003	SLC6A4/5-HTT, 5-HTTLPR (genótipos)	Caso-controle	30 (de 314) alcoolistas com TDAH + personalidade anti-social vs 180 alcoolistas sem comorbidade vs 220 controles	Caso-controle: valor de p= NS
V De Luca, JL Kennedy (Toronto, Canadá) 2004a	ADRA2C, $[TG]_n$ 15 kb *upstream* do primeiro códon (alelos)	TDT	128 trios	TDT para TG_{16}: χ^2 = 0,06, p= 0,79, sem efeito de origem parental detectado TDT para TG_{17}: χ^2 = 0,06, p= 0,80, sem efeito de origem parental detectado
V De Luca, JL Kennedy (Toronto, Canadá) 2004b	SLC6A2/NET1, rs998424, íntron 9 (alelos)	*One-way* ANOVA TDT qualitativo e quantitativo	128 trios	ANOVA: BADDS escore total: F(2,168)=0,61, p= 0,17 TDT: não-significativo para ambos
B Inkster, JL Kennedy (Toronto, Canadá) 2004	DBH, *TaqI* (íntron 5) (alelos)	TDT Caso-controle	97 trios 112 casos, controles pareados	TDT: χ^2 = 1,03, p= 0,31 Caso-controle: χ^2 = 3,63, p= 0,057, mas alelo de risco menos freqüente nos casos
DE Lynn, SL Smalley (Los Angeles, EUA) 2005	DRD4, VNTR no éxon III (genótipos 7R vs não-7R)	Modelo de ajustamento no TCI e genótipo DRD4	171 pacientes de 96 famílias (= pais de pares de irmãos com TDAH; 33% com TDAH vitalício, 50% deles com TDAH atualmente), TCI	Modelo de ajustamento: procura por novidades correlacionado com diagnóstico TDAH na vida (r^2=0,26), DRD4 correlacionado com sintomas TDAH (r^2=0,05), mas não há correlação do DRD4 com novelty seeking.

NS = não-significante; TDT= Teste de desequilíbrio de transmissão; TCI = Inventário de Caráter e Temperamento.

3.2.2.1.1. Gene DAT1

O gene DAT1 (SLC6A3, 5p15.3, Figura 1) foi o primeiro candidato para o TDAH, visto ser o principal alvo do metilfenidato e outras medicações psicoestimulantes (Volkow e cols., 1998; Seeman e Madras, 1998). No nível molecular, a proteína DAT contém uma grande alça extracelular, com sítios consenso para a glicosilação (Figura 2), que funcionam regulando a estabilidade e o transporte da proteína (Li e cols., 2004). A proteína DAT limita a duração da atividade sináptica e difusão através do seqüestro da dopamina em neurônios (Cragg e Rice, 2004) e é expressa seletivamente em todos os neurônios dopaminérgicos (Ciliax e cols., 1995).

Cook e cols. (1995) mostraram uma associação entre o alelo de 480 pb (10 repetições ou 10R) de um polimorfismo do tipo VNTR (*Variable Number of Tandem Repeats*) do DAT1, localizado na região 3' não-traduzida (UTR) do gene. Desde então, esse achado tem sido replicado por vários autores; porém, das meta-análises realizadas em crianças até o momento, a maioria concluiu que não haveria associação entre o alelo 10R e o TDAH (Maher e cols., 2002; Purper-Ouakil e cols., 2005; Wohl e cols., 2005). Somente Faraone e cols. (2005), reunindo estudos baseados em famílias, verificaram uma associação pequena, porém significante, reafirmando a necessidade de mais investigações envolvendo o DAT1. Os trabalhos de associação caso-controle e baseados em famílias publicados entre o gene DAT1 e o TDAH estão na Tabela 4, incluindo aqueles publicados antes e depois das meta-análises citadas. Vale destacar ainda que, de todos esses trabalhos, apenas um (Muglia e cols., 2002b) analisou uma amostra de adultos com TDAH, aumentando a importância de estudos com esse tipo de pacientes, já que os dados com crianças ainda são inconclusivos.

Os resultados dos estudos iniciais sobre a funcionalidade do VNTR 3'UTR no DAT1 são bastante contraditórios, apesar de diversas análises *in vitro* já terem sido

realizadas. Foi mostrado que o alelo 10R estaria associado com níveis elevados de RNA mensageiro (mRNA) em tecidos cerebrais pós-morte (Mill e cols., 2002). A hipótese do alelo 10R sendo mais funcional que os demais apoia os resultados de Fuke e cols. (2001) em linhagens celulares através de luciferase. Por outro lado, Michelhaugh e cols. (2001) demonstraram que o alelo 9R aumentaria a transcrição de DAT em células derivadas de cérebro de camundongos. Esse resultado foi observado também por Miller e Madras (2002), em uma análise de expressão celular. Existem ainda autores que não observaram diferença na expressão entre os dois alelos mais comuns desse polimorfismo (Greenwood e Kelsoe, 2003; Mill e cols., 2005a). Recentemente, uma investigação delineada visando evitar limitações metodológicas presentes nos estudos anteriores apoiou a idéia de que o alelo 10R exibiria uma concentração de DAT maior que o 9R. Esse resultado foi reafirmado por diversas evidências e experimentos convergentes, sustentando esse polimorfismo como sendo funcional (Van Ness e cols., 2005).

Os estudos *in vivo*, usando Tomografia Computadorizada por Emissão de Fóton Único (SPECT; *Single Photon Computerized Emission Tomography*), também são contraditórios ou sem efeito diferencial entre os alelos (Martinez e cols., 2001). Heinz e cols. (2000) observaram que indivíduos com o genótipo 9/10 possuíam uma redução da proteína quando comparados com aqueles 10/10. Contudo, Van Dyck e cols. (2005), analisando uma amostra de 96 americanos euro-descendentes, relataram maiores níveis de DAT em portadores do alelo 9R em comparação aos homozigotos para 10R, em concordância com Jacobsen e cols. (2000). Vale destacar, no entanto, que esses estudos apresentam um poder estatístico muito baixo, tendo em conta a heterogeneidade das amostras e o pequeno efeito presumido do polimorfismo.

Embora esse VNTR no DAT1 fique na região 3'UTR do gene e está no final oposto das seqüências promotoras à montante de DAT1, as seqüências 3'UTR são conhecidas pelo seu papel na regulação da expressão gênica (Asherson e cols., 2004). Essas seqüências podem controlar especificamente a exportação nuclear, a poliadenilação e a taxa de tradução e de degradação deste mRNA (Conne e cols., 2000). A região 3' UTR pode, então, ser vista como uma região regulatória que é essencial para a expressão apropriada de muitos genes.

Os estudos de farmacogenética envolvendo o TDAH e o metilfenidato tem se concentrado principalmente nesse gene e nesse VNTR de 40 pb. Winsberg e Comings (1999) foram os primeiros a fazer tal tipo de análise, na qual observaram uma resposta diminuída em portadores do genótipo homozigoto para o alelo 10R, dado replicado por Roman e cols. (2002b) e por Cheon e cols. (2005). No entanto, Kirley e cols. (2003), Stein e cols. (2005) e Bellgrove e cols. (2005a) observaram uma resposta aumentada em pacientes com o alelo 10R, enquanto que dados publicados em McGough (2005) e Langley e cols. (2005) não demonstraram efeito entre o genótipo desse polimorfismo e a resposta ao metilfenidato. Os dados de farmacogenética envolvendo esse gene e outros no TDAH permanecem inconclusivos. Os resultados contraditórios podem ser devidos à estratificação populacional nos diferentes grupos de estudo, também sendo necessária a análise em amostras maiores que as relatadas até o momento (McGough, 2005).

Mais recentemente, diversos SNPs foram identificados e têm sido estudados em amostras de crianças com TDAH. Um polimorfismo no éxon 15 (G352A) do DAT1 foi analisado em uma amostra de crianças de origem chinesa (Qian e cols., 2004a), com uma tendência do alelo G em meninas com TDAH. Barr e cols. (2001b) investigaram, juntamente com o VNTR na região 3'UTR, dois polimorfismos de sítio de restrição no

íntron 9 e no éxon 9 no gene DAT1, verificando associação apenas quando a análise haplotípica continha o alelo 10R. Galili-Weisstub e cols. (2005) encontraram os mesmos resultados para um SNP no éxon 15. Hawi e cols. (2003) analisaram dois polimorfismos na região codificadora (éxon 9 e 15) e três polimorfismos na região flanqueadora do gene (um na região 5' e dois na região 3'), com uma transmissão preferencial para aqueles da região 5' (alelo 3 do marcador D5S1981) e da 3' (alelo 2 do marcador DS52005) em uma análise de HRR. Através da análise de haplótipos, foram encontradas quatro combinações associadas ao TDAH, todas com o alelo 10R. Feng e cols. (2005b) estudaram, além do VNTR 3'UTR, polimorfismos *MspI* (rs27072, localizado 480 pb a montante do VNTR), *DraI* (T/C, localizado a 136 pb a jusante do VNTR), *BstUI* (rs3863145, localizado a 852 pb mais a montante do VNTR que DraI), no éxon 9 (rs6347) e íntron 9 (rs8179029). Somente o alelo G de *MspI* foi transmitido preferencialmente para os pacientes com TDAH; porém, quando em haplótipo, o alelo 10R era significativo. Os dados de haplótipos desses autores, sempre envolvendo o 10R, são compatíveis com os de Brookes e cols. (2006), que analisaram 10 polimorfismos da região 5' à 3' do gene DAT1. De todos eles, somente foi demonstrada transmissão preferencial do alelo 10R e do alelo 3 do VNTR de 30 pb do íntron 8, e quando estes dois alelos estavam juntos em haplótipos em duas populações diferentes. Por outro lado, Langley e cols. (2005) testaram o VNTR 3'UTR e 3 SNPs na provável região promotora do DAT1 em 263 trios e também em estudo caso-controle, e não puderam detectar associação entre nenhum desses marcadores ou haplótipos e o TDAH.

Figura 1. Localização do polimorfismo tipo VNTR de 40 pb na região 3'UTR do gene DAT1 (adaptado de Madras e cols., 2005).

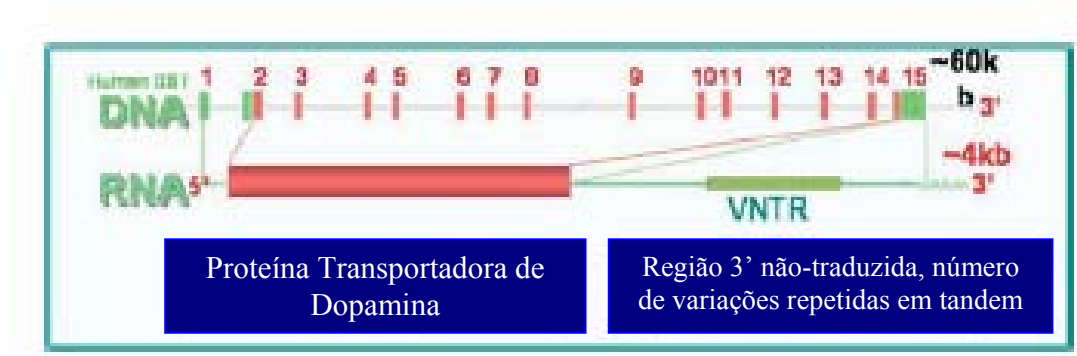

Figura 2. Modelo da proteína DAT (adaptação de Madras e cols., 2005).

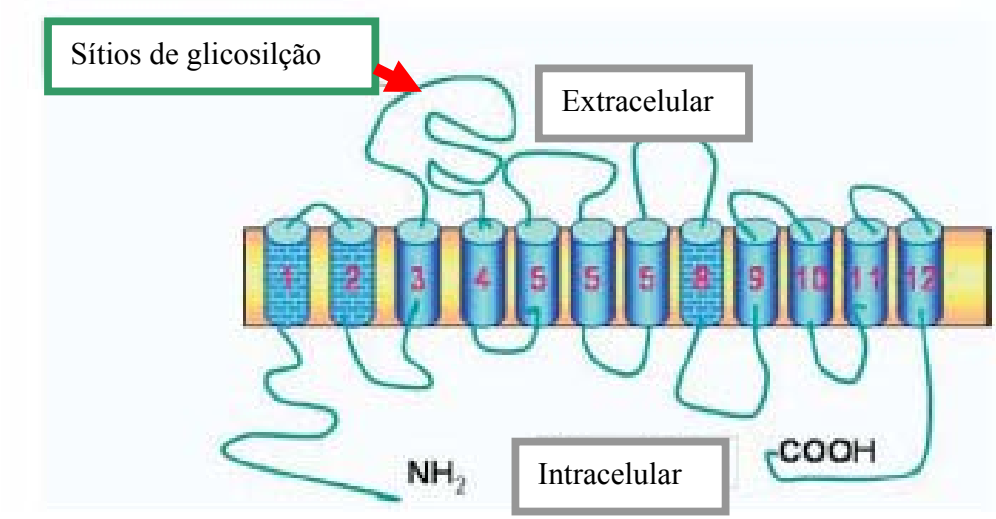

Tabela 4. Estudos de associação caso-controle e baseados em família entre o TDAH e o alelo 10R do VNTR 3'UTR do gene DAT1.

Primeiro Autor	Ano	Método	Tamanho amostral	Crianças / Adultos	Achado	Local
Cook e cols.	1995	HRR	49 trios	Crianças	Positivo	EUA
Gill e cols.	1997	HRR	40 trios	Crianças	Positivo	Irlanda
Waldman e cols.	1998	TDT e subtipos	117 famílias	Crianças	Positivo, principalmente para sintomas de hiperatividade.	EUA
Daly e cols.	1999	TDT e HRR	118 famílias	Crianças	Positivo	Irlanda
Palmer e cols.	1999	TDT	124 famílias	Crianças	Negativo	EUA
Holmes e cols.	2000	Caso-controle e TDT	137 Crianças TDAH	Crianças	Negativo	Reino Unido
Swanson e cols.	2000a	TDT e HRR	80 famílias	Crianças	Negativo	Canadá
Barr e cols.	2001b	TDT	102 famílias	Crianças	Negativo; positivo para haplótipo contendo alelo 10R.	Canadá
Curran e cols.	2001a	TDT	*	Crianças	Positivo para amostra do Reino Unido; negativo para amostra da Turquia.	Reino Unido e Turquia
Payton e cols.	2001	Gêmeos	Gêmeos: 50 pares com escores muito concordantes e 42 pouco	Crianças	Negativo	Inglaterra
Roman e cols.	2001	Caso-controle e HRR	81 famílias, 100 controles	Crianças	Negativo.	Brasil
Rowe e cols.	2001	Caso-controle	Pais de TDAH: 80 pais, 107 mães vs. Pais de controles: 42 pais e 51 mães	Adultos com filhos com TDAH, análise retrospectiva	Positivo para mães 10/10 e maiores níveis de desatenção.	EUA
Todd e cols.	2001a	TDT	523 gêmeos	Crianças	Negativo, inclusive com subtipos.	EUA
Muglia e cols.	2002b	TDT	152 TDAH	Adultos	Negativo	Canadá
Chen e cols.	2003	TDT	110 trios	Crianças	Positivo	Taiwan
Hawi e cols.	2003	HRR	118 crianças com TDAH	Crianças	Positivo	Irlanda
Oh e cols.	2003	Medidas de TDAH	44 meninos	Crianças	Positivo com 10/10 e medidas de TDAH.	Coréia
Smith e cols.	2003	Caso-controle	105 ADHD, 68 controles	Crianças	Negativo	EUA
Carrasco e cols.	2004	Caso-controle	26 famílias com TDAH, 25 controles	Crianças	Negativo	Chile
Kustanovich e cols.	2004	TDT	293 famílias com 535 crianças afetadas	Crianças	Negativo	EUA
Qian e cols.	2004b	Caso-controle, HRR	340 TDAH, 226 controles e	Crianças	Negativo, porém alelos mais	China

		e TDT	202 trios		longos aumentam risco.	
Bakker e cols.	2005	TDT	236 famílias	Crianças	Negativo	Holanda
Bellgrove e cols.	2005a	Medidas de desatenção	43 famílias	Crianças	Positivo para 10R e desatenção do lado esquerdo do campo de visão.	Irlanda
Bellgrove e cols.	2005b	Caso-controle	22 TDAH, 20 controles	Crianças	Positivo para 10R e prejuízo neuropsicológico no TDAH.	Irlanda
Bobb e cols.	2005	Caso-controle e TDT	163 TDAH, 192 pais e 129 controles	Crianças	Negativo	EUA
Cornish e cols.	2005	Caso-controle	58 meninos com mais de 90% de sintomas de TDAH e 68 meninos com menos de 10%	Crianças	Positivo para genótipo 10/10 com sintomas de TDAH e inibição de resposta, e amostra geral da população.	Canadá
Feng e cols.	2005b	TDT	178 famílias	Crianças	Negativo	Canadá
Galili-Weisstub e cols.	2005	TDT	76 famílias	Crianças	Positivo	Israel
JW Kim e cols.	2005	Caso-controle	85 TDAH	Crianças	Negativo para análise geral; porém positivo entre impulsividade e 9R.	Coréia
Langley e cols.	2005	Caso-controle e FBAT	263 trios e 287 controles	Crianças	Negativo	Reino Unido
Mill e cols.	2005b	Gêmeos	329 homens gêmeos dizigóticos	*	Positiva	Reino Unido
Simsek e cols.	2005	Caso-controle	92 crianças com TDAH e 110 controles	Crianças	Negativo para caso-controle, meninos com TDAH tem mais 10R que meninas com TDAH (diferença não vista em controles).	Oman
Todd e cols.	2005	TDT e subtipos	2090 TDAH multicêntrico	Crianças	Negativo para análise geral; positivo entre 10R e subtipo combinado severo.	EUA
YS Kim e cols.	2005	TDT	126 trios	Crianças	Negativo	EUA e Coréia
Brookes e cols.	2006	TDT	180 famílias inglesas e 216 famílias taiwanesas	Crianças	Positivo em ambas as amostras.	Reino Unido e Taiwan
Carrasco e cols.	2006	Família	*	Crianças	Negativo	Chile
Cheuk e cols.	2006	Caso-controle e TDT	64 famílias, 64 controles	Crianças	Negativo	China

HRR: risco relativo de haplótipos; TDT: teste de desequilíbrio de transmissão; Tamanho amostral: em famílias (trios, duplas e/ou pares de irmãos) para HRR e TDT ou em número de probandos e controles para estudos caso-controle.

* Dados não obtidos durante a confecção das tabelas.

** Positivo: $p < 0.05$; negativo: $p > 0.05$

3.2.2.1.2. Gene DRD4

Outro gene do sistema dopaminérgico intensamente investigado no TDAH é o gene DRD4. Essa proteína está presente em redes frontal-subcorticais implicadas na fisipatologia do TDAH através de estudos de neuroimagem e neuropsicológicos (Faraone e Biederman, 1998). Em humanos, o gene DRD4 está localizado no cromossomo 11p15.5 (Gelernter, 1992; Figura 3). Esse gene é um membro da família de receptores de dopamina tipo D2 por ter um grande anel intracelular, diminuir a formação de AMP cíclico (cAMP) e ativar a abertura de canais de potássio e inibir os de cálcio (Ceresér e Vianna, 2004).

Os pesquisadores têm focado predominantemente um VNTR de 48 pb no éxon III do gene, visto que estudos *in vitro* mostraram que a variante de 7 repetições (7R) produziria uma proteína com menor resposta à dopamina (Van Tol e cols., 1992; Asghari e cols., 1995). Asghari e cols. (1995) verificaram que esse VNTR causaria pequenas mudanças na habilidade do DRD4 bloquear a produção de cAMP, tendo o alelo 7R uma potência de dobramento de 2-3 vezes menor para o acoplamento de dopamina e adenil ciclase em comparação com os alelos 2R ou 4R. Dados genéticos mais atuais, entretanto, sugerem que a maioria dos alelos 2R são derivados do 7R (Wang e cols., 2004). A variante 2R também teria uma resposta cAMP enfraquecida, intermediária aos alelos 4R e 7R (Figura 4; Wang e cols., 2004). Essa resposta seria "não-linear" (i.e., a capacidade reduzida de cAMP não seria linearmente relacionada ao tamanho do VNTR) (Asghari e cols., 1995; Jovanovic e cols., 1999). As meta-análises de crianças com TDAH têm mostrado associação positiva entre o alelo 7R e crianças com TDAH em estudos caso-controle e baseados em família (Faraone e cols., 2001, 2005; Maher e cols., 2002; Wohl e cols., 2005).

Um número menor de estudos tem analisado outros polimorfismos nesse gene. Um exemplo é uma repetição de 120 pb na região 5' (região não-codificadora), localizada a

1,2kb do ponto de início de tradução do DRD4 (Seaman e cols., 1999). McCracken e cols. (2000) relataram uma associação entre o alelo de 240 pb (L, com a duplicação) e o TDAH, porém, a maioria dos outros autores não conseguiu o mesmo resultado (Barr e cols., 2001c; Todd e cols., 2001b; Frank e cols., 2004; Kirley e cols., 2004) ou apenas o observou em haplótipos envolvendo também outros polimorfismos deste gene, principalmente com o alelo 7R do éxon III (Mill e cols., 2003; Arcos-Burgos e cols., 2004b). Somente Kustanovich e cols. (2004) conseguiram replicar a associação direta entre esse polimorfismo e o TDAH. Posteriormente, foi demonstrado que este seria um polimorfismo funcional, com o alelo L tendo uma função diminuída em relação ao S (D'Souza e cols., 2004). Trabalhos caso-controle e baseados em família referentes a esse polimorfismo e ao VNTR no éxon III estão na Tabela 5. Novamente, destaca-se o pequeno número de trabalhos com adultos.

Outros polimorfismos na região promotora do gene DRD4 têm sido estudados. Barr e cols. (2001c) analisaram, além da duplicação de 120 pb, uma mudança C/A em -521, a qual mostrou previamente influenciar o nível de transcrição do gene (Okuyama e cols., 1999), além de uma substituição C/G em -616. Porém, nenhum desses polimorfismos mostrou-se associado ao TDAH. Resultados negativos para os mesmos polimorfismos e para uma repetição de poli-G no íntron I foram também descritos por Mill e cols. (2003) e Kirley e cols. (2004). Este último grupo verificou uma tendência de associação entre pacientes com TDAH e o alelo A da região -521, também associado com pacientes com desempenho prejudicado em testes de atenção (Bellgrove e cols., 2005c).

