UNIVERSIDADE FEDERAL DE MINAS GERAIS

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VARIABILIDADE DE DESEMPENHO EM FUNÇÕES DE AUTORREGULAÇÃO: INFLUÊNCIA DO POLIMORFISMO VAL^{158}MET DO GENE *COMT*, ESTABILIDADE E DESFECHOS ASSOCIADOS

BELO HORIZONTE

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Medicina Molecular, Faculdade de Medicina, Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Doutora em Medicina Molecular.

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RESUMO

COSTA, Danielle de Souza. **Variabilidade de desempenho em funções de Autorregulação: influência do polimorfismo Val^{158}Met do gene *COMT*, Estabilidade e Desfechos associados.** Belo Horizonte, 2017. Tese (Doutorado em Medicina Molecular) - Faculdade de Medicina, Universidade Federal de Minas Gerais, 2017.

Autorregulação seria o processo pelo qual as pessoas mudam ou controlam seus pensamentos, sentimentos ou ações de modo a satisfazer objetivos ou padrões pessoais e sociais, permitindo-as fazer planos, controlar seus impulsos, escolher entre alternativas, inibir pensamentos indesejados e regular o comportamento instintual. Entender os fatores associados a variabilidade de desempenho em funções de autorregulação e suas consequências é crítico para o avanço na compreensão dessa que está entre as maiores habilidades adaptativas de uma espécie eminentemente social. Aqui, investigamos a associação do polimorfismo Val^{158}Met do gene *COMT* com dois aspectos cognitivos da autorregulação e observamos a estabilidade e a associação de características comportamentais da autorregulação com variáveis biológicas e acadêmicas. Quatro trabalhos foram apresentados. 1) Testou-se se o polimorfismo Val^{158}Met do gene *COMT* estaria associado ao desempenho em tarefas de memória operacional e aritmética em 50 indivíduos com Neurofibromatose tipo 1 (NF1), uma doença neurogenética marcada por déficits cognitivos variados. Mesmo nessa doença monogênica, encontramos que o perfil homozigoto Met/Met parece favorecer a manipulação de informações verbais na memória operacional. 2) Exploramos se a associação do polimorfismo Val^{158}Met do gene *COMT* com o desempenho numa tarefa de tomada de decisão sob ambiguidade e risco (Iowa Gambling Task – IGT) seria modificada de acordo com o sexo (84 homens e 108 mulheres hígidos). Observamos um efeito do sexo, com mulheres Val/Val demonstrando desempenho mais vantajoso na dimensão de tomada de decisão sob risco do IGT. 3) Testamos se o comprimento telomérico (CT), um marcador associado com estresse psicológico e exposição precoce a adversidades ambientais estaria associado às dimensões comportamentais do Transtorno de Déficit de Atenção/Hiperatividade (TDAH), em 61 crianças com TDAH e seus pais. Encontramos que o CT seria altamente herdável e associado à gravidade dos sintomas de hiperatividade-impulsividade, sendo um potencial biomarcador do impacto do TDAH nas famílias afetadas. 4) Avaliamos a estabilidade do perfil de funções executivas (FE) comportamentais e os desfechos acadêmicos associados, em 180 adolescentes australianos nascidos com prematuridade extrema (<28 semanas) que foram avaliados com 8 e 18 anos com a versão parental do BRIEF- *Behavior Rating Inventory of Executive Function*. Verificamos que a frequência de indivíduos por grupo, em ordem decrescente foi: desenvolvimento típico (BRI 61%, MCI 53%), dificuldade persistente (BRI 15%, MCI 16%), dificuldade tardia (BRI 12%, MCI 19%) e em remissão (BRI 12%, MCI 13%). Esses grupos apresentaram desfechos acadêmicos distintos e demonstram que a variabilidade de perfil intragrupo deveria ser considerada para estudos futuros. Concluindo, reúne-se aqui evidências da complexidade do estudo de funções de autorregulação, enfatizando-se o papel adaptativo das mesmas numa espécie em que o controle dos impulsos parece essencial no suprimento de uma necessidade humana inata: a necessidade de pertencer ao grupo.

Palavras-Chave: Autorregulação, Funções Executivas, Memória Operacional, Tomada de Decisão Afetiva, Polimorfismo Val^{158}Met do gene *COMT*, Sexo, Telômero, Desempenho acadêmico, Estabilidade longitudinal, Neurofibromatose tipo 1, Transtorno de Déficit de Atenção/Hiperatividade, Prematuro.

ABSTRACT

COSTA, Danielle de Souza. ***Performance variability in Self-regulation: influence of the Val^{158}Met COMT polymorphism, performance stability, and related outcomes*** *[*Variabilidade de desempenho em funções de Autorregulação: influência do polimorfismo Val^{158}Met do gene *COMT*, Estabilidade e Desfechos associados]. Belo Horizonte, 2017. Tese (Doutorado em Medicina Molecular) - Faculdade de Medicina, Universidade Federal de Minas Gerais, 2017.

Self-regulation allows people to make plans, choose from alternatives, control impulses, inhibit unwanted thoughts, and regulate appetitive behavior. To study factors associated with self-regulation performance variability and related outcomes is critical for advancing in understanding one of the most adaptive human functions. Here, we sought to investigate the association of the Val^{158}Met COMT polymorphism with two cognitive aspects of self-regulation. We also investigated behavioral features of self-regulation and biological and academic outcomes. Four papers were presented. 1) We investigated the association between the catechol-O-methyltransferase (COMT) Val158Met polymorphism and working memory and arithmetic performance in 50 Neurofibromatosis type I (NF1) individuals. A significant association of the COMT polymorphism was observed with verbal working memory with an advantageous performance for Met/Met carriers. 2) We investigated the influence of the COMT Val158Met polymorphism on Iowa Gambling Task (IGT) performance depending on sex in a healthy adult sample. Participants were 192 healthy adults (84 men and 108 women). The results revealed a sex-dependent effect of COMT Va l158Met polymorphism on decision-making as measured by the IGT. Val/Val women showed the best performance in the last trials of the IGT. 3) We evaluated the telomere length (TL) of 61 ADHD children and their parents. We found general heritability to be the major mechanism explaining interindividual TL variation in ADHD, and hyperactive-impulsive dimension of ADHD was related with children's TL. TL was shown to be a potential biomarker of the ADHD symptoms burden in families affected by this neurodevelopmental disorder. 4) Four distinct developmental groups regarding Executive Functions from 8 to 18 years of age were described in 180 children born extremely preterm. Academic performance in adolescence was related to EF stability, with those with typical and remitting EF profiles showing better outcomes. In conclusion, there was evidence of the self-regulation complexity, emphasizing the adaptive role of impulsive control through cognitive and affective-motivational mechanisms.

Keywords: Self-regulation, Executive Functions, Working Memory, Affective Decision-making, Val^{158}Met *COMT* polymorphism, Sex, Telomere, Academics, Longitudinal stability, Neurofibromatosis type I, Attention Deficit/Hyperactive Disorder, Preterm

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

ADHD	Attention Deficit/Hyperactive Disorder
ADHD-C	ADHD combined subtype
ADHD-H	ADHD hyperactive subtype
ADHD-I	ADHD inattentive subtype
BRI	Behavioral Regulation Index
BRIEF	Behavior Rating Inventory of Executive Function
CCA	Córtex cingulado anterior
CCEB	Brazilian Criterion for Economic Classification
COMT	Catecol O-metiltransferase
COMT	Catechol-O-methyltransferase
CPF	Córtex pré-frontal
CPFvm	Córtex pré-frontal ventromedial
CT	Comprimento telomérico
DA	Dopamina
EF	Executive function
ELBW	Extremo baixo peso ao nascimento
ELBW	Extremely low birth weight
EP	Extremamente prematuros
EP	Extremely preterm
FEs	Funções Executivas
GDT	Game of Dice Task
HI	Hyperactivity-impulsivity
IGT	Iowa Gambling Task
MCI	Metacognition Index
Met	Metionina
NF1	Neurofibromatose tipo 1
NF1	Neurofibromatosis type 1
NIH	National Institutes of Health
PFC	Prefrontal córtex
SES	Socioeconomic status
T/S	Telomere repeat length (T) to a single-copy rerence gene (36B4) (S) ratio
TDAH	Transtorno de déficit de atenção/ hiperatividade
TL	Telomere length
Val	Valina
VIBeS	Victorian Infant Brain Studies
WRAT4	Wide Range Achievement Test: Fourth Edition

SUMÁRIO

1 - INTRODUÇÃO

Os seres humanos possuem uma necessidade fundamental de pertencimento que encoraja comportamentos consistentes com ser um bom membro do grupo e desencoraja comportamentos que levariam à exclusão do grupo (Heatherton, 2011). Isso provavelmente se daria por pressões evolutivas, visto que muitos dos desafios adaptativos de nossos ancestrais seriam sociais por natureza (p.ex., distinguir amigos de inimigos, identificar e avaliar possíveis parceiros, entender a estrutura e a natureza de bandos e seus papéis sociais). Ser um bom membro do grupo, contudo, não é uma tarefa fácil. Existe um conflito inerente entre o que é prazeroso no nível individual (regra hedonista) e o que é melhor para o grupo, o que faz da capacidade de autorregulação um componente essencial do convívio social (Heatherton, 2011).

Autorregulação seria o processo pelo qual as pessoas mudam ou controlam seus pensamentos, sentimentos ou ações de modo a satisfazer objetivos ou padrões pessoais e sociais, permitindo-as fazer planos, controlar seus impulsos, escolher entre alternativas, inibir pensamentos indesejados e regular o comportamento instintual (Heatherton, 2011). Embora seja uma habilidade impressionante dos seres humanos, falhas na autorregulação são muito comuns e contribuem para inúmeros problemas sociais incluindo obesidade, predação sexual, vício e outros (Kelley, Wagner, & Heatherton, 2015). Essas falhas aumentam diante da exposição a estímulos tentadores (p.ex., comida, drogas), humor negativo ou estresse emocional e social, e depleção de sistemas de autorregulação (exaustão) (Kelley, Wagner, & Heatherton, 2015). A autorregulação, centralmente, envolve um equilíbrio crítico entre a força de um impulso e a capacidade do indivíduo de inibir um comportamento desejado. Sem autorregulação as pessoas poderiam ser impulsivas, descontroladas emocionalmente, explodir a menor das provocações, falar qualquer coisa que passasse pela cabeça e fazer qualquer coisa que parecesse prazeroso de imediato. Muito do que sabemos hoje sobre essa extraordinária capacidade adaptativa vem dos modelos e estudos de caso neuropsicológicos das funções executivas (Wagner, Demos, & Heatherton, 2011).

As funções executivas (FEs) são um conjunto de habilidades que controlam o pensamento e o comportamento orientado a objetivos, envolvendo domínios necessários ao controle consciente ou por esforço sobre nossos instintos ou comportamentos automáticos (Diamond, 2013). O interesse científico sobre as FEs ultrapassa mais de um século, mas apenas recentemente uma distinção teórica e empírica surgiu dentro desse domínio cognitivo geral, as funções executivas quentes e frias (Peterson, & Welsh, 2014; Zelazo & Muller, 2002). As FEs frias se referem ao conjunto de habilidades que permitem o comportamento orientado a objetivos e ao futuro como planejamento, inibição, flexibilidade, memória operacional e monitoramento, as quais se manifestam em condições de testagem de forma relativamente descontextualizada, não emocional e analítica (Miyake et al., 2000; Stuss & Benson, 1984; Welsh & Pennington, 1988). Em contraste, as FEs quentes seriam um conjunto de habilidades que permitem o comportamento orientado a objetivos e ao futuro eliciados em contextos emocionais, motivacionais e de tensão entre a gratificação imediata e recompensas de longo-prazo (Zelazo & Muller, 2002; Zelazo et al., 2005). Essa distinção, portanto, surge de um alvo crescente em neurociências: a interconexão entre cognição e emoção na saúde mental e funcionamento adaptativo do indivíduo em desenvolvimento (Peterson, & Welsh, 2014).

O conhecimento das FEs foi fortemente influenciado pelas observações clínicas e neuropsicológicas de indivíduos com lesões corticais frontais, principalmente em tempos de guerra (Welsh et al., 2006). O que se destacava para os teóricos da época da revolução cognitiva (p.ex., Tueber, 1964; Luria, 1973; Norman & Shallice, 1986), contudo, não era a distinção entre os aspectos cognitivos e emocionais da autorregulação, mas a determinação de aspectos que eram preservados e aspectos funcionais irrevogavelmente danificados com foco nas

funções analíticas ou deliberativas, frias (Peterson, & Welsh, 2014). Até hoje, por exemplo, discutimos a validade de modelos unitários e multifacetados das FEs frias, os quais, com alta frequência, dependem dos testes neuropsicológicos usados para suporte de um ou outro (Peterson, & Welsh, 2014). Um dos modelos mais influentes foi sumarizado nos estudos de Miyake e colaboradores (2000) com uma amostra de adultos, no qual ele observa estatisticamente a unicidade (correlação entre os fatores) e a diversidade (três fatores específicos: memória operacional, inibição e flexibilidade) intrínseca das FEs frias. Evidências sobre o desenvolvimento dessas funções também dependeriam do tipo de medida empregada. Rudimentos das FEs surgiriam precocemente (perto do primeiro ano de vida) (Diamond, 1990), mas seu desenvolvimento se tornaria mesmo evidente entre os anos pré-escolares e os anos iniciais da idade escolar (muito evidente entre os 5 e os 8 anos de idade) (Carlson & Moses, 2001; Zelazo, Muller, Frye, & Marcovitch, 2003). Mesmo assim, aspectos mais complexos das FEs não amadureceriam até a adolescência ou mesmo até o início da vida adulta (Best, & Miller, 2010). A avaliação do desenvolvimento das FEs usando medidas que podem ser empregadas sem modificações substanciais ao longo de grandes intervalos de tempo pode ser útil no esclarecimento da estabilidade e das mudanças dessas funções no ciclo vital (Peterson, & Welsh, 2014). Outra crítica imposta a esses modelos é a modesta associação de medidas laboratoriais ou do desempenho em testes propriamente com funções da "vida real" (Peterson, & Welsh, 2014). Em uma revisão sobre o assunto, Diamond (2013) busca sintetizar um modelo relativamente consensual, descrevendo seu desenvolvimento no ciclo vital e os principais métodos disponíveis para sua avaliação. Novamente, é proposta a existência de ao menos três processos cognitivos associados às FEs: memória operacional, controle inibitório (incluindo aspectos comportamentais, cognitivos e atencionais) e flexibilidade cognitiva (ou flexibilidade mental). Ela propõe que essas funções se integrariam num nível mais básico para dar suporte a aspectos mais complexos do funcionamento executivo, incluindo a capacidade de planejamento, raciocínio e solução de problemas. Juntas, essas funções estariam em alguma medida associada a diversos aspectos funcionais incluindo saúde mental (p.ex., alterações observadas nos transtornos de abuso de substância, déficit de atenção/hiperatividade, de conduta, depressivo, obsessivo compulsivo, esquizofrenia etc), saúde física (p.ex., obesidade, adesão a tratamento), qualidade de vida, prontidão para a aprendizagem, desempenho em matemática e lectoescrita, produtividade no trabalho e empregabilidade, harmonia conjugal e segurança pública (p.ex., taxa de criminalidade, comportamento de risco, violência, explosões emocionais etc) (Diamond, 2013).

As FEs frias também são um importante componente da capacidade do ser humano de tomar decisões, a qual envolve ainda os aspectos quentes das FEs. Tomada de decisão se refere aos processos envolvidos na escolha de uma entre várias alternativas das quais se esperaria consequências diferentes (Lee, 2013). A capacidade de tomar decisões surge de uma combinação de processos intuitivos (emocionais ou quentes) e analíticos (deliberativos ou frios) (Evans, 2003), bem como do contexto homeostático de cada pessoa (Paulus, 2007). A capacidade de tomada de decisão ao longo do desenvolvimento é um aspecto importante do funcionamento social adaptativo (Garon, & Moore, 2004). As escolhas que fazemos no dia-a-dia (e suas consequências) dependem de fatores complexos incluindo maximização de ganhos subjetivos (*utilidade* econômica), equilíbrio entre oferta e demanda, aprendizagem ou experiência (condicionamento por reforço, atitudes e memórias de decisões anteriores, assim como suas consequências) e leis de controle ótimo (custo funcional), por exemplo (Lee, 2013). Hoje sabemos que nossas ações são escolhidas a partir de múltiplos sistemas cerebrais coordenados: cada sistema implementa uma sequência diferente e finita de instruções neurais bem definidas (algoritmos). Como consequência, alterações no processo de tomada de decisão são frequentemente observadas em diversos transtornos neurológicos e psiquiátricos (Lee, 2013).