Depois do gene DAT1, o VNTR do éxon III do DRD4 tem sido o mais estudado no TDAH com relação à resposta ao metilfenidato. Mesmo assim, o número de trabalhos ainda é bastante reduzido. O primeiro estudo não conseguiu mostrar efeito do gene (Winsberg e

Comings, 1999). Hamarman e cols. (2004) concluíram que crianças portadoras do alelo 7R necessitavam de doses mais altas do medicamento para melhoria e normalização dos sintomas. Esse estudo apoia os dados de Seeger e cols. (2001), no qual o 7R estaria associado a uma melhora terapêutica menos intensa.

Figura 3. Esquema do gene DRD4 e seus polimorfismos mais estudados, entre outros (adaptado de Wang e cols., 2004).

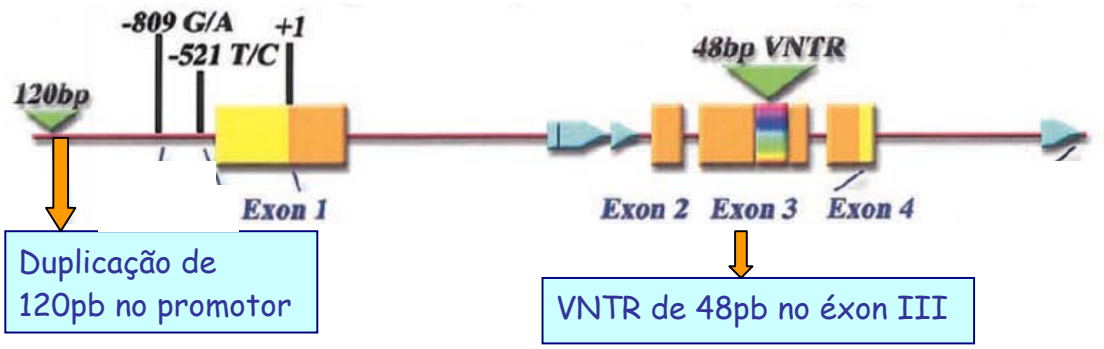

Figura 4. Gráfico mostrando a funcionalidade reduzida dos alelos 7R e 2R do VNTR de 48 pb do éxon III do gene DRD4 em comparação ao alelo mais comum, 4R, sugerindo que o alelo 2R seja uma mistura dos alelos 7R e 4R (adaptação de Wang e cols., 2004).

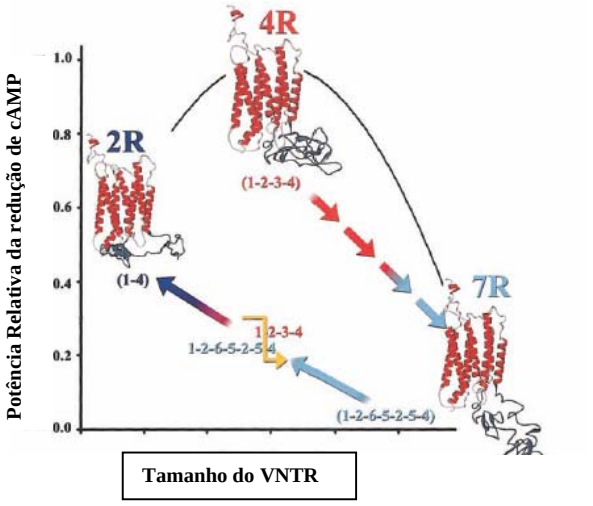

Tabela 5. Estudos de associação caso-controle e baseados em família entre o TDAH e polimorfismos do gene DRD4.

Primeiro e último autor	Ano	Polimorfismos	Método	Tamanho amostral	Crianças / Adultos	Achado	Local
Lahoste e cols.	1996	Éxon III	Caso-controle	*	Crianças	Positivo para 7R	EUA
Castellanos e cols.	1998	Éxon III	Caso-controle	41 TDAH e 56 controles	Crianças	Negativo	EUA
Rowe e cols.	1998	Éxon III	Caso-controle	*	Crianças	Positivo entre subtipo desatento e alelo 7R	EUA
Smalley e cols.	1998	Éxon III	TDT	52 famílias	Crianças	Positivo para alelo 7R	EUA
Swanson e cols.	1998b	Éxon III	Caso-controle e HRR	52 famílias	Crianças	Positivo para alelo 7R	EUA
Faraone e cols.	1999a	Éxon III	TDT	27 trios com adulto com TDAH, esposa e filho TDAH	Crianças e Adultos	Positivo para alelo 7R	EUA
Barr e cols.	2000b	Éxon III	TDT	*	Crianças	Positivo para 2 haplótipos	Canadá
Eisenberg e cols.	2000	Éxon III	TDT	*	Crianças	Negativo	Israel
Hawi e cols.	2000	Éxon III	HRR e caso-controle	78 trios com TDAH, 22 trios sem	Crianças	Negativo	Irlanda
Holmes e cols.	2000	Éxon III	TDT e caso-controle	137 Crianças TDAH	Crianças	Positivo para alelo 7R	Reino Unido
Kotler e cols.	2000	Éxon III	HRR	49 trios	Crianças	Negativo	Israel
McCracken e cols.	2000	Duplicação 120 pb no promotor	TDT e subtipos	371 Crianças TDAH e pais	Crianças	Positivo para alelo de 240 pb	EUA
Muglia e cols.	2000	Éxon III	TDT e caso-controle	44 famílias, 66 ADHD e 66 controles	Adultos	Positivo para caso-controle, tendência para estudo de família	Canadá
Sunohara e cols.	2000	Éxon III	TDT	*	Crianças	Positivo para 7R	Canadá
Swanson e cols.	2000	Éxon III	Subgrupos de TDAH	96 TDAH, 48 controles	Crianças	Positivo entre 7R e comportamento extremo; negativo para testes de atenção	EUA
Tahir e cols.	2000	Éxon III	TDT e comorbidades	104 trios	Crianças	Positivo para alelo 7R	Turquia
Auerbach e cols.	2001	Éxon III	Medidas de temperamento e inteligência	64 crianças de 1 ano	Crianças	Positivo para processamento de informação e atenção sustentada cedo	Israel
Barr e cols.	2001c	Duplicação 120 pb no promotor	TDT	82 famílias	Crianças	Negativo	Canadá
Curran e cols.	2001b	Éxon III	Análise de	*	Crianças	Positivo entre 7R e altos escores de	Reino Unido

			sintomas de TDAH em Crianças da população			TDAH	
Mill e cols.	2001	Éxon III	HRR e caso-controle	132 TDAH, 189 controles e 85 trios	Crianças	Negativo	Reino Unido
Payton e cols.	2001	Éxon III	TDT	150 TDAH	Crianças	Negativo	Inglaterra
Roman e cols.	2001	Éxon III	HRR e Caso-controle	81 famílias	Crianças	Positivo para estudo caso-controle; negativo para HRR	Brasil
Rowe e cols.	2001	Éxon III	Caso-controle e comorbidades	TDAH: 80 pais, 107 mães; 93 pais controles	Pais de TDAH X Pais de controles	Positivo para pais com 7R e altos níveis de desatenção e transtorno de conduta.	EUA
Todd e cols.	2001b	Éxon III e duplicação 120 pb no promotor	Subtipos	*	Crianças	Negativo	EUA
Holmes e cols.	2002	Éxon III	TDT e comorbidades	67 famílias com ADHD e transtorno de conduta	Crianças	Positivo entre 7R e Transtorno de Conduta em TDAH.	Reino Unido
Manor e cols.	2002b	Éxon III	TDT	178 trios	Crianças	Positiva	Israel
Kustanovich e cols.	2004	Éxon III e duplicação 120 pb no promotor	TDT	293 famílias, 535 pacientes	Crianças	Positivo para alelo 120 pb, negativo para 7R.	EUA
Lakatos e cols.	2003	Éxon III	Temperamento de Crianças de 12 meses	90 crianças	Crianças	Negativo	Hungaria
Mill e cols.	2003	Duplicação 120 pb no promotor, C-616G, C-521A, íntron 1, éxon III	família	188 famílias	Crianças	Positivo para haplótipo	Reino Unido
Smith e cols.	2003	Éxon III	Caso-controle	105 ADHD, 68 controles	Adultos	Negativo	EUA
Arcos-Burgos e cols.	2004b	Éxon III e duplicação 120 pb promotor	TDT	14 famílias	Crianças	Positivo para 7R e haplótipo 7R+240 pb	EUA
Carrasco e cols.	2004	Éxon III	Famílias	26 famílias com TDAH, 25 sem	Crianças	Positiva	Chile
El-Faddagh e cols.	2004	Éxon III	Estudo retrospectivo	265 crianças da comunidade	Crianças	Positivo entre 7R e TDAH	Alemanha
Frank e cols.	2004	Duplicação 120 pb no promotor	Caso-controle	81 TDAH e 24 controles	Crianças	Negativa	EUA

Kirley e cols.	2004	Duplicação 120 pb no promotor, -616, -521, -376, éxon III.	Comorbidades e subtipos	178 famílias	Crianças	Positiva entre 7R e ODD	Irlanda
Langley e cols.	2004	Éxon III	Medidas Neuropsicológicas	133 TDAH	Crianças	Alelo 7R associado com respostas impulsivas.	EUA
Lowe e cols.	2004b	Marcadores adicionais na parte 5' do gene, como o SNP -521	TDT	*	Crianças	Positiva associação entre -521 e TDAH.	Irlanda
Qian e cols.	2004b	Éxon III	HRR e caso-controle	202 famílias, 340 TDAH, 226 controles	Crianças	Positivo para alelos longos, negativo com alelos específicos.	China
Seeger e cols.	2004	Éxon III	Caso-controle	64 TDAH, 163 controles	Crianças	Positiva para alelo 7R com interação ambiental.	Alemanha
Bakker e cols.	2005	Éxon III	TDT	236 famílias	Crianças	Negativo	Holanda
Bellgrove e cols.	2005c	Éxon III e dois SNP (-521 e -616) no promotor	Caso-controle e medidas de atenção	54 crianças com TDAH, mais grupo controle	Crianças	Positiva para alelo 7 do Éxon III e melhor perfomance no teste de atenção, e alelo T do -521 com resposta pior.	Irlanda
Bobb e cols.	2005	Éxon III	TDT e caso-controle	163 TDAH, 192 pais e 129 controles	Crianças	Negativo	EUA
Brookes e cols.	2005	Éxon III e duplicação 120 pb no promotor	TDT e HHRR	216 famílias	Crianças	Negativo	Reino Unido e Taiwan
Kim e cols.	2005	Éxon III	TDT	126 trios	Crianças	Negativo	EUA e Coréia
Leung e cols.	2005	Éxon III	Caso-controle	32 TDAH e controles	Crianças	Positiva para 2R	China
Lynn e cols.	2005	Éxon III	TDT	171 pais e 96 probandos	Adultos	Associado com uma história de vida de TDAH.	EUA
Mill e cols.	2005b	Éxon III	Gêmeos	329 homens gêmeos dizigóticos	*	Negativo	Reino Unido
Todd e cols.	2005	Éxon III	TDT e subtipos	2090 TDAH multicêntrico	Crianças	Negativo, tendência entre 7R e subtipo combinado severo.	EUA
Bhaduri e cols.	2006	Éxon III, duplicação 120 pb no promotor e éxon 1 (12pb)	HRR e TDT	50 famílias	Crianças	Positivo entre 6R e 7R do exon 3 e TDAH, negativo para outros polimorfismos.	Índia
Carrasco e cols.	2006	Éxon III	Famílias	*	Crianças	Negativo	Chile

HRR: risco relativo de haplótipos.
TDT: teste de desequilíbrio de transmissão.
Tamanho amostral: em famílias (trios, duplas e/ou pares de irmãos) para HRR e TDT ou em número de probandos e controles para estudos caso-controle.
* Dados não obtidos durante a confecção das tabelas.
** Positivo: $p < 0.05$; negativo: $p > 0.05$

II. OBJETIVOS

1. Geral

- Investigar possíveis associações entre polimorfismos nos genes DAT1 e DRD4 em uma amostra de pacientes adultos com TDAH, estudados em comparação com um grupo controle da população.

2. Específicos

- Analisar as freqüências gênicas dos polimorfismos de duplicação de 120 pb no promotor do gene DRD4, VNTR de 48 pb no éxon III do gene DRD4 e VNTR de 40 pb na região 3'UTR do gene DAT1 em uma amostra de adultos euro-descendentes com TDAH, comparando com as freqüências gênicas de uma amostra controle da mesma etnia.
- Investigar se as freqüências do polimorfismo 3'UTR VNTR do gene DAT1 estão associadas aos subtipos do TDAH, gravidade e comorbidades.
- Avaliar se as freqüências dos polimorfismos do gene DRD4 estão associadas aos subtipos do TDAH, gravidade, comorbidades e idade de início dos sintomas.

III. MANUSCRITO I

Bidirectional and Gender-Related Effects of the DAT1 3'UTR VNTR in the Subtypes of Adults with Attention-Deficit/Hyperactivity Disorder

FZC Marques, EH Grevet, CAI Salgado, AG Fischer, K Kalil, MM Victor, CR Garcia, NO Sousa, P Belmonte-de-Abreu and CHD Bau.

A ser submetido à revista *Molecular Psychiatry*

ORIGINAL RESEARCH ARTICLE

Bidirectional and Gender-Related Effects of the DAT1 3'UTR VNTR in the Subtypes of Adults with Attention-Deficit/Hyperactivity Disorder

FZC Marques[1,3], EH Grevet [1], CAI Salgado [1], AG Fischer [1], K Kalil [1], MM Victor [1], CR Garcia [1], NO Sousa [1], P Belmonte-de-Abreu[1, 2] and CHD Bau[1,3].

[1] Adult ADHD Outpatient Clinic, Clinical Hospital of Porto Alegre, Porto Alegre, RS, Brazil.

[2] Department of Psychiatry, Medical School, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

[3] Department of Genetics, Institute of Biosciences, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Running title: DAT1 in adult ADHD subtypes.

Correspondence to Claiton H. D. Bau, Departamento de Genética, Instituto de Biociências, UFRGS, Caixa Postal 15053, 91501-970 Porto Alegre, RS, Brazil.
Tel: (55) 51 3316 6718 Fax: (55) 51 3316 7311
E-mail: claiton.bau@ufrgs.br

Funding sources: CNPq, FINEP, PRONEX, FAPERGS

Abstract

The 10-repeats (10R) allele of the 3'UTR VNTR of the dopamine transporter gene (DAT1) has been reported to be associated with attention-deficit/hyperactivity disorder (ADHD) in several children samples. There is growing evidence from in vitro studies indicating that variability in the repeat number of the polymorphism may influence the transporter density. The diagnoses of ADHD and subtypes were based on DSM-IV criteria. The polymorphic site of DAT1 was genotyped in 308 adult patients and 233 controls, all of them Brazilians of European descent. The 10/10 genotype was significantly underrepresented among ADHD patients (χ^2= 6.06, P= 0.014). The post hoc analysis revealed that genotypes containing the 9R allele are more common among male patients of the inattentive subtype and among female patients of both subtypes. Males of the combined subtype and controls had similar genotype frequencies. Subjects with the 10/10 genotype presented more hyperactivity symptoms than those with other genotypes. These data suggest that the DAT1 3'UTR VNTR effects in the ADHD subtypes are bidirectional and influenced by gender. Children and adult ADHD samples provide apparently contradictory results. However, considering the subtype and gender differences of the samples, the overall available data suggests that different alleles could be associated to different aspects of the disorder, whose preponderance depends on the sample characteristics.

Keywords: attention deficit disorder, dopamine transporter gene, inattentive, combined, hyperactivity.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a prevalent, highly heritable[1] childhood-onset disorder that is characterized by age-inappropriate levels of inattention, hyperactivity and impulsivity.[2] Although it is commonly recognized as a childhood disorder, it is estimated that 30-70% of individuals have persistent symptoms into adulthood.[3-5] The decline of the prevalence with increasing age may be advantageous to genetic investigations because the persistent form of the disorder is less common and could have a greater genetic liability.[6] Adult samples are also more suitable to the investigation of gender effects, since the proportion of affected females, very small in the childhood, increases to approximately 1:1 among adults.[7,8] The age increase is also accompanied by a higher prevalence of the inattentive subtype.[9]

The human dopamine transporter gene (DAT1; SLC6A3) was initially considered as a suitable candidate gene for ADHD because stimulant medications are know to block the transporter as one mechanism of action for achieving their therapeutic effects.[10] This treatment significantly down-regulates elevated DAT density in the brain of ADHD children and adults.[11,12] Imaging studies have revealed a relatively increased density of the dopamine transporter in the striatal regions of ADHD adults compared with non-ADHD subjects.[13]

DAT1 has been mapped to chromosome 5p15.3,[14,15] and is comprised of 15 exons. It contains a 40 base pair (bp) variable number of tandem repeats (VNTR) in the 3'-untranslated region (3'UTR).[15] Alleles with 3 to 13 repeats have been described, but those with 10 and 9 repeats (10R and 9R, respectively) are the most frequent across several populations.[16,17] Many groups have investigated the functional role of the DAT1 3'UTR VNTR in vitro and in vivo, both with conflicting and inconclusive results, sustaining either the 9R[18-21] or the 10R[22-24] alleles with a higher transcription, or no

differential effect between these two alleles.[25-27] The most recent study, designed to avoid the pitfalls of previous investigations, utilized a targeted stable integration protocol followed by radioligand binding and immunoblotting techniques.[28] The 10R allele displayed a higher concentration of the dopamine transporter protein than the 9R allele.[28] The study provides multiple lines of converging in vitro evidence for the DAT1 VNTR's modulatory effect on DAT expression, thus supporting it as a functional polymorphism that may contribute to the recognized inter-individual differences in DAT density and dopaminergic function.

The first study to detect an association between the 10R allele of the 3'UTR VNTR of DAT1 and children ADHD was based on 49 families.[29] After that, many investigations were performed, some of them replicating the initial finding.[30-38] Two meta-analyses pooled eleven and thirteen family based studies and did not support the involvement of the dopamine transporter gene in ADHD liability.[39,40] Another meta-analysis[1] showed a weak association between this allele and the disorder (OR= 1.13), suggesting that DAT1 merits further analysis. As opposed to the large number of children studies, up to now there is only one investigation of this polymorphism in adult ADHD.[41] This study found no significant association between the 3'UTR VNTR and ADHD, considering the disorder either as categorical or as a continuous trait, although there was no focus on subtypes.

The overall findings regarding the possible role of the DAT1 3'UTR VNTR on ADHD suggest that a possible small effect could be masked by factors related to the heterogeneity of the problem. The successful treatment of the disorder with pharmacological agents that target the dopamine transporter and the functionality of this polymorphism strongly suggest that DAT1 could influence some facet of the disorder. Up to now, few works had sufficient statistical power to explore the effects of gender

and subtypes. The aim of the present study is test for the association between the DAT1 3'UTR VNTR polymorphism and adult ADHD in a relatively large sample, separately by gender and subtypes.

Materials and Methods

Subjects

Patients with ADHD were drawn from the ADHD outpatient clinic of the Hospital de Clinicas de Porto Alegre (HCPA, a major teaching hospital in the south of Brazil) in the period from September 2002 to December 2005. The exclusion criteria were evidence of clinically significant neurological diseases and current or past history of psychosis and IQ $\leq$ 70.[42] All of them were older than eighteen years old. Patients were investigated and treated after a screening interview that confirmed ADHD diagnosis. The interviewers in this research were all psychiatrists extensively trained in the application of all instruments in the research protocol.

This study included 308 adult ADHD patients, 163 males (52.9%) and 145 females (47.1%). The three subtypes were found in the sample (39.0% inattentive, 6.5% hyperactive/impulsive and 54.5% combined), and the male/female ratio in the subtypes did not differ. All patients were Brazilians of European descent. Unlike other Brazilian regions, the African contribution to the gene pool in this region of the country is relatively small, about 7.2%.[43] The classification was based in skin color, morphologic characters and self-reported ethnicity.

The control group for allele and genotype frequencies is composed of 233 blood donor males assessed in a blood bank. Controls were therefore not interviewed for psychiatric diagnoses. The expected frequency of ADHD in this group is the same as in

the general population. This sample was designed to be non-screened, representative of the gene frequencies of individuals of European descent in Porto Alegre. These individuals are replacement donors, that is, they are people that replaced the blood used by a hospitalized family member or friend. For this reason, a behavior-related bias is not likely. Careful matching for ethnicity with the ADHD patients was performed.

This investigation was approved by the Ethical Committee of HCPA and by the Federal University of Rio Grande do Sul. All individuals sampled (patients and controls) provided written informed consent.

Diagnostic assessment of ADHD

The diagnostic procedures for ADHD are extensively described elsewhere.[8] The diagnoses followed the DSM-IV criteria,[2] using the respective sections of the Portuguese version of K-SADS-E[44] for children and adolescents. The Kappa coefficients of interrater agreement for the K-SADS-E were higher than 0.90 for current and lifetime diagnoses of ADHD and its subtypes.[45]

Current and lifetime psychiatric comorbidities (alcohol, drug and nicotine dependences, antisocial personality, oppositional defiant, anxiety, major depressive and bipolar disorders) were assessed using SCID-IV-R.[46] The severity of current ADHD symptoms was assessed by the SNAP-IV Rating Scale[47] and K-SADS-E.[44] Temperament dimensions (novelty seeking, harm avoidance, reward dependence and persistence) were evaluated using the Temperament and Character Inventory (TCI).[48,49]

Laboratory Methods

High-molecular-weigh genomic DNA was extracted from whole blood by an adaptation of Lahiri and Nurnberger.[50] PCR amplification of the 40 bp VNTR of DAT1

was performed according to adaptations of Sano *et al*.[51] The products were electrophoresed on 2.5% agarose gel, stained with ethidium bromide. One hundred base pair ladders were used to score the various repeat alleles, and positive controls were always analyzed together with the PCR products.

Statistical Analysis

Allele frequencies were estimated by gene counting. The analysis of Hardy-Weinberg equilibrium and comparisons between patients and controls were performed with the chi-square test. One-way analysis of variance (ANOVA) was used to analyze the number of ADHD symptoms and TCI scores.

Results

Genotype distribution was in Hardy-Weinberg equilibrium both in control subjects (χ^2= 14.04, P= 0.522) and patients (χ^2= 5.17, P= 0.99). The allele and genotype frequencies for the DAT1 polymorphism in patients and controls are shown in Table 1. Patients of the hyperactive/impulsive subtype (N= 20) were not included in the statistical analysis due to the small number of subjects. ADHD patients differed significantly from controls in the genotype frequencies. The 9R containing genotypes were more frequent among cases. The same result was verified in the comparison between female patients of both subtypes and controls, and males of the inattentive subtype compared with controls (Table 1). Males of the combined subtype presented similar genotype frequencies as compared to controls.

Insert Table 1 about here

Table 2 shows the number of K-SADS-E hyperactivity symptoms for the inattentive and combined subtypes and the DAT1 genotypes. Individuals with the 10/10 genotype have higher hyperactivity scores than those with other genotypes, independently of subtype. There is no genotype effect in the inattention or impulsivity scores. Severity as measured by the SNAP-IV Rating Scale and TCI Temperament dimensions are not associated to DAT1 genotypes.

Discussion

The overall pattern of results in this study disagrees with those obtained in children samples. Nevertheless, when we restrict the comparison to males of the combined subtype, results are more compatible with those of children studies, in this case, no difference between cases and controls. Interestingly, this group is also the most similar with the disorder profile in childhood, composed mainly of combined boys.[52-54] This is consistent with the observation that, in this sample, the 10/10 genotype is associated with a higher number of hyperactivity symptoms. The interesting finding is the contrasting influence of each genotype, since the 9R containing genotypes are associated with the inattentive subtype.

We hypothesize that the contrasting results obtained in this study compared to children ADHD investigations might be related to the characteristics of the study populations (age, comorbidities and different compositions regarding to gender and subtypes). The association with the 9R allele might not be detected in children samples because girls are subdiagnosed in childhood.[55,56] They have lower risk of disruptive behavior disorders[55] and are less likely to be associated with problems in school than boys with ADHD.[56] In addition to the small percentage of girls, children ADHD

samples usually have a smaller fraction of patients of the inattentive subtype, what could possibly prevent the obtention of the results reported here. Maybe there are peculiarities in the genetic profile of the ADHD children who persist with the disorder into adulthood. On the other hand, there could be ADHD cases not diagnosed in the childhood (mostly women and inattentives) that are more likely to be referred as adults. These patients might also have a different genetic profile for ADHD. Thus, our results could be either related to the persistent form of the disorder or to new cases not available in children ADHD studies.

Insert Table 2 about here

There are few studies analyzing children ADHD subtypes and the DAT1 3'UTR VNTR. They are consistent with an association between the 10R allele and hyperactivity/impulsivity symptoms [31] and the severe combined subtype.[57] Oh *et al*[58] used the Continuous Performance Test, as an endophenotypic measure, and concluded that the 10/10 genotype was associated with less attention deficits than genotypes carrying the 9R allele. This last study is consistent with our finding that the 9R allele could be associated with inattention.

There is previous evidence that the 9R allele may be associated with other psychiatric disorders or phenotypes. These include withdrawal seizures or delirium and more severe effects of alcohol withdrawal in alcohol dependent subjects;[59-61] type 2 alcoholism, with early onset and heavy behavioral problems;[62] paranoia symptoms in cocaine dependents;[63] posttraumatic stress disorder;[64] cigarette smoking;[65] externalizing behavior in children;[66] and antisocial–violent behaviour and aggressiveness in heroin-dependent males.[67] However, when we analyzed the most frequent comorbidities in our

ADHD patients, there was no association with the 9R allele. Therefore, in this sample the 9R allele does not seem to be associated with any more specific phenotype than ADHD inattention.

DAT1 is not the first gene for which there is suggestive evidence that different alleles may predispose to different psychiatric phenotypes. The two alleles of the polymorphism that changes a valine to methionine at position 158 (Val158Met) of Catechol-O-methyltransferase (COMT) gene seem to increase the predisposition to symptoms of schizophrenia or anxiety, respectively.[68] It has been hypothesized that the inattentive and combined ADHD subtypes could in fact be to two separate disorders, probably with distinct etiologies.[69] Both subtypes are characterized by dissociable cognitive and behavioral profiles, and different patterns of comorbidities, responses to medication and underlying neurobiological problems.[70] In this case, the DAT1 9R and 10R alleles might in fact be small added parts of phenotypes or dimensions that, under the influence of other genetic or environmental factors, would predispose to inattention or hyperactivity.

Many investigations have analyzed other polymorphisms in the 3' region of DAT1, with data that suggest linkage disequilibrium with the 3'UTR VNTR.[36-38,71] Haplotype analysis is a valuable approach to detect additional functional polymorphisms associated to a particular disorder.[72] However, the one-at-a-time study of each functional polymorphism is a necessary and more conservative approach for association studies, especially in scarcely studied phenotypes as adult ADHD. Obviously, further studies are required to isolate additional relevant functional sequences and demonstrate the way in which they alter DAT1 function and the possible predisposition to ADHD.

The interpretation of our results should consider some methodological aspects. Case-control studies should always be aware of the risk of population stratification. Our control group was carefully matched for ethnicity, and the allele and genotype frequencies are nearly identical to those found in a Chilean population sample (10R: 74%, 9R: 23%)[73] and in a US Mixed population (10R: 76.2%, 9R: 22.6%)[29]. This control group is composed only of men, but the allele and genotype frequencies did not differ from another control group from Porto Alegre genotyped for the DAT1 3'UTR VNTR that included women.[74] The present sample size is not as big as has been suggested for state-of-the-art case control studies,[68] but is one of the biggest in ADHD genetics studies, especially adult ADHD. These results were obtained in a clinical-based sample of Brazilians of European descent. However, the clinical presentation of adult ADHD in Brazil is very similar to other published samples.[8] Another limitation is the small sample size of the pure hyperactive/impulsive subtype. Future studies should have statistical power to analyze this subtype separately. These analyses should be replicated in independent studies to further clarify the role of DAT1 in adult ADHD.

To the best of our knowledge, this is the first study to evaluate the relationships between the DAT1 genotypes, subtypes and genders in adult ADHD. The results support the hypothesis that DAT1 has an important but complex role in ADHD. This role seems to be remarkably influenced by factors related to gender, subtype and age of the sample.

Acknowledgements

Thanks are due to the following Brazilian funding agencies: The National Council for Scientific and Technological Development (CNPq), Coordination for the

Improvement of Higher Education Personnel (CAPES), Foundation for the Support of Research of the State of Rio Grande do Sul (FAPERGS), the Fund for the Support of Research of the Clinical Hospital of Porto Alegre (FIPE-HCPA). Dr. Sidia Callegari-Jacques helped in the statistical analyses.

References

1. Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA *et al.* Molecular Genetics of Attention-Deficit/Hyperactivity Disorder. *Biol Psychiatry* 2005; **57**: 1313-23.

2. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders.* 4th edition. Washington DC: APA, Revised (DSM-IV), 1994.

3. Gittelman R, Mannuzza S, Shenker R, Bonagura N. Hyperactive boys almost grown up: I Psychiatric status. *Arch Gen Psychiatry* 1985; **42**: 937–947.

4. Mannuzza S, Klein RG. Long-term prognosis in attention-deficit/hyperactivity disorder. *Child Adolesc Psychiatr Clin N Am* 2000; **9**: 711-26.

5. Wender PH, Wolf LE, Wasserstein J. Adults with ADHD. An overview. *Ann N Y Acad Sci* 2001; **931**: 1-16.

6. Faraone SV, Biederman J, Monuteaux MC. Toward guidelines for pedigree selection in genetic studies of attention deficit hyperactivity disorder. *Genet Epidemiol* 2000; **18**: 1–16.

7. Biederman J, Faraone SV, Spencer T, Wilens T, Mick E, Lapey KA. Gender differences in a sample of adults with attention deficit hyperactivity disorder. *Psychiatry Res* 1994; **53**: 13-29.

8. Grevet EH, Bau CH, Salgado CA, Ficher A, Kalil K, Victor MM *et al*. Lack of gender effects on subtype outcomes in adults with attention-deficit/hyperactivity disorder: Support for the validity of subtypes. *Eur Arch Psychiatry Clin Neurosci, in press.*

9. Applegate B, Lahey BB, Hart EL, Biederman J, Hynd GW, Barkley RA *et al.* Validity of the age-of-onset criterion for ADHD: a report from the DSM-IV field trials. *J Am Acad Child Adolesc Psychiatry* 1997; **36**: 1211-21.

10. Spencer T, Biederman J, Wilens T. Pharmacotherapy of attention deficit hyperactivity disorder. *Child Adolesc Psychiatr Clin North Am* 2000; **9**: 77–97

11. Vles JS, Feron FJ, Hendriksen JG, Jolles J, van Kroonenburgh MJ, Weber WE. Methylphenidate down-regulates the dopamine receptor and transporter system in children with attention deficit hyperkinetic disorder (ADHD). *Neuropediatrics* 2003; **34**: 77-80.

12. Dresel S, Krause J, Krause KH, LaFougere C, Brinkbaumer K, Kung HF *et al*. Attention deficit hyperactivity disorder: binding of [99mTc]TRODAT-1 to the dopamine transporter before and after methylphenidate treatment. *Eur J Nucl Med* 2000; **27:** 1518–24.

13. Spencer TJ, Biederman J, Madras BK, Faraone SV, Dougherty DD, Bonab AA *et al*. In vivo neuroreceptor imaging in attention-deficit/hyperactivity disorder: a focus on the dopamine transporter. *Biol Psychiatry* 2005; **57**: 1293-300.

14. Giros B, el Mestikawy S, Godinot N, Zheng K, Han H, Yang-Feng T *et al*. Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. *Mol Pharmacol* 1992; **42**: 383-90.

15. Vandenbergh DJ, Persico AM, Hawkins AL, Griffin CA, Li X, Jabs EW *et al.* Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. *Genomics* 1992; **14:** 1104–1106.

16. Kang AM, Palmatier MA, Kidd KK. Global variation of a 40 bp VNTR in the 3'-untranslated region of the dopamine transporter gene (SLC6A3). *Biol Psychiatry* 1999; **46**: 151-60.

17. Mitchell RJ, Howlett S, Earl L, White NG, McComb J, Schanfield MS *et al*. Distribution of the 3' VNTR polymorphism in the human dopamine transporter gene in world populations. *Hum Biol* 2000; **72**: 295-304.

18. Jacobsen LK, Staley JK, Zoghbi SS, Seibyl JP, Kosten TR, Innis RB *et al.* Prediction of dopamine transporter binding availability by genotype: a preliminary report. *Am J Psychiatry* 2000; **157**: 1700-1703.

19. Michelhaugh SK, Fiskerstrand C, Lovejoy E, Bannon MJ, Quinn JP. The dopamine transporter gene (SLC6A3) variable number of tandem repeats domain enhances transcription in dopamine neurons. *J Neurochem* 2001; **79**: 1033-1038.

20. Miller GM, Madras BK. Polymorphisms in the 3'-untranslated region of human and monkey dopamine transporter genes affect reporter gene expression. *Mol Psychiatry* 2002; **7**: 44-55.

21. Van Dyck CH, Malison RT, Jacobsen LK, Seibyl JP, Staley JK, Laruelle M *et al.* Increased dopamine transporter availability associated with the 9-repeat allele of the SLC6A3 gene. *J Nucl Med* 2005; **46**: 745-51.

22. Heinz A, Goldman D, Jones DW, Palmour R, Hommer D, Gorey JG. Genotype influences in vivo dopamine transporter availability in human striatum. *Neuropsychopharmacology* 2000; **22**: 133-9.

23. Fuke S, Suo S, Takahashi N, Koike H, Sasagawa N, Ishiura S. The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression. *Pharmacogenomics* J 2001; **1**: 152-156.

24. Mill J, Asherson P, Browes C, D'souza U, Craig I. Expression of the dopamine transporter gene is regulated by the 3' UTR VNTR: Evidence from brain and lymphocytes using quantitative RT-PCR. *Am J Med Genet* 2002; **114**: 975–979.

25. Martinez D, Gelernter J, Abi-Dargham A, van Dyck CH, Kegeles L, Innis RB *et al.* The Variable Number of Tandem Repeats Polymorphism of the Dopamine Transporter Gene Is Not Associated with Significant Change in Dopamine Transporter Phenotype in Humans. *Neuropsychopharmacology* 2001; **24**: 553-560.

26. Greenwood TA, Kelsoe JR. Promoter and intronic variants affect the transcriptional regulation of the human dopamine transporter gene. *Genomics* 2003; **82**: 511-520.

27. Mill J, Asherson P, Craig I, D'Souza UM. Transient expression analysis of allelic variants of a VNTR in the dopamine transporter gene (DAT1). *BMC Genetics* 2005; **6**: 3.

28. Van Ness SH, Owens MJ, Kilts CD. The variable number of tandem repeats element in DAT1 regulates in vitro dopamine transporter density. *BMC Genetics* 2005; **6**: 55.

29. Cook EH, Stein MA, Krasowski MD, Cox NJ, Olkon DM, Kieffer JE *et al.* Association of attention-deficit disorder and the dopamine transporter gene. *Am J Hum Genet* 1995; **56**: 993-8.

30. Gill M, Daly G, Heron S, Hawi Z, Fitzgerald M. Confirmation of association between attention deficit hyperactivity disorder and a dopamine transporter polymorphism. *Mol Psychiatry* 1997; **2**: 311-3.

31. Waldman ID, Rowe DC, Abramowitz A, Kozel ST, Mohr JH, Sherman SL *et al.* Association and linkage of the dopamine transporter gene and attention-deficit hyperactivity disorder in children: heterogeneity owing to diagnostic subtype and severity. *Am J Hum Genet* 1998; **63**: 1767-76.

32. Daly G, Hawi Z, Fitzgerald M, Gill M. Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children. *Mol Psychiatry* 1999; **4**:192-196.

33. Curran S, Mill J, Tahir E, Kent L, Richards S, Gould A *et al.* Association study of a dopamine transporter polymorphism and attention deficit hyperactivity disorder in UK and Turkish samples. *Mol Psychiatry* 2001; **6**: 425-8.

34. Rowe DC, Stever C, Chase D, Sherman S, Abramowitz A, Waldman ID. Two dopamine genes related to reports of childhood retrospective inattention and conduct disorder symptoms. *Mol Psychiatry* 2001; **6**: 429-33.

35. Chen CK, Chen SL, Mill J, Huang YS, Lin SK, Curran S *et al.* The dopamine transporter gene is associated with attention deficit hyperactivity disorder in a Taiwanese sample. *Mol Psychiatry* 2003; **8**: 393-6.

36. Hawi Z, Lowe N, Kirley A, Gruenhage F, Nothen M, Greenwood T. Linkage disequilibrium mapping at DAT1, DRD5 and DBH narrows the search for ADHD susceptibility alleles at these loci. *Mol Psychiatry* 2003; **8**: 299-308.

37. Galili-Weisstub E, Levy S, Frisch A, Gross-Tsur V, Michaelovsky E, Kosov A *et al*. Dopamine transporter haplotype and attention-deficit hyperactivity disorder. *Mol Psychiatry* 2005; **10**: 617-8.

38. Brookes KJ, Mill J, Guindalini C, Curran S, Xu X, Knight J *et al.* A common haplotype of the dopamine transporter gene associated with attention-deficit/hyperactivity disorder and interacting with maternal use of alcohol during pregnancy. *Arch Gen Psychiatry* 2006; **63**: 74-81.

39. Maher BS, Marazita ML, Ferrell RE, Vanyukov MM. Dopamine system genes and attention deficit hyperactivity disorder: a meta-analysis. *Psychiatr Genet* 2002; **12**: 207-15.

40. Purper-Ouakil D, Wohl M, Mouren MC, Verpillat P, Ades J, Gorwood P. Meta-analysis of family-based association studies between the dopamine transporter gene and attention deficit hyperactivity disorder. *Psychiatr Genet* 2005; **15**: 53-9.

41. Muglia P, Jain U, Inkster B, Kennedy JL. A quantitative trait locus analysis of the dopamine transporter gene in adults with ADHD. *Neuropsychopharmacology* 2002; **27**: 655-62.

42. Kaplan E, Fein D, Morris R, Delis DC. *WAIS-R: Manual.* Psychological Corporation San Antonio, 1991.

43. Dornelles CL, Callegari-Jacques SM, Robinson WM, Weimer TA, Franco MHLP, Hickmann AC *et al.* Genetics, surnames, grandparents nationalities, and ethnic admixture in southern Brazil - Do the patterns of variation coincide? *Genetics and Molecular Biology* 1999; **22**: 151-161.

44. Mercadante MT, Asbahr F, Rosário-Campos MC, Ayres AM, Karman L, Ferrari MC *et al. K-SADS - Entrevista semi-estruturada para diagnóstico em psiquiatria da*

infância, versão epidemiológica. 1st edition. Editora São Paulo, PROTOC – Hospital das Clínicas da FMUSP, São Paulo, 1995.

45. Grevet EH, Bau CH, Salgado CA, 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.

46. First MB, Spitzer RL, Gibbon M, Williams JB. *Structured clinical interview for DSM-IV axis I disorders, patient edition* (SCID-I/P, Version 2.0, 8/98 revision). Biometric Research Department, New York State, Psychiatric Institute, New York, 1998.

47. Swanson JM. *School-based assessments and interventions for add students*. KC Publishing Irvine, 1992.

48. Cloninger CR, Svrakic DM, Przybeck TR. A psychobiological model of temperament and character. *Arch Gen Psychiatry* 1993; **50:** 975–990.

49. Cloninger CR, Przybeck TR, Svrakic D M, Wetzel RD. The temperament and character inventory (TCI). *A guide to its development and use.* St.Louis, MO: Center for Psychobiology of Personality, Washington University, 1994.

50. Lahiri DK, Nurnberger JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991; **19**: 5444.

51. Sano A, Kondoh K, Kakimoto Y, Kondo I. A 40-nucleotide repeat polymorphism in the human dopamine transporter gene. *Hum Genet* 1993; **91**: 405-6.

52. Leung AK, Lemay JF. Attention deficit hyperactivity disorder: an update. *Adv Ther* 2003; **20**: 305-18.

53. Montiel-Nava C, Pena JA, Montiel-Barbero I. Epidemiological data about attention deficit hyperactivity disorder in a sample of Marabino children. *Rev Neurol* 2003; **37**: 815-9.

54. Cardo E, Servera-Barcelo M. The prevalence of attention deficit hyperactivity disorder. *Rev Neurol* 2005; **40**: S11-5.

55. Gaub M, Carlson CL. Gender differences in ADHD: a meta-analysis and critical review. *J Am Acad Child Adolesc Psychiatry* 1997; **36**: 1783-92.

56. Biederman J, Mick E, Faraone SV, Braaten E, Doyle A, Spencer T *et al.* Influence of gender on attention deficit hyperactivity disorder in children referred to a psychiatric clinic. *Am J Psychiatry* 2002; **159**: 36-42.

57. Todd RD, Huang H, Smalley SL, Nelson SF, Willcutt EG, Pennington BF *et al*. Collaborative analysis of DRD4 and DAT genotypes in population-defined ADHD subtypes. *J Child Psychol Psychiatry* 2005; **46**: 1067-73.

58. Oh KS, Shin DW, Oh GT, Noh KS. Dopamine transporter genotype influences the attention deficit in Korean boys with ADHD. *Yonsei Med J* 2003; **44**: 787-92.

59. Sander T, Harms H, Podschus J, Finckh U, Nickel B, Rolfs A *et al.* Allelic association of a dopamine transporter gene polymorphism in alcohol dependence with withdrawal seizures or delirium. *Biol Psychiatry* 1997; **41**: 299–304.

60. Schmidt LG, Harms H, Kuhn S, Rommelspacher H, Sander T. Modification of Alcohol Withdrawal by the A9 Allele of the Dopamine Transporter Gene. *Am J Psychiatry 1998*; **155**: 474–478.

61. Gorwood P, Limosin F, Batel P, Hamon M, Ades J, Boni C. The A9 allele of the dopamine transporter gene is associated with delirium tremens and alcohol-withdrawal seizure. *Biol Psychiatry* 2003; **53**: 85–92.

62. Galeeva AR, Iur'ev EB, Khusnutdinova EK. The evaluation of VNTR-polymorphism in the gene of dopamine transporter in men of different nationalities with acute alcoholic psychosis. *Zh Nevrol Psikhiatr Im S S Korsakova* 2001; **101**: 43–45.

63. Gelernter J, Kranzler HR, Satel Sl, Rao PA. Genetic association between dopamine transporter protein alleles and cocaine-induced paranoia. *Neuropsychopharmacology* 1994; **11**: 195–200.

64. Segman RH, Cooper-Kazaz R, Macciardi F, Goltser T, Halfon Y, Dobroborski T *et al*. Association between the dopamine transporter gene and posttraumatic stress disorder. *Mol Psychiatry* 2002; **7**: 903-7.

65. Vandenbergh DJ, Bennett CJ, Grant MD, Strasser AA, O'Connor R, Stauffer RL *et al.* Smoking status and the human dopamine transporter variable number of tandem repeats (VNTR) polymorphism: failure to replicate and finding that never-smokers may be different. *Nicotine Tob Res* 2002; **4**: 247 –249.

66. Young SE, Smolen A, Corley RP, Krauter KS, DeFries JC, Crowley TJ *et al*. Dopamine transporter polymorphism associated with externalizing behavior problems in children. *Am J Med Genet* 2002; **114**: 144-9.

67. Gerra G, Garofano L, Pellegrini C, Bosari S, Zaimovic A, Moi G *et al*. Allelic association of a dopamine transporter gene polymorphism with antisocial behaviour in heroin-dependent patients. *Addiction Biology* 2005; **10**: 275-81.

68. Gunzerath L, Goldman D. G x E: a NIAAA workshop on gene-environment interactions. *Alcohol Clin Exp Res* 2003; **27**: 540-62.

69. Sagvolden T, Aase H, Johansen EB, VA Russell. A dynamic developmental theory of attention-deficit/hyperactivity disorder (ADHD) predominantly

hyperactive/impulsive and combined subtypes. *Behavioral and Brain Sciences* 2005; **28**: 397– 468.