A retomada do interesse pelos aspectos emocionais ou "quentes" da autorregulação e da tomada de decisão se dá de forma mais aparente após os estudos de Damasio e colaboradores (Damasio, 1994) com pacientes com lesões ventromediais no córtex pré-frontal (CPF), demonstrando preservação de funções complexas como inteligência em detrimento de graves prejuízos sociais. As regiões orbitofrontal e ventromedial do CPF são intensamente conectadas a áreas límbicas associadas ao processamento emocional e social (Bechara, 2004; Beer et al., 2006). O estudo sistemático de pacientes com danos orbitais e ventromediais com preservação da região dorsolateral do córtex pré-frontal (associada a FEs frias) levou ao suporte da noção de que a tomada de decisão e o comportamento orientado a objetivos não poderia ser inteiramente explicado por fatores cognitivos ou "frios". Nesta época, o grupo criou um paradigma de testagem da tomada de decisão chamado Iowa Gambling Task (IGT; Bechara et al., 1994). Nele, os participantes precisam lidar com ganhos e perdas monetárias probabilísticas escolhendo entre quatro baralhos. Consideremos, por um momento, apenas o aspecto "utilidade/valor subjetivo" na tomada de decisão, ou seja, a força da preferência individual por uma opção particular. Sabe-se que a mesma seria influenciada por fatores como a magnitude ou o valor em jogo de dada escolha, o tempo de espera (imediato ou não) e o esforço envolvido, além da probabilidade de uma consequência esperada ser realmente alcançada (ganhos e perdas incertas) (Williams, & Taylor, 2004). O aspecto probabilístico é central no IGT e diz sobre a tomada de decisão num contexto de incerteza, pois não se sabe se a recompensa de fato será ganha. O contexto de incerteza ou probabilístico guarda ainda no teste dois outros aspectos: decisões sob ambiguidade, em que não conhecemos a probabilidade dos desfechos esperados, e decisões sob risco, em que a probabilidade dos desfechos associados a cada escolha é sabido (Kahneman, & Tversky, 1979). Os pacientes com as lesões estudadas apresentavam um perfil de escolhas chamadas desvantajosas ou arriscadas no IGT, com uma tendência a escolher cartas dos montes com maiores ganhos e perdas, em detrimento de cartas dos montes com menor ganhos e perdas, mas que gerariam maior ganho final (em longo prazo). Observando esses achados, os pesquisadores desenvolveram uma hipótese do marcador somático: os circuitos que envolvem o CPF ventromedial estariam envolvidos na associação de respostas ou estados somáticos (corporais) com várias situações, levando a decisões futuras enviesadas pela experiência prévia. Esses estados somáticos são chamados afetivos e estariam associados ao desenvolvimento do comportamento moral e a tendência a emitir comportamentos de risco (Peterson, & Welsh, 2014). A entrada das neurociências como método de estudo da tomada de decisão somou fortemente às teorias que argumentavam sobre a falha de modelos da tomada de decisão e autorregulação baseados na premissa da superioridade do comportamento racional, consistente e lógico na tomada de decisão (i.e., independente das contingências da escolha), visto que grande parte das decisões humanas não parecem necessariamente ótimas de uma ótica racional (De Martino et al., 2006; Kahneman, & Frederick, 2007).

O córtex pré-frontal (CPF) estaria particularmente envolvido na capacidade de autorregulação (Kelley, Wagner, & Heatherton, 2015). Comparado ao de outros animais, o CPF humano é desproporcionalmente maior, possui uma conectividade aumentada via sustância branca e leva mais tempo para amadurecer (Rilling 2006; Sakaiet al. 2011; Schoenemann et al. 2005). Para a autorregularão, três sub-regiões do CPF parecem importantes: o CPF ventromedial (CPFvm), o CPF lateral (incluindo as convexidades dorsal e ventral) e o córtex cingulado anterior (CCA). O CPFvm, incluindo o córtex orbitofrontal, compartilha conexões recíprocas com estruturas límbicas subcorticais como a amígdala e regiões associadas ao processamento de recompensas como o estriado ventral, sendo associado a regulação emocional e a autorregulação social e apetitiva (Fehr & Camerer 2007, Hare et al. 2009, Lin et al. 2012; Quirk & Beer 2006). O CPF lateral está conectado a regiões motoras secundárias, núcleos da base, CCA e CPFvm (McDonald et al. 1996; Nambu 2008; Petrides & Pandya 1999). Além de estar

associado a funções de linguagem, o CPF lateral está relacionado com FEs frias centrais como a memória operacional e inibição (Curtis & D'Esposito 2003; Garavan et al. 1999; Smith & Jonides 1999), sendo essencial para funções complexas necessárias a uma autorregulação bem-sucedida (Cohen & Lieberman 2010). Pacientes com lesões no CPF lateral têm dificuldade para planejar, coordenar e manter objetivos complexos, falham em adaptar o comportamento frente a mudanças de objetivos ou circunstâncias e têm problema para filtrar estímulos irrelevantes (Petrides & Milner 1982, Shallice 1982, Shallice & Burgess 1996). Já o CCA parece estar fortemente associado com controle cognitivo e monitoramento de conflitos cognitivos (Bush et al. 2000). Entre os neurotransmissores, a dopamina (DA) é conhecida por ter um papel funcional importante em funções cognitivas complexas como a memória operacional e no controle cognitivo, mas os mecanismos dessa influência não são tão bem compreendidos, visto que o efeito dopaminérgico varia muito entre as funções avaliadas e de pessoa para pessoa, de modo geral (Cools, & D'Esposito, 2011). As catecolaminas, incluindo a DA, são cruciais para o funcionamento de funções associadas ao CPF, mas apresenta um papel paradoxal dependendo de um equilíbrio complexo entre a capacidade de atualização flexível e, por outro lado, estabilização cognitiva (Cools, & D'Esposito, 2011). A relação entre desempenho cognitivo e DA não é linear e provavelmente segue a forma de um U-invertido, significando que tanto o excesso quanto níveis insuficientes de DA se associam a prejuízos funcionais. Além disso, o efeito da DA depende da região de concentração com nível aumentado de DA no corpo estriado associado a melhora da capacidade de atualização flexível, mas no CPF atuando na estabilização cognitiva (estabiliza e sustenta representações ativas na memória protegendo-as de distratores) (Cools, & D'Esposito, 2011). Não fosse o bastante, a atividade dopaminérgica em uma estrutura (p.ex., CPF) induz mudanças adaptativas na atividade dopaminérgica em outra estrutura (p.ex., corpo estriado) (Cools, 2008). Falhas na autorregulação incluindo impulsividade e inflexibilidade está associada, por exemplo, a alterações em processos motivacionais que implicam o estriado ventral (com destaque para o núcleo accumbens) e a porção ventromedial do CPF, à qual é fortemente conectado (Cools, 2008). Já outra parte do circuito frontoestriatal que conecta a parte dorsal do corpo estriado (núcleo caudado e putâmen) com o CPF dorsolateral teria uma influência distinta na autorregulação, provavelmente incluindo depleção da capacidade deliberativa de autorregulação. Simplificando, o efeito mais evidente parece ser o de níveis aumentados de DA no CPF associado a melhora de funções frias, em detrimento de pior desempenho em funções quentes e vice-versa.

As dificuldades na compreensão da variabilidade intra e interindividual dos efeitos dopaminérgicos na cognição aumenta quando consideramos outros fatores como o nível basal de DA. Por exemplo, há evidências de que hormônios sexuais como o estrogênio aumenta a atividade da DA aumentando a síntese, liberação e turnover da DA, bem como modificando as taxas de disparo de neurônios DA via receptores de membrana de estrogênio, enfatizando o sexo como um fator de variabilidade interindividual da ação da DA (Becker, 2000). A DA basal pode variar, também, de acordo com diferenças genéticas individuais. O gene *COMT*, codificador da enzima catecol O-metiltransferase (COMT), é um dos mais investigados entre os genes que influenciam funções mediadas pela DA como as FEs (Dickinson & Elvevag, 2009). A COMT media a degradação de catecolaminas (dopamina, norepinefrina e epinefrina), sendo um mecanismo fundamental ao fluxo dopaminérgico no córtex pré-frontal (CPF; Chen et al., 2004). O polimorfismo funcional mais estudado do gene é a variante Val^{158}Met (rs4680). Neste polimorfismo, ocorre a substituição de uma adenina por uma guanina no códon 158 do gene que codifica a COMT resultando em 2 alelos: o alelo VAL (valina) e o alelo MET (metionina). O alelo VAL codifica 3 vezes mais a enzima COMT e tem sido associado a uma diminuição da atividade dopaminérgica (Barnet, Jones, Robbins & Muller, 2007; Chen et al., 2004). Enquanto o alelo Met favoreceria o desempenho em tarefas de FEs frias, o alelo

Val favoreceria o desempenho em paradigmas afetivos da tomada de decisão (Mier, Kirsch, & Meyer-Lindenberg, 2010). Para esse gene, a complexidade não para por aí, visto que ele poderia contribuir ainda para dimorfismos sexuais no funcionamento do cérebro (Harrison, & Tumbridge, 2008).

Entender os fatores associados a variabilidade de desempenho em funções de autorregulação e suas consequências, portanto, é crítico para o avanço na compreensão dessa que está entre as maiores habilidades adaptativas de uma espécie eminentemente social (Heatherton, 2011; Kelley, Wagner, & Heatherton, 2015). Enquanto uma capacidade pobre de autorregulação colocaria as pessoas sob risco para vários problemas de saúde e de relacionamentos interpessoais, aqueles que conseguem controlar melhor seus comportamentos demonstram melhor saúde mental e menor risco de engajamento em comportamento sexual de risco e problemas de adição (Duckworth & Seligman 2005; Quinn & Fromme 2010; Tangney et al. 2004). Nesta tese, abordamos fatores associados a variabilidade em funções de autorregulação e possíveis desfechos, em quatro trabalhos individuais.

Num dos trabalhos, investigamos a associação da variante Val^{158}Met do gene *COMT* com o desempenho em tarefas medindo memória operacional numa amostra de participantes com Neurofibromatose tipo 1 (NF1). Além disso, verificamos se diferenças de desempenho na memória operacional estaria associada ao desempenho em aritmética dos participantes. Esta é uma população particularmente interessante para o estudo de fenótipos cognitivos e comportamentais complexos (Shilyansky, Lee & Silva, 2010). A neurofibromatose tipo 1 (NF1) é uma doença genética autossômica dominante multissistêmica com prevalência estimada de 1 em 3500 (Kayl & Moore, 2000). Indivíduos afetados são heterozigotos para a mutação do gene tumor supressor *NF1* (Friedman, 1999), localizado no braço longo do cromossoma 17 (17q11.2). O diagnóstico é baseado em critérios clínicos e o prognóstico da doença ainda é relativamente imprevisível com a possibilidade de complicações que afetem vários órgãos, sendo altamente variável quanto à severidade (Huson, Compston, Clark & Harper, 1989).

Mesmo sendo uma doença de gene único, a NF1 apresenta um quadro complexo de déficits cognitivo-comportamentais. Apesar da neurofibromina, produto do gene *NF1*, se expressar em diversos sistemas encefálicos (Gutmann, Geist, Wright & Snider, 1995) e interagir com uma série de reguladores da proteína RAS, tendo potencial para desempenhar múltiplos papéis na sinalização intracelular, a drástica variabilidade na expressão dos sintomas na NF1 (Clements-Stephens, Rimrodt, Gaur & Cutting, 2008; Dilts et al., 1996; Hyman, Shores & North, 2005; Hyman & Shores, 2006) parece ser em grande parte determinada por modificadores genéticos herdados independentemente (Easton, Ponder, Huson & Ponder, 1993, Sabbagh et al. 2009). Em modelos animais, por exemplo, alguns estudos apontam que o efeito fenotípico da mutação em NF1 depende de outros fatores genéticos previamente presentes à mutação, sugerindo ainda que a força da relação entre o gene modificador e o fenótipo correspondente é aumentada na presença dessa mutação (Costa et al., 2002; Hawes, Tuskan & Reilly, 2007; Shilyansky, Lee, & Silva, 2010). Assim, a identificação de loci específicos que codificam modificadores que alteram a expressão fenotípica na NF1 tem grande potencial no sentido de esclarecer o espectro de severidade entre seus diversos grupos de sintomas. Isso evitaria também que características devido à variação genética propriamente dita fossem equivocadamente atribuídas a uma determinada mutação.

A despeito do foco inicial nos sintomas somáticos da NF1 (manchas café-com-leite, nódulos de Lisch, neurofibromas etc.), diversas pesquisas têm investigado o fenótipo comportamental da doença relatando uma frequente ocorrência de transtornos de aprendizagem (30%-60%) e comportamentais como o Transtorno de déficit de atenção/ hiperatividade (TDAH) (30%-50) nesta população (Hyman, Shores & North, 2005; Hyman & Shores, 2006; Shilyansky, Lee & Silva, 2010). Os déficits cognitivos subjacentes a esses transtornos têm sido considerados a maior fonte de limitação nas atividades de vida diária de crianças e adolescentes portadores da NF1(North *et al*.,

1997), ainda assim, são escassos os estudos que visam elucidar a natureza desses sintomas (Shilyansky, Lee & Silva, 2010). Déficits nas FEs frias são prevalentes e podem ser graves na NF1. Quando subdomínios específicos são testados em baterias neuropsicológicas a taxa de indivíduos afetados é elevada (63%) (Hyman, Shores & North, 2005). Os déficits executivos mais proeminentes podem ser vistos em relação à memória de trabalho, flexibilidade cognitiva e controle inibitório (Rowbotham, Pit-tem, Sonuga-Barke & Hujibregts, 2009). Por hora, não se encontraram estudos publicados a respeito da relação entre polimorfismos e fenótipos cognitivos ou comportamentais em humanos portadores de NF1. A investigação da existência de genes modificadores que alterem a expressão dos diversos sintomas que caracterizariam a NF1 não apenas permite conclusões mais precisas em relação ao fenótipo estritamente relacionado ao gene *NF1* como também poderá acrescentar informações a respeito da influência de variações genotípicas comuns na capacidade de autorregulação, em populações complexas.

Ainda investigando a influência da variante Val^{158}Met (rs4680) do gene *COMT* em funções de autorregulação, verificamos a associação da mesma com o desempenho na tomada de decisão incerta como medida pelo IGT, em adultos hígidos. Contudo, incluímos o fator sexo no estudo, observando se uma possível associação não dependeria dessa variável. Como discutido acima, o sexo pode ser um dos fatores contribuindo para a variabilidade de desempenho em funções de autorregulação com o gene *COMT* podendo influenciar essas diferenças (Harrison, & Tumbridge, 2008). Todavia, não encontramos nenhum estudo investigando essa associação do gene *COMT* com o desempenho em uma das tarefas mais clássicas na investigação da capacidade de tomada de decisão afetiva, o IGT, dependendo do sexo. Diferenças sexuais no comportamento humano demonstram papeis adaptativos complementares: homens podem apresentar maior capacidade em habilidades motoras e espaciais, além de proclividade para agressão física, enquanto mulheres teriam maior capacidade de memória verbalmente mediada e cognição social o que se reflete na força de padrões diferenciados de conectividade encefálica (Ingalhalikar et al., 2014). Mesmo assim, sexo ainda é uma variável surpreendentemente negligenciada em neurociências (Shansky, & Woolley, 2016).

Caminhando para a avaliação de possíveis desfechos associados a variabilidade em funções de autorregulação, trabalhamos com outras duas populações com perfis cognitivos e comportamentais complexos. Num dos trabalhos, investigamos um aspecto novo na literatura do Transtorno de Déficit de Atenção/Hiperatividade (TDAH): a investigação da associação das dimensões comportamentais do TDAH com o comprimento telomérico de crianças com TDAH e de seus pais. O TDAH é um dos transtornos do neurodesenvolvimento mais comuns na infância, acometendo mais de 5% da população infantil mundial (Polanczyk et al., 2007). O transtorno se caracteriza por sintomas marcantes de desatenção-desorganização, hiperatividade e impulsividade, configurando entre os principais transtornos da autorregulação (incluindo funções executivas e seus componentes frios e quentes) (Nigg, 2005; Willcutt et al., 2012). A etiologia do TDAH é complexa e ainda existem muitas lacunas no conhecimento sobre os mecanismos específicos que conectam genótipos, processos neurais e sintomas cognitivos e comportamentais (Purper-Ouakil et al., 2011). O TDAH representa um grupo de alta vulnerabilidade genética com vários genes explicando uma pequena parte da variância de seus sintomas (Gizer, Ficks, & Waldman, 2009). A literatura recente dá suporte, particularmente, ao papel de genes que codificam receptores dopaminérgicos (DRD4, DRD5) e serotoninérgicos (HTR1B), transportadores de dopamina (DAT -SLC6A3) e proteínas envolvidas na regulação da liberação de neurotransmissores (SNAP25) na etiologia do transtorno (Faraone, & Mick, 2010). Essas crianças com elevado risco para comportamentos externalizantes são mais sensíveis aos efeitos de uma educação parental hiper-reativa do que outras crianças, por

exemplo (Lipscomb et al., 2014). A interação mais negativa sobre a criança parece ser eliciada pelos problemas externalizantes herdados (Marceau et. al., 2013). Crianças com TDAH, usualmente, falam demais, são mais distraídas, demandantes, emburradas e pouco cooperativas o que é mais desafiador para os pais e os tornam mais estressados (van Steijn et al., 2014). O estresse parental, por sua vez, contribui para a adoção de estratégias parentais negativas como o autoritarismo, bem como para problemas disruptivos nas crianças e implementação ineficiente de intervenções (Harpin 2005; Hastings 2002). As consequências poderiam se manifestar em diferentes níveis, por exemplo, no encurtamento telomérico.

Entre os marcadores biológicos que seriam regulados genética e epigeneticamente está o telômero (Blasco, 2007), um marcador associado com estresse psicológico e exposição precoce a adversidades ambientais (Drury et al., 2011; Mitchell et. al., 2014). Os telômeros são complexos DNA-proteína encontrados nas extremidades dos cromossomos lineares, que os protegem da degradação, da recombinação e da fusão robertsoniana, estabilizando-os. Devido à observação de que seu tamanho regride ao longo das duplicações celulares até um tamanho mínimo que interrompe a proliferação celular, criou-se a hipótese de que o telômero funcionaria como um relógio celular. O encurtamento progressivo dos telômeros durante a replicação celular estaria ligado a patofisiologia de diversas doenças humanas, não apenas devido à perda da estabilidade cromossômica, senescência e apoptose, mas ainda devido a alterações de expressão gênica dentro da região subtelomérica (Blasco, 2007). Do ponto de vista da interação gene-ambiente no desenvolvimento humano, o telômero tem sido apontado como biomarcador do efeito de experiências adversas e de menor potencial psicológico para lidar com a adversidade (Drury et al., 2012; Zalli et al., 2014). Em função dessas associações, o comprimento telomérico (CT) passou a ser investigado como um possível biomarcador do impacto biológico do ambiente em sujeitos com sintomas psiquiátricos.