70. Diamond A. Attention-deficit disorder hyperactivity disorder without hyperactivity: A neurobiologically and behaviorally distinct disorder from attention-deficit hyperactivity disorder with hyperactivity. *Development and Psychopathology* 2005; **17**: 807–825.

71. Barr CL, Xu C, Kroft J, Feng Y, Wigg K, Zai G *et al.* Haplotype study of three polymorphisms at the dopamine transporter locus confirm linkage to attention-deficit/hyperactivity disorder. *Biol Psychiatry* 2001; **49**: 333-9.

72. Sklar P. Principles of haplotype mapping and potential applications to attention-deficit/hyperactivity disorder. *Biol Psychiatry* 2005; **57**: 1357-66.

73. Vieyra GS, Moraga MV, Henríquez HB, Aboitiz FD, Rothhammer FE. Distribution of alleles of DRD4 and DAT1 genes of the dopaminergic system in the mixed Chilean population. *Rev Méd Chile* 2003; **131**: 135-143.

74. Bau CH, Almeida S, Costa FT, Garcia CE, Elias EP, Ponso AC *et al.* DRD4 and DAT1 as modifying genes in alcoholism: interaction with novelty seeking on level of alcohol consumption. *Mol Psychiatry* 2001; **6**: 7-9.

Tables

Table 1. Genotype and allele frequencies of the DAT1 3'UTR VNTR.

Sample	*Alleles frequencies*								*Genotypes frequencies*					
	9R		10R		Others				10/10		Others			
	N	%	N	%	N	%	$\chi 2$	P	N	%	N	%	$\chi 2$	P
Controls (N=233)	102	21.9	355	76.2	9	1.9			142	60.9	91	39.8		
ADHD (N= 308)	171	27.8	433	70.3	12	1.9	4.88	0.087	155	50.3	153	49.7	6.06	0.014
Males (N= 163)	89	27.3	234	71.8	3	0.9	4.12	0.127	86	52.8	77	47.2	2.63	0.105
Females (N= 145)	83	28.6	198	68.3	9	3.1	5.87	0.053	69	47.6	76	52.4	6.47	0.034
Inattentive (N=120)	72	30.0	164	68.3	4	1.7	5.16	0.060	58	48.3	62	51.7	5.13	0.024
Males (N=67)	39	29.1	94	70.1	1	0.8	3.69	0.158	32	47.8	35	52.2	3.71	0.054
Females (N= 53)	33	31.2	70	66.0	3	2.8	4.65	0.098	26	49.1	27	50.9	2.51	0.113
Hyperactive/Impulsive (N= 20)	17	42.5	23	57.5	0	0			7	35.0	13	65.0		
Combined (N= 168)	83	24.7	245	72.9	8	2.3	1.13	0.567	90	53.6	78	46.4	2.18	0.140
Males (N= 84)	38	22.6	128	76.2	2	1.2	0.42	0.810	50	59.5	34	40.5	0.05	0.819
Females (N=84)	45	26.8	117	69.6	6	3.6	3.39	0.184	40	47.6	44	52.4	4.48	0.034

Table 2. K-SADS-E hyperactivity scores and the DAT1 3' UTR VNTR.

Subtype	*DAT1 genotypes*			
	10/10		Others	
	N	Mean (± SE)	N	Mean (± SE)
Inattentive	58	2.15 (± 1.41)	62	1.64 (± 1.45)
Combined[1]	89	4.98 (± 0.92)	78	4.79 (± 0.93)
Total	147	3.87 (± 1.79)	140	3.40 (± 1.97)

[1]This variable was not available in one individual.

DAT1 genotypes vs. hyperactivity scores: $F = 6.36$, $P = 0.012$

ADHD subtypes vs. hyperactivity scores: $F = 460.03$, $P < 0.0001$

DAT1 genotypes vs. subtypes interaction: $F = 1.28$, $P = 0.26$

IV. MANUSCRITO II

DRD4 in an adult sample of Attention-Deficit/Hyperactivity Disorder: Possible influence in the age of onset of symptoms and comorbid bipolar disorder

Francine Z. C. Marques, Maria Paula Quiroga, Eugenio H. Grevet, Carlos A. I. Salgado, Aline G. Fischer, Katiane Kalil, Marcelo M. Victor, Christiane R. Garcia, Nyvia O. Sousa, Paulo Belmonte-de-Abreu and Claiton H. D. Bau

A ser submetido à revista *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics)*

RESEARCH ARTICLE

DRD4 in an adult sample of Attention-Deficit/Hyperactivity Disorder: Possible influence in the age of onset of symptoms and comorbid bipolar disorder

Francine Z. C. Marques[1,3], Maria Paula Quiroga[3], Eugenio H. Grevet [1], Carlos A. I. Salgado [1], Aline G. Fischer[1], Katiane Kalil [1], Marcelo M. Victor [1], Christiane R. Garcia [1], Nyvia O. Sousa [1], Paulo Belmonte-de-Abreu[1, 2] and Claiton H. D. Bau[1,3]

[1] Adult ADHD Outpatient Clinic, Clinical Hospital of Porto Alegre, Porto Alegre, RS, Brazil.
[2] Department of Psychiatry, Medical School, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.
[3] Department of Genetics, Institute of Biosciences, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Running title: DRD4 in an adult sample of ADHD

Correspondence to Claiton H. D. Bau, Departamento de Genética, Instituto de Biociências, UFRGS, Caixa Postal 15053, 91501-970 Porto Alegre, RS, Brazil.
Tel: (55) 51 3316 6718 Fax: (55) 51 3316 7311
E-mail: claiton.bau@ufrgs.br

Grant sponsor: CNPq; Grant sponsor: FINEP; Grant sponsor: PRONEX; Grant sponsor: FAPERGS.

ABSTRACT

Recent meta-analyses have indicated that the 7-repeat (7R) allele of a VNTR in the third exon of dopamine D4 receptor gene (DRD4) is associated with children attention-deficit/hyperactivity disorder (ADHD), although single studies frequently failed to show significant association. More recently, the long (L allele) of a 120-base pair repeat in the 5' region of DRD4 has been associated to the disorder. Despite of childhood studies, adult samples were little explored. We genotyped 308 adult ADHD patients and 230 controls, all of them Brazilians of European descent. The diagnoses of ADHD and comorbid disorders were based in the DSM-IV criteria. The risk alleles were not associated with adult ADHD or its subtypes. However, the 7R allele was associated with an earlier age of onset of the disorder (F= 5.21, P= 0.023) and with comorbid bipolar disorder in the ADHD sample (χ^2= 4.43, P=0.035). These findings support a small, complex influence of DRD4 in the development and heterogeneity of ADHD.

Keywords: comorbidities; dopaminergic; endophenotype; exon III VNTR; haplotype; multifactorial; promoter.

INTRODUCTION

Progress in identifying some of the genes involved in attention-deficit/hyperactivity disorder (ADHD) susceptibility has been relatively fruitful over the past decade by screening genetic variants that lie within or close to genes that regulate neurotransmitter systems, particularly dopamine pathway genes [Asherson et al., 2004]. One of the more consistent findings, reported in several meta-analyses in children ADHD [Faraone et al., 2001, 2005; Maher et al., 2002; Wohl et al., 2005], is the association with the 7-repeat allele (7R) of a 48-base pair (bp) variable number of tandem repeats (VNTR) in exon III of the dopamine D4 receptor gene (DRD4). However, there is also a number of negative reports and discrepancies between case-control and family studies [Castellanos et al., 1998; Eisenberg et al., 2000; Hawi et al., 2000; Kotler et al., 2000; Mill et al., 2001; Payton et al., 2001; Todd et al., 2001; Kustanovich et al., 2004; Smith et al., 2003; Frank et al., 2004; Bakker et al., 2005; Bobb et al., 2005; Brookes et al., 2005; Carrasco et al., 2006]. Functional in vitro studies suggested that the 7R allele produces a blunted response to dopamine [Van Tol et al., 1992; Asghari et al., 1995; Wang et al., 2004].

Genetic variants in the DRD4 5'-regulatory region have also been reported to be associated with ADHD. A 120 bp duplication located 1.2 kb upstream of the initiation codon provided positive association findings with ADHD [McCracken et al., 2000; Kustanovich et al., 2004], although negative studies have also been reported [Barr et al., 2001; Todd et al., 2001; Frank et al., 2004; Kirley et al., 2004]. An expression study showed that the L allele (549 bp) conferred lower transcriptional activity than the S allele (429bp) in mammalian cell lines [D'Souza et al., 2004].

Several follow-up studies that evaluated the persistence of the disorder in adulthood have revealed that one to two thirds of ADHD children have significant,

persistent symptoms of the disorder [Weiss et al., 2000; Faraone et al., 2006]. Despite the positive meta-analyses in children involving the exon III VNTR, studies in adult ADHD samples are scarce and therefore insufficient for a definitive conclusion about a possible association. An initial association was suggested between adult ADHD and the 7R allele [Muglia et al., 2000], but afterwards the same association was restricted to a haplotype composed by the 7R and L alleles in a sample combining adults and children with the disorder [Arcos-Burgos et al., 2004]. In addition, the 7R variant was associated with a lifetime history of ADHD in a sample of parents of ADHD children [Lynn et al., 2005].

Both clinical [Biederman et al., 2004] and genetic [Doyle et al., 2005] studies have shown that ADHD is a highly heterogeneous disorder. This heterogeneity manifests itself as the subtypes of the disorder as well as in a range of frequent comorbidities [Biederman et al., 2004]. Although some investigations focused the heterogeneity issue in genetic studies of children ADHD [Rowe et al., 1998, 2001; Swanson et al., 2000; Auerbach et al., 2001; Todd et al., 2001, 2005; Holmes et al., 2002; Kirley et al., 2004; Langley et al., 2004; Bellgrove et al., 2005], there are no similar studies with adult samples. The aim of the present work is to investigate the association between two polymorphisms in the DRD4 gene in a relatively large sample of adult ADHD. In order to verify if the association could be restricted to some relevant aspect of ADHD that could explain the weak effect of the 7R allele in children meta-analyses and persistent ADHD, we also analyzed the ADHD subtypes, temperament dimensions, age of onset of ADHD symptoms and prevalent comorbidities.

MATERIALS AND METHODS

Subjects

Patients with ADHD were drawn from the ADHD outpatient clinic of the Hospital de Clinicas de Porto Alegre (HCPA, a major teaching hospital in the south of Brazil) in the period from September 2002 to December 2005. The exclusion criteria were evidence of clinically significant neurological diseases and current or past history of psychosis and IQ ≤ 70 [Kaplan et al., 1991]. All of them were older than eighteen years old. Patients were investigated and treated after a screening interview that confirmed ADHD diagnosis. The interviewers in this research were all psychiatrists extensively trained in the application of all instruments in the research protocol.

A total of 308 ADHD probands were included in the analysis, 164 males (53.25%) and 144 females (46.75%). The majority (54.7%) of probands were diagnosed with ADHD combined type, while 38.8% were primarily inattentives and 6.5% presented the hyperactive/impulsive subtype.

Patients were all Brazilians of European descent. Unlike other Brazilian regions, the African contribution to the gene pool in this region of the country is small, about 7.2% [Dornelles et al., 1999]. The classification was based in skin color, morphologic characters and self-reported ethnicity.

The control group for allele and genotype frequencies is composed of 230 Brazilian blood donor males carefully matched for ethnicity. Controls were therefore not interviewed for psychiatric diagnoses. The expected frequency of ADHD in this group is the same as in the general population. This sample was designed to be non-screened, representative of the gene frequencies of individuals of European descent in Porto Alegre. These individuals are replacement donors, that is, they are people that

replaced the blood used by a hospitalized family member or friend. For this reason, a behavior-related bias is not likely.

This investigation was approved by the Ethical Committee of HCPA and by the Federal University of Rio Grande do Sul. All individuals sampled (patients and controls) provided written informed consent.

Diagnosis

The diagnostic procedures for ADHD followed the DSM-IV criteria [American Psychiatric Association, 1994], using the respective sections of the Portuguese version of K-SADS-E [Mercadante et al., 1995] for children and adolescents. The only adaptation to adulthood symptoms [Grevet et al., 2005] was the adjustment of the criterion for onset of symptoms to age 12 or earlier instead of 7 or earlier as reported by others [Murphy and Barkley, 1996; Murphy et al., 2002]. In the clinical practice, adolescents and adults frequently fail to provide precise recall on age of onset [Rohde et al., 2000]. This adjustment is justified because no evidence exists to show that this criterion of onset by age 7 distinguishes valid from invalid cases [Barkley and Biederman, 1997; Rohde et al., 2000]. The age of onset was investigated asking patients or some close family member who knew the patient during childhood when they presented impairments in at least two settings related to inattention, impulsivity and/or hyperactivity. The earlier referred age for each patient was included in the analysis. This variable was not available in four individuals, decreasing the final sample size for this analysis to 304.

Current and lifetime psychiatric comorbidities (alcohol, drug and nicotine dependences, antisocial personality, oppositional defiant, anxiety, major depressive and bipolar disorders) were assessed using SCID-IV-R [First et al., 1998]. In order to

increase the sample size in some cells, some diagnoses were grouped. Therefore, as anxiety disorder we considered any anxiety disorder (panic, agoraphobia, social phobia, general anxiety and obsessive compulsive) and as bipolar disorder we included any bipolar disorder (type I, type II and cyclothymic disorder). The severity of current ADHD symptoms was assessed by the number of K-SADS-E symptoms [Mercadante et al, 1995]. The Kappa coefficients of interrater agreement for the K-SADS-E were higher than 0.90 for current and lifetime diagnoses of ADHD and its subtypes [Grevet et al., 2005]. Temperament dimensions (novelty seeking, harm avoidance, reward dependence and persistence) were evaluated using the Temperament and Character Inventory (TCI) [Cloninger et al., 1993; Cloninger et al., 1994].

Laboratory Methods

The DNA was extracted from whole blood following the Lahiri and Nurnberger method [1991]. PCR amplification of the exon III VNTR was performed according to adaptations of Lichter et al. [1993] e Chang et al. [1996], and for the promoter polymorphism, of Seaman et al. [1999]. The products were electrophoresed on 3.5% (exon III VNTR) and 1.5% (promoter polymorphism) agarose gel stained with ethidium bromide. One hundred base pair ladders were used to score the various repeat alleles, and positive controls were always analyzed with the PCR products.

Statistical Analysis

Allele frequencies were estimated by gene counting. The analysis of Hardy-Weinberg equilibrium and comparisons between patients and controls were performed with the chi-square test. One-way analysis of variance (ANOVA) was used to analyze the age of onset of ADHD symptoms. Haplotypes were set by the MLOCUS program

[Long et al., 1995; Long, 1999; Peterson et al., 1999]. Linkage disequilibrium between the markers was estimated with the 3LOCUS [Long, 1999] and the Arlequin [Schneider et al., 2000] softwares.

RESULTS

Genotype distribution was in Hardy-Weinberg equilibrium both in controls (exon III VNTR: $\chi 2$= 20.79, P= 0.472; promoter: $\chi 2$= 1.13, P= 0.29) and patients (exon III VNTR: $\chi 2$= 15.57, P= 0.793; promoter: $\chi 2$= 0.15, P= 0.70). The allele and genotype frequencies for the exon III VNTR and for the 120 bp duplication of DRD4 for controls and patients are shown in Table I. Since the sample size of the hyperactive/impulsive subtype was very small (N=20), this group was not included in the statistical analyses. None of the polymorphisms revealed significant differences between patients and controls, even when considering ADHD subtypes or gender effects (Table I). There is no significant linkage disequilibrium between both loci ($\chi 2$= 9.20; P= 0.16). However, there is a strong linkage between the alleles L and 7R, 97% of 7R alleles are in a chromosome that bears the L allele. While the overall D' is 0.26, the pairwise D' between L and 7R is 0.46. The haplotype-based results did not provide additional information in relation to single locus analyses, and therefore are not presented.

The 7R allele of exon III VNTR was associated with an earlier age of onset of ADHD symptoms (Table II) and the presence of comorbid bipolar disorder in ADHD patients (Table III). In relation to the 5' polymorphism, there is a trend towards association with bipolar disorder (Table III), but not with age of onset (Table II). No association was detected between the polymorphisms and temperament dimensions and the other comorbidities investigated.

During the course of DRD4 genotyping, one ADHD patient was found to have an allele of 729 bp for the polymorphism in the 5' region. To facilitate statistical analyzes, this individual was excluded from the initial sample (N=309). This allele was classified as 4 repeats of 120 bp duplication. This variant was confirmed by independent PCR and genotyping. To our knowledge, this allele has not been reported previously.

DISCUSSION

This is one of the few available association studies between adult ADHD and the DRD4 gene. Although no direct association was observed, some potentially relevant findings should be taken into consideration. The first regards to the possible effect of the 7R allele in the age of onset of ADHD symptoms, a variable that usually is not investigated in children samples. The second is the suggestive association of the same allele with comorbid bipolar disorder.

Although most ADHD studies pull together the three ADHD subtypes, one investigation found that the 7R allele was present more frequently in children with the ADHD inattentive type [Rowe et al., 1998], while another showed marginally significant evidence for over-transmission of the same allele in the severe combined ADHD subtype [Todd et al., 2005]. None of the former association studies between DRD4 and adult ADHD focused on subtypes. In our search for clinically relevant aspects of ADHD influenced by DRD4, subtypes were not associated to genotypes. Likewise, we did not detect associations between DRD4 and the number of symptoms of inattention or hyperactivity/impulsivity.

To the best of our knowledge, there are no previous association studies between a genetic polymorphism and the age of onset of ADHD symptoms. In the present sample, the 7R allele of DRD4 was associated with an earlier onset of problems.

Interestingly, in our sample an earlier age of onset of symptoms is associated with a higher number of symptoms of hyperactivity (r= -0.18; P= 0.002), but not with symptoms of inattention. These results suggest that the age of onset of symptoms might related to some hyperactivity-related endophenotype not assessed in the current study. This result supports a previous finding that linked the 7R-allele with hyperactivity symptoms in ADHD [Roman et al., 2001].

There are no previous adult ADHD studies focusing on the association between DRD4 and ADHD comorbidities. Children studies concentrated in conduct disorder and oppositional defiant disorder, suggesting an association with the 7R allele of exon III VNTR and these comorbidities [Rowe et al., 2001; Holmes et al., 2002; Kirley et al., 2004].

Although there are no previous genetic studies focusing on bipolar disorder as a comorbidity in ADHD, many studies have investigated the DRD4 exon III VNTR in bipolar disorder patients. While most studies did not find a positive association with the 7R allele [De Bruyn et al., 1994; Lim et al., 1994; Manki et al., 1996; Oruc et al., 1997; Staner et al., 1998; Bochetta et al., 1999; Muglia et al., 2002; Serretti et al., 2004], it was shown that this allele was associated with higher scores of delusional symptoms in mood disorders, including bipolar disorder [Serretti et al., 1998]. It could be hypothesized that a fraction of ADHD children that persist with symptoms in the adulthood could share both the 7R allele and a predisposition to bipolar disorder. Future studies in children ADHD should verify the possible association between the 7R allele and bipolar disorder. Moreover, longitudinal, follow-up studies could tell if ADHD children with the 7R allele are prone to bipolar disorder during the course of their development. It is noteworthy that bipolar disorder is a prevalent comorbidity in adult ADHD [Biederman, 2004; Secnick et al., 2005; Grevet et al., *in press*].

Despite the 120 bp duplication polymorphism is known as a functional one [D'Souza et al., 2004], no significant association was observed between the L allele and the ADHD diagnosis, subtypes, comorbidities or another endophenotype. This result reinforces the view that the exon III VNTR is associated with ADHD-related phenotypes. Chance, false positive associations are expected to occur in all polymorphisms, but positive results are concentrated, in this and previous studies, in the VNTR.

The interpretation of our results should consider some methodological aspects. Case-control studies should always be aware of the risk of population stratification. Our control group was carefully matched for ethnicity, and the allele and genotype frequencies are nearly identical to those found in European populations [Chang et al., 1996; Paterson et al., 1996; Seaman et al., 1999]. This control group is composed only of men, but the allele and genotype frequencies of exon III VNTR did not differ from another control group from Porto Alegre genotyped for the DRD4 gene that included women [Bau et al., 2001]. Assuming the positive meta-analyses reported for the candidate gene and the small presumed effect size, we regarded the Bonferroni correction as excessively conservative, and prefer a cautious interpretation. The associations observed should be tested in independent samples of adults with ADHD. These results are valid only for a clinical-based sample of Brazilians of European descent. However, the clinical presentation of adult ADHD in Brazil is very similar to other published samples [Grevet et al., in press]. Another limitation is the small size of hyperactive/impulsive subtype sample. Future studies should have statistical power to analyze this subtype separately.

In conclusion, our results from an adult sample did not support a direct association between the DRD4 gene and ADHD, differently from the meta-analyses of

children studies. However, the overall findings support a small influence of the gene in ADHD, when aspects of the heterogeneity (bipolar disorder and age of onset) of the disorder are considered.

ACKNOWLEDGEMENTS

Thanks are due to the following Brazilian funding agencies: The National Council for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES), Foundation for the Support of Research of the State of Rio Grande do Sul (FAPERGS), the Fund for the Support of Research of the Clinical Hospital of Porto Alegre (FIPE-HCPA). Dr. Marilu Fiegenbaum and Dr. Sídia Callegari-Jacques helped in the statistical analyses.

REFERENCES

American Psychiatric Association. 1994. Diagnostic and statistical manual of mental disorders. 4th edition. Washington DC: APA, Revised (DSM-IV).

Arcos-Burgos M, Castellanos FX, Konecki D, Lopera F, Pineda D, Palacio JD, Rapoport JL, Berg K, Bailey-Wilson J, Muenke M. 2004. Pedigree Disequilibrium Test (PTD) Replicates Association and Linkage Between DRD4 and ADHD in Multigenerational and Extended Pedigree From Genetic Isolate. Mol Psychiatry 9:252-259.

Asghari V, Sanyal S, Buchwaldt S, Paterson A, Jovanovic V, Van Tol HH. 1995. Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. J Neurochemistry 65:1157–1165.

Asherson P, IMAGE Consortium. 2004. Attention-Deficit Hyperactivity Disorder in the post-genomic era. Eur Child Adolesc Psychiatry 13:50-70.

Auerbach JG, Benjamin J, Faroy M, Geller V, Ebstein R. 2001. DRD4 related to infant attention and information processing: a developmental link to ADHD? Psychiatr Genet 11:31-5.

Bakker SC, Van Der Meulen EM, Oteman N, Schelleman H, Pearson PL, Buitelaar JK, Sinke RJ. 2005. DAT1, DRD4 and DRD5 polymorphisms are not associated with ADHD in Dutch families. Am J Med Genet 132:50-2.

Barkley RA, Biederman J. 1997. Toward a broader definition of the age-of-onset criterion for attention-deficit hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 36:1204-10.

Barr CL, Feng Y, Wigg KG, Schachar R, Tannock R, Roberts W, Malone M, Kennedy JL. 2001. 5'-untranslated region of the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. Am J Med Genet 105:84-90.

Bau CH, Almeida S, Costa FT, Garcia CE, Elias EP, Ponso AC, Spode A, Hutz MH. 2001. DRD4 and DAT1 as modifying genes in alcoholism: interaction with novelty seeking on level of alcohol consumption. Mol Psychiatry 6:7-9.

Bellgrove MA, Hawi Z, Lowe N, Kirley A, Robertson IH, Gill M. 2005. DRD4 gene variants and sustained attention in attention deficit hyperactivity disorder (ADHD): effects of associated alleles at the VNTR and -521 SNP. Am J Med Genet 136:81-6.

Biederman J. 2004. Impact of comorbidity in adults with attention-deficit/hyperactivity disorder. J Clin Psychiatry 65:3-7.

Biederman J, Faraone SV, Monuteaux MC, Bober M, Cadogen E. 2004. Gender Effects on Attention-Deficit/Hyperactivity Disorder in Adults, Revisited. Biol Psychiatry 55:692–700.

Bobb AJ, Addington AM, Sidransky E, Gornick MC, Lerch JP, Greenstein DK, Clasen LS, Sharp WS, Inoff-Germain G, Wavrant DE, Vrieze F, Arcos-Burgos M, Straub RE, Hardy JA, Castellanos FX, Rapoport JL. 2005. Support for association between ADHD and two candidate genes: NET1 and DRD1. Am J Med Genet 134:67-72.

Bocchetta A, Piccardi MP, Palmas MA, Chillotti C, Oi A, Del Zompo M. 1999. Family-based association study between bipolar disorder and DRD2, DRD4, DAT, and SERT in Sardinia. Am J Med Genet 88:522-6.

Brookes KJ, Xu X, Chen CK, Huang YS, Wu YY, Asherson P. 2005. No evidence for the association of DRD4 with ADHD in a Taiwanese population within-family study. BMC Med Genet 6:31.

Carrasco X, Rothhammer P, Moraga M, Henriquez H, Chakraborty R, Aboitiz F, Rothhammer F. 2006. Genotypic interaction between DRD4 and DAT1 loci is a high risk factor for attention-deficit/hyperactivity disorder in Chilean families. Am J Med Genet 141:51-4.

Castellanos FX, Lau E, Tayebi N, Lee P, Long RE, Giedd JN, Sharp W, Marsh WL, Walter JM, Hamburger SD, Ginns EI, Rapoport JL, Sidransky E. 1998. Lack of an association between a dopamine-4 receptor polymorphism and attention-deficit/hyperactivity disorder: genetic and brain morphometric analyses. Mol Psychiatry 3:431-4.

Chang FM, Kidd JR, Livak KJ, Pakstis AJ, Kidd KK. 1996. The world-wide distribution of allele frequencies at the human dopamine D4 receptor locus. Hum Genet 98:91-101.

Cloninger CR, Svrakic DM, Przybeck TR. 1993. A psychobiological model of temperament and character. Arch Gen Psychiatry 50:975-–990.

Cloninger CR, Przybeck TR, Svrakic D M, Wetzel RD. 1994. The temperament and character inventory (TCI). A guide to its development and use. St.Louis, MO: Center for Psychobiology of Personality, Washington University.

D'souza UM, Russ C, Tahir E, Mill J, Mcguffin P, Asherson PJ, Craig IW. 2004. Functional Effects of a Tandem Duplication Polymorphism in the 5' Flanking Region of the DRD4 Gene. Biol Psychiatry 56:691–697.

De Bruyn A, Mendelbaum K, Sandkuijl LA, Delvenne V, Hirsch D, Staner L, Mendlewicz J, Van Broeckhoven C. 1994. Nonlinkage of bipolar illness to tyrosine hydroxylase, tyrosinase, and D2 and D4 dopamine receptor genes on chromosome 11. Am J Psychiatry 151:102-6.

Dornelles CL, Callegari-Jacques SM, Robinson WM, Weimer TA, Franco MHLP, Hickmann AC, Geiger C, Salzano FM. 1999. Genetics, surnames, grandparents nationalities, and ethnic admixture in southern Brazil - Do the patterns of variation coincide? Genetics Mol Biology 22:151-161.

Doyle AE, Willcutt EG, Seidman LJ, Biederman J, Chouinard VA, Silva J, Faraone SV. 2005. Attention-deficit/hyperactivity disorder endophenotypes. Biol Psychiatry 57:1324-35.

Eisenberg J, Zohar A Mei-Tal G Steinberg A Tartakovsky E Gritsenko I Newmanov L Ebstein R. 2000. A Haplotype Relative Risk Study of the Dopamine D4 Receptor (DRD4) Exon III Repeat Polymorphism and Attention Deficit Hyperactivity Disorder (ADHD). J Med Genet 96:258-261.

Faraone SV, Doyle AE, Mick E, Biederman J. 2001. Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. Am J Psychiatry 158:1052-7.

Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA, Sklar P. 2005. Molecular Genetics of Attention-Deficit/Hyperactivity Disorder. Biol Psychiatry 57:1313-23.

Faraone SV, Biederman J, Mick E. 2006. The age-dependent decline of attention deficit hyperactivity disorder: a meta-analysis of follow-up studies. Psychol Med 36:159-65.

First MB, Spitzer RL, Gibbon M, Williams JB. 1998. Structured clinical interview for DSM-IV axis I disorders, patient edition (SCID-I/P, Version 2.0, 8/98 revision). Biometric Research Department, New York State, Psychiatric Institute, New York.

Frank Y, Pergolizzi RG, Perilla MJ. 2004. Dopamine D4 receptor gene and attention deficit hyperactivity disorder. Pediatr Neurol 31:345-8.

Grevet EH, Bau CH, Salgado CA, Ficher A, Victor MM, Garcia C, Sousa NO, Nerung L, Abreu PB. 2005. Interrater reliability for diagnosis in adults of attention deficit hyperactivity disorder and oppositional defiant disorder using K-SADS-E. Arq Neuropsiquiatr 63:307-10.

Grevet EH, Bau CH, Salgado CA, Ficher A, Kalil K, Victor MM, Garcia CR, Sousa NO, Rohde LA, Abreu PB. In press. Lack of gender effects on subtype outcomes in adults with attention-deficit/hyperactivity disorder: Support for the validity of subtypes. Eur Arch Psychiatry Clin Neurosci.

Hawi Z, McCarron M, Kirley A, Daly G, Fitzgerald M, Gill M. 2000. No Association of the Dopamine DRD4 Receptor (DRD4) Gene Polymorphism With Attention Deficit Hyperactivity Disorder (ADHD) in the Irish Population. Am J Med Genet 96:268-272.

Holmes J, Payton A, Barrett J, Harrington R, McGuffin P, Ollier W, Worthington J, Gill M, Kirley A, Hawi Z, Fitzgerald M, Asherson P, Curran S, Mill J, Gould A, Taylor E, Kent L, Craddock N, Thapar A. 2002. Association of DRD4 in Children With ADHD and Comorbid Conduct Problems. Am J Med Genet 114:150-153.

Kaplan E, Fein D, Morris R, Delis DC. 1991. WAIS-R: Manual. Psychological Corporation San Antonio.

Kirley A, Lowe N, Mullins C, Mccarron M, Daly G, Waldman I, Fitzgerald M, Gill M, Hawi Z. 2004. Phenotype studies of the DRD4 gene polymorphisms in ADHD: association with oppositional defiant disorder and positive family history. Am J Med Genet 131:38-42.

Kotler M, Manor I, Sever Y, Eisenberg J, Cohen H, Ebstein RP, Tyano S. 2000. Failure to Replicate an Excess of the Long Dopamine D4 Exon III Repeat Polymorphism in ADHD in a Family-Based Study. Am J Med Genet 96:278-281.

Kustanovich V, Ishii J, Crawford L, Yang M, Mcgough JJ, Mccracken JT, Smalley SL, Nelson SF. 2004. Transmission disequilibrium testing of dopamine-related candidate gene polymorphisms in ADHD: confirmation of association of ADHD with DRD4 and DRD5. Mol Psychiatry 9:711-7.

Lahiri DK, Nurnberger JI. 1991. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 19:5444.

Langley K, Marshall L, Van Den Bree M, Thomas H, Owen M, O'donovan M, Thapar A. 2004. Association of the dopamine D4 receptor gene 7-repeat allele with neuropsychological test performance of children with ADHD. Am J Psychiatry 161:133-8.

Lichter JB, Barr CL, Kennedy JL, Van Tol HH, Kidd KK, Livak KJ. 1993. A hypervariable segment in the human dopamine receptor D4 (DRD4) gene. Mol Genetics 2:767-73.

Lim LC, Nothen MM, Korner J, Rietschel M, Castle D, Hunt N, Propping P, Murray R, Gill M. 1994. No evidence of association between dopamine D4 receptor variants and bipolar affective disorder. Am J Med Genet 54:259-63.

Long JC, Williams RC, Urbanek M. 1995. An E-M algorithm and testing strategy for multiple locus haplotypes. Am J Human Genet 56:799-810.

Long JC. 1999. Multiple Locus Haplotype Analysis, version 2.0. Software and documentation distributed by the author. Section on Population Genetics and Linkage, Laboratory of Neurogenetics, NIAAA. National Institutes of Health, Bethesda, M.D.

Lynn DE, Lubke G, Yang M, McCracken JT, McGough JJ, Ishii J, Loo SK, Nelson SF, Smalley SL. 2005. Temperament and character profiles and the dopamine D4 receptor gene in ADHD. Am J Psychiatry 162:906-13.

Maher BS, Marazita ML, Ferrell RE, Vanyukov MM. 2002. Dopamine system genes and attention deficit hyperactivity disorder: a meta-analysis. Psychiatr Genet 12:207-15.

Manki H, Kanba S, Muramatsu T, Higuchi S, Suzuki E, Matsushita S, Ono Y, Chiba H, Shintani F, Nakamura M, Yagi G, Asai M. 1996. Dopamine D2, D3 and D4

receptor and transporter gene polymorphisms and mood disorders. J Affect Disord 40:7-13.

McCracken JT, Smalley SL, McGough JJ, Crawford L, Del'Homme M, Cantor RM, Liu A, Nelson SF. 2000. Evidence for Linkage of a Tandem Duplication Polymorphism Upstream of the Dopamine D4 Receptor Gene (DRD4) With Attention Deficit Hyperactivity Disorder (ADHD). Mol Psychiatry 5:551-556.

Mercadante MT, Asbahr F, Rosário-Campos MC, Ayres AM, Karman L, Ferrari MC et al. 1995. K-SADS - Entrevista semi-estruturada para diagnóstico em psiquiatria da infância, versão epidemiológica. 1st edition. Editora São Paulo, PROTOC – Hospital das Clínicas da FMUSP, São Paulo, 1995.

Mill J, Curran S, Kent L, Richards S, Gould A, Virdee V, Huckett L, Sharp J, Batten C, Fernado S, Simanoff E, Thompson M, Zha J, Sham P, Taylor E, Asherson P. 2001. Attention Deficit Hyperactivity Disorder (ADHD) and the Dopamine D4 Receptor Gene: Evidence of Association but No Linkage in a UK Sample. Mol Psychiatry 6:440-444.

Muglia P, Jain U, Macciardi F, Kennedy JL. 2000. Adult attention deficit hyperactivity disorder and the dopamine D4 receptor gene. Am J Med Genet 96:273-7.

Muglia P, Petronis A, Mundo E, Lander S, Cate T, Kennedy JL. 2002. Dopamine D4 receptor and tyrosine hydroxylase genes in bipolar disorder: evidence for a role of DRD4. Mol Psychiatry 7:860-6.

Murphy K, Barkley RA. 1996. Attention Deficit Hyperactivity Disorder Adults: Comorbidities and Adaptive Impairments. Comp Psychiatry 6:393-401.

Murphy KR, Barkley RA, Bush T. 2002. Young adults with attention deficit hyperactivity disorder: subtype differences in comorbidity, educational, and clinical history. J Nerv Ment Dis 190:147-57.

Oruc L, Verheyen GR, Furac I, Jakovljevic M, Ivezic S, Raeymaekers P, Van Broeckhoven C. 1997. Analysis of the tyrosine hydroxylase and dopamine D4 receptor genes in a Croatian sample of bipolar I and unipolar patients. Am J Med Genet 74:176-8.

Paterson AD, Sunohara GA, Kennedy JL. 1999. Dopamine D4 receptor gene: novelty or nonsense? Neuropsychopharmacology 21:3-16.

Payton A, Holmes J, Barrett JH, Hever T, Fitzpatrick H, Trumper AL, Harrington R, McGuffin P, O'Donovan M, Owen M, Ollier W, Worthington J, Thapar A. 2001. Examining for Association Between Candidate Gene Polymorphisms in the Dopamine Pathway and Attention-Deficit Hyperactivity Disorder: a Family-Based Study. Am J Med Genet 105:464-470.

Peterson RJ, Goldman D, Long JC. 1999. Nucleotide sequence diversity in non-coding regions of ALDH2 as revealed by restriction enzyme and SSCP analysis. Human Genetics. 104: 177-187.

Rohde LA, Biederman J, Zimmermann H, Schmitz M, Martins S, Tramontina S. 2000. Exploring ADHD age-of-onset criterion in Brazilian adolescents. Eur Child Adolesc Psychiatry 9:212-8.

Roman T, Schmitz M, Polanczyk G, Eizirik M, Rohde LA, Hutz MH. 2001. Attention-deficit hyperactivity disorder: a study of association with both the dopamine transporter gene and the dopamine D4 receptor gene. Am J Med Genet 105:471-8.

Rowe DC, Stever C, Giedinghagen LN, Gard JM, Cleveland HH, Terris ST, Mohr JH, Sherman S, Abramowitz A, Waldman ID. 1998. Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder. Mol Psychiatry 3:419-26.

Rowe DC, Stever C, Chase D, Sherman S, Abramowitz A, Waldman ID. 2001. Two dopamine genes related to reports of childhood retrospective inattention and conduct disorder symptoms. Mol Psychiatry 6:429-33.

Schneider S, Roessli D, Excoffier L. 2000. Arlequin: A software for population genetics data analysis Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Seaman MI, Fisher JB, Chang F, Kidd KK. 1999. Tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4). Am J Med Genet 88:705-9.

Secnik K, Swensen A, Lage MJ. 2005. Comorbidities and costs of adult patients diagnosed with attention-deficit hyperactivity disorder. Pharmacoeconomics 23:93-102.

Serretti A, Macciardi F, Cusin C, Lattuada E, Lilli R, Smeraldi E. 1998. Dopamine receptor D4 gene is associated with delusional symptomatology in mood disorders. Psychiatry Res 80:129-36.

Serretti A, Lorenzi C, Mandelli L, Cichon S, Schumacher J, Nothen MM, Rietschel M, Tullius M, Ohlraun S. 2004. DRD4 exon 3 variants are not associated with symptomatology of major psychoses in a German population. Neurosci Lett 368:269-73.

Smith KM, Daly M, Fischer M, Yiannoutsos CT, Bauer L, Barkley R, Navia A. 2003. Association of the dopamine beta hydroxylase gene with attention deficit

hyperactivity disorder: genetic analysis of the Milwaukee longitudinal study. Am J Med Genet 119:77-85.

Staner L, Hilger C, Hentges F, Monreal J, Hoffmann A, Couturier M, Le Bon O, Stefos G, Souery D, Mendlewicz J. 1998. Association between novelty-seeking and the dopamine D3 receptor gene in bipolar patients: a preliminary report. Am J Med Genet 81:192-4.

Swanson J, Oosterlaan J, Murias M, Schuck S, Flodman P, Spence MA, Wasdell M, Ding Y, Chi HC, Smith M, Mann M, Carlson C, Kennedy JL, Sergeant JA, Leung P, Zhang YP, Sadeh A, Chen C, Whalen CK, Babb KA, Moyzis R, Posner MI. 2000. Attention deficit/hyperactivity disorder children with a 7-repeat allele of the dopamine receptor D4 gene have extreme behavior but normal performance on critical neuropsychological tests of attention. Proc Natl Acad Sci USA 97:4754-9.

Todd RD, Neuman RJ, Lobos EA, Jong YJ, Reich W, Heath AC. 2001. Lack of association of dopamine D4 receptor gene polymorphisms with ADHD subtypes in a population sample of twins. Am J Med Genet 105:432-8.

Todd RD, Huang H, Smalley SL, Nelson SF, Willcutt EG, Pennington BF, Smith SD, Faraone SV, Neuman RJ. 2005. Collaborative analysis of DRD4 and DAT genotypes in population-defined ADHD subtypes. J Child Psychol Psychiatry 46:1067-73.

Van Tol HH, Wu CM, Guan HC, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB, Jovanovic V. 1992. Multiple dopamine D4 receptor variants in the human population. Nature 358:149-52.

Wang E, Ding YC, Flodman P, Kidd JR, Kidd KK, Grady DL, Ryder OA, Spence MA, Swanson JM, Moyzis RK. 2004. The Genetic Architecture of Selection at the

Human Dopamine Receptor D4 (DRD4) Gene Locus. Am J Hum Genet 74:931–944.

Weiss M, Hechtman L, Weiss G. 2000. ADHD in parents. J Am Acad Child Adolesc Psychiatry 39:1059-61.

Wohl M, Purper-Ouakil D, Mouren MC, Ades J, Gorwood P. 2005. Meta-analysis of candidate genes in attention-deficit hyperactivity disorder. Encephale 4:437-47.

TABLES

TABLE I. DRD4 gene frequencies and adult ADHD.

Sample	Controls		ADHD (Total)		ADHD Subtypes													
					Inattentive					Hyperactive				Combined				
					Males		Females		Total	(Total)		Males		Females		Total		
Sample size	230		308		67		52		119	20		85		84		169		
Frequencies	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
EXON III																		
2R	39	8.5	52	8.5	15	11.2	6	5.8	21	8.8	3	7.5	15	8.8	13	7.7	28	8.3
4R	300	65.2	392	63.6	84	62.7	69	66.3	153	64.3	32	80.0	104	61.2	103	61.3	207	61.2
7R	110	23.9	138	22.4	26	19.4	24	23.1	50	21.0	5	12.5	40	23.5	43	25.6	83	24.6
Others	11	2.4	34	5.5	9	6.7	5	4.8	14	5.9	0	0	11	6.5	9	5.4	20	5.9
χ^2, P			6.52	0.09	7.63	0.05	2.57	0.46	5.95	0.11			3.64	0.30	3.92	0.27	6.76	0.08
Presence of 7R	102	44.3	126	40.9	24	35.8	21	40.4	45	37.8	4	20.0	38	44.7	39	46.4	77	45.6
Absence of 7R	128	55.7	182	59.1	43	64.2	31	59.6	74	62.2	16	80.0	47	55.3	45	53.6	92	54.4
χ^2, P			0.64	0.42	1.54	0.21	0.27	0.60	1.37	0.24			0.00	0.95	0.11	0.74	0.05	0.81
PROMOTER																		
SS	12	5.2	13	4.2	4	6.0	2	3.8	6	5.1	2	10.0	3	3.5	2	2.4	5	3.0
SL	69	30.0	106	34.4	17	25.4	16	30.8	33	27.7	11	55.0	30	35.3	32	38.1	62	36.7
LL	149	64.8	189	61.4	46	68.6	34	65.4	80	67.2	7	35.0	52	61.2	50	59.5	102	60.3
χ^2, P			1.32	0.52	0.55	0.76	0.17	0.92	0.21	0.90			1.1	0.59	2.63	0.27	2.79	0.25
Presence of S	81	35.2	119	38.6	21	31.3	18	34.6	39	32.8	13	65.0	33	38.8	34	40.5	67	39.6
Absence of S	149	64.8	189	61.4	46	68.7	34	65.4	80	67.2	7	35.0	52	61.2	50	59.5	102	60.4
χ^2, P			0.55	0.45	0.40	0.53	0.1	0.90	0.26	0.61			0.30	0.59	0.66	0.42	0.72	0.40

All comparisons between ADHD patients and controls resulted non-significant.
Comparisons between ADHD subtypes and controls also resulted non-significant.
There are no significant gender differences in allele and genotype frequencies.

TABLE II. DRD4 gene and age of onset of ADHD symptoms.

Allele	Average age of onset (±SE)	F	P
Exon III (N=304)		5.210	0.023
Presence of 7R (N=125)	6.048 (±2.741)		
Absence (N=179)	6.824 (±3.033)		
Promoter (N=304)		0.877	0.350
Presence of S (N=119)	6.703 (±3.034)		
Absence (N=185)	6.378 (±2.873)		

TABLE III. DRD4 gene and Bipolar Disorder in adult ADHD.

	ADHD without Bipolar Disorder		ADHD with Bipolar Disorder			
	N	%	N	%	χ^2	P
Exon III (N= 308)	254	82.5	54	17.5	4.434	0.035
Presence of 7R	97	38.2	29	53.7		
Absence	157	61.8	25	46.3		
Promoter (N= 308)	254	82.5	54	17.5	2.240	0.134
Presence of S	103	40.6	16	29.6		
Absence	151	59.4	38	70.4		

V. DISCUSSÃO

O conjunto de resultados da presente dissertação deve ser avaliado no contexto da linha de pesquisa voltada para o TDAH em adultos em desenvolvimento na Universidade Federal do Rio Grande do Sul. Os genes DRD4 e DAT1 foram escolhidos para esse esforço inicial porque são os genes mais estudados em amostras de TDAH em crianças. Ambos contam com evidências de diferentes tipos apontando para um papel no transtorno. Como pouquíssimos estudos genéticos de associação foram publicados com o TDAH em adultos (ver Tabela 3 da introdução), decidimos começar os estudos genéticos da nossa amostra por esses genes. De maneira geral, os resultados não são de fácil interpretação. Ao longo dos dois artigos, discutimos aspectos específicos de cada um dos genes, tentando alcançar uma convergência entre os resultados obtidos e aqueles já publicados em amostras de crianças. Nesta seção, serão abordadas questões mais gerais sobre a metodologia empregada e será feita uma tentativa de correlacionar e integrar os resultados obtidos, bem como discutir as perspectivas para a continuidade deste trabalho. A amostra está sendo ampliada para futuras análises genéticas e confirmação do efeito dos genes aqui analisados no TDAH em adultos.