Finalmente, investigamos o desempenho acadêmico em tarefas de escrita, leitura e matemática em adolescentes que nasceram extremamente prematuros (<28 semanas; EP) ou com extremo baixo peso (<1000g; ELBW) dependendo da estabilidade ou das mudanças de dificuldades em funções executivas comportamentais dos 8 aos 18 anos de idade. Esta foi uma oportunidade para a avaliação de diferenças na estabilidade de perfis variados de desempenho em FEs usando exatamente a mesma medida em 10 anos de intervalo, algo raro no estudo do desenvolvimento das FEs (Peterson, & Welsh, 2014). Em geral, o grau de prematuridade é inversamente associado a desfechos cognitivos e comportamentais com indivíduos nascidos EP/ELBW caracterizando o grupo de maior vulnerabilidade (Johnson, 2007). Em estudos usando medidas comportamentais das FEs como o inventário *Behavior Rating Inventory of Executive Function (BRIEF)*, por exemplo, a população prematura parece apresentar mais problemas comparativamente à população controle (Anderson et al., 2004, 2011). Um pior desempenho acadêmico também já foi documentado para esta população (Litt et al., 2012; Lee et al., 2011; Saigal et al., 2000). Embora as FEs estejam fortemente associadas a desfechos acadêmicos (Diamond, 2013), até o momento, nenhum estudo havia investigado a frequência do perfil de estabilidade e mudanças em dificuldades executivas comportamentais e seus desfechos acadêmicos, num estudo longitudinal.

Portanto, nesta tese abordamos aspectos que nos ajudam a avançar no entendimento de alguns aspectos associados a variabilidade, estabilidade e desfechos dessa extraordinária capacidade adaptativa humana: a autorregulação.

2 - OBJETIVOS

Objetivo Geral

Investigar a associação do polimorfismo Val^{158}Met do gene *COMT* com dois aspectos cognitivos da autorregulação, bem como verificar a estabilidade e a associação de características comportamentais da autorregulação com variáveis biológicas e acadêmicas.

Objetivos específicos

- Investigar se há associação entre o polimorfismo Val^{158}Met do gene *COMT* com o desempenho em tarefas de memória operacional (manutenção e manipulação verbal e visuoespacial) e sua relação com o desempenho aritmético, em participantes com NF1;

- Investigar a associação entre o polimorfismo Val^{158}Met do gene *COMT* com o desempenho em duas dimensões de uma tarefa de tomada de decisão sob incerteza (ambiguidade e risco) dependendo do sexo, em participantes hígidos adultos;

- Investigar a associação entre o comprimento telomérico (CT) de pais e filhos numa amostra de crianças e adolescentes com TDAH; suas relações com dimensões comportamentais do TDAH e fatores sociodemográficos;

- Avaliar a estabilidade de duas dimensões de funções executivas comportamentais (regulação comportamental e metacognição) e seus desfechos acadêmicos, dos 8 aos 18 anos, numa amostra de adolescentes nascidos extremamente prematuros (<28 semanas).

3 - MÉTODOS

Esta tese foi estruturada em torno de quatro artigos científicos que compõem a sessão resultados. Optou-se por essa estrutura, pois parte do material que compõe a tese já se encontra publicado em periódicos científicos e as metodologias empregadas são específicas de cada estudo.

- Costa, D. S., de Paula, J. J., Alvim-Soares Jr, A. M., Pereira, P. A., Malloy-Diniz, L. F., Rodrigues, L. O., ... & de Miranda, D. M. (2016). COMT Val158Met Polymorphism Is Associated with Verbal Working Memory in Neurofibromatosis Type 1. *Frontiers in Human Neuroscience*, 10.

A Neurofibromatose tipo 1 (NF1) é uma doença genética autossômica dominante multissistêmica. Mesmo sendo uma doença de gene único, a NF1 apresenta um quadro complexo de déficits cognitivo-comportamentais incluindo déficits nas funções executivas que são prevalentes e podem ser graves na NF1. Entre as hipóteses para

a drástica variabilidade na expressão do fenótipo da NF1 está aquela que sugere que grande parte dessa variabilidade seria determinada por modificadores genéticos herdados independentemente. A hipótese foi testada para outras características da NF1, mas nunca para os aspectos cognitivos. Mesmo com uma amostra pequena (n=50) para testar esse tipo de associação, observamos um efeito moderado do gene *COMT* (Val^{158}Met) na capacidade de manipulação verbal da memória operacional dos participantes. O estudo aponta para o uso da abordagem de gene candidato como uma possível ferramenta para reduzir a imprevisibilidade dos perfis cognitivos na NF1 no nível individual e reforça a influência dessa variante do gene *COMT* na memória operacional.

> Costa, D. S., Bechara, A., de Paula, J. J., Romano-Silva, M. A., Correa, H., Lage, G. M., ... & Malloy-Diniz, L. F. (2016). Influence of COMT Val158Met polymorphism on emotional decision-making: A sex-dependent relationship? *Psychiatry Research*, 246, 650-655.

Neste trabalho, testamos se o sexo teria um papel moderador numa possível associação entre o gene *COMT* (variante Val^{158}Met) e a tomada de decisão sob incerteza. O estudo consistiu numa nova análise dos dados de 192 adultos hígidos coletados pelo pesquisador Guilherme Lage e equipe, professor na UFMG. Neste trabalho, apontamos para a interação sexo**COMT* Val^{158}Met como uma fonte de variabilidade na tomada de decisão sob risco.

- Costa, D. S., Rosa, D. V. F., Barros, A. G. A., Romano-Silva, M. A., Malloy-Diniz, L. F., Mattos, P., & de Miranda, D. M. (2015). Telomere length is highly inherited and associated with hyperactivity-impulsivity in children with attention deficit/hyperactivity disorder. *Frontiers in molecular neuroscience*, 8.

O artigo foi o primeiro publicado na literatura investigando a associação de sintomas das dimensões comportamentais do TDAH com comprimento telomérico (CT), além de determinar a variância explicada do CT das crianças e adolescentes investigados pelo CT de seus pais. Este foi um trabalho realizado em parceria com pesquisadores da Universidade Federal do Rio de Janeiro (UFRJ). Dele participaram 61 crianças e adolescentes e seus pais. O telômero parece ser um marcador promissor da reatividade ao estresse ou de como cada indivíduo traduz eventos de vida em impacto biológico. Contudo, muitos estudos na área são transversais e associam o impacto de determinadas doenças à redução do CT. Aqui, ressaltamos que essa conclusão não deveria ser apenas baseada na comparação entre grupos, visto que diversos fatores ambientais afetariam o CT numa geração que, por sua vez, em grande medida influenciaria o CT de sua prole. Ainda, encontramos que o CT de crianças e adolescentes com TDAH poderia também estar inversamente associado com a intensidade dos sintomas de hiperatividade-impulsividade e diretamente com à escolaridade de suas mães. Esse estudo é frágil em vários aspectos, mas encoraja a investigação do CT como um marcador do impacto do TDAH, um transtorno eminentemente da autorregulação, no nível individual e familiar.

- Costa, D. S., Miranda, D. M., Burnett, A. C., Doyle, L. W., Cheong, J. L. Y., Anderson, P. J.; Victorian Infant Collaborative Study Group. (2017). Executive Function and Academic Outcomes in Children Who Were Extremely Preterm. *Pediatrics.* 140(3):e20170257.

Este trabalho foi produto dos nove meses que estive com a equipe do *Victorian Infant Brain Studies (VIBeS)*, no *Murdoch Children's Research Institute*, em Melboune, na Austrália, em 2016. Esta foi uma das experiências mais enriquecedoras do doutorado. Meu orientador, prof. Peter Anderson, havia colocado como um dos objetivos do estágio sanduíche com sua equipe a produção de artigos científicos. O desenho longitudinal dos projetos do VIBeS e as várias coortes de pesquisa em andamento nos abriu a oportunidade de trabalhar com diversos aspectos complexos do desenvolvimento de crianças e adolescentes nascidos prematuros. Depois de meses de discussão, desenhamos o objetivo desse artigo que levava em conta a variabilidade do perfil executivo de prematuros extremos. Trabalhamos com uma amostra geográfica de Victoria com 180 participantes que tiveram seu desempenho executivo medido pelo mesmo instrumento com 10 anos de intervalo, em média, buscando verificar a frequência das trajetórias possíveis e o desfecho acadêmico associado. Uma discussão ainda corrente na literatura com prematuros seria se os déficits cognitivos associados à prematuridade configurariam um déficit genuíno ou um atraso no desenvolvimento com posterior normalização. Este estudo evidencia que ambas as hipóteses são observadas, no caso das funções executivas, e que os desfechos associados são diferentes dependendo da capacidade avaliada. Mais interessante, contudo, é verificar que a maioria dos participantes tiveram trajetórias estáveis e favoráveis.

4 – RESULTADOS

4.1 ARTIGO 1: Anexo 1

Costa, D. S., de Paula, J. J., Alvim-Soares Jr, A. M., Pereira, P. A., Malloy-Diniz, L. F., Rodrigues, L. O., ... & de Miranda, D. M. (2016). COMT Val158Met Polymorphism Is Associated with Verbal Working Memory in Neurofibromatosis Type 1. Frontiers in Human Neuroscience, 10.

doi: 10.3389/fnhum.2016.00334

4.2 ARTIGO 2: Artigo suprimido da tese online por copyright.

Costa, D. S., Bechara, A., de Paula, J. J., Romano-Silva, M. A., Correa, H., Lage, G. M., ... & Malloy-Diniz, L. F. (2016). Influence of COMT Val158Met polymorphism on emotional decision-making: A sex-dependent relationship? Psychiatry Research, 246, 650-655.

doi: 10.1016/j.psychres.2016.10.073

4.3 ARTIGO 3: Anexo 2

Costa, D. S., Rosa, D. V. F., Barros, A. G. A., Romano-Silva, M. A., Malloy-Diniz, L. F., Mattos, P., & de Miranda, D. M. (2015). Telomere length is highly inherited and associated with hyperactivity-impulsivity in children with attention deficit/hyperactivity disorder. Frontiers in Molecular Neuroscience, 8.

doi: 10.3389/fnmol.2015.00028

4.4 ARTIGO 4: Artigo suprimido da tese online por copyright.

Costa, D. S., Miranda, D. M., Burnett, A. C., Doyle, L. W., Cheong, J. L. Y., Anderson, P. J.; Victorian Infant Collaborative Study Group. (2017). Executive Function and Academic Outcomes in Children Who Were Extremely Preterm. Pediatrics. 140(3):e20170257.

doi: 10.1542/peds.2017-0257.

5. CONSIDERAÇÕES FINAIS

Através de quatro artigos científicos, investigamos a associação do polimorfismo Val^{158}Met do gene *COMT* com dois aspectos cognitivos da autorregulação, memória operacional e tomada de decisão afetiva, em populações diferentes. Observamos também a associação das dimensões comportamentais do TDAH, um transtorno da autorregulação, com o comprimento do telômero de crianças com TDAH e de seus pais. Finalmente, observamos o impacto de diferentes trajetórias desenvolvimentais de funções executivas comportamentais no desempenho acadêmico de adolescentes nascidos extremamente prematuros.

No primeiro artigo, somamos ao corpo de evidência que sugere que o desempenho em tarefas de memória operacional, uma função executiva fria, parece influenciado pelo gene *COMT* (p.ex., Aguilera et al., 2008; Bruder et al., 2005). Em relação ao polimorfismo Val^{158}Met, observamos que participantes com NF1 com o genótipo Met/Met apresentou vantagem na capacidade de manipulação de informações verbais na memória de curto-prazo. Cada vez mais, a NF1 tem sido reconhecida como um transtorno do neurodesenvolvimento conferindo risco para diversos problemas cognitivos incluindo rebaixamento da capacidade intelectual, déficits executivos, prejuízos no processamento visuoespacial e atrasos motores; além disso, é uma condição com taxas excepcionalmente elevadas de TDAH, transtornos de aprendizagem, transtorno do espectro autista e outras condições psiquiátricas (Vogel, Gutmann, & Morris, 2017). A elevada variabilidade dos perfis individuais, contudo, é uma barreira para a proposição de intervenções específicas. Neste estudo, sugerimos que a variabilidade de desempenho numa função complexa como a memória operacional, conhecida por atenuar desfechos pouco adaptativos, principalmente na performance acadêmica, pode começar a ser desvelada na NF1 a partir do estudo de genes candidatos associados a funções cognitivas em populações não afetadas pela NF1.

Aqui também demonstramos a influência do polimorfismo Val^{158}Met do gene *COMT* sobre a tomada de decisão afetiva como medida pelo IGT, uma função executiva quente. Estudos prévios, incluindo do nosso grupo de pesquisa, já haviam ressaltado que o genótipo da *COMT* teria um papel na variabilidade de desempenho nos processos cognitivos avaliados pelo IGT (Malloy-Diniz et al., 2013). O que fizemos foi questionar se esses efeitos poderiam ser moderados pelo sexo e vimos que, em nossa amostra, sim. Entre os homens adultos investigados, o genótipo não trouxe diferenças significativas de desempenho entre os grupos. Já entre as mulheres, aquelas com o genótipo Val/Val apresentaram melhor desempenho na fase de tomada de decisão sob risco do IGT. Ainda não se sabe, contudo, quais são os mecanismos que subjazem essa interação *COMT* e sexo em funções cerebrais. É surpreendente como sexo é uma variável frequentemente desconsiderada em neurociências (Shansky, & Woolley, 2016). Muito do que sabemos em neurociências básica, por exemplo, advém de estudos com animais do sexo masculino (Beery, & Zucker; 2011; Mogil, & Chanda, 2005; Yoon et al., 2014). Na tentativa de amenizar esse viés, nos Estados Unidos, pesquisadores que buscarem financiamento do *National Institutes of Health (NIH)* para estudar animais vertebrados devem deixar explícito em seus projetos como considerarão o sexo como uma variável biológica (Shansky, & Woolley, 2016). Diferenças de sexo em aspectos cognitivos das funções de autorregulação são pouco estudados, mas alguns estudos indicam vantagem de desempenho para o sexo feminino em funções como inibição e postergação da gratificação *(*Hansen, 2011; Yuan et al., 2008).

Em relação ao terceiro artigo, certamente o aspecto mais consistente é o da alta herdabilidade do comprimento telomérico (CT). Mas, aqui, focaremos na associação do CT com a dimensão hiperativa-impulsiva do TDAH. É interessante notar que, de todos os déficits cognitivos associados ao TDAH (i.e., atenção sustentada e vigilância, funções executivas frias e quentes, processamento temporal etc) a que parece mais especificamente

associada à dimensão hiperativa-impulsiva no TDAH seria a capacidade de postergação da gratificação ou desconto temporal (Willcutt et al., 2012). Há evidências de que um telômero mais curto estaria associado a menor capacidade de postergação ou a maiores descontos temporais (Kang et al., 2017; Yim et al., 2016). Portanto, apesar do foco inicial da relação do CT com eventos e transtornos mentais mais diretamente associados ao estresse, é possível que funções de autorregulação como as funções executivas atuem aumentando a capacidade individual para lidar com situações estressantes (Johnson, 2012) e, assim, tenha impacto no CT.

Finalmente, verificamos como a estabilidade ou as mudanças em funções executivas comportamentais estaria associada ao desempenho escolar em adolescentes prematuros. Indivíduos nascidos extremamente prematuros (EP) consistem num grupo de risco para déficits em diversos aspectos do neurodesenvolvimento (Anderson et al., 2003). Contudo, a variabilidade de desempenho intragrupo é enorme o que não é contemplado na maior parte dos estudos, levando a um foco exagerado em prejuízos em detrimento da investigação dos fatores que poderiam influenciar o funcionamento ótimo do ser humano (Pluess, & Belsky, 2013). Neste estudo, também tivemos a chance de empregar a mesma medida de FEs num intervalo de 10 anos. Vimos que grande parte de adolescentes EP mantém trajetórias estáveis (previsíveis) de FEs ao longo dos anos investigados. Dentre aqueles que saem da faixa de risco quanto ao desempenho em funções relacionadas à regulação comportamental (incluindo controle emocional), os desfechos acadêmicos parecem similares (ou até melhores!) aos daqueles que já tinham um desempenho típico na idade escolar. Contudo, o desempenho acadêmico, principalmente em matemática, daqueles que tinham dificuldades metacognitivas (similares aquelas observadas no TDAH) na idade escolar, são piores, mesmo quando essas dificuldades são amenizadas com o passar do tempo. Portanto, é possível que o impacto precoce sobre funções metacognitivas tenham um impacto mais duradouro do que aquelas relacionadas ao controle emocional.

Nesta tese, identificamos evidências da associação do polimorfismo Val^{158}Met do gene *COMT* com dois aspectos cognitivos da autorregulação. Sugerimos que o comprimento telomérico pode ser um potencial candidato na mensuração de como recursos de autorregulação amenizariam o impacto de eventos estressantes num nível biológico. Além disso, vimos que diferentes trajetórias desenvolvimentais nas FEs comportamentais estariam associadas a desfechos acadêmicos distintos. As limitações especificas desses estudos foram abordadas em cada artigo. Aqui, buscamos oferecer evidências da complexidade do estudo de funções de autorregulação enfatizando o papel adaptativo das mesmas numa espécie em que o controle dos impulsos parece essencial no suprimento de uma necessidade humana inata: a necessidade de pertencer ao grupo.

6. REFERÊNCIAS

Aguilera, M., Barrantes-Vidal, N., Arias, B., Moya, J., Villa, H., Ibanez, M. I., ... & Fananas, L. (2008). Putative role of the COMT gene polymorphism (Val158Met) on verbal working memory functioning in a healthy population. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 147(6), 898-902.

Anderson, P. J., & Doyle, L. W. (2004). Executive functioning in school-aged children who were born very preterm or with extremely low birth weight in the 1990s. Pediatrics, 114(1), 50-57.

Anderson, P. J., De Luca, C. R., Hutchinson, E., Spencer-Smith, M. M., Roberts, G., Doyle, L. W., & Victorian Infant Collaborative Study Group*. (2011). Attention problems in a representative sample of extremely preterm/extremely low birth weight children. Developmental neuropsychology, 36(1), 57-73.