Nas amostras de crianças, há alguma convergência na literatura no sentido de que o alelo 7R do DRD4 estaria associado ao TDAH (Faraone e cols., 2001, 2005; Maher e cols., 2002; Wohl e cols., 2005) e sugestões menos robustas de que o 10R do DAT1 estaria envolvido com os sintomas de hiperatividade (Waldman e cols., 1998). Nos dois artigos, buscamos avaliar as mesmas associações em adultos, uma vez que a prevalência e as características demográficas e clínicas não são equivalentes nas duas faixas etárias. Além disso, a comparação dos resultados obtidos em adultos com aqueles da infância pode permitir inferências novas sobre o papel destes genes na história natural do transtorno. De

fato, os dois artigos aqui apresentados sugerem que o TDAH avaliado em pacientes que buscam atendimento na idade adulta apresenta diferenças clínicas e genéticas substanciais em relação às amostras de crianças. Existe, no entanto, ao menos uma concordância absoluta entre os presentes resultados e os já publicados: a de que o efeito individual de cada gene envolvido tende a ser muito pequeno. Abre-se, assim, uma ampla perspectiva de investigação na área, já que a análise comparativa do efeito de outros genes, envolvidos em outros fenótipos associados ao TDAH, poderá facilitar a elucidação do panorama geral do transtorno nas diferentes faixas etárias.

São poucos os polimorfismos em genes candidatos para o TDAH com um efeito funcional bem esclarecido. Um bom exemplo é o 5-HTTLPR, uma inserção/deleção de 44 pb no promotor do gene 5-HTT (Collier e cols., 1996; Heils e cols., 1996). Um maior esclarecimento do significado funcional dos polimorfismos é imprescindível para esclarecer os reais papéis dos genes na suscetibilidade ao TDAH. Pouco pode ser inferido sobre a contribuição de um gene sobre os mecanismos biológicos de uma doença se não for conhecido o significado da variação genética estudada. Nenhum dos três polimorfismos estudados nessa dissertação apresenta evidências de significado funcional tão robustas quanto o 5-HTTLPR, mas ainda assim destacam-se nesse quesito quando comparados a outros polimorfismos nos mesmos genes.

A possibilidade do efeito atribuído aos polimorfismos estudados ser devido a outros *loci* em desequilíbrio de ligação já foi brevemente considerada nos capítulos anteriores. Apesar do efeito isolado de outros polimorfismos ter sido detectado em alguns estudos de haplótipos para ambos os genes (Barr e cols., 2001b, 2001c; Hawi e cols., 2003; Mill e cols., 2003; Kirley e cols., 2004; Lowe e cols., 2004; Bellgrove e cols., 2005c; Feng e cols., 2005b; Galili-Weisstub e cols., 2005; Langley e cols., 2005; Brookes e cols., 2006),

nenhum deles foi replicado e nenhum estudo foi realizado em amostras envolvendo adultos. Contudo, não se pode descartar a possibilidade de que outros polimorfismos funcionais estejam mascarando o papel real daqueles analisados no presente estudo, incluindo os anteriormente descritos. Objetiva-se, futuramente, estudar a influência de outros polimorfismos nos genes DRD4 e DAT1 em adultos com TDAH.

Existem duas explicações principais para as inconsistências dos resultados dos estudos de genética molecular em condições complexas como o TDAH: o baixo poder estatístico para detectar genes de efeito pequeno e a heterogeneidade (Faraone e cols., 1999b; Doyle e cols., 2005). A análise de subgrupos visando desvendar a heterogeneidade não é comum, sendo que o principal motivo para a falta de estudos com essa abordagem é a rara disponibilidade de um tamanho amostral adequado. É importante ter em mente que muitos transtornos psiquiátricos tendem a estar associados entre si, mais freqüentemente do que seria esperado por acaso (Kessler e cols., 2005). Essa peculiaridade faz com que a complexidade vinculada aos transtornos mentais seja muito grande, demandando uma caracterização fenotípica muito detalhada e a consideração da mesma nas análises genéticas. Alguns estudos moleculares têm começado a explorar fontes de heterogeneidade nos genes aqui analisados (Rowe e cols., 1998, 2001; Waldman e cols., 1998; McCracken e cols., 2000; Auerbach e cols., 2001; Todd e cols., 2001b, 2005; Holmes e cols., 2002; Oh e cols., 2003; Kirley e cols., 2004; Langley e cols., 2004; Bellgrove e cols., 2005a, 2005b; Kim e cols., 2005); porém, os resultados não tem sido conclusivos em função da necessidade de amostras ainda maiores para análise de subgrupos. A análise de endofenótipos é potencialmente uma ferramenta útil na determinação do efeito de genes em doenças complexas (Almasy e Blangero, 2001; Gottesman e Gould, 2003). Já que um endofenótipo seria influenciado por menos fatores genéticos (e ambientais) que o transtorno

como um todo, o seu uso poderia resultar, teoricamente, em um poder estatístico maior para detectar o efeito de genes individuais (Doyle e cols., 2005). No entanto, ainda muito deve ser investigado para que se chegue a uma lista consistente de endofenótipos para estudo no TDAH.

A heterogeneidade clínica existente nas amostras de crianças e adultos pode ter influenciado a não replicação de alguns dos achados positivos na infância. Como já foi bastante enfatizado, a representatividade de cada sexo e subtipo varia significativamente entre os dois tipos de amostras. Além disso, embora na maioria dos estudos o diagnóstico do TDAH e comorbidades seja baseado nos critérios do DSM-IV, o uso de instrumentos diversos pode levar à obtenção de amostras clinicamente diferentes. O modo de recrutamento dos pacientes também pode interferir, assim como o treinamento dos entrevistadores. A origem dos indivíduos, se da comunidade ou de hospitais, também parece influenciar bastante na caracterização clínica das amostras (The ADHD Molecular Genetics Network, 2000).

A heterogeneidade genética também pode ter contribuído para as inconsistências nos resultados entre os nossos estudos e os anteriores. A grande variabilidade nas freqüências alélicas na maioria dos *loci* polimórficos entre as populações (Cavalli-Sforza e cols., 1994) sugere que amostras de países com diferentes origens étnicas podem ter um *background* genético diferente com influência nos transtornos mentais. É importante considerar ainda que, em doenças complexas, muitas combinações diferentes de genes e alelos podem produzir o mesmo fenótipo (State e cols., 2000; Swanson e cols., 2001). Quando a estratificação populacional não é controlada adequadamente em uma investigação, este fator de confusão poderia gerar resultados falso-positivos. No nosso estudo, tal problema foi minimizado pelo pareamento entre controles e pacientes quanto à

etnia. Ainda que não se tenha estudado marcadores genéticos para confirmar essa similaridade, estudos na mesma população não puderam demonstrar uma estratificação populacional significativa na população de Porto Alegre (Zembrzuski e cols., no prelo).

Alguns estudos têm se focado na interação gene X ambiente. Em relação ao DAT1, Kahn e cols. (2003) demonstraram um efeito do genótipo 10/10 quando associado ao tabagismo na gestação, enquanto que Brookes e cols. (2006) evidenciaram que um haplótipo envolvendo o alelo 10R moderaria o risco de desenvolver o TDAH associado ao uso de álcool durante a gravidez. Outro estudo mostrou uma interação entre o peso ao nascer e o genótipo do polimorfismo Val158Met da COMT em pacientes com TDAH (Thapar e cols., 2005b). Além desses estudos, trabalhos envolvendo a interação gene X gene são muito interessantes, mas ainda pouco explorados, dada a necessidade de amostras muito maiores que as analisadas até o momento (Gunzerath e Goldman, 2003). Roman e cols. (2001) mostraram uma interação entre o alelo 10R do VNTR 3'UTR de DAT1 e o alelo 7R do VNTR do éxon III do DRD4 no aumento do número de sintomas de hiperatividade/impulsividade, interação observada por Carrasco e cols. (2006) no aumento da predisposição para o desenvolvimento do TDAH. Essa interação será testada futuramente na nossa amostra. Além disso, estudos envolvendo a resposta ao tratamento com metilfenidato também serão realizados envolvendo ambos os genes. No presente estudo, optamos por não avaliar essas interações até que o efeito individual de cada gene esteja melhor esclarecido entre os adultos com TDAH. Essa estratégia visa reduzir o risco de obtenção de resultados falso-positivos.

Por fim, entendemos que os resultados aqui obtidos apontam para a existência de diferenças substanciais no perfil genético das amostras de TDAH em crianças e adultos. Nesse sentido, entendemos que o estudo dos polimorfismos escolhidos ajuda a demonstrar

(embora não exatamente elucidar) a enorme complexidade do transtorno. O surgimento de tantas hipóteses de pesquisa a partir do estudo de apenas dois genes permite vislumbrar uma enorme potencialidade elucidativa quando muitos outros fatores genéticos e clínicos puderem ser adicionados ao modelo, caso exista uma amostra disponível com tamanho suficiente.

VI. CONCLUSÕES

Os principais resultados e conclusões do presente trabalho foram:

1. O polimorfismo tipo VNTR do éxon III e o de duplicação de 120 pb no promotor do gene DRD4 parecem não ter um papel direto no TDAH. Porém, o alelo 7R do VNTR no éxon III parece predispor ao transtorno bipolar em pacientes adultos com TDAH e a uma idade de início de sintomas mais precoce.
2. O VNTR na região 3'UTR do gene DAT1 parece ter um papel mais complexo que o visto até o momento, com um efeito aparentemente bidirecional sobre o TDAH: genótipos contendo o alelo 9R predisporiam ao subtipo desatento de TDAH entre os pacientes do sexo masculino e ao TDAH em geral entre as mulheres, enquanto que o genótipo 10/10 estaria associado a um maior número de sintomas de hiperatividade.

VII. REFERÊNCIAS BIBLIOGRÁFICAS

Almasy L and Blangero J (2001) Endophenotypes as quantitative risk factors for psychiatric disease: rationale and study design. Am J Med Genet 105:42-4.

Alsobrook JP and Pauls DL (1998) Molecular approaches to child psychopathology. Hum Biol 70:413-32.

Amara SG and Kuhar MJ (1993) Neurotransmitter transporters: recent progress. Annu Rev Neurosci 16:73-93.

American Academy of Child and Adolescent Psychiatry (AACAP) (1997) Practice parameters for the assessment and treatment of children adolescents and adults with Attention-deficit/Hyperactivity Disorder. J Am Acad Child Adolesc Psychiatry 36:10 suppl 85S-121S.

American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders. 4th edition. Washington DC: APA, Revised (DSM-IV).

Arcos-Burgos M, Castellanos FX, Pineda D, Lopera F, Palacio JD, Palacio LG, Rapoport JL, Berg K, Bailey-Wilson JE and Muenke M (2004a) 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, Castellanos FX, Konecki D, Lopera F, Pineda D, Palacio JD, Rapoport JL, Berg K, Bailey-Wilson J and Muenke M (2004b) Pedigree disequilibrium test (PDT) replicates association and linkage between DRD4 and ADHD in multigenerational and extended pedigrees from a genetic isolate. Mol Psychiatry 9:252-9.

Arnold L (1996) Sex differences in ADHD: conference summary. J Abnorm Child Psychol 24:555–569.

Arnsten AF, Steere JC and Hunt RD (1996) The contribution of alpha 2-noradrenergic mechanisms of prefrontal cortical cognitive function. Potential significance for attention-deficit hyperactivity disorder. Arch Gen Psychiatry 53:448-55.

Arnsten AF (2000) Genetics of childhood disorders: XVIII ADHD Part 2: Norepinephrine has a critical modulatory influence on prefrontal cortical function. J Am Acad Child Adolesc Psychiatry 39:1201-3.

Asghari V, Sanyal S, Buchwaldt S, Paterson A, Jovanovic V and Van Tol HH (1995) Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. J Neurochemistry 65:1157–1165.

Asherson P and IMAGE Consortium (2004) Attention-Deficit Hyperactivity Disorder in the post-genomic era. Eur Child Adolesc Psychiatry 13:50-70.

Auerbach JG, Benjamin J, Faroy M, Geller V and Ebstein R (2001) DRD4 related to infant attention and information processing: a developmental link to ADHD? Psychiatr Genet 11:31-5.

Bakker SC, van der Meulen EM, Buitelaar JK, Sandkuijl LA, Pauls DL, Monsuur AJ, van 't Slot R, Minderaa RB, Gunning WB, Pearson PL and Sinke RJ (2003) A whole-genome

scan in 164 Dutch sib pairs with attention-deficit/hyperactivity disorder: suggestive evidence for linkage on chromosomes 7p and 15q. Am J Hum Genet 72:1251-60.

Bakker SC, Van Der Meulen EM, Oteman N, Schelleman H, Pearson PL, Buitelaar JK and Sinke RJ (2005) DAT1, DRD4 and DRD5 polymorphisms are not associated with ADHD in Dutch families. Am J Med Genet 132:50-2.

Barr CL, Feng Y, Wigg K, Bloom S, Roberts W, Malone M, Schachar R, Tannock R and Kennedy JL (2000a) Identification of DNA variants in the SNAP-25 gene and linkage study of these polymorphisms and attention-deficit hyperactivity disorder. Mol Psychiatry 5:405– 409.

Barr CL, Wigg KG, Bloom S, Schachar R, Tannock R, Roberts W, Malone M and Kennedy JL (2000b) Further evidence from haplotype analysis for linkage of the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. Am J Med Genet 96:262-7.

Barr CL, Wigg KG, Zai G, Roberts W, Malone M, Schachar R, Tannock R and Kennedy JL (2001a) Attention-deficit hyperactivity disorder and the adrenergic receptors alpha 1C and alpha 2C. Mol Psychiatry 6:334-7.

Barr CL, Xu C, Kroft J, Feng Y, Wigg K, Zai G, Tannock R, Schachar R, Malone M, Roberts W, Nothen MM, Grunhage F, Vandenbergh DJ, Uhl G, Sunohara G, King N and Kennedy JL (2001b) Haplotype study of three polymorphisms at the dopamine transporter locus confirm linkage to attention-deficit/hyperactivity disorder. Biol Psychiatry 49:333-9.

Barr CL, Feng Y, Wigg KG, Schachar R, Tannock R, Roberts W, Malone M and Kennedy JL (2001c) 5'-untranslated region of the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. Am J Med Genet 105:84-90.

Barr CL, Kroft J, Feng Y, Wigg K, Roberts W, Malone M, , Ickowicz A, Schachar R, Tannock R and Kennedy JL (2002) The norepinephrine transporter gene and attention-deficit hyperactivity disorder. Am J Med Genet 114:255–259.

Bellgrove MA, Hawi Z, Kirley A, Fitzgerald M, Gill M and Robertson IH (2005a) Association between dopamine transporter (DAT1) genotype, left-sided inattention, and an enhanced response to methylphenidate in attention-deficit hyperactivity disorder. Neuropsychopharmacology 30:2290-7.

Bellgrove MA, Hawi Z, Kirley A, Gill M and Robertson IH (2005b) Dissecting the attention deficit hyperactivity disorder (ADHD) phenotype: sustained attention, response variability and spatial attentional asymmetries in relation to dopamine transporter (DAT1) genotype. Neuropsychologia 43:1847-57.

Bellgrove MA, Hawi Z, Lowe N, Kirley A, Robertson IH and Gill M (2005c) DRD4 gene variants and sustained attention in attention deficit hyperactivity disorder (ADHD): effects of associated alleles at the VNTR and -521 SNP. Am J Med Genet 136:81-6.

Bhaduri N, Das M, Sinha S, Chattopadhyay A, Gangopadhyay PK, Chaudhuri K, Singh M and Mukhopadhyay K (2006) Association of dopamine D4 receptor (DRD4) polymorphisms with attention deficit hyperactivity disorder in Indian population. Am J Med Genet 141:61-6.

Biederman J, Faraone SV, Keenan K, Knee D and Tsuang MT (1990) Family genetic and psychosocial risk factors in DSM-III attention deficit disorder.r J Am Acad Child Adolesc Psychiatry 29:526 –533.

Biederman J, Faraone SV, Keenan K, Benjamin J, Krifcher B, Moore C, Sprich-Buckminster S, Ugaglia K, Jellinek MS and Steingard R (1992) Further evidence for family-genetic risk factors in attention deficit hyperactivity disorder Patterns of comorbidity in probands and relatives psychiatrically and pediatrically referred samples. Arch Gen Psychiatry 49:728-38.

Biederman J, Faraone SV, Spencer T, Wilens T, Norman D, Lapey KA, Mick E, Lehman BK and Doyle A (1993) Patterns of psychiatric comorbidity cognition and psychosocial functioning in adults with attention deficit hyperactivity disorder. Am J Psychiatry 150:1792-8.

Biederman J, Faraone SV, Mick E, Williamson S, Wilens TE, Spencer TJ, Weber W, Jetton J, Kraus I, Pert J and Zallen B (1999) Clinical correlates of ADHD in females: findings from a large group of girls ascertained from pediatric and psychiatric referral sources. J Am Acad Child Adolesc Psychiatry 38:966-75.

Biederman J and Spencer T (1999) Attention-deficit/hyperactivity disorder (ADHD) as a noradrenergic disorder. Biol Psychiatry 46:1234-42.

Biederman J, Mick E, Faraone SV, Braaten E, Doyle A, Spencer T, Wilens TE, Frazier E and Johnson MA (2002) Influence of gender on attention deficit hyperactivity disorder in children referred to a psychiatric clinic. Am J Psychiatry 159:36-42.

Biederman J (2004) Impact of comorbidity in adults with attention-deficit/hyperactivity disorder. J Clin Psychiatry 65:3-7.

Bobb AJ, Addington AM, Sidransky E, Gornick MC, Lerch JP, Greenstein DK, Clasen LS, Sharp WS, Inoff-Germain G, Wavrant DE, Vrieze F, Arcos-Burgos M, Straub RE, Hardy JA, Castellanos FX and Rapoport JL (2005) Support for association between ADHD and two candidate genes: NET1 and DRD1. Am J Med Genet 134:67-72.

Brookes KJ, Xu X, Chen CK, Huang YS, Wu YY and Asherson P (2005) No evidence for the association of DRD4 with ADHD in a Taiwanese population within-family study. BMC Med Genet 6:31.

Brookes KJ, Mill J, Guindalini C, Curran S, Xu X, Knight J, Chen CK, Huang YS, Sethna V, Taylor E, Chen W, Breen G and Asherson P (2006) A common haplotype of the dopamine transporter gene associated with attention-deficit/hyperactivity disorder and interacting with maternal use of alcohol during pregnancy. Arch Gen Psychiatry 63:74-81.

Button TM, Thapar A and McGuffin P (2005) Relationship between antisocial behaviour, attention-deficit hyperactivity disorder and maternal prenatal smoking. Br J Psychiatry. 87:155-60.

Cantwell DP (1975) Genetics of hyperactivity. J Child Psychol Psychiatry 16: 261–264.

Cantwell DP (1996) Attention Deficit disorder: a review of the past 10 years. J Am Acad Child Adolesc Psychiatry 35:978-87.

Carrasco X, Rothhammer P, Moraga M, Henriquez H, Aboitiz F and Rothhammer F (2004) Presence of DRD4/7R and DAT1/10R allele in Chilean family members with attention deficit hyperactivity disorder. Rev Med Chil 132:1047-52.

Carrasco X, Rothhammer P, Moraga M, Henriquez H, Chakraborty R, Aboitiz F and Rothhammer F (2006) Genotypic interaction between DRD4 and DAT1 loci is a high risk factor for attention-deficit/hyperactivity disorder in Chilean families. Am J Med Genet 141:51-4.

Castellanos FX, Lau E, Tayebi N, Lee P, Long RE, Giedd JN, Sharp W, Marsh WL, Walter JM, Hamburger SD, Ginns EI, Rapoport JL and Sidransky E (1998) Lack of an association between a dopamine-4 receptor polymorphism and attention-deficit/hyperactivity disorder: genetic and brain morphometric analyses. Mol Psychiatry 3:431-4.

Cavalli-Sforza LL, Manozzi P and Piazza A (1994) The history and geography of human genes. New Jersey: Princenton University Press, Princenton.

Ceresér KM and Vianna MR (2004) Neurotransmissores. In: Kapzinski F, Quevedo J and Izquierdo I (eds). Bases biológicas dos transtornos psiquiátricos. Editora Artmed, Porto Alegre, pp 35-52.

Chen CK, Chen SL, Mill J, Huang YS, Lin SK, Curran S, Purcell S, Sham P and Asherson P (2003) The dopamine transporter gene is associated with attention deficit hyperactivity disorder in a Taiwanese sample. Mol Psychiatry 8:393-6.

Cheon KA, Ryu YH, Kim JW and Cho DY (2005) The homozygosity for 10-repeat allele at dopamine transporter gene and dopamine transporter density in Korean children with attention deficit hyperactivity disorder: relating to treatment response to methylphenidate. Eur Neuropsychopharmacol 15:95-101.

Cheuk DK, Li SY and Wong V (2006) No association between VNTR polymorphisms of dopamine transporter gene and attention deficit hyperactivity disorder in Chinese children. Am J Med Genet 141B:123-125.

Ciliax BJ, Heilman C, Demchyshyn LL, Pristupa ZB, Ince E, Hersch SM, Niznik HB and Levey AI (1995) The dopamine transporter: immunochemical characterization and localization in brain. J Neurosci 15:1714-23.

Collier DA, Stober G, Li T, Heils A, Catalano M, Di Bella D, Arranz MJ, Murray RM, Vallada HP, Bengel D, Muller CR, Roberts GW, Smeraldi E, Kirov G, Sham P and Lesch KP (1996) A novel functional polymorphism within the promoter of the serotonin transporter gene: possible role in susceptibility to affective disorders. Mol Psychiatry 1:453-60.

Comings DE, Gade-Andavolu R, Gonzalez N, Blake H, Wu S and Macmurray JP (1999) Additive effect of three noradrenergic genes (ADRA2A, ADRA2C, DBH) on attention-deficit hyperactivity disorder and learning disabilities in Tourette syndrome subjects. Clin Genet 55:160-72.

Comings DE, Gade-Andavolu R, Gonzalez N, Wu S, Muhleman D, Blake H, Dietz G, Saucier G and MacMurray (2000) JP Comparison of the role of dopamine, serotonin,

and noradrenaline genes in ADHD, ODD and conduct disorder: multivariate regression analysis of 20 genes. Clin Genet 57:178-96.

Conne B, Stutz A and Vassalli JD (2000) The 3' untranslated region of messenger RNA: A molecular 'hotspot' for pathology? Nat Med 6:637-41.

Cook EH Jr, Stein MA, Krasowski MD, Cox NJ, Olkon DM, Kieffer JE and Leventhal BL (1995) Association of attention-deficit disorder and the dopamine transporter gene. Am J Hum Genet 56:993-8.

Cornish KM, Manly T, Savage R, Swanson J, Morisano D, Butler N, Grant C, Cross G, Bentley L and Hollis CP (2005) Association of the dopamine transporter (DAT1) 10/10-repeat genotype with ADHD symptoms and response inhibition in a general population sample. Mol Psychiatry 10:686-98.

Cragg SJ and Rice ME (2004) Dancing past the DAT at a DA synapse. Trends Neurosci 27:270-7.

Curran S, Mill J, Tahir E, Kent L, Richards S, Gould A, Huckett L, Sharp J, Batten C, Fernando S, Ozbay F, Yazgan Y, Simonoff E, Thompson M, Taylor E and Asherson P (2001a) Association study of a dopamine transporter polymorphism and attention deficit hyperactivity disorder in UK and Turkish samples. Mol Psychiatry 6:425-8.

Curran S, Mill J, Sham P, Rijsdijk F, Marusic K, Taylor E and Asherson P (2001b) QTL association analysis of the DRD4 exon 3 VNTR polymorphism in a population sample of children screened with a parent rating scale for ADHD symptoms. Am J Med Genet 105:387-93.

D'souza UM, Russ C, Tahir E, Mill J, Mcguffin P, Asherson PJ and Craig IW (2004) Functional Effects of a Tandem Duplication Polymorphism in the 5' Flanking Region of the DRD4 Gene. Biol Psychiatry 56:691–697.

Daly G, Hawi Z, Fitzgerald M and Gill M (1999) Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children. Mol Psychiatry 4:192-196.

De Luca V, Muglia P, Vincent JB, Lanktree M, Jain U and Kennedy JL (2004a) Adrenergic alpha 2C receptor genomic organization: association study in adult ADHD. Am J Med Genet 127:65-7.

De Luca V, Muglia P, Jain U and Kennedy JL (2004b) No evidence of linkage or association between the norepinephrine transporter (NET) gene MnlI polymorphism and adult ADHD. Am J Med Genet 124:38-40.

Dougherty DD, Bonab AA, Spencer TJ, Rauch SL, Madras BK and Fischman AJ (1999) Dopamine transporter density in patients with attention deficit hyperactivity disorder. Lancet 354:2132-3.

Doyle AE, Willcutt EG, Seidman LJ, Biederman J, Chouinard VA, Silva J and Faraone SV (2005) Attention-deficit/hyperactivity disorder endophenotypes. Biol Psychiatry 57:1324-35.

Eisenberg J, Zohar A, Mei-Tal G, Steinberg A, Tartakovsky E, Gritsenko I, Nemanov L and Ebstein RP (2000) A haplotype relative risk study of the dopamine D4 receptor

(DRD4) exon III repeat polymorphism and attention deficit hyperactivity disorder (ADHD). Am J Med Genet 96:258-61.

El-Faddagh M, Laucht M, Maras A, Vohringer L and Schmidt MH (2004) Association of dopamine D4 receptor (DRD4) gene with attention-deficit/hyperactivity disorder (ADHD) in a high-risk community sample: a longitudinal study from birth to 11 years of age. J Neural Transm 111:883-9.

Faraone SV, Biederman J, Chen WJ, Krifcher B, Keenan K, Moore C, Sprich S and Tsuang MT (1992) Segregation analysis of attention deficit hyperactivity disorder: Evidence for single gene transmission. Psychiatr Genet 2:257-275.

Faraone SV, Biederman J, Weber W and Russell RL (1998) Psychiatric, neuropsychological, and psychosocial features of DSM-IV subtypes of attention-deficit/hyperactivity disorder: results from a clinically referred sample. J Am Acad Child Adolesc Psychiatry 37:185-93.

Faraone SV and Biederman J (1998) Neurobiology of attention-deficit hyperactivity disorder. Biol Psychiatry 44:951-8.

Faraone SV, Biederman J, Weiffenbach B, Keith T, Chu MP, Weaver A, Spencer TJ, Wilens TE, Frazier J, Cleves M and Sakai J (1999a) Dopamine D4 gene 7-repeat allele and attention deficit hyperactivity disorder. Am J Psychiatry 156:768-70.

Faraone SV, Tsuang D and Tsuang MT (1999b) Genetics and Mental Disorders: A Guide for Students, Clinicians, and Researchers. New York: Guilford Press.

Faraone SV and Doyle AE (2000) Genetic influences on attention deficit hyperactivity disorder. Curr Psychiatry Rep 2:143-6.

Faraone SV, Doyle AE, Mick E and Biederman J (2001) Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. Am J Psychiatry 158:1052-7.

Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA and Sklar P (2005) Molecular Genetics of Attention-Deficit/Hyperactivity Disorder. Biol Psychiatry 57:1313-23.

Faraone SV, Biederman J and Mick E (2006) The age-dependent decline of attention deficit hyperactivity disorder: a meta-analysis of follow-up studies. Psychol Med 36:159-65.

Feng Y, Crosbie J, Wigg K, Pathare T, Ickowicz A, Schachar R, Tannock R, Roberts W, Malone M, Swanson J, Kennedy JL and Barr CL (2005a) The SNAP-25 gene as a susceptibility gene contributing to attention-deficit hyperactivity disorder. Mol Psychiatry 10:998-1005.

Feng Y, Wigg KG, Makkar R, Ickowicz A, Pathare T, Tannock R, Roberts W, Malone M, Kennedy JL, Schachar R and Barr CL (2005b) Sequence variation in the 3'-untranslated region of the dopamine transporter gene and attention-deficit hyperactivity disorder (ADHD). Am J Med Genet 139:1-6.

Fisher SE, Francks C, McCracken JT, McGough JJ, Marlow AJ, MacPhie IL, Newbury DF, Crawford LR, Palmer CG, Woodward JA, Del'Homme M, Cantwell DP, Nelson SF,

Monaco AP and Smalley SL (2002) A genomewide scan for loci involved in attention-deficit/hyperactivity disorder. Am J Hum Genet 70:1183-96.

Frank Y, Pergolizzi RG and Perilla MJ (2004) Dopamine D4 receptor gene and attention deficit hyperactivity disorder. Pediatr Neurol 31:345-8.

Fuke S, Suo S, Takahashi N, Koike H, Sasagawa N and Ishiura S (2001) The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression. Pharmacogenomics J 1:152-156.

Gainetdinov RR, Wetsel WC, Jones SR, Levin ED, Jaber M and Caron MG (1999) Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. Science 283:397-401.

Galili-Weisstub E, Levy S, Frisch A, Gross-Tsur V, Michaelovsky E, Kosov A, Meltzer A, Goltser T, Serretti A, Cusin C, Darvasi A, Inbar E, Weizman A and Segman RH (2005) Dopamine transporter haplotype and attention-deficit hyperactivity disorder. Mol Psychiatry 10:617-8.

Gelernter J, Kennedy JL, Van Tol HH, Civelli O and Kidd KK (1992) The D4 dopamine receptor (DRD4) maps to distal 11p close to HRAS. Genomics 13:208-10.

Gill M, Daly G, Heron S, Hawi Z and Fitzgerald M (1997) Confirmation of association between attention deficit hyperactivity disorder and a dopamine transporter polymorphism. Mol Psychiatry 2:311-3.

Giros B, Jaber M, Jones SR, Wightman RM and Caron MG (1996) Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379:606-12.

Gittelman R, Mannuzza S, Shenker R and Bonagura N (1985) Hyperactive boys almost grown up: I Psychiatric status. Arch Gen Psychiatry 42:937–947.

Gottesman II and Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160:636-45.

Greenwood TA and Kelsoe JR (2003) Promoter and intronic variants affect the transcriptional regulation of the human dopamine transporter gene. Genomics 82:511-520.

Gunzerath L and Goldman D (2003) G x E: a NIAAA workshop on gene-environment interactions. Alcohol Clin Exp Res 27:540-62.

Hamarman S, Fossella J, Ulger C, Brimacombe M and Dermody J (2004) Dopamine receptor 4 (DRD4) 7-repeat allele predicts methylphenidate dose response in children with attention deficit hyperactivity disorder: a pharmacogenetic study. J Child Adolesc Psychopharmacol 14:564-74.

Hawi Z, Mccarron M, Kirley A, Daly G, Fitzgerald M and Gill M (2000) No association of the dopamine DRD4 receptor (DRD4) gene polymorphism with attention deficit hyperactivity disorder (ADHD) in the Irish population. Am J Med Genet 96:268-72.

Hawi Z, Lowe N, Kirley A, Gruenhage F, Nothen M, Greenwood T, Kelsoe J, Fitzgerald M and Gill M (2003) Linkage disequilibrium mapping at DAT1, DRD5 and DBH narrows the search for ADHD susceptibility alleles at these loci. Mol Psychiatry 8:299-308.

Hebebrand J, Dempfle A, Saar K, Thiele H, Herpertz-Dahlmann B, Linder M, Kiefl H, Remschmidt H, Hemminger U, Warnke A, Knolker U, Heiser P, Friedel S, Hinney A, Schafer H, Nurnberg P and Konrad K (2006) A genome-wide scan for attention-deficit/hyperactivity disorder in 155 German sib-pairs. Mol Psychiatry 11:196-205.

Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D and Lesch KP (1996). Allelic variation of human serotonin transporter gene expression. J Neurochem 66:2621-4.

Heinz A, Goldman D, Jones DW, Palmour R, Hommer D, Gorey JG, Lee KS, Linnoila M and Weinberger DR (2000) Genotype influences in vivo dopamine transporter availability in human striatum. Neuropsychopharmacology 22:133-9.

Holmes J, Payton A, Barrett JH, Hever T, Fitzpatrick H, Trumper AL, Harrington R, Mcguffin P, Owen M, Ollier W, Worthington J and Thapar A (2000) A family-based and case-control association study of the dopamine D4 receptor gene and dopamine transporter gene in attention deficit hyperactivity disorder. Mol Psychiatry 5:523-30.

Holmes J, Payton A, Barrett J, Harrington R, Mcguffin P, Owen M, Ollier W, Worthington J, Gill M, Kirley A, Hawi Z, Fitzgerald M, Asherson P, Curran S, Mill J, Gould A, Taylor E, Kent L, Craddock N and Thapar A (2002) Association of DRD4 in children with ADHD and comorbid conduct problems. Am J Med Genet 114:150-3.

Inkster B, Muglia P, Jain U and Kennedy JL (2004) Linkage disequilibrium analysis of the dopamine beta-hydroxylase gene in persistent attention deficit hyperactivity disorder. Psychiatr Genet 14:117-20.

Jaber M, Dumartin B, Sagne C, Haycock JW, Roubert C, Giros B, Bloch B and Caron MG (1999) Differential regulation of tyrosine hydroxylase in the basal ganglia of mice lacking the dopamine transporter. Eur J Neurosci 11:3499-511.

Jacobsen LK, Staley JK, Zoghbi SS, Seibyl JP, Kosten TR, Innis RB and Gelernter J (2000) Prediction of dopamine transporter binding availability by genotype: a preliminary report. Am J Psychiatry 157:1700-3.

Johann M, Bobbe G, Putzhammer A and Wodarz N (2003) Comorbidity of alcohol dependence with attention-deficit hyperactivity disorder: differences in phenotype with increased severity of the substance disorder, but not in genotype (serotonin transporter and 5-hydroxytryptamine-2c receptor). Alcohol Clin Exp Res 27:1527-34.

Jovanovic V, Guan HC and Van Tol HH (1999) Comparative pharmacological and functional analysis of the human dopamine D42 and D410 receptor variants. Pharmacogenetics 9:561-8.

Kahn RS, Khoury J, Nichols WC and Lanphear BP (2003) Role of dopamine transporter genotype and maternal prenatal smoking in childhood hyperactive-impulsive, inattentive, and oppositional behaviors. J Pediatr 143:104-10.

Kent L, Green E, Holmes J, Thapar A, Gill M, Hawi Z, Fitzgerald M, Asherson P, Curran S, Mills J, Payton A and Craddock N (2001a) No association between CHRNA7 microsatellite markers and attention-deficit hyperactivity disorder. Am J Med Genet 105:686-9.

Kent L, Middle F, Hawi Z, Fitzgerald M, Gill M, Feehan C and Craddock N (2001b) Nicotinic acetylcholine receptor alpha4 subunit gene polymorphism and attention deficit hyperactivity disorder. Psychiatr Genet 11:37-40.

Kent L, Green E, Hawi Z, Kirley A, Dudbridge F, Lowe N, Raybould R, Langley K, Bray N, Fitzgerald M, Owen MJ, O'Donovan MC, Gill M, Thapar A and Craddock N (2005) Association of the paternally transmitted copy of common Valine allele of the Val66Met polymorphism of the brain-derived neurotrophic factor (BDNF) gene with susceptibility to ADHD. Mol Psychiatry 10:939-43.

Kessler RC, Chiu WT, Demler O, Merikangas KR and Walters EE (2005) Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 62:617-27.

Kim JW, Kim BN and Cho SC (2005) The dopamine transporter gene and the impulsivity phenotype in attention deficit hyperactivity disorder: A case-control association study in a Korean sample. J Psychiatr Res.

Kim YS, Leventhal BL, Kim SJ, Kim BN, Cheon KA, Yoo HJ, Kim SJ, Badner J and Cook EH (2005) Family-based association study of DAT1 and DRD4 polymorphism in Korean children with ADHD. Neurosci Lett 390:176-81.

Kirley A, Lowe N, Hawi Z, Mullins C, Daly G, Waldman I, McCarron M, O'Donnell D, Fitzgerald M and Gill M (2003) Association of the 480 bp DAT1 allele with methylphenidate response in a sample of Irish children with ADHD. Am J Med Genet 121:50-4.

Kirley A, Lowe N, Mullins C, Mccarron M, Daly G, Waldman I, Fitzgerald M, Gill M and Hawi Z (2004) Phenotype studies of the DRD4 gene polymorphisms in ADHD: association with oppositional defiant disorder and positive family history. Am J Med Genet 131:38-42.

Kotler M, Manor I, Sever Y, Eisenberg J, Cohen H, Ebstein RP and Tyano S (2000) Failure to replicate an excess of the long dopamine D4 exon III repeat polymorphism in ADHD in a family-based study. Am J Med Genet 96:278-81.

Krause KH, Dresel SH, Krause J, Kung HF and Tatsch K (2000) Increased striatal dopamine transporter in adult patients with attention deficit hyperactivity disorder: effects of methylphenidate as measured by single photon emission computed tomography. Neuroscience Letters 285:107–110.

Kustanovich V, Ishii J, Crawford L, Yang M, Mcgough JJ, Mccracken JT, Smalley SL and Nelson SF (2004) Transmission disequilibrium testing of dopamine-related candidate gene polymorphisms in ADHD: confirmation of association of ADHD with DRD4 and DRD5. Mol Psychiatry 9:711-7.

Lahoste GJ, Swanson JM, Wigal SB, Glabe C, Wigal T, King N and Kennedy JL (1996) Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder. Mol Psychiatry 1:121-4.

Lakatos K, Nemoda Z, Birkas E, Ronai Z, Kovacs E, Ney K, Toth I, Sasvari-Szekely M and Gervai J (2003) Association of D4 dopamine receptor gene and serotonin

transporter promoter polymorphisms with infants' response to novelty. Mol Psychiatry 8:90-7.

Langley K, Marshall L, Van Den Bree M, Thomas H, Owen M, O'donovan M and Thapar A (2004) Association of the dopamine D4 receptor gene 7-repeat allele with neuropsychological test performance of children with ADHD. Am J Psychiatry 161:133-8.

Langley K, Turic D, Peirce TR, Mills S, Van Den Bree MB, Owen MJ, O'Donovan MC and Thapar A (2005) No support for association between the dopamine transporter (DAT1) gene and ADHD. Am J Med Genet 139:7-10.

Laucht M and Schmidt MH (2004) Maternal smoking during pregnancy: risk factor for ADHD in the offspring? Z Kinder Jugendpsychiatr Psychother 32:177-85.

Laurin N, Misener VL, Crosbie J, Ickowicz A, Pathare T, Roberts W, Malone M, Tannock R, Schachar R, Kennedy JL and Barr CL (2005) Association of the calcyon gene (DRD1IP) with attention deficit/hyperactivity disorder. Mol Psychiatry 10:1117-25.

Lawson DC, Turic D, Langley K, Pay HM, Govan CF, Norton N, Hamshere ML, Owen MJ, O'Donovan MC and Thapar A (2003) Association analysis of monoamine oxidase A and attention deficit hyperactivity disorder. Am J Med Genet B 116:84-9.

Leung PW, Lee CC, Hung SF, Ho TP, Tang CP, Kwong SL, Leung SY, Yuen ST, Lieh-Mak F, Oosterlaan J, Grady D, Harxhi A, Ding YC, Chi HC, Flodman P, Schuck S, Spence Ma, Moyzis R and Swanson J (2005) Dopamine receptor D4 (DRD4) gene in Han Chinese children with attention-deficit/hyperactivity disorder (ADHD): increased prevalence of the 2-repeat allele. Am J Med Genet 133:54-6.

Li J, Wang YF, Zhou RL, Yang L, Zhang HB and Wang B (2003) Association between tryptophan hydroxylase gene polymorphisms and attention deficit hyperactivity disorder with or without learning disorder. Zhonghua Yi Xue Za Zhi 83:2114 –2118.

Li J, Wang Y, Zhou R, Zhang H, Yang L, Wang B and Faraone SV (2006) Association between tryptophan hydroxylase gene polymorphisms and attention deficit hyperactivity disorder in Chinese Han population. Am J Med Genet 141:126-129.

Li LB, Chen N, Ramamoorthy S, Chi L, Cui XN, Wang LC and Reith ME (2004) The role of N-glycosylation in function and surface trafficking of the human dopamine transporter. J Biol Chem 279:21012-20.

Linnet KM, Dalsgaard S, Obel C, Wisborg K, Hendriksen TB, Rodriguez A, Kotimaa A, Moilanen I, Thomsen PH, Olsen J and Jarvelin MR (2003) Maternal life style factors in pregnancy risk of attention deficit hyperactivity disorder and associated behaviors: review of the current evidence. Am J Psychiatry 160:1028–1040.

Lowe N, Kirley A, Hawi Z, Sham P, Wickham H, Kratochvil CJ, Smith SD, Lee SY, Levy F, Kent L, Middle F, Rohde LA, Roman T, Tahir E, Yazgan Y, Asherson P, Mill J, Thapar A, Payton A, Todd RD, Stephens T, Ebstein RP, Manor I, Barr CL, Wigg KG, Sinke RJ, Buitelaar JK, Smalley SL, Nelson SF, Biederman J, Faraone SV and Gill M (2004a) Joint analysis of the DRD5 marker concludes association with attention-deficit/hyperactivity disorder confined to the predominantly inattentive and combined subtypes. Am J Hum Genet 74:348-56.

Lowe N, Kirley A, Mullins C, Fitzgerald M, Gill M and Hawi Z (2004b) Multiple marker analysis at the promoter region of the DRD4 gene and ADHD: evidence of linkage and association with the SNP -616. Am J Med Genet 131:33-7.

Lynn DE, Lubke G, Yang M, McCracken JT, McGough JJ, Ishii J, Loo SK, Nelson SF and Smalley SL (2005) Temperament and character profiles and the dopamine D4 receptor gene in ADHD. Am J Psychiatry 162:906-13.

Madras BK, Miller MM and Fischman AJ (2005) The Dopamine Transporter and Attention-Deficit/Hyperactivity Disorder. Biol Psych 57:1397–1409.

Maher BS, Marazita ML, Ferrell RE and Vanyukov MM (2002) Dopamine system genes and attention deficit hyperactivity disorder: a meta-analysis. Psychiatr Genet 12:207-15.

Mannuzza S, Klein RG and Addalli KA (1991) Young adult mental status of hyperactive boys and their brothers: a prospective follow-up study. J Am Acad Child Adolesc Psychiatry 30:743-51.

Manor I, Tyano S, Mel E, Eisenberg J, Bachner-Melman R, Kotler M and Ebstein RP (2002a) Family-based and association studies of monoamine oxidase A and attention deficit hyperactivity disorder (ADHD): preferential transmission of the long promoter-region repeat and its association with impaired performance on a continuous performance test (TOVA). Mol Psychiatry 7:626-32

Manor I, Tyano S, Eisenberg J, Bachner-Melman R, Kotler M and Ebstein RP (2002b) The short DRD4 repeats confer risk to attention deficit hyperactivity disorder in a family-based design and impair performance on a continuous performance test (TOVA) Mol Psychiatry 7:790-4.

Martinez D, Gelernter J, Abi-Dargham A, van Dyck CH, Kegeles L, Innis RB and Laruelle M (2001) The variable number of tandem repeats polymorphism of the dopamine transporter gene is not associated with significant change in dopamine transporter phenotype in humans. Neuropsychopharmacology 24:553-60.

Mattos P, Abreu PB and Grevet E (2003) O TDAH no Adulto: Dificuldades Diagnósticas e de Tratamento. In: Rohde LA, Mattos P, *et al.* (eds) Princípios e práticas em transtorno de déficit de atenção/hiperatividade. 1st ed. Artmed, Porto Alegre, pp 219-235.