Anderson, P., Doyle, L. W., & Victorian Infant Collaborative Study Group. (2003). Neurobehavioral outcomes of school-age children born extremely low birth weight or very preterm in the 1990s. JAMA, 289(24), 3264-3272.

Barnett JH, Jones PB, Robbins TW, Muller U. (2007). Effects of the catechol-O-methyltransferase Val^{158}Met polymorphism on executive function: a meta-analysis of the Wisconsin Card Sort Test in schizophrenia and healthy controls. Molecular Psychiatry, 12, 502-509.

Bechara, A. (2004). The role of emotion in decision-making: evidence from neurological patients with orbitofrontal damage. Brain and cognition, 55(1), 30-40.

Becker, J. B. (2000). Oestrogen effects on dopaminergic function in striatum. Neuronal and Cognitive Effects of Oestrogens, 230, 134-145.

Beer, J. S., John, O. P., Scabini, D., & Knight, R. T. (2006). Orbitofrontal cortex and social behavior: integrating self-monitoring and emotion-cognition interactions. Journal of cognitive neuroscience, 18(6), 871-879.

Beery, A. K., & Zucker, I. (2011). Sex bias in neuroscience and biomedical research. Neuroscience & Biobehavioral Reviews, 35(3), 565-572.

Blasco, M. A. (2007). The epigenetic regulation of mammalian telomeres. Nature reviews. Genetics, 8(4), 299.

Bruder, G. E., Keilp, J. G., Xu, H., Shikhman, M., Schori, E., Gorman, J. M., & Gilliam, T. C. (2005). Catechol-O-methyltransferase (COMT) genotypes and working memory: associations with differing cognitive operations. Biological psychiatry, 58(11), 901-907.

Bush, G., Luu, P., & Posner, M. I. (2000). Cognitive and emotional influences in anterior cingulate cortex. Trends in cognitive sciences, 4(6), 215-222.

Chen, J., Lipska, B. K., Halim, N., Ma, Q. D., Matsumoto, M., Melhem, S., ... & Egan, M. F. (2004). Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. The American Journal of Human Genetics, 75(5), 807-821.

Clements-Stephens AM, Rimrodt SL, Gaur P, Cutting LE. (2008). Visuospatial processing in children with neurofibromatosis type 1. *Neuropsychologia*, 46 (2), 690-697.

Cohen, J. R., & Lieberman, M. D. (2010). The common neural basis of exerting self-control in multiple domains. Self control in society, mind, and brain, 1, 141-162.

Cools, R. (2008). Role of dopamine in the motivational and cognitive control of behavior. The Neuroscientist, 14(4), 381-395.

Cools, R., & D'Esposito, M. (2011). Inverted-U shaped dopamine actions on human working memory and cognitive control. Biological Psychiatry, 69(12), e113–e125.

Costa, R. M., Federov, N. B., Kogan, J. H., Murphy, G. G., Stern, J., Ohno, M., ... & Silva, A. J. (2002). Mechanism for the learning deficits in a mouse model of neurofibromatosis type 1. nature, 415(6871), 526-530.

Curtis, C. E., & D'Esposito, M. (2003). Persistent activity in the prefrontal cortex during working memory. Trends in cognitive sciences, 7(9), 415-423.

Dickinson, D., & Elvevåg, B. (2009). Genes, cognition and brain through a COMT lens. Neuroscience, 164(1), 72-87.

Dilts, C. V., Carey, J. C., Kircher, J. C., Hoffman, R. O., Creel, D., Ward, K., ... & Leonard, C. O. (1996). Children and adolescents with neurofibromatosis 1: a behavioral phenotype. Journal of developmental & behavioral pediatrics, 17(4), 229-239.

Drury, S. S., Theall, K., Gleason, M. M., Smyke, A. T., De Vivo, I., Wong, J. Y. Y., ... & Nelson, C. A. (2012). Telomere length and early severe social deprivation: linking early adversity and cellular aging. Molecular psychiatry, 17(7), 719.

Duckworth, A. L., & Seligman, M. E. (2005). Self-discipline outdoes IQ in predicting academic performance of adolescents. Psychological science, 16(12), 939-944.

Easton DF, Ponder MA, Huson SM, Ponder BA. (1993). An analysis of variation in expression of neurofibromatosis (NF) type 1 (NF1): evidence for modifying genes. *American Journal of Human Genetics*, 53, 305–13.

Faraone, S.V., & Mick, E. (2010). Molecular Genetics of Attention Deficit Hyperactivity Disorder. Psychiatric Clinics of North America,33 (1),159-180.

Fehr, E., & Camerer, C. F. (2007). Social neuroeconomics: the neural circuitry of social preferences. Trends in cognitive sciences, 11(10), 419-427.

Friedman, J. M. (1999). Epidemiology of neurofibromatosis type 1. American Journal of Medical Genetics Part A, 89(1), 1-6.

Garavan, H., Ross, T. J., & Stein, E. A. (1999). Right hemispheric dominance of inhibitory control: an event-related functional MRI study. Proceedings of the National Academy of Sciences, 96(14), 8301-8306.

Garon, N., & Moore, C. (2004). Complex decision-making in early childhood. Brain and Cognition, 55 1, 158-70.

Gizer, I. R., Ficks, C., & Waldman, I. D. (2009). Candidate gene studies of ADHD: a meta-analytic review. Human genetics, 126(1), 51-90.

Gutmann, D. H., Geist, R. T., Wright, D. E., & Snider, W. D. (1995). Expression of the neurofibromatosis 1 (NF1) isoforms in developing and adult rat tissues. Cell Growth and Differentiation-Publication American Association for Cancer Research, 6(3), 315-324.

Hansen, S. (2011). Inhibitory control and empathy-related personality traits: Sex-linked associations. Brain and cognition, 76(3), 364-368.

Hare, T. A., Camerer, C. F., & Rangel, A. (2009). Self-control in decision-making involves modulation of the vmPFC valuation system. Science, 324(5927), 646-648.

Harpin, V. A. (2005). The effect of ADHD on the life of an individual, their family, and community from preschool to adult life. Archives of Disease in Childhood, 90, i2

Harrison, P. J., & Tunbridge, E. M. (2008). Catechol-O-methyltransferase (COMT): a gene contributing to sex differences in brain function, and to sexual dimorphism in the predisposition to psychiatric disorders. Neuropsychopharmacology, 33(13), 3037.

Hastings, R. P. (2002). Parental stress and behaviour problems of children with developmental disability. Journal of Intellectual and Developmental Disability, 27(3), 149-160.

Hawes JJ, Tuskan RG, Reilly KM. (2007). Nf1 expression is dependent o n s train background: implications for tumor suppressor haploinsufficiency studies. *Neurogenetics*, 8, 121-130.

Heatherton, T. F. (2011). Neuroscience of self and self-regulation. Annual review of psychology, 62, 363-390.

Huson, S. M., Compston, D. A., Clark, P., & Harper, P. S. (1989). A genetic study of von Recklinghausen neurofibromatosis in south east Wales. I. Prevalence, fitness, mutation rate, and effect of parental transmission on severity. Journal of medical genetics, 26(11), 704-711.

Hyman, S. L., Arthur, E., & North, K. N. (2006). Learning disabilities in children with neurofibromatosis type 1: subtypes, cognitive profile, and attention-deficit-hyperactivity disorder. Developmental Medicine & Child Neurology, 48(12), 973-977.

Hyman SL, Shores A, North KN. (2005). The nature and frequency of cognitive deficits in children with neurofibromatosis type 1. *Neurology*, 65, 1037–1044.

Johnson, S. (2007). Cognitive and behavioural outcomes following very preterm birth. In Seminars in Fetal and Neonatal Medicine, 12(5), 363-373). WB Saunders.

Kang, J. I., Hwang, S. S., Choi, J. R., Lee, S. T., Kim, J., Hwang, I. S., ... & Kim, S. J. (2017). Telomere length in alcohol dependence: A role for impulsive choice and childhood maltreatment. Psychoneuroendocrinology, 83, 72-78.

Kayl, A. E., & Moore, B. D. (2000). Behavioral phenotype of neurofibromatosis, type 1. Developmental Disabilities Research Reviews, 6(2), 117-124.

Kelley, W. M., Wagner, D. D., & Heatherton, T. F. (2015). In search of a human self-regulation system. Annual review of neuroscience, 38, 389-411.

Lee, D. (2013). Decision making: from neuroscience to psychiatry. Neuron, 78(2), 233-248.

Lee, E. S., Yeatman, J. D., Luna, B., & Feldman, H. M. (2011). Specific language and reading skills in school-aged children and adolescents are associated with prematurity after controlling for IQ. Neuropsychologia, 49(5), 906-913.

Lin, A., Adolphs, R., & Rangel, A. (2011). Social and monetary reward learning engage overlapping neural substrates. Social cognitive and affective neuroscience, 7(3), 274-281.

Litt, J. S., Gerry Taylor, H., Margevicius, S., Schluchter, M., Andreias, L., & Hack, M. (2012). Academic achievement of adolescents born with extremely low birth weight. Acta Paediatrica, 101(12), 1240-1245.

Luria, A. R. (1973). The working brain. Penguin Books Harmondsworth.

Malloy-Diniz, L. F., Lage, G. M., Campos, S. B., de Paula, J. J., de Souza Costa, D., Romano-Silva, M. A., ... & Correa, H. (2013). Association between the catechol O-methyltransferase (COMT) Val158met polymorphism and different dimensions of impulsivity. PLoS One, 8(9), e73509.

Marceau, K., Horwitz, B. N., Narusyte, J., Ganiban, J. M., Spotts, E. L., Reiss, D., & Neiderhiser, J. M. (2013). Gene–environment correlation underlying the association between parental negativity and adolescent externalizing problems. Child development, 84(6), 2031-2046.

McDonald, A. J., Mascagni, F., & Guo, L. (1996). Projections of the medial and lateral prefrontal cortices to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat. Neuroscience, 71(1), 55-75.

Mier, D., Kirsch, P., & Meyer-Lindenberg, A. (2010). Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. Molecular psychiatry, 15(9), 918.

Mitchell, C., Hobcraft, J., McLanahan, S. S., Siegel, S. R., Berg, A., Brooks-Gunn, J., ... & Notterman, D. (2014). Social disadvantage, genetic sensitivity, and children's telomere length. Proceedings of the National Academy of Sciences, 111(16), 5944-5949.

Miyake, A., Friedman, N. P., Emerson, M. J., Witzki, A. H., Howerter, A., & Wager, T. D. (2000). The unity and diversity of executive functions and their contributions to complex "frontal lobe" tasks: A latent variable analysis. Cognitive psychology, 41(1), 49-100.

Mogil, J. S., & Chanda, M. L. (2005). The case for the inclusion of female subjects in basic science studies of pain. Pain, 117(1-2), 1-5.

Nambu, A. (2008). Seven problems on the basal ganglia. Current opinion in neurobiology, 18(6), 595-604.

Nigg, J. T. (2005). Neuropsychologic theory and findings in attention-deficit/hyperactivity disorder: the state of the field and salient challenges for the coming decade. Biological psychiatry, 57(11), 1424-1435.

Norman, D. A., & Shallice, T. (1986). Attention to action. In Consciousness and self-regulation (pp. 1-18). Springer US.

Paulus, M. P. (2007). Decision-making dysfunctions in psychiatry—altered homeostatic processing?. Science, 318(5850), 602-606.

Peterson, E., & Welsh, M. C. (2014). The development of hot and cool executive functions in childhood and adolescence: Are we getting warmer?. In Handbook of executive functioning (pp. 45-65). Springer New York.

Petrides, M., & Milner, B. (1982). Deficits on subject-ordered tasks after frontal-and temporal-lobe lesions in man. Neuropsychologia, 20(3), 249-262.

Polanczyk, G., Lima, M.S., Horta, B.L., Biederman, J., & Rohde, L.A. (2007). The world-wide prevalence of ADHD: a systematic review and metaregression analysis. American Journal of J Psychiatry, 164(6), 942–948.

Purper-Ouakil, D., Ramoz, N., Lepagnol-Bestel, AM., Gorwood, P., & Simonneau, M. (2011). Neurobiology of Attention Deficit/Hyperactivity Disorder. Pediatric Research, 69 (5), 69R-76R.

Quinn, P. D., & Fromme, K. (2010). Self-regulation as a protective factor against risky drinking and sexual behavior. Psychology of Addictive Behaviors, 24(3), 376.

Quirk, G. J., & Beer, J. S. (2006). Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. Current opinion in neurobiology, 16(6), 723-727.

Rilling, J. K. (2006). Human and nonhuman primate brains: are they allometrically scaled versions of the same design?. Evolutionary Anthropology: Issues, News, and Reviews, 15(2), 65-77.

Rowbotham, I., Pit-ten Cate, I. M., Sonuga-Barke, E. J. S., & Huijbregts, S. C. J. (2009). Cognitive control in adolescents with neurofibromatosis type 1. Neuropsychology, 23(1), 50.

Sabbagh, A., Pasmant, E., Laurendeau, I., Parfait, B., Barbarot, S., Guillot, B., ... & Vidaud, D. (2009). Unravelling the genetic basis of variable clinical expression in neurofibromatosis 1. Human molecular genetics, 18(15), 2768-2778.

Saigal, S., Hoult, L. A., Streiner, D. L., Stoskopf, B. L., & Rosenbaum, P. L. (2000). School difficulties at adolescence in a regional cohort of children who were extremely low birth weight. Pediatrics, 105(2), 325-331.

Sakai, T., Mikami, A., Tomonaga, M., Matsui, M., Suzuki, J., Hamada, Y., ... & Matsuzawa, T. (2011). Differential prefrontal white matter development in chimpanzees and humans. Current Biology, 21(16), 1397-1402.

Schoenemann, P. T., Sheehan, M. J., & Glotzer, L. D. (2005). Prefrontal white matter volume is disproportionately larger in humans than in other primates. Nature neuroscience, 8(2).

Shallice, T. (1982). Specific impairments of planning. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 199-209.

Shallice, T. I. M., & Burgess, P. W. (1991). Deficits in strategy application following frontal lobe damage in man. Brain, 114(2), 727-741.

Shansky, R. M., & Woolley, C. S. (2016). Considering Sex as a Biological Variable Will Be Valuable for Neuroscience Research. The Journal of Neuroscience, 36(47), 11817–11822.

Shilyansky, C., Lee, Y. S., & Silva, A. J. (2010). Molecular and cellular mechanisms of learning disabilities: a focus on NF1. Annual review of neuroscience, 33, 221-243.

Smith, E. E., & Jonides, J. (1999). Storage and executive processes in the frontal lobes. Science, 283(5408), 1657-1661.

Stuss, D. T., & Benson, D. F. (1984). Neuropsychological studies of the frontal lobes. Psychological bulletin, 95(1), 3.

Tangney, J. P., Baumeister, R. F., & Boone, A. L. (2004). High self-control predicts good adjustment, less pathology, better grades, and interpersonal success. Journal of personality, 72(2), 271-324.

Teuber, H. L. (1964). The riddle of frontal lobe function in man. The frontal granular cortex and behavior, 410-444.

Van Steijn, D. J., Oerlemans, A. M., Van Aken, M. A., Buitelaar, J. K., & Rommelse, N. N. (2014). The reciprocal relationship of ASD, ADHD, depressive symptoms and stress in parents of children with ASD and/or ADHD. Journal of autism and developmental disorders, 44(5), 1064-1076.

Vogel, A. C., Gutmann, D. H., & Morris, S. M. (2017). Neurodevelopmental disorders in children with neurofibromatosis type 1. Developmental Medicine & Child Neurology.

Wagner, D. D., Demos, K. E., & Heatherton, T. F. (2011). Staying in control: the neural basis of self-regulation and its failure. The Oxford handbook of social neuroscience, 360-377.

Welsh, M. C., & Pennington, B. F. (1988). Assessing frontal lobe functioning in children: Views from developmental psychology. Developmental neuropsychology, 4(3), 199-230.

Welsh, M. C., Friedman, S. L., & Spieker, S. J. (2006). Executive Functions in Developing Children: Current Conceptualizations and Questions for the Future.

Willcutt, E. G., Nigg, J. T., Pennington, B. F., Solanto, M. V., Rohde, L. A., Tannock, R., ... & Lahey, B. B. (2012). Validity of DSM-IV attention deficit/hyperactivity disorder symptom dimensions and subtypes. Journal of Abnormal Psychology, 121(4), 991-1010.

Yim, O.-S., Zhang, X., Shalev, I., Monakhov, M., Zhong, S., Hsu, M., … Ebstein, R. P. (2016). Delay discounting, genetic sensitivity, and leukocyte telomere length. Proceedings of the National Academy of Sciences of the United States of America, 113(10), 2780–2785.

Yuan, J., He, Y., Qinglin, Z., Chen, A., & Li, H. (2008). Gender differences in behavioral inhibitory control: ERP evidence from a two-choice oddball task. Psychophysiology, 45(6), 986-993.

Zalli, A., Carvalho, L. A., Lin, J., Hamer, M., Erusalimsky, J. D., Blackburn, E. H., & Steptoe, A. (2014). Shorter telomeres with high telomerase activity are associated with raised allostatic load and impoverished psychosocial resources. Proceedings of the National Academy of Sciences, 111(12), 4519-4524.

Zelazo, P. D., & Müller, U. (2002). Executive function in typical and atypical development. The Wiley-Blackwell Handbook of Childhood Cognitive Development, First edition, 574-603.

Zelazo, P. D., Qu, L., Müller, U., & Schneider, W. (2005). Hot and cool aspects of executive function: Relations in early development. Young children's cognitive development: Interrelationships among executive functioning, working memory, verbal ability, and theory of mind, 71-93.

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COMT Val^{158}Met Polymorphism Is Associated with Verbal Working Memory in Neurofibromatosis Type 1

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Neurofibromatosis type I (NF1) is a neurogenetic disease marked by multiple cognitive and learning problems. Genetic variants may account for phenotypic variance in NF1. Here, we investigated the association between the catechol-*O*-methyltransferase (*COMT*) Val^{158}Met polymorphism and working memory and arithmetic performance in 50 NF1 individuals. A significant association of the *COMT* polymorphism was observed only with verbal working memory, as measured by the backward digit-span task with an advantageous performance for Met/Met carriers. To study how genetic modifiers influence NF1 cognitive performance might be of importance to decrease the unpredictability of the cognitive profile among NF1 patients.

Keywords: neurofibromatosis type I, *COMT* Val^{158}Met polymorphism, working memory, arithmetic, genetic modifiers, neuropsychology, executive functions

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INTRODUCTION

Neurofibromatosis type 1 (NF1) is a common neurogenetic disorder affecting 1 in each 3500 individuals (Friedman, 1999; Ferner, 2007). The *NF1* gene includes 63 exons and encodes a 220–250 kDa protein termed neurofibromin (Cawthon et al., 1990; Viskochil et al., 1990). NF1 is caused by mutations only in the *NF1* gene and have an autosomal dominant inheritance (Easton et al., 1993; Ward and Gutmann, 2005; Sabbagh et al., 2009). This single-gene disease is marked by cognitive, learning, and behavioral problems and is a potential model for the investigation of the biological mechanisms related to these complex phenotypes (Shilyansky et al., 2010).

Cognitive impairment and academic failure are the most common reported problems in the clinical care of NF1 individuals (Hyman et al., 2005). Executive function impairments impact overall academic achievement and quality of life with up to 80% of NF1 children experiencing moderate to severe deficits. NF1 affects planning, visuospatial processing, reading and vocabulary skills, and courses with an observed higher rate of attention-deficit/hyperactivity disorder and a mildly lower IQ score (Hachon et al., 2011; Lehtonen et al., 2013). However, there is a high variation among NF1 patients regarding the specific cognitive domain affected or the extension of the cognitive deficit (Lehtonen et al., 2013). In fact, NF1 phenotype varies from minimal to maximal presentation in all clinical characteristics, and cognitive and behavioral aspects are not an exception (Shilyansky et al., 2010).

Phenotype variability in NF1 is not easily explained. There are thousands of mutations described in the *NF1* gene with unsatisfactory genotype–phenotype associations (Pasmant et al., 2012). Even in the same family with multiple cases, a phenotypic variation of NF1 is present (Pasmant et al., 2012). It is possible that genetic variants also account for phenotypic variance in NF1 with the same mutation being modified concerning genotype–phenotype associations depending on different genetic backgrounds (Shilyansky et al., 2010).

Genetics has a significant influence on individual differences in cognitive function with dopamine-related polymorphisms among the most studied candidate genes (Savitz et al., 2006; Bellander et al., 2015). Dopamine level is essential for prefrontal function and cognition (Cools and Robbins, 2004), which is well documented for working memory and other aspects of cognitive control (Cools and D'Esposito, 2011). The catechol-*O*-methyltransferase (*COMT*) gene is the most investigated of the genes influencing dopamine-mediated functions (Dickinson and Elvevag, 2009). A commonly explored *COMT* variant, the Val^{158}Met (rs4680), consists in a 158Val (*G*) to Met (*A*) polymorphism that reduces the activity of the COMT enzyme leading to a higher extracellular dopamine level mostly in the prefrontal cortex (PFC; Chen et al., 2004; Dickinson and Elvevag, 2009). Met allele carriers and conditions with intermediary values in a U-shape distribution of the dopaminergic synaptic availability in the PFC generally are favored in measures of cognitive control (Mier et al., 2010), though this is still a matter of controversy.

Different cognitive subprocesses may be differentially affected by the *COMT* alleles (Barnett et al., 2008; Mier et al., 2010). An example of the differential effect of the COMT alleles is on working memory. Working memory involves processes of maintenance and updating of information. It is an important cognitive function and is closely related to executive functions (Diamond, 2013). Regarding the *COMT* influence on working memory, performance requiring maintenance seems to be favored by the Met allele while the Val allele may be advantageous in updating tasks (Bellander et al., 2015). Testing different components of working memory (i.e., simple retention of information, content or modalities of information, and active manipulation of information) studies have shown that only mental manipulation of information is sensitive to the *COMT* dopaminergic modulation with Met/Met participants showing the best performance (Bruder et al., 2005; Aguilera et al., 2008). There are also investigations showing no significant association between the *COMT* gene and cognitive measures. Recently, a study using a multi-task approach found no effect of the COMT genotype on performance at highly demanding working memory loads (Ihne et al., 2016). Searching for evidence of a COMT genotype effect on working memory-related activation, a meta-analytic imaging study identified expected regions, namely the right inferior parietal lobe and the right dorsolateral PFC, as showing the highest likelihood for activation in both healthy controls and schizophrenia patients, but the significance of these results did not survive correction for a whole-brain approach (Nickl-Jockschat et al., 2015). On the other hand, many individual studies were able to find an association between the *COMT* alleles and performance in working memory tasks with activation of areas of the prefrontal–parietal–striatal network (Tan et al., 2007; Stokes et al., 2011; Kondo et al., 2015). Still, the association of the *COMT* gene with working memory is one of the best replicated so far (Mier et al., 2010; Ihne et al., 2016).

Cognitive impairment in NF1 has significant consequences in daily life, including prominent deficits in school abilities, which may occur in 75% of NF1 patients (Krab et al., 2008). Impairment in working memory and executive functions is a common feature of NF1, and might be an underlying contribute factor for the impairment in academic abilities (Hyman et al., 2005; Krab et al., 2008; Rowbotham et al., 2009). Working memory is highly involved in academic skills including reading, writing, and arithmetic (Baddeley, 2003; Geary, 2011). As stated before, working memory is a dopamine-mediated function. Dopamine homeostasis contributes to learning, memory, and attention, however, the mechanisms by which NF1 modulates dopamine signaling is still unknown (Diggs-Andrews and Gutmann, 2013). Therefore, in a multilevel perspective, *COMT* genotype (neurobiological level) might modulate working memory (cognitive level) and reflects on low academic achievement (functional level). To date, we found no study investigating the association of this specific genetic polymorphism with cognitive performance in an NF1 population. In this study, we aim to unravel the association between the *COMT* genotype, working memory performance, and school achievement (using a basic arithmetic test) in a heterogeneous NF1 sample. The study has the potential to provide insight into the mechanisms underlying phenotypic variability in NF1.

METHODS AND PROCEDURES

Participants

Fifty participants with NF1 [19 subjects from 6- to 18-year-old (11.89 ± 4.11 years; 11 male) and 31 adults from 19- to 50-year-old (30.97 ± 8.81 years; 13 male)] were enrolled in this study. All individuals were recruited from a specialized clinic in neurofibromatosis at the Hospital of the Federal University of Minas Gerais. NF1 diagnosis followed the criteria specified by the National Institutes of Health statement [NIH] (1988) statement. Besides NF1, it was not reported by the participants or their families any history of genetic, neurological, or psychiatric disorders. This study is part of a research project that seeks to investigate molecular mechanisms of NF1 approved by the Federal University of Minas Gerais ethics committee. Written informed consent was obtained from all participants and/or from their parents according to the Declaration of Helsinki.

Working Memory Assessment

All participants completed the age-appropriate digit-span subtest of the Wechsler Adult and Children Intelligence Scales (Wechsler, 2002, 2004) and the Corsi block-tapping task (Kessels et al., 2000, 2008). Both are span tasks where the examiner presents a growing sequence of numbers (digit-span) or moves on a wooden board (Corsi block-tapping). The subject must

repeat the same sequence (*forward* versions of the tasks) or say/do it from the last to the first item (a *backward* version of the tasks). For each span (starting at two items), the examiner presented two different sequences. The tasks are stopped when the subject is not able to correctly repeat two sequences of same span length. We used the product of the maximum span length and number of correct trials as test measures (Kessels et al., 2008). This strategy usually produces more representative measures of working memory variability than the number of correct trials or the maximum span achieved.

IQ Assessment

General intellectual functioning was assessed by the third version of the Brazilian Wechsler Intelligence Scales (WAIS-III or WISC-III for adults and children, respectively; Wechsler, 2002, 2004).

School Performance Assessment

We adopted the arithmetic subtest from the School Achievement Test (Stein, 1994), as an objective measure of school performance. The School Achievement Test is a standard measure of academic skills including reading, writing, and arithmetic. The test was developed for the Brazilian population following the country educational agenda and have adequate normative data for grades 1–6. Participants' scores on arithmetic were categorized in low-achievement or normal-high-achievement according to the guidelines proposed by Oliveira-Ferreira et al. (2012) and the total years of formal education showed by each participant.

Socioeconomic Status Assessment

Socioeconomic status (SES) was assessed using the Brazilian Criterion for Economic Classification (CCEB) according to the criteria established by the Brazilian Research Enterprises Association (Associação Brasileira de Empresas de Pesquisa [ABEP], 2013). The CCEB estimates the purchasing power of families living in urban areas. It includes nine items that measure the available resources at home and one item that judges the education level of the householder, resulting in a scale ranging from 0 to 46 points, and segmentation into eight economic classes. These economic classes can be divided into three larger classes: "high" (A and B classes; median monthly household income from U$2349 to U$4152), "middle" (C class; median monthly household income from U$514 to U$1190) and "low" (D and E classes; median monthly household income of U$348). Eighteen NF1 participants (36%) were classified as high class, 27 (54%) as middle class, and five (10%) as low class.

COMT Genotyping

The polymorphism was assessed by a standard procedure previously reported (Pereira et al., 2012). Genomic DNA was extracted from blood samples using the high salt method (Lahiri and Nurnberger, 1991). The COMT functional polymorphism (val158met, *rs4680*) was purchased in a made-to-order from Applied Biosystems®. Genotyping was performed using a real-time PCR system in the allelic discrimination mode (Stratagene Mx3005 – MxPro QPCR-Software, 2007) using the TaqMan Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA). PCR parameters included an initial denaturation at 95°C for 10 min, followed by 50 cycles at 95°C for 15 s and 60°C for 1 min. Each reaction contained 3.5 μl of mix, 0.1 μl of the probe, 3.4 μl of deionized water, and 1.0 μl of DNA. Researchers involved in genotyping were blind to neuropsychological results, and researchers participating in neuropsychological assessments were blind to the genotyping results. COMT genotype was coded as a categorical variable (Val/Val, Met/Val, and Met/Met) for further analysis.

Statistical Procedures

Most of our data was non-normally distributed. The use of data transformation procedures (square, cube, square root, and logarithm) did not succeed in normalizing the data distribution. We then adopted non-parametric tests for the following procedures. Non-parametric univariate comparisons performed by the Kruskal–Wallis tests did not show differences between age ($\chi^2 = 3.21$, $p = 0.201$), years of formal education ($\chi^2 = 0.65$, $p = 0.721$), SES ($\chi^2 = 1.03$, $p = 0.596$), or intelligence ($\chi^2 = 1.83$, $p = 0.400$) between the genotype groups. In this sense, we compared the three *COMT* genotypes (Val/Val, Val/Met, Met/Met) in the digit-span and Corsi block-tapping tasks by the same statistical procedure. To ensure results' consistency, we analyzed the *p*-values along with the effect sizes ("*r*" conversion computed by dividing the resulting "*Z*" by the square root of the total sample size). This method can be interpreted as a correlational coefficient, and effect sizes higher than 0.3 can be considered moderate and larger than 0.5 interpreted as large according to Cohen's (1988) guidelines. *Post hoc* comparisons between each *COMT* genotype were corrected by the Dunn–Bonferroni method. The comparison between the two groups defined by the school achievement and the *COMT* genotype was performed by a chi-square test. A secondary analysis investigated the association between *COMT* genotype, IQ, and working memory with arithmetic's performance. We stratified the participants based on the School Achievement Test performance and used multinomial stepwise logistic regression models to assess whether low school achievement was associated with neurobiological and cognitive measures. All statistical procedures were performed in SPSS 20.0.

RESULTS

Demographic and cognitive characteristics of the participants are shown in **Table 1**. There were no differences between the genotype groups regarding sociodemographic features. No significant differences in performance according to *COMT* genotype were found for the digit-span forward ($\chi^2 = 1.06$, $p = 0.587$), Corsi block-tapping task forward ($\chi^2 = 4.29$, $p = 0.117$) and backward ($\chi^2 = 3.27$, $p = 0.195$). In the digit-span backward condition, significant group differences were found ($\chi^2 = 6.65$, $p = 0.036$). The Met/Met group outperformed the

TABLE 1 | Demographic and cognitive characteristics of the participants.

	Val/Val ($N = 16$)			Val/Met ($N = 23$)			Met/Met ($N = 11$)			KW[1]	
	Pc.25	Pc.50	Pc.75	Pc.25	Pc.50	Pc.75	Pc.25	Pc.50	Pc.75	χ^2	*p*
Age (years)	17	26	32	10	19	29	18	28	44	3.21	0.201
Formal education (years)	8	9	11	3	11	11	8	9	11	0.65	0.721
Socioeconomic status	16	21	26	15	19	25	16	20	33	1.03	0.596
Full scale IQ	89	95	103	77	94	106	89	98	106	1.73	0.400
Digit-span (forward)	22	31	47	18	24	35	20	33	35	1.06	0.587
Digit-span (backward)*	9	12	20	4	9	20	12	20	25	6.65	0.036
Corsi block-tapping (forward)	34	35	44	16	30	40	35	40	48	4.29	0.117
Corsi block-tapping (backward)	14	21	38	4	12	24	4	18	48	3.27	0.195

[1]*Kruskal–Wallis Test, Pc=Percentile, *significant at $p < 0.05$.*

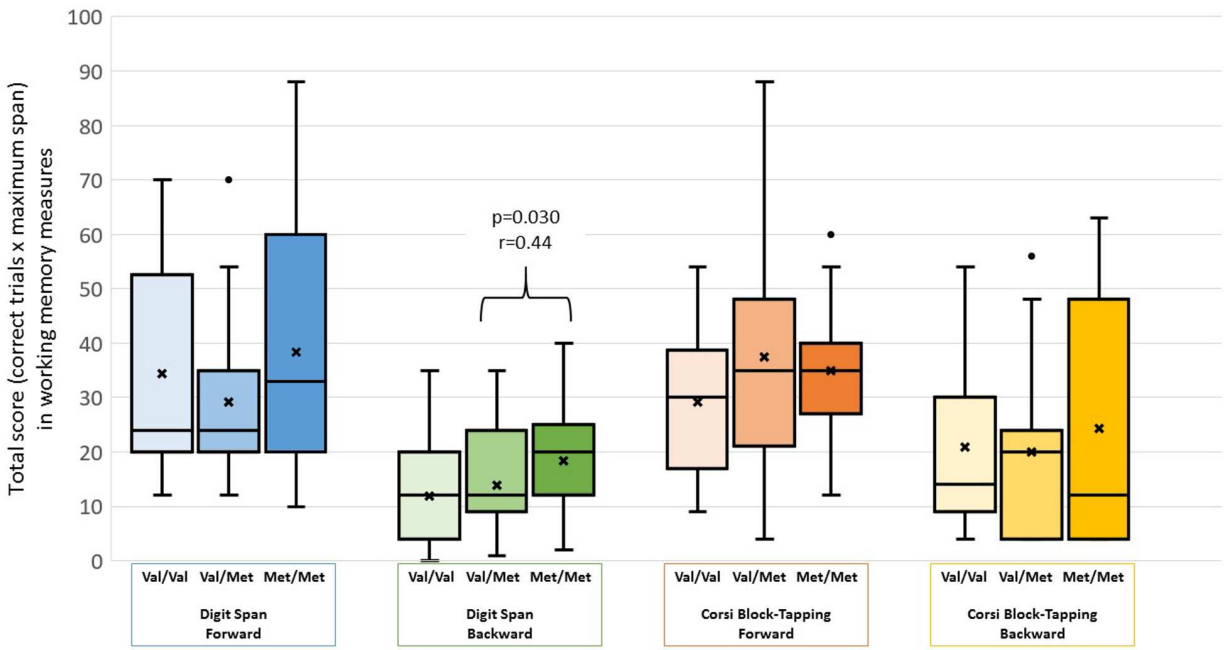

FIGURE 1 | Box-plots showing participants performance on working memory measures stratified by COMT genotype. We found no significant differences in the digit-span forward ($\chi^2 = 1.06$, $p = 0.587$), Corsi block-tapping task forward ($\chi^2 = 4.29$, $p = 0.117$) and backward ($\chi^2 = 3.27$, $p = 0.195$). In the digit-span backward condition, significant group differences were found ($\chi^2 = 6.65$, $p = 0.036$). The Met/Met group outperformed the Val/Met group ($Z = 2.58$, $p = 0.030$, $r = 0.44$) but not the Val/Val group ($Z = 0.87$, $p = 1.000$). There were no differences between the Val/Val and Val/Met groups ($Z = 1.69$, $p = 0.273$). "x" represents test means. The dots represent outlier observation points.

Val/Met group ($Z = 2.58$, $p = 0.030$, $r = 0.44$) but not the Val/Val group ($Z = 0.87$, $p = 0.999$). There was no difference in performance between the Val/Val and Val/Met groups ($Z = 1.69$, $p = 0.273$). Group differences are represented in **Figure 1**.

The school performance analysis showed that 40% of our sample had difficulties in basic arithmetic skills according to the cut-offs of the School Achievement Test (i.e., performance below the 25 percentile). However, we found no significant difference between low-achievement and normal-high-achievement groups regarding *COMT* genotypes distribution ($\chi^2 = 0.952$, $p = 0.621$). The final step of the backward logistic regression model was significant ($\chi^2 = 26.30$, df = 2, $p < 0.001$) and showed a moderate sensitivity (83%) and specificity (75%) for individual classification. IQ ($p = 0.007$) and working memory assessed by the backward digit-span task ($p = 0.020$) were directly associated with lower arithmetic performance, but not *COMT* genotype neither the remaining working memory measures.

DISCUSSION

Our preliminary results support an advantageous working memory performance in NF1 Met/Met carriers, which strengthens the hypothesis of genetic variants accounting for phenotypic variability in NF1. Considering the well-established

COMT polymorphism effect on working memory (Mier et al., 2010; Bellander et al., 2015), we add into this line of evidence showing a *COMT* Val^{158}Met genotype effect on cognitive control even in a sample of subjects with a monogenic disorder with compromising of behavior and cognition.

The COMT effect on working memory in our sample, however, was only observed for performance on the backward condition of the digit-span task. This result is in line with studies showing a COMT effect on measures demanding an active process of manipulation, but not on measures that only require maintenance of information (Bruder et al., 2005; Aguilera et al., 2008). Therefore, the lack of a COMT influence on the forward conditions of the working memory tasks that we observed is not without precedents. The absence of a COMT association with the backward version of the Corsi block-tapping task could lead us to hypothesize about a content-dependent (i.e., verbal vs. visuospatial) effect. Nevertheless, other studies have not shown such modality-dependent differences (Bruder et al., 2005; Aguilera et al., 2008; Ihne et al., 2016). Moreover, the backward Corsi block-tapping task have failed to demonstrate the same level of difficulty compared to the backward digit-span task and participants reach the same performance on both the forward and the backward versions of the Corsi block-tapping task (Mammarella and Cornoldi, 2005; Kessels et al., 2008). Thus, it seems more likely that our findings reflect a major COMT influence on measures demanding greater mental manipulation of information in working memory (Bruder et al., 2005).

In two different animal models of NF1, an inverted relation between the reduction of dopamine levels and the impairments of spatial learning were observed (Anastasaki et al., 2015) suggesting the importance of dopamine activity for NF1 cognition. It has been shown that neurofibromin modulates inhibitory networks in prefrontal and striatal regions, impacting working memory performance (Shilyansky et al., 2010), but our results suggest that variability in cognitive level expression between NF1 individuals may occur as a result of variability in their genetic background. It has been hypothesized that genetic modifiers could interact on a more functional level to exacerbate or compensate for the signaling changes caused by loss of *NF1* (Shilyansky et al., 2010). Future studies are needed to show whether NF1 may moderate known effects of other specific genetic polymorphisms on cognition.

Although we have found no direct effect of the COMT genotype on NF1 arithmetic performance, the backward digit-span was predictive of lower arithmetic performance, together with IQ, in our sample. It is important to emphasize that 40% of the subjects in this study were classified as showing difficulties in basic arithmetic abilities. Working memory is known to be important for numerical processing (González-Giraldo et al., 2014). Despite controversies regarding which allele would be advantageous to numerical abilities, the *COMT* Val^{158}Met has been associated with arithmetical functioning (Júlio-Costa et al., 2013; González-Giraldo et al., 2014) with dopamine playing a key role in updating new information at the neural systems level (Tan et al., 2007). It is possible that the lack of association at a functional level (academic performance) with the COMT polymorphism in our study reflects a bias of sample power, but future studies are needed to investigate the existence of a more direct effect of *COMT* on arithmetic in NF1.

To our knowledge, this is the first study finding associations of a polymorphism in the *COMT* gene with cognitive measures in NF1 participants. This result may have practical implications since it may add evidence to the usefulness of dopamine-targeted therapies for some NF1 individuals with executive impairments (Diggs-Andrews and Gutmann, 2013). For example, the pharmacological response of NF1 individuals to methylphenidate, a psychostimulant medication that increases extracellular dopamine availability in dopaminergic neurons, might results in improved attention and working memory and consequent better academic performance (Mautner et al., 2002; Lion-François et al., 2014). In this sense, the genetic background may be useful to determine the sensibility of specific groups to distinct therapy methods. Additionally, to study how genetic modifiers influence NF1 cognitive performance might be of importance to decrease the unpredictability of the cognitive profile among NF1 patients. In this context, we have to emphasize that it is a preliminary data in a small sample, but with some potential consequences. In conclusion, we found a preliminary data identifying modifier genes such as COMT polymorphism being associated with working memory performance in an NF1 sample.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: DS, JP, DM, and MR-S. Neuropsychological data collection and supervision: DS, JP, and LM-D. Clinical NF1 data collection and supervision: DM and LR. Genetic data analysis: AA-S and PP. Analyzed the data: DS and JP. Contributed reagents/materials/analysis tools: DM, LM-D, MR-S, and LR. Wrote the paper: DS, JP, AA-S, PP, LR, LM-D, MR-S, and DM.

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REFERENCES

Aguilera, M., Barrantes-Vidal, N., Arias, B., Moya, J., Villa, H., Ibáñez, M. I., et al. (2008). Putative role of the COMT gene polymorphism (Val158Met) on verbal working memory functioning in a healthy population. *Am. J. Med. Genet. Part B* 147B, 898–902. doi: 10.1002/ajmg.b.30705

Anastasaki, C., Woo, A. S., Messiaen, L. M., and Gutmann, D. H. (2015). Elucidating the impact of neurofibromatosis-1 germline mutations on neurofibromin function and dopamine-based learning. *Hum. Mol. Genet.* 24, 3518–3528. doi: 10.1093/hmg/ddv103

Associação Brasileira de Empresas de Pesquisa [ABEP] (2013). *Critério de Classificação Econômica Brasil*. Available at: http://www.abep.org/new/criterioBrasil.aspx

Baddeley, A. (2003). Working memory: looking back and looking forward. *Nat. Rev. Neurosci.* 4, 229–239. doi: 10.1038/nrn1201

Barnett, J. H., Scoriels, L., and Munafo, M. R. (2008). Meta-analysis of the cognitive effects of the catechol-O-methyltransferase gene val158/108met polymorphism. *Biol. Psychiatry* 64, 137–144. doi: 10.1016/j.biopsych.2008.01.005

Bellander, M., Bäckman, L., Liu, T., Schjeide, B. M., Bertram, L., Schmiedek, F., et al. (2015). Lower baseline performance but greater plasticity of working memory for carriers of the val allele of the COMT Val^{158}Met polymorphism. *Neuropsychology* 29, 247–254. doi: 10.1037/neu0000088

Bruder, G. E., Keilp, J. G., Xu, H., Shikhman, M., Schori, E., Gorman, J. M., et al. (2005). Catechol-O-methyltransferase (COMT) genotypes and working memory: associations with differing cognitive operations. *Biol. Psychiatry* 58, 901–907. doi: 10.1016/j.biopsych.2005.05.010

Cawthon, R. M., Weiss, M., Xu, G., Viskochil, D., Culver, M., Stevens, J., et al. (1990). A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure and point mutations. *Cell* 62, 193–201. doi: 10.1016/0092-8674(90)90253-B

Chen, J., Lipska, B. K., Halim, N., Ma, Q. D., Matsumoto, M., Melhem, S., et al. (2004). Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am. J. Hum. Genet.* 75, 807–821. doi: 10.1086/425589

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences,* 2 Edn. New York, NY: Lawrence Erlbaum Associates.

Cools, R., and D'Esposito, M. (2011). Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biol. Psychiatry* 69, e113–e125. doi: 10.1016/j.biopsych.2011.03.028

Cools, R., and Robbins, T. W. (2004). Chemistry of the adaptive mind. *Philos. Trans. A Math. Phys. Eng. Sci.* 362, 2871–2888. doi: 10.1098/rsta.2004.1468

Diamond, A. (2013). Executive functions. *Annu. Rev. Psychol.* 64, 135–168. doi: 10.1146/annurev-psych-113011-143750

Dickinson, D., and Elvevag, B. (2009). Genes, cognition and brain through a COMT lens. *Neuroscience* 164, 72–87. doi: 10.1016/j.neuroscience.2009.05.014

Diggs-Andrews, K. A., and Gutmann, D. H. (2013). Modeling cognitive dysfunction in neurofibromatosis-1. *Trends Neurosci.* 36, 237–247. doi: 10.1016/j.tins.2012.12.002

Easton, D. F., Pondert, M. A., Huson, S. M., and Ponder, B. A. J. (1993). An analysis of variation in expression of neurofibromatosis (NF) Type I (NF I): evidence for modifying genes. *Am. J. Hum. Genet.* 53, 305–313.

Ferner, R. E. (2007). Neurofibromatosis 1. *Eur. J. Hum. Gene.* 15, 131–138. doi: 10.1038/sj.ejhg.5201676

Friedman, J. M. (1999). Epidemiology of neurofibromatosis type. *Am. J. Med. Genet.* 89, 1–6. doi: 10.1002/(SICI)1096-8628(19990326)89:1<1::AID-AJMG3>3.0.CO;2-8

Geary, D. C. (2011). Cognitive predictors of achievement growth in mathematics: a five year longitudinal study. *Dev. Psychol.* 47, 1539–1552. doi: 10.1037/a0025510

González-Giraldo, Y., Rojas, J., Novoa, P., Mueller, S. T., Piper, B. J., Adan, A., et al. (2014). Functional polymorphisms in BDNF and COMT genes are associated with objective differences in arithmetical functioning in a sample of young adults. *Neuropsychobiology* 70, 152–157. doi: 10.1159/000366483

Hachon, C., Iannuzzi, S., and Chaix, Y. (2011). Behavioural and cognitive phenotypes in children with neurofibromatosis type 1 (NF1): the link with the neurobiological level. *Brain Dev.* 33, 52–61. doi: 10.1016/j.braindev.2009.12.008

Hyman, S. L., Shores, E. A., and North, K. N. (2005). The nature and frequency of cognitive deficits in children with neurofibromatosis type 1. *Neurology* 65, 1037–1044. doi: 10.1212/01.wnl.0000179303.72345.ce

Ihne, J. L., Gallagher, N. M., Sullivan, M., Callicott, J. H., and Green, A. E. (2016). Is less really more: does a prefrontal efficiency genotype actually confer better performance when working memory becomes difficult? *Cortex* 74, 79–95. doi: 10.1016/j.cortex.2015.10.025

Júlio-Costa, A., Antunes, A. M., Lopes-Silva, J. B., Moreira, B. C., Vianna, G. S., Wood, G., et al. (2013). Count on dopamine: influences of COMT polymorphisms on numerical cognition. *Front. Psychol.* 4:531. doi: 10.3389/fpsyg.2013.00531

Kessels, R. P., van den Berg, E., Ruis, C., and Brands, A. M. (2008). The backward span of the Corsi Block-Tapping Task and its association with the WAIS-III Digit Span. *Assessment* 15, 426–434. doi: 10.1177/1073191108315611

Kessels, R. P., Van Zandvoort, M. J., Postma, A., Kappelle, L. J., and De Haan, E. H. (2000). The Corsi block-tapping task: standardization and normative data. *App. Neuropsychol.* 7, 252–258. doi: 10.1207/S15324826AN0704_8

Kondo, H. M., Nomura, M., and Kashino, M. (2015). Different roles of COMT and HTR2A genotypes in working memory subprocesses. *PLoS ONE* 10:e0126511. doi: 10.1371/journal.pone.0126511

Krab, L. C., Aarsen, F. K., de Goede-Bolder, A., Catsman-Berrevoets, C. E., Arts, W. F., Moll, A. H., et al. (2008). Impact of neurofibromatosis type 1 on school performance. *J. Child Neurol.* 23, 1002–1010. doi: 10.1177/0883073808316366

Lahiri, D. K., and Nurnberger, J. I. Jr. (1991). A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res.* 19:5444. doi: 10.1093/nar/19.19.5444

Lehtonen, A., Howie, E., Trump, D., and Huson, S. M. (2013). Behaviour in children with neurofibromatosis type 1: cognition, executive function, attention, emotion, and social competence. *Dev. Med. Child Neurol.* 55, 111–125. doi: 10.1111/j.1469-8749.2012.04399.x

Lion-François, L., Gueyffier, F., Mercier, C., Gérard, D., Herbillon, V., Kemlin, I., et al. (2014). NF1 Rhône Alpes Auvergne-France. The effect of methylphenidate on neurofibromatosis type 1: a randomised, double-blind, placebo-controlled, crossover trial. *Orphanet J. Rare Dis.* 9:142. doi: 10.1186/s13023-014-0142-4

Mammarella, I. C., and Cornoldi, C. (2005). Sequence and space: the critical role of a backward spatial span in the working memory deficit of visuospatial learning disabled children. *Cogn. Neuropsychol.* 22, 1055–1068. doi: 10.1080/02643290442000509

Mautner, V. F., Kluwe, L., Thakker, S. D., and Leark, R. A. (2002). Treatment of ADHD in neurofibromatosis type 1. *Dev. Med. Child Neurol.* 44, 164–170. doi: 10.1111/j.1469-8749.2002.tb00780.x

Mier, D., Kirsch, P., and Meyer-Lindenberg, A. (2010). Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. *Mol. Psychiatry* 15, 918–927. doi: 10.1038/mp.2009.36

National Institutes of Health statement [NIH] (1988). Neurofibromatosis conference statement. National Institute of Health Consensus Development Conference. *Arch. Neurol.* 45, 576–578.

Nickl-Jockschat, T., Janouschek, H., Eickhoff, S. B., and Eickhoff, C. R. (2015). Lack of meta-analytic evidence for an impact of COMT Val158Met genotype on brain activation during working memory tasks. *Biol. Psychiatry* 78, e43–e46. doi: 10.1016/j.biopsych.2015.02.030

Oliveira-Ferreira, F., Costa, D. S., Micheli, L. R., Oliveira, L. F. S., Pinheiro-Chagas, P., and Haase, V. G. (2012). School achievement test: normative data for a representative sample of elementary school children. *Psychol. Neurosci.* 5, 157–164. doi: 10.3922/j.psns.2012.2.05

Pasmant, E., Vidaud, M., Vidaud, D., and Wolkenstein, P. (2012). Neurofibromatosis type 1: from genotype to phenotype. *J. Med. Genet.* 49, 483–489. doi: 10.1136/jmedgenet-2012-100978

Pereira, P. A., Bicalho, M. A., Nicolato, R., Malloy-Diniz, L. F., Romano-Silva, M. A., and Miranda, D. M. (2012). Catechol-O-Methyltransferase genetic variant and associated with risk of Alzheimer Disease in Brazilian Population. *Dement. Geriatr. Cogn. Disord.* 34, 90–95. doi: 10.1159/000341578

Rowbotham, I., Pit-ten Cate, I. M., Sonuga-Barke, E. J., and Huijbregts, S. C. (2009). Cognitive control in adolescents with neurofibromatosis type 1. *Neuropsychology* 23, 50–60. doi: 10.1037/a0013927

Sabbagh, A., Pasmant, E., Laurendeau, I., Parfait, B., Barbarot, S., Guillot, B., et al. (2009). Unravelling the genetic basis of variable clinical

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Telomere length is highly inherited and associated with hyperactivity-impulsivity in children with attention deficit/hyperactivity disorder

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Telomere length (TL) is highly heritable, and a shorter telomere at birth may increase the risk of age-related problems. Additionally, a shorter TL may represent a biomarker of chronic stress and has been associated with psychiatric disorders. However, no study has explored whether there is an association between TL and the symptoms of one of the most common neurodevelopmental disorders in childhood: Attention Deficit/Hyperactive Disorder (ADHD). We evaluated 61 (range, 6–16 years) ADHD children and their parents between 2012 and 2014. TL was measured with a quantitative polymerase chain reaction method with telomere signal normalized to the signal from a single copy gene (*36B4*) to generate a T/S ratio. Family data was processed through a generalized estimated equations (GEE) model to determine the effect of parental TL on children TL. Inattentive and hyperactive-impulsive symptoms were also evaluated in relation to TL. For the first time, we found general heritability to be the major mechanism explaining interindividual TL variation in ADHD (father-child: 95% CI = 0.35/0.91, $p < 0.001$; mother-child: 95% CI = 0.38/0.74, $p < 0.001$). The hyperactive-impulsive dimension of ADHD was related with children's TL ($r = -339$, $p = 0.008$) and maternal TL ($r = -264$, $p = 0.047$), but not with paternal TL ($p > 0.05$). The ADHD inattentive dimension was not significant associated with TL in this study ($p > 0.05$). TL was shown to be a potential biomarker of the ADHD symptoms burden in families affected by this neurodevelopmental disorder. However, it is crucial that future studies investigating the rate of telomere attrition in relation to psychiatric problems to consider the strong determination of TL at birth by inheritance.

Keywords: inattention, hyperactivity-impulsivity, ADHD, biological aging, telomere length, inheritance

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Introduction

Telomeres are DNA structures that protect a chromosome's end from deterioration or from fusion with neighboring chromosomes, maintaining genomic stability (Blackburn and Gall, 1978). Over time, due to each cell division, the telomere ends become shorter. Telomere shortening during life is modulated by factors such as oxidative stress and

adversity (Drury et al., 2012). However, telomere length (TL) at birth is highly determined by inheritance (De Meyer et al., 2011; Broer et al., 2013). Therefore, a shorter telomere at birth may increase the risk of age-related problems, and it is very important to know more about the mechanisms and consequences of TL inheritance (De Meyer et al., 2011).

TL is heritable (estimates ranging from 34 to 82%) with most of the shared variance among relatives resulting from genetic factors (Broer et al., 2013). The genetic mechanisms of telomere inheritance have been discussed in the literature, but the topic is still controversial. A study by Nawrot et al. (2004) suggested a maternal inheritance based on the X-link mechanism, resulting in a parent-of-origin effect. However, there is increasing evidence that father age at birth also influences TL (Nordfjäll et al., 2005; Vasa-Nicotera et al., 2005; Njajou et al., 2007; Atzmon et al., 2010; Chiang et al., 2010; Broer et al., 2013). By combining data from six different populations, Broer et al. (2013) recently showed a stronger mother–offspring correlation than father–offspring correlation. Although this study had a sample size larger than all previous published samples combined, Eisenberg (2014) showed no significant difference between mother–offspring and father–offspring TL after combining correlation coefficients across all previous human studies, including estimates of father–offspring and mother–offspring correlations of blood TL. Eisenberg points to the high heterogeneity across studies on both genetic and environmental determinants of TL and to the necessity of more examination of specific parental effects on this matter.

Shorter TL has been associated with psychiatric disorders such as Bipolar type II disorder and other mood disorders marked by depressive symptoms (Simon et al., 2006; Lung et al., 2007; Hartmann et al., 2010). Additionally, shorter TL was related to Posttraumatic Stress Disorder (O'Donovan et al., 2011) and schizophrenia (Kao et al., 2008). However, there is evidence suggesting that shorter TL may not be an intrinsic feature of mental disorders (Savolainen et al., 2012; Shaffer et al., 2012) but a biomarker of the effect of experienced adversity (Drury et al., 2012) and an impoverishment of psychological resources to address adverse situations (Zalli et al., 2014). In fact, the dynamics of biological sensitivity to context is calibrated by developmental experience in addition to heritable variation (Boyce and Ellis, 2005). Attention Deficit/Hyperactive Disorder (ADHD) represents a group of high genetic vulnerability, with *dopaminergic* genes among the most important components in the etiology of the disorder (Gizer et al., 2009). Because genetic vulnerability is important to stress reactivity by altering the magnitude of the association between social environment, children's well-being and TL (Mitchell et al., 2014), ADHD is of particular interest to study the putative association between TL and psychiatric disorders. However, according to our knowledge, no empirical research has addressed TL and ADHD.

ADHD is substantially inherited (more than 70% of heritability) and is characterized by two complex behavioral traits: inattention and hyperactivity-impulsivity (HI; Doyle et al., 2005). ADHD is a major health condition and should not be viewed only as a disorder affecting children's behavior and learning (Barbaresi et al., 2013). The cumulative burden of ADHD through the lifespan is considerable, including mortality (in addition to increased risk for early death due to suicide), social adversity [(risk for later criminal behavior and increased rates (∼84%) of other mental health problems (e.g., substance abuse disorder, anxiety disorder, mood disorder, personality disorders, and disruptive behavior disorders; Barkley et al., 2008; Barbaresi et al., 2013)]. Compared with typically developing peers, ADHD children have additional social disadvantages (lower income, lower educational attainment, higher rates of school dropout; Biederman and Faraone, 2005).

In this paper, we investigated the association between parent–offspring TL in ADHD. We further sought to investigate whether child inattentive and/or hyperactive-impulsive symptoms were related to child or parental TL. Age, parental education and economic class were also evaluated in relation to TL.

Materials and Methods

Participants

Subjects were 61 (range, 6–16 years) ADHD children and their parents from two Southeast Brazilian Capitals: Rio de Janeiro (n = 32) and Belo Horizonte (n = 29). Thirty-three children had both parents enrolled in the study, four children had only fathers, and 24 children only mothers. Overall, 37 fathers (range, 30–55 years at the time of assessment) and 57 (range, 25–62 years at the time of assessment) mothers were enrolled. The Ethics Committees from the Federal University of Rio de Janeiro and the Federal University of Minas Gerais approved the study. All subjects provided written informed consent before enrollment. Families from both cities had spontaneously sought the psychiatry services of the University's Hospitals concerned by children's inattentive and/or externalizing behavior. At the time of the diagnostic interview, parents and children were invited to participate in an inter-university consortium for ADHD research. Those families who consented biological data extraction were included in this study. All family assessment was performed between 2012 and 2014.

Parents underwent a semi-structured psychiatric diagnostic interview with the Brazilian version of the K-SADS-PL (Brasil, 2003), and the diagnosis of ADHD was established in accordance with the DSM-IV criteria [American Psychiatric Association (APA), 1994]. Thirty-three (54%) children were diagnosed with the inattentive subtype (ADHD-I), five (8%) with the hyperactive subtype (ADHD-H), and 23 (38%) with the combined subtype (ADHD-C). One child had both parents reporting as being diagnosed with ADHD, and seven other children had at least one parent reporting as being diagnosed with ADHD (three fathers and four mothers).

Measures

Relative Telomere Length

Peripheral blood samples were collected in tube containing ethylenediaminetetraacetic acid (EDTA), followed by DNA

extraction with high salt method (Lahiri and Nurnberger, 1991). DNA was quantified Using NanoDrop Spectrophotometer and diluted to 75 ng in 96 well plates. TL was measured using a relative quantification method described previously (Drury et al., 2012). Telomere repeat length (T) to a single-copy reference gene (36B4) (S) ratio (T/S) reflects the size of telomere for each sample. The *36B4* gene, which encodes acidic ribosomal phosphoprotein PO, is located on chromosome 12 (Boulay et al., 1999). The telomere reaction proceed for one cycle at 95°C for 10 min, followed by 18 cycles at 95°C for 15 s and 54°C for 2 min and primers used were Tel-1 primer (GGT TTT TGA GGG TGA GGG TGA GGG TGA GGG TGA GGG T) and Tel-2 primer (TCC CGA CTA TCC CTA TCC CTA TCC CTA TCC CTA TCC CTA). The 36B4 reaction proceeded for one cycle at 95°C for 10 min, followed by 30 cycles at 95°C for 15 s and 58°C for 1 min 10 s and primers used were 36B4u (CAG CAA GTG GGA AGG TGT AAT CC), 36B4d (CCC ATT CTA TCA TCA ACG GGT ACA A). Primers specifically designed to amplify telomeric hexamer repeats, without generating primer dimer-derived products, were used as described previously (Cawthon, 2002). Briefly, primers were planned to allow DNA polymerase to extend from its 3′-ends only when hybridized with genomic telomeric regions. Additionally, modifications on the primers 5′-ends and a mismatch strategically placed inside each one, block DNA synthesis starting in the middle of amplification products or from primer dimers. All samples for both the telomere and single-copy gene (36B4) reactions were performed in triplicate on different plates in the same well position. Interplate coefficients of variations for the threshold cycle (Ct) values were below one percent for both the telomere and single gene reaction Cycle thresholds for each telomere and control gene 36B4 PCR reactions were calculated using the ABI software algorithm. Considering the exponential kinetics of the PCR reaction, this ratio may be expressed as the following equation: 2-ΔCt where ΔCt = Cttelomere-Ct control gene 36B4 of sample *n*. For PCR reactions, PlatinumTaq (Invitrogen) was used and amplicon formation was monitored using SYBR-Green fluorescent dye (Invitrogen). All PCR reactions and fluorescence collection were carried out in an ABI-7500 real-time PCR machine (ABI).

Assessment of Socioeconomic Status

Socioeconomic status was assessed using the Brazilian Criteria of Economic Classification (CCEB) according to the criteria established by the Brazilian Research Enterprises Association (Associação Brasileira de Empresas de Pesquisa (ABEP), 2008). The CCEB estimates the purchasing power of families living in urban areas. It includes nine items that measure the available resources at home and one item that considers the education level of the householder, resulting in a scale ranging from 0–46 points and classification into eight economic classes. These economic classes are divided into three classes that are more heterogeneous: "high" (A and B classes; median monthly household income of U$1048 to U$5308), "middle" (C class; median monthly household income of U$418 to U$629), and "low" (D and E classes; median monthly household income of U$194 to U$291).

ADHD Symptoms

As stated before, parents underwent a semi-structured psychiatric diagnostic interview with the Brazilian version of the K-SADS-PL (Brasil, 2003) and current Inattentive and Hyperactive-Impulsive symptoms were registered. The sum of Inattentive and Hyperactive-Impulsive symptoms can vary from 0–9 for each dimension.

Statistics

Parental TL effect on children's TL (family data) was calculated using generalized estimated equations (GEE) with a linear regression model, robust estimators, and exchangeable structure for working correlation matrices. The dependent variable was children's TL. Independent variables were maternal and paternal TL. Effects of maternal and paternal TL on children's TL were controlled for parental age and education. For the GEE model, variables were transformed into z-scores using the means and SDs of the sample to facilitate effect sizes interpretation.

Bivariate relationships between ADHD symptoms (i.e., Inattention and HI) and TL (relative T/S ratio) were assessed through Spearman correlations. Due to the small sample size associations between variables were investigated by a resampling approach (*bootstrapping k* = 5000). In addition, a general linear model was performed to test the effect of socioeconomic class on TL (relative T/S ratio). We conducted all statistical procedures in SPSS 20.0.

Results

Children were 48 (79%) boys and 13 (21%) girls from 6 to 16 years old (11.03 ± 2.44, median = 11) with average inattentive level of 7.54 symptoms (SD = 1.61, median = 8) and with average hyperactive-impulsive level of 5.33 symptoms (SD = 2.45, median = 5). Fathers had a mean age of 40.92 (SD = 6.85, median = 39) and 13.10 (SD = 3.02, median = 15) average level of education. The mean age of mothers was 41.00 (SD = 7.88, median = 44) and the mean education level was 11.86 (SD = 2.81, median = 13). Participants' mean relative TL (T/S ratio) were 1173.02 (SD = 806.00, median = 1764.58), 1379.51 (SD = 766.06, median = 1357.86) and 1088.93 (SD = 480.50, median = 1230.51) for children, fathers and mothers, respectively. In this study, 16 (26%) children were from the high class (all at the B stratum), 39 (64%) were from the middle class, and six (10%) from the low class. There was no significant difference in TL among socioeconomic classes for children ($F_{(2,61)} = 0.648$, $p = 0.530$), fathers ($F_{(2,37)} = 0.522$, $p = 0.599$), or mothers ($F_{(2,57)} = 0.602$, $p = 0.554$).

As reported in **Table 1**, controlled for parental age and education, paternal ($B = 0.63$, CI = 0.35/0.91, $p < 0.001$) and maternal ($B = 0.56$, CI = 0.38/0.74, $p < 0.001$) relative TL were positively and highly related to TL in offspring. Maternal age ($B = 0.20$, CI = −0.15/0.28, $p = 0.008$) and education ($B = 0.25$,

TABLE 1 | Effects of parental telomere length and sociodemographic variables on ADHD children telomere length (T/S ratio).

Outcome	Predictor	*B*	95% CI	*p-value*
TL_{child}	$TL_{\_father}$	0.63	0.35/0.91	0.001
	$TL_{\_mother}$	0.56	0.38/0.74	0.001
	$Age_{\_child}$	0.06	−0.16/0.27	0.606
	$Age_{\_father}$	−0.01	−0.18/0.16	0.895
	$Age_{\_mother}$	0.20	0.05/0.35	0.008
	$Education_{\_father}$	0.07	−0.15/0.28	0.542
	$Education_{\_mother}$	0.25	0.10/0.40	0.001

Note: TL, Telomere length; CI, Confidence Interval.

CI = 0.10/0.40, $p < 0.001$) were the only demographic variables related to children's TL.

Table 2 shows the association between inattentive and hyperactive-impulsive symptoms and TL. We observed that higher levels of HI were associated with shorter relative TL in ADHD children ($r = -0.34$, $p = 0.008$) and in their mothers ($r = -0.26$, $p = 0.047$), but there was no significant association between children's HI and fathers' TL ($p > 0.05$). Inattentive symptoms were not related to TL in this study ($p > 0.05$). The association between children's hyperactive-impulsive symptoms and TL is graphically shown in **Figure 1**.

Discussion

In this data set, correlates parameter estimates from GEE model showed high effects of paternal TL and maternal TL on ADHD children TL, suggesting the majority of variance in ADHD children's TL to be accounted for by relatedness. Additionally, we found the hyperactive-impulsive dimension of ADHD to be related with children's TL and maternal TL, but not with paternal TL. The ADHD inattentive dimension was not significant associated with TL in this study. These results offer further support for the hypothesis that TL (or alterations in cellular aging) may be a pathway by which individual experiences during development get "under the skin" in high genetic sensitivity groups such as ADHD and impact health outcomes (Boyce and Ellis, 2005; Drury et al., 2012; Mitchell et al., 2014).

Previous results have associated TL with early childhood adverse experiences or adverse environment (Kananen et al., 2010; Tyrka et al., 2010; Drury et al., 2012; Mitchell et al., 2014) and with psychiatric conditions in childhood such as autism (Li et al., 2014). Notwithstanding, this is the first study extending these findings to include HI in childhood as a factor related to TL, at least in an ADHD context. An association between ADHD symptoms and TL is expected if the individual rate of TL attrition is established epigenetically, and early childhood is a critical period for the interaction of stress, cellular aging, and neurodevelopment (Cameron and Demerath, 2002; Mitchell et al., 2014). Some previous studies have shown the presence of adversity in ADHD (Biederman et al., 2002; Mulligan et al., 2013). If inattention is classically associated with academic failure, HI seems to be a dimension that is more associated with the development of oppositional defiant disorder and lack of support at home (Counts et al., 2005; Mulligan et al., 2013). A study by Mulligan et al. (2013) showed that among ADHD children aged less than 10 years, a poor physical home environment or less learning opportunities are associated with more hyperactive-impulsive symptoms than the children with a better environment. Pingault et al. (2011) found that hyperactivity was strongly correlated with physical aggression and opposition but not with final graduation degree. Hyperactive symptoms are most common in earlier ages and decreases in most ADHD children after 8 years old (Larsson et al., 2011; Pingault et al., 2011), which suggests that HI may begin to be translated into related life adversities too early in ADHD. It is important to note that executive function deficits, often described in ADHD children, were related to impairment in coping with stressful situations (Johnson, 2012) and heightening the impact of adversity in biological systems (Tomalski and Johnson, 2010). This finding stresses the importance of precocious treatment and development of coping abilities in ADHD. By succeeding with treatment and decreasing symptoms, we would expect a decrease in psychosocial stress and smaller shortening of telomeres. Further knowledge of the understanding of the effects of environmental and other epigenetic factors on the association between ADHD and TL is needed. However, reducing hyperactive-impulsive behaviors in early

TABLE 2 | Association between ADHD symptoms and relative telomere length (T/S ratio) (k = 5000).

	$Inattention_{\_child}$				$Hyperactivity\text{-}impulsivity_{\_child}$			
	r	*p-value*	SE_{boot}	CI	*r*	*p-value*	SE_{boot}	CI
$TL_{\_child}$ (*N* = 61)	0.144	0.268	0.126	−0.12/0.37	−0.339*	0.008	0.118	−0.55/−0.08
$TL_{\_father}$ (*N* = 37)	0.107	0.527	0.158	−0.22/0.40	−0.234	0.164	0.142	−0.50/0.06
$TL_{\_mother}$ (*N* = 57)	0.177	0.189	0.129	−0.09/0.42	−0.264*	0.047	0.126	−0.50/−0.01

*Note: TL, Telomere length; SE_{boot}, Standard Error; CI, Confidence Interval. *Significant at $p < 0.05$.*

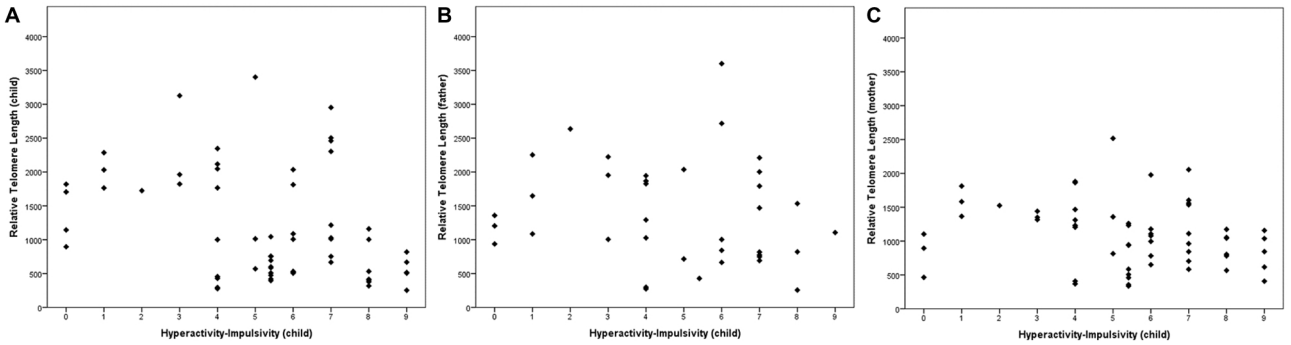

FIGURE 1 | Telomere length (TL) in relation to child's hyperactivity-impulsivity (HI). (A) The children's HI level was associated with their TL ($r = -0.339$, $p = 0.008$). In parents, **(B)** we found a non-significant correlation between HI and paternal TL ($r = -0.234$, $p = 0.164$) and a **(C)** significant association between HI and maternal TL ($r = -0.264$, $p = 0.047$). Correlations were performed with a bootstrapping strategy *(k = 5000)*.

childhood (telomere attrition is more rapid in the first decade of life; Frenck et al., 1998) might be important to decrease the influence of stressful events and adversity on ADHD on a biological level through telomere maintenance, as it occurs in other therapeutic interventions designed to mitigate adverse effects of psychosocial stress (Price et al., 2013).

The influence of maternal age and education on children's TL is also worth noting. Paternal age has been shown to be a main predictor of offspring TL (Kimura et al., 2008; Broer et al., 2013), yet only maternal age was positively associated with TL in our ADHD sample. Unlike TL in sperm which increases with age, TL in oocytes decreases with age (Wright et al., 1996, 2001; Liu et al., 2007). Therefore, a positive association between maternal age and larger TL in offspring seems implausible and a sampling bias is likely to explain our results, but further studies are needed to verify possible specificities of ADHD TL determinants. Maternal education is predictive of many indicators of child health, such as children minimal growth in regions of slums (Lartey et al., 2000) or prevention of obesity in developed countries (Matthiessen et al., 2014). In children with social disadvantages, maternal education is a predictive factor of telomere shortening (Mitchell et al., 2014). If TL is considered a biomarker of adversity, mother education can be understood as an epigenetic factor that decreases the effect of telomere shortening and possibly its clinical consequences.

In our sample, mothers were likely to be more affected by the ADHD children's symptoms than fathers were. Being the caretaker of individuals with chronic illnesses, including caregivers of children with disabilities, has been associated with shortening of TL (Damjanovic et al., 2007; Chen et al., 2015). Mothers of ADHD children usually have higher levels of depressive disorders (Margari et al., 2013) and report more stress in parenting their children than fathers (van Steijn et al., 2014). Because parenting stress is related to negative parent-child interactions (Hastings, 2002; Harpin, 2005) parent-mediated interventions should include maternal stress reduction (Herring et al., 2006). In ADHD, genetic sensitive adolescents who experienced less responsiveness and stimulating early maternal care exhibit more symptoms of ADHD (Nikitopoulos et al., 2014), but positive parenting interaction predicts fewer future conduct problems (Chronis et al., 2007).

Finally, we would like to stress the genetic component of TL. In ADHD populations, similarities between relatives are almost completely due to genetic influence. Shared environmental influences also generally result in similarities between relatives and may moderately contribute to several developmental disorders, but ADHD exceptionally almost does not suffer shared environmental influences (Burt, 2009, 2010; Burt et al., 2012). Because TL is highly heritable, a reduced TL, at a specific time point, does not indicate a greater rate of TL decrease over time due to epigenetically established regulation of TL attrition. In a cross-sectional study design may be important to control for parental TL to ensure that a psychiatric population has a shorter TL, if compared to a healthy one, due to more psychosocial stress during life and not due to inherited shorter TL. Zhang et al. (2014), for example, also suggested that age effects were not detectable in a Posttraumatic Stress Disorder group because they already had shorter telomeres.

We should note limitations of the current study. Differences in sample composition or TL assessments may lead to divergent estimates (Horn et al., 2010), and this study is the first to investigate an ADHD population, which increases the need for studies with larger samples and different methods to strengthen our results. An important limitation is the lack of evaluation of parental psychopathology, which might contribute to the observed effect on TL. Additionally, children evaluated in this study had a mean age of 11 years old; therefore, a possible association of the inattentive symptoms with TL could not be observed in this study because stressful events related to inattention, such as chronic academic failure (Willcutt et al., 2012), may be found on TL later in life. Specific home environment confounders such as violence, child previous exposure to institutional care, marital status of parents and number of partners of the mothers until the time of children's TL assessment were not investigated. Moreover, despite of

clinical ADHD diagnosis, children were not characterized for any specific gene variants to determine genetic vulnerability.

In this study, for the first time, we found general heritability to be the major mechanism explaining interindividual TL variation in ADHD. Moreover, we demonstrated that the association between HI and TL is detectable during childhood. Our results also suggest a relationship between child hyperactive-impulsive symptoms and maternal TL. Additionally, the higher the maternal education the longer the ADHD children TL. In conclusion, TL could be a useful biomarker of the burden of psychopathology in families affected by neurodevelopmental disorders. However, it is crucial that future studies investigating the rate of telomere attrition in relation to psychiatric problems to consider the strong determination of TL at birth by inheritance.

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References

American Psychiatric Association (APA). (1994). *Diagnostic and Statistical Manual of Mental Disorders–DSM-IV-TR®*, 4th Edn. Washington: American Psychiatric Publishing.

Associação Brasileira de Empresas de Pesquisa (ABEP). (2008). *Critério de Classificação Econômica Brasil [Internet]*. Available online at: http://www.abep.org/criterio-brasil

Atzmon, G., Cho, M., Cawthon, R. M., Budagov, T., Katz, M., Yang, X., et al. (2010). Evolution in health and medicine Sackler colloquium: genetic variation in human telomerase is associated with telomere length in Ashkenazi centenarians. *Proc. Natl. Acad. Sci. U S A* 107(Suppl. 1), 1710–1717. doi: 10.1073/pnas.0906191106

Barbaresi, W. J., Colligan, R. C., Weaver, A. L., Voigt, R. G., Killian, J. M., and Katusic, S. K. (2013). Mortality, ADHD and psychosocial adversity in adults with childhood ADHD: a prospective study. *Pediatrics* 131, 637–644. doi: 10.1542/peds.2012-2354d

Barkley, R. A., Murphy, K. R., and Fischer, M. (2008). *ADHD in Adults: What the Science Says*. New York: Guilford Press.

Biederman, J., and Faraone, S. V. (2005). Attention-deficit hyperactivity disorder. *Lancet* 366, 237–248. doi: 10.1016/S0140-6736(05)66915-2

Biederman, J., Faraone, S. V., and Monuteaux, M. C. (2002). Differential effect of environmental adversity by gender: Rutter's index of adversity in a group of boys and girls with and without ADHD. *Am. J. Psychiatry* 159, 1556–1562. doi: 10.1176/appi.ajp.159.9.1556

Blackburn, E. H., and Gall, J. G. (1978). A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena. *J. Mol. Biol.* 120, 33–53. doi: 10.1016/0022-2836(78)90294-2

Boulay, J. L., Reuter, J., Ritschard, R., Terracciano, L., Herrmann, R., and Rochlitz, C. (1999). Gene dosage by quantitative real-time PCR. *Biotechniques* 27, 228–230, 232.

Boyce, W. T., and Ellis, B. J. (2005). Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. *Dev. Psychopathol.* 17, 271–301. doi: 10.1017/s0954579405050145

Brasil, H. H. A. (2003). *Desenvolvimento da Versão Brasileira da K-SADS-PL (Schedule for Affective Disorders and Schizophrenia for Scholl Aged Children Present and Lifetime Version) e Estudo de Suas Propriedades Psicométricas [thesis]*. São Paulo: Universidade Federal de São Paulo.

Broer, L., Codd, V., Nyholt, D. R., Deelen, J., Mangino, M., Willemsen, G., et al. (2013). Meta-analysis of telomere length in 19 713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. *Eur. J. Hum. Genet.* 21, 1163–1168. doi: 10.1038/ejhg.2012.303

Burt, S. A. (2009). Rethinking environmental contributions to child and adolescent psychopathology: a meta-analysis of shared environ-mental influences. *Psychol. Bull.* 135, 608–637. doi: 10.1037/a0015702

Burt, S. A. (2010). Are there shared environmental influences on attention-deficit/hyperactivity disorder? Reply to Wood, Buitelaar, Rijsdijk, Asherson, and Kuntsi [corrected]. *Psychol. Bull.* 136, 341–343. doi: 10.1037/a0019116

Burt, S. A., Larsson, H., Lichtenstein, P., and Klump, K. L. (2012). Additional evidence against shared environmental contributions to attention-deficit/hyperactivity problems. *Behav. Genet.* 42, 711–721. doi: 10.1007/s10519-012-9545-y

Cameron, N., and Demerath, E. W. (2002). Critical periods in human growth and their relationship to diseases of aging. *Am. J. Phys. Anthropol.* 119, 159–184. doi: 10.1002/ajpa.10183

Cawthon, R. M. (2002). Telomere measurement by quantitative PCR. *Nucleic Acids Res.* 30:e47. doi: 10.1093/nar/30.10.e47

Chen, X., Velez, J. C., Barbosa, C., Pepper, M., Andrade, A., Stoner, L., et al. (2015). Smoking and perceived stress in relation to short salivary telomere length among caregivers of children with disabilities. *Stress* 18, 20–28. doi: 10.3109/10253890.2014.969704

Chiang, Y. J., Calado, R. T., Hathcock, K. S., Lansdorp, P. M., Young, N. S., and Hodes, R. J. (2010). Telomere length is inherited with resetting of the telomere set-point. *Proc. Natl. Acad. Sci. U S A* 107, 10148–10153. doi: 10.1073/pnas.0913125107

Chronis, A. M., Lahey, B. B., Pelham, W. E. Jr., Williams, S. H., Baumann, B. L., Kipp, H., et al. (2007). Maternal depression and early positive parenting predict future conduct problems in young children with attention-deficit/hyperactivity disorder. *Dev. Psychol.* 43, 70–82. doi: 10.1037/0012-1649.43.1.70

Counts, C. A., Nigg, J. T., Stawicki, J. A., Rappley, M. D., and von Eye, A. (2005). Family adversity in DSM-IV ADHD combined and inattentive subtypes and associated disruptive behavior problems. *J. Am. Acad. Child Adolesc. Psychiatry* 44, 690–698. doi: 10.1097/01.chi.0000162582.87710.66

Damjanovic, A. K., Yang, Y., Glaser, R., Kiecolt-Glaser, J. K., Nguyen, H., Laskowski, B., et al. (2007). Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. *J. Immunol.* 179, 4249–4254. doi: 10.4049/jimmunol.179.6.4249

De Meyer, T., Rietzschel, E. R., De Buyzere, M. L., Van Criekinge, W., and Bekaert, S. (2011). Telomere length and cardiovascular aging: the means to the ends? *Ageing Res. Rev.* 10, 297–303. doi: 10.1016/j.arr.2010.11.001

Doyle, A. E., Willcutt, E. G., Seidman, L. J., Biederman, J., Chouinard, V. A., Silva, J., et al. (2005). Attention-deficit/hyperactivity disorder endophenotypes. *Biol. Psychiatry* 57, 1324–1335. doi: 10.1016/j.biopsych.2005.03.015

Drury, S. S., Theall, K., Gleason, M. M., Smyke, A. T., De Vivo, I., Wong, J. Y., et al. (2012). Telomere length and early severe social deprivation: linking early adversity and cellular aging. *Mol. Psychiatry* 17, 719–727. doi: 10.1038/mp.2011.53

Eisenberg, D. T. A. (2014). Inconsistent inheritance of telomere length (TL): is offspring TL more strongly correlated with maternal or paternal TL? *Eur. J. Hum. Genet.* 22, 8–9. doi: 10.1038/ejhg.2013.202

Frenck, R. W. Jr., Blackburn, E. H., and Shannon, K. M. (1998). The rate of telomere sequence loss in human leukocytes varies with age. *Proc. Natl. Acad. Sci. U S A* 95, 5607–5610. doi: 10.1073/pnas.95.10.5607

Gizer, I. R., Ficks, C., and Waldman, I. D. (2009). Candidate genes studies of ADHD: a meta-analytic review. *Hum. Genet.* 126, 51–90. doi: 10.1007/s00439-009-0694-x

Harpin, V. A. (2005). The effect of ADHD on the life of an individual, their family and community from preschool to adult life. *Arch. Dis. Child.* 90, i2–i7. doi: 10.1136/adc.2004.059006

Hartmann, N., Boehner, M., Groenen, F., and Kalb, R. (2010). Telomere length of patients with major depression is shortened but independent from therapy and severity of the disease. *Depress. Anxiety* 27, 1111–1116. doi: 10.1002/da.20749

Hastings, R. P. (2002). Parental stress and behavior problems of children with developmental disability. *J. Intell. Dev. Disabil.* 27, 149–160. doi: 10.1080/1366825021000008657

Herring, S., Gray, K., Taffe, J., Tonge, B., Sweeney, D., and Einfeld, S. (2006). Behaviour and emotional problems in toddlers with pervasive developmental disorders and developmental delay: associations with parental mental health and family functioning. *J. Intellect. Disabil. Res.* 50, 874–882. doi: 10.1111/j.1365-2788.2006.00904.x

Horn, T., Robertson, B. C., and Gemmell, N. J. (2010). The use of telomere length in ecology and evolutionary biology. *Heredity (Edinb)* 105, 497–506. doi: 10.1038/hdy.2010.113

Johnson, M. H. (2012). Executive function and developmental disorders: the flip side of the coin. *Trends Cogn. Sci.* 16, 454–457. doi: 10.1016/j.tics.2012.07.001

Kananen, L., Surakka, I., Pirkola, S., Suvisaari, J., Lönnqvist, J. F.-W., Peltonen, L., et al. (2010). Childhood adversities are associated with shorter telomere length at adult age both in individuals with an anxiety disorder and controls. *PLoS One* 5:e10826. doi: 10.1371/journal.pone.0010826

Kao, H. T., Cawthon, R. M., Delisi, L. E., Bertisch, H. C., Ji, F., Gordon, D., et al. (2008). Rapid telomere erosion in schizophrenia. *Mol. Psychiatry* 13, 118–119. doi: 10.1038/sj.mp.4002105

Kimura, M., Cherkas, L. F., Kato, B. S., Demissie, S., Hjelmborg, J. B., Brimacombe, M., et al. (2008). Offspring's leukocyte telomere length, paternal age and telomere elongation in sperm. *PLoS Genet.* 4:e37. doi: 10.1371/journal.pgen.0040037

Lahiri, D. K., and Nurnberger, J. I. Jr. (1991). A rapid nonenzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res.* 19:5444. doi: 10.1093/nar/19.19.5444

Larsson, H., Dilshad, R., Lichtenstein, P., and Barker, E. D. (2011). Developmental trajectories of DSM-IV symptoms of attention-deficit/hyperactivity disorder: genetic effects, family risk and associated psychopathology. *J. Child Psychol. Psychiatry* 52, 954–963. doi: 10.1111/j.1469-7610.2011.02379.x

Lartey, A., Manu, A., Brown, K. H., Peerson, J. M., and Dewey, K. G. (2000). Predictors of growth from 1 to 18 months among breast-fed Ghanaian infants. *Eur. J. Clin. Nutr.* 54, 41–49. doi: 10.1038/sj.ejcn.1600891

Li, Z., Tang, J., Li, H., Chen, S., He, Y., Liao, Y., et al. (2014). Shorter telomere length in peripheral blood leukocytes is associated with childhood autism. *Sci. Rep.* 4:7073. doi: 10.1038/srep07073

Liu, L., Bailey, S. M., Okuka, M., Muñoz, P., Li, C., Zhou, L., et al. (2007). Telomere lengthening early in development. *Nat. Cell Biol.* 9, 1436–1441. doi: 10.1038/ncb1664

Lung, F.-W., Chen, N. C., and Shu, B. C. (2007). Genetic pathway of major depressive disorder in shortening telomeric length. *Psychiatr. Genet.* 17, 195–199. doi: 10.1097/ypg.0b013e32808374f6

Margari, F., Craig, F., Petruzzelli, M. G., Lamanna, A., Matera, E., and Margari, L. (2013). Parents psychopathology of children with attention deficit hyperactivity disorder. *Res. Dev. Disabil.* 34, 1036–1043. doi: 10.1016/j.ridd.2012.12.001

Matthiessen, J., Stockmarr, A., Fagt, S., Knudsen, V. K., and Biltoft-Jensen, A. (2014). Danish children born to parents with lower levels of education are more likely to become overweight. *Acta Paediatr.* 103, 1083–1088. doi: 10.1111/apa.12722

Mitchell, C., Hobcraft, J., McLanahan, S. S., Siegel, S. R., Berg, A., Brooks-Gunn, J., et al. (2014). Social disadvantage, genetic sensitivity and children's telomere length. *Proc. Natl. Acad. Sci. U S A* 111, 5944–5949. doi: 10.1073/pnas.1404293111

Mulligan, A., Anney, R., Butler, L., O'Regan, M., Richardson, T., Tulewicz, E. M., et al. (2013). Home environment: association with hyperactivity/impulsivity in children with ADHD and their non-ADHD siblings. *Child Care Health Dev.* 39, 202–212. doi: 10.1111/j.1365-2214.2011.01345.x

Nawrot, T. S., Staessen, J. A., Gardner, J. P., and Aviv, A. (2004). Telomere length and possible link to X chromosome. *Lancet* 363, 507–510. doi: 10.1016/s0140-6736(04)15535-9

Nikitopoulos, J., Zohsel, K., Blomeyer, D., Buchmann, A. F., Schmid, B., Jennen-Steinmetz, C., et al. (2014). Are infants differentially sensitive to parenting? Early maternal care, DRD4 genotype and externalizing behavior during adolescence. *J. Psychiatr. Res.* 59, 53–59. doi: 10.1016/j.jpsychires.2014.08.012

Njajou, O. T., Cawthon, R. M., Damcott, C. M., Wu, S.-H., Ott, S., Garant, M. J., et al. (2007). Telomere length is paternally inherited and is associated with parental lifespan. *Proc. Natl. Acad. Sci. U S A* 104, 12135–12139. doi: 10.1073/pnas.0702703104

Nordfjäll, K., Larefalk, A., Lindgren, P., Holmberg, D., and Roos, G. (2005). Telomere length and heredity: indications of paternal inheritance. *Proc. Natl. Acad. Sci. U S A* 102, 16374–16378. doi: 10.1073/pnas.0501724102

O'Donovan, A., Epel, E., Lin, J., Wolkowitz, O., Cohen, B., Maguen, S., et al. (2011). Childhood trauma associated with short leukocyte telomere length in posttraumatic stress disorder. *Biol. Psychiatry* 70, 465–471. doi: 10.1016/j.biopsych.2011.01.035

Pingault, J. B., Tremblay, R. E., Vitaro, F., Carbonneau, R., Genolini, C., Falissard, B., et al. (2011). Childhood trajectories of inattention and hyperactivity and prediction of educational attainment in early adulthood: a 16-year longitudinal population-based study. *Am. J. Psychiatry* 168, 1164–1170. doi: 10.1176/appi.ajp.2011.10121732

Price, L. H., Kao, H. T., Burgers, D. E., Carpenter, L. L., and Tyrka, A. R. (2013). Telomeres and early-life stress: an overview. *Biol. Psychiatry* 73, 15–23. doi: 10.1016/j.biopsych.2012.06.025

Savolainen, K., Räikkönen, K., Kananen, L., Kajantie, E., Hovatta, I., Lahti, M., et al. (2012). History of mental disorders and leukocyte telomere length in late adulthood: the Helsinki Birth Cohort Study (HBCS). *J. Psychiatr. Res.* 46, 1346–1353. doi: 10.1016/j.jpsychires.2012.07.005

Shaffer, J. A., Epel, E., Kang, M. S., Ye, S., Schwartz, J. E., Davidson, K. W., et al. (2012). Depressive symptoms are not associated with leukocyte telomere length: findings from the nova scotia health survey (NSHS95), a population-based study. *PLoS One* 7:e48318. doi: 10.1371/journal.pone.0048318

Simon, N. M., Smoller, J. W., McNamara, K. L., Maser, R. S., Zalta, A. K., Pollack