McCracken JT, Smalley SL, Mcgough JJ, Crawford L, Del'homme M, Cantor RM, Liu A and Nelson SF (2000) Evidence for linkage of a tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4) with attention deficit hyperactivity disorder (ADHD). Mol Psychiatry 5:531-6.

McEvoy B, Hawi Z, Fitzgerald M and Gill M (2002) No evidence of linkage or association between the norepinephrine transporter (NET) gene polymorphisms and ADHD in the Irish population. Am J Med Genet 114:665-6.

McGough JJ (2005) Attention-deficit/hyperactivity disorder pharmacogenomics. Biol Psychiatry 57:1367-73.

Michelhaugh SK, Fiskerstrand C, Lovejoy E, Bannon MJ and Quinn JP (2001) The dopamine transporter gene (SLC6A3) variable number of tandem repeats domain enhances transcription in dopamine neurons. J Neurochem 79:1033-1038.

Mill J, Curran S, Kent L, Richards S, Gould A, Virdee V, Huckett L, Sharp J, Batten C, Fernando S, Simanoff E, Thompson M, Zhao J, Sham P, Taylor E and Asherson P (2001) Attention deficit hyperactivity disorder (ADHD) and the dopamine D4 receptor gene: evidence of association but no linkage in a UK sample. Mol Psychiatry 6:440-4.

Mill J, Asherson P, Browes C, D'souza U and Craig I (2002) Expression of the dopamine transporter gene is regulated by the 3' UTR VNTR: Evidence from brain and lymphocytes using quantitative RT-PCR. Am J Med Genet 114:975–979.

Mill J, Fisher N, Curran S, Richards S, Taylor E and Asherson P (2003) Polymorphisms in the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. Neuroreport 14:1463-6.

Mill J, Richards S, Knight J, Curran S, Taylor E and Asherson P (2004) Haplotype analysis of SNAP-25 suggests a role in the aetiology of ADHD. Mol Psychiatry 9:801-10.

Mill J, Asherson P, Craig I and D'Souza UM (2005a) Transient expression analysis of allelic variants of a VNTR in the dopamine transporter gene (DAT1). BMC Genetics 6:3.

Mill J, Xu X, Ronald A, Curran S, Price T, Knight J, Craig I, Sham P, Plomin R and Asherson P (2005b) Quantitative trait locus analysis of candidate gene alleles associated with attention deficit hyperactivity disorder (ADHD) in five genes: DRD4, DAT1, DRD5, SNAP-25, and 5HT1B. Am J Med Genet 133:68-73.

Miller GM and Madras BK (2002) Polymorphisms in the 3'-untranslated region of human and monkey dopamine transporter genes affect reporter gene expression. Mol Psychiatry 7:44-55.

Morrison JR and Stewart (1973) The psychiatric status of the legal families of adopted hyperactive children. Arch Gen Psychiatry 28:888–891.

Muglia P, Jain U, Macciardi F and Kennedy JL (2000) Adult attention deficit hyperactivity disorder and the dopamine D4 receptor gene. Am J Med Genet 96:273-7.

Muglia P, Jain U and Kennedy JL (2002a) A transmission disequilibrium test of the Ser9/Gly dopamine D3 receptor gene polymorphism in adult attention-deficit hyperactivity disorder. Behav Brain Res 130:91-5.

Muglia P, Jain U, Inkster B and Kennedy JL (2002b) A quantitative trait locus analysis of the dopamine transporter gene in adults with ADHD. Neuropsychopharmacology 27:655-62.

Murphy K and Barkley RA (1996) Attention Deficit Hyperactivity Disorder Adults: Comorbidities and Adaptive Impairments. Comp Psychiatry 6:393-401.

Noland JS, Singer LT, Short EJ, Minnes S, Arendt RE, Kirchner HL and Bearer C (2005) Prenatal drug exposure and selective attention in preschoolers. Neurotoxicol Teratol 27:429-38.

Ogdie MN, Macphie IL, Minassian SL, Yang M, Fisher SE, Francks C, Cantor RM, McCracken JT, McGough JJ, Nelson SF, Monaco AP and Smalley SL (2003) A genomewide scan for attention-deficit/hyperactivity disorder in an extended sample: suggestive linkage on 17p11. Am J Hum Genet 72:1268-79.

Ogdie MN, Fisher SE, Yang M, Ishii J, Francks C, Loo SK, Cantor RM, McCracken JT, McGough JJ, Smalley SL and Nelson SF (2004) Attention deficit hyperactivity disorder: fine mapping supports linkage to 5p13, 6q12, 16p13, and 17p11. Am J Hum Genet 75:661-8.

Oh KS, Shin DW, Oh GT and Noh KS (2003) Dopamine transporter genotype influences the attention deficit in Korean boys with ADHD. Yonsei Med J 44:787-92.

Okuyama Y, Ishiguro H, Toru M and Arinami T (1999) A genetic polymorphism in the promoter region of DRD4 associated with expression and schizophrenia. Biochem Biophys Res Commun 258:292-5.

Palmer CG, Bailey JN, Ramsey C, Cantwell D, Sinsheimer JS, Del'Homme M, McGough J, Woodward JA, Asarnow R, Asarnow J, Nelson S and Smalley SL (1999) No evidence of linkage or linkage disequilibrium between DAT1 and attention deficit hyperactivity disorder in a large sample. Psychiatr Genet 9:157-60.

Payton A, Holmes J, Barrett JH, Hever T, Fitzpatrick H, Trumper AL, Harrington R, Mcguffin P, O'donovan M, Owen M, Ollier W, Worthington J and Thapar A (2001) Examining for association between candidate gene polymorphisms in the dopamine pathway and attention-deficit hyperactivity disorder: a family-based study. Am J Med Genet 105:464-70.

Pericak-Vance MA (1998) Linkage disequilibrium and allelic association. In: Haines JL and Pericak-Vance MA (eds). Approaches to Gene Mapping in Complex Human Diseases. Wiley-Liss, New York, pp323-334.

Pliszka SR, Mccracken JT and Maas JW (1996) Catecholamines in attentiondeficit/ hyperactivity disorder: current perspectives. J Am Acad Child Adolesc Psychiatry 35:254-71.

Purper-Ouakil D, Wohl M, Mouren MC, Verpillat P, Ades J and Gorwood P (2005) Meta-analysis of family-based association studies between the dopamine transporter gene and attention deficit hyperactivity disorder. Psychiatr Genet 15:53-9.

Qian QJ, Wang YF, Zhou RL, Yang L and Li J (2004a) Association studies of G352A polymorphism of dopamine transporter gene in Han Chinese attention deficit hyperactivity disorder patients. Beijing Da Xue Xue Bao 36:626-9.

Qian Q, Wang Y, Zhou R, Yang L and Faraone SV (2004b) Family-based and case-control association studies of DRD4 and DAT1 polymorphisms in Chinese attention deficit hyperactivity disorder patients suggest long repeats contribute to genetic risk for the disorder. Am J Med Genet 128:84-9.

Quist JF and Kennedy JL (2001) Genetics of childhood disorders: XXIII ADHD, Part 7: The serotonin system. J Am Acad Child Adolesc Psychiatry 40:253-6.

Rohde LA, Biederman J, Busnello EA, Zimmermann H, Schmitz M, Martins S and Tramontina S (1999) ADHD in a school sample of Brazilian adolescents: a study of prevalence, comorbid conditions, and impairments. J Am Acad Child Adolesc Psychiatry 38:716-22.

Roman T, Schmitz M, Polanczyk G, Eizirik M, Rohde LA and Hutz MH (2001) Attention-deficit hyperactivity disorder: a study of association with both the dopamine transporter gene and the dopamine D4 receptor gene. Am J Med Genet 105:471-8.

Roman T, Schmitz M, Polanczyk GV, Eizirik M, Rohde LA and Hutz MH (2002a) Further evidence for the association between attention-deficit/hyperactivity disorder and the dopamine-beta-hydroxylase gene. Am J Med Genet 114:154-8.

Roman T, Szobot C, Martins S, Biederman J, Rohde LA and Hutz MH (2002b) Dopamine transporter gene and response to methylphenidate in attention-deficit/hyperactivity disorder. Pharmacogenetics 12:497-9.

Roman T, Schmitz M, Polanczyk GV, Eizirik M, Rohde LA and Hutz MH (2003) Is the alpha-2A adrenergic receptor gene (ADRA2A) associated with attention-deficit/hyperactivity disorder? Am J Med Genet 120:116-20.

Roman T, Polanczyk GV, Zeni C, Genro JP, Rohde LA and Hutz MH (2006) Further evidence of the involvement of alpha-2A-adrenergic receptor gene (ADRA2A) in inattentive dimensional scores of attention-deficit/hyperactivity disorder. Mol Psychiatry 11:8-10.

Rowe DC, Stever C, Giedinghagen LN, Gard JM, Cleveland HH, Terris ST, Mohr JH, Sherman S, Abramowitz A and Waldman ID (1998) Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder. Mol Psychiatry 3:419-26.

Rowe DC, Stever C, Chase D, Sherman S, Abramowitz A and Waldman ID (2001) Two dopamine genes related to reports of childhood retrospective inattention and conduct disorder symptoms. Mol Psychiatry 6:429-33.

Russell V, Allie S and Wiggins T (2000) Increased noradrenergic activity in prefrontal cortex slices of an animal model for attention-deficit hyperactivity disorder--the spontaneously hypertensive rat. Behav Brain Res 117:69-74.

Russell VA, Sagvolden T and Johansen EB (2005) Animal models of attention-deficit hyperactivity disorder. Behav Brain Funct 1:9.

Satterfield JH and Dawson ME (1971) Electrodermal correlates of hyperactivity in children. Psychophysiology 8:191-197.

Seaman MI, Fisher JB, Chang F and Kidd KK (1999) Tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4). Am J Med Genet 88:705-9

Seeger G, Schloss P and Schmidt MH (2001) Marker gene polymorphisms in hyperkinetic disorder--predictors of clinical response to treatment with methylphenidate? Neurosci Lett 313:45-8.

Seeger G, Schloss P, Schmidt MH, Ruter-Jungfleisch A and Henn FA (2004) Gene-environment interaction in hyperkinetic conduct disorder (HD + CD) as indicated by season of birth variations in dopamine receptor (DRD4) gene polymorphism. Neurosci Lett 366:282-6.

Seeman P and Madras BK (1998) Anti-hyperactivity medication: methylphenidate and amphetamine. Mol Psychiatry 3:386-96.

Sheehan K, Lowe N, Kirley A, Mullins C, Fitzgerald M, Gill M and Hawi Z (2005) Tryptophan hydroxylase 2 (TPH2) gene variants associated with ADHD. Mol Psychiatry 10:944-9.

Simsek M, Al-Sharbati M, Al-Adawi S, Ganguly SS and Lawatia K (2005) Association of the risk allele of dopamine transporter gene (DAT1*10) in Omani male children with attention-deficit hyperactivity disorder. Clin Biochem 38:739-42.

Smalley SL, Bailey JN, Palmer CG, Cantwell DP, Mcgough JJ, Del'homme MA, Asarnow JR, Woodward JA, Ramsey C and Nelson SF (1998) Evidence that the dopamine D4 receptor is a susceptibility gene in attention deficit hyperactivity disorder. Mol Psychiatry 3:427-30.

Smalley SL, Kustanovich V, Minassian SL, Stone JL, Ogdie MN, McGough JJ, McCracken JT, MacPhie IL, Francks C, Fisher SE, Cantor RM, Monaco AP and Nelson SF (2002) Genetic linkage of attention-deficit/hyperactivity disorder on chromosome 16p13, in a region implicated in autism. Am J Hum Genet 71:959-63.

Smith KM, Daly M, Fischer M, Yiannoutsos CT, Bauer L, Barkley R and Navia A (2003) Association of the dopamine beta hydroxylase gene with attention deficit hyperactivity disorder: genetic analysis of the Milwaukee longitudinal study. Am J Med Genet 119:77-85.

Smoller JW, Biederman J, Arbeitman L, Doyle AE, Fagerness J, Perlis RH, Sklar P and Faraone SV (2005) Association Between the 5HT1B Receptor Gene (HTR1B) and the Inattentive Subtype of ADHD. Biol Psychiatry.

Sprich S, Biederman J, Crawford MH, Mundy E and Faraone SV (2000) Adoptive and biological families of children and adolescents with ADHD. J Am Acad Child Adolesc Psychiatry 39:1432–1437.

State MW, Lombroso PJ, Pauls DL and Leckman JR (2000) The genetics of childhood psychiatric disorders: A decade of progress. J Child Psychol Psychiatry 39:946-962.

Stein MA, Waldman ID, Sarampote CS, Seymour KE, Robb AS, Conlon C, Kim SJ and Cook EH (2005) Dopamine transporter genotype and methylphenidate dose response in children with ADHD. Neuropsychopharmacology 30:1374-82.

Stevenson J, Langley K, Pay H, Payton A, Worthington J, Ollier W and Thapar A (2005) Attention deficit hyperactivity disorder with reading disabilities: preliminary genetic findings on the involvement of the ADRA2A gene. J Child Psychol Psychiatry 46:1081-8.

Sunohara GA, Roberts W, Malone M, Schachar RJ, Tannock R, Basile VS, Wigal T, Wigal SB, Schuck S, Moriarty J, Swanson JM, Kennedy JL and Barr CL (2000) Linkage of the dopamine D4 receptor gene and attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 39:1537-42.

Swanson J, Castellanos FX, Murias M, Lahoste G and Kennedy J (1998a) Cognitive neuroscience of attention deficit hyperactivity disorder and hyperkinetic disorder. Curr Opin Neurobiol 8:263-71.

Swanson J, Sunohara GA, Kennedy JL, Regino R, Fineberg E, Wigal T, Lerner M, Williams L, Lahoste GJ and Wigal S (1998b) Association of the dopamine receptor D4 (DRD4) gene with a refined phenotype of attention deficit hyperactivity disorder (ADHD): a family-based approach. Mol Psychiatry 3:38-41.

Swanson J, Oosterlaan J, Murias M, Schuck S, Flodman P, Spence MA, Wasdell M, Ding Y, Chi HC, Smith M, Mann M, Carlson C, Kennedy JL, Sergeant JA, Leung P, Zhang YP, Sadeh A, Chen C, Whalen CK, Babb KA, Moyzis R and Posner MI (2000) Attention deficit/hyperactivity disorder children with a 7-repeat allele of the dopamine receptor D4 gene have extreme behavior but normal performance on critical neuropsychological tests of attention. Proc Natl Acad Sci USA 97:4754-9.

Swanson J, Posner M, Fusella J, Wasdell M, Sommer T and Fan J (2001) Genes and attention deficit hyperactivity disorder. Curr Psychiatry Rep 3:92-100.

Tahir E, Yazgan Y, Cirakoglu B, Ozbay F, Waldman I and Asherson PJ (2000) Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children. Mol Psychiatry 5:396-404.

Tang G, Ren D, Xin R, Qian Y, Wang D and Jiang S (2001) Lack of association between the tryptophan hydroxylase gene A218C polymorphism and attention-deficit hyperactivity disorder in Chinese Han population. Am J Med Genet 105:485-8.

Tannock R (1998) Attention deficit hyperactivity disorder: advances in cognitive, neurobiological, and genetic research. J Child Psychol Psychiatry 39:65-99.

Thapar A, Holmes J, Poulton K and Harrington R (1999) Genetic basis of attention deficit and hyperactivity. Br J Psychiatry 174:105-11.

Thapar A, Langley K, Fowler T, Rice F, Turic D, Whittinger N, Aggleton J, Van den Bree M, Owen M and O'Donovan M (2005) Catechol O-methyltransferase gene variant and birth weight predict early-onset antisocial behavior in children with attention-deficit/hyperactivity disorder. Arch Gen Psychiatry 62:1275-8.

The ADHD Molecular Genetics Network (2000) Collaborative possibilities for molecular genetic studies of attention deficit hyperactivity disorder: report from an international conference. Am J Med Genet 96:251-7.

Todd RD, Jong YJ, Lobos EA, Reich W, Heath AC and Neuman RJ (2001a) No association of the dopamine transporter gene 3' VNTR polymorphism with ADHD subtypes in a population sample of twins. Am J Med Genet 105:745-8.

Todd RD, Neuman RJ, Lobos EA, Jong YJ, Reich W and Heath AC (2001b) Lack of association of dopamine D4 receptor gene polymorphisms with ADHD subtypes in a population sample of twins. Am J Med Genet 105:432-8.

Todd RD, Huang H, Smalley SL, Nelson SF, Willcutt EG, Pennington BF, Smith SD, Faraone SV and Neuman RJ (2005) Collaborative analysis of DRD4 and DAT genotypes in population-defined ADHD subtypes. J Child Psychol Psychiatry 46:1067-73.

Turic D, Langley K, Mills S, Stephens M, Lawson D, Govan C, Williams N, Van Den Bree M, Craddock N, Kent L, Owen M, O'Donovan M and Thapar A (2004) Follow-up of

genetic linkage findings on chromosome 16p13: evidence of association of N-methyl-D aspartate glutamate receptor 2A gene polymorphism with ADHD. Mol Psychiatry 9:169-73.

Turic D, Langley K, Williams H, Norton N, Williams NM, Moskvina V, Van den Bree MB, Owen MJ, Thapar A and O'Donovan MC (2005) A family based study implicates solute carrier family 1-member 3 (SLC1A3) gene in attention-deficit/hyperactivity disorder. Biol Psychiatry 57:1461-6.

Vaidya CJ, Austin G, Kirkorian G, Ridlehuber HW, Desmond JE, Glover GH and Gabrieli JD (1998) Selective effects of methylphenidate in attention deficit hyperactivity disorder: a functional magnetic resonance study. Proc Natl Acad Sci USA 95:14494-9.

Van Dyck CH, Malison RT, Jacobsen LK, Seibyl JP, Staley JK, Laruelle M, Baldwin RM, Innis RB and Gelernter J (2005) Increased dopamine transporter availability associated with the 9-repeat allele of the SLC6A3 gene. J Nucl Med 46: 745-51.

Van Tol HH, Wu CM, Guan HC, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB and Jovanovic V (1992) Multiple dopamine D4 receptor variants in the human population. Nature 358:149-52.

VanNess SH, Owens MJ and Kilts CD (2005) The variable number of tandem repeats element in DAT1 regulates in vitro dopamine transporter density. BMC Genet 6:55.

Volkow ND, Wang GJ, Fowler JS, Gatley SJ, Logan J, Ding YS, Hitzemann R and Pappas N (1998) Dopamine transporter occupancies in the human brain induced by therapeutic doses of oral methylphenidate. Am J Psychiatry 155:1325-31.

Waldman ID, Rowe DC, Abramowitz A, Kozel ST, Mohr JH, Sherman SL, Cleveland HH, Sanders ML, Gard JM and Stever C (1998) Association and linkage of the dopamine transporter gene and attention-deficit hyperactivity disorder in children: heterogeneity owing to diagnostic subtype and severity. Am J Hum Genet 63:1767-76.

Walitza S, Renner TJ, Dempfle A, Konrad K, Wewetzer Ch, Halbach A, Herpertz-Dahlmann B, Remschmidt H, Smidt J, Linder M, Flierl L, Knolker U, Friedel S, Schafer H, Gross C, Hebebrand J, Warnke A and Lesch KP (2005) Transmission disequilibrium of polymorphic variants in the tryptophan hydroxylase-2 gene in attention-deficit/hyperactivity disorder. Mol Psychiatry 10:1126-32.

Wang E, Ding YC, Flodman P, Kidd JR, Kidd KK, Grady DL, Ryder OA, Spence MA, Swanson JM and Moyzis RK (2004) The Genetic Architecture of Selection at the Human Dopamine Receptor D4 (DRD4) Gene Locus. Am J Hum Genet 74:931–944.

Wang B, Wang Y, Zhou R, Li J, Qian Q, Yang L, Guan L and Faraone SV (2006) Possible association of the alpha-2A adrenergic receptor gene (ADRA2A) with symptoms of attention-deficit/hyperactivity disorder. Am J Med Genet 141:130-134.

Weiss G, Hechtman L, Milroy T and Perlman T (1985) Psychiatric status of hyperactives as adults: A controlled prospective 15-year follow-up of 63 hyperactive children. J Am Acad Child Adolesc Psychiatry 24:211–220.

Wender PH, Reimherr FW and Wood DR (1981) Attention deficit disorder ('minimal brain dysfunction') in adults: a replication study of diagnosis and drug treatment. Arch Gen Psychiatry 38:449-56.

Wender PH, Wood DR and Reimherr FW (1985) Pharmacological treatment of attention deficit disorder, residual type (ADD,RT, "minimal brain dysfunction," "hyperactivity") in adults. Psychopharmacol Bull 21:222-31.

Wender PH, Wolf LE and Wasserstein J (2001) Adults with ADHD. An overview. Ann N Y Acad Sci 931:1-16.

Winsberg BG and Comings DE (1999) Association of the dopamine transporter gene (DAT1) with poor methylphenidate response. J Am Acad Child Adolesc Psychiatry 38:1474-7.

Wohl M, Purper-Ouakil D, Mouren MC, Ades J and Gorwood P (2005) Meta-analysis of candidate genes in attention-deficit hyperactivity disorder. Encephale 4:437-47.

Woodruff TJ, Axelrad DA, Kyle AD, Nweke O, Miller GG and Hurley BJ (2004) Trends in environmentally related Childhood Illnesses. Pediatrics 113: 1133-1140.

Zabetian CP, Anderson GM, Buxbaum S, Elston RS, Ichinose H, Nagatsu T, Kim K, Kim C, Malison RT, Gelernter J and Cubells JF (2001) A Quantitative-Trait Analysis of Plasma-Dopamine β- Hydroxylase Activity: Evidence for a major Functional Polymorphism at the DBH Locus. Am J Hum Genet 68:515-522.

Zametkin AJ and Rapoport JL (1987) Neurobiology of attention deficit disorder with hyperactivity: where have we come in 50 years? J Am Acad Child Adolesc Psychiatry 26:676-686.

Zembrzuski VM, Callegari-Jacques SM, Hutz MH (2006) Application of an African Ancestry Index as a Genomic Control approach in a Brazilian population. Annals of Human Genetics, no Prelo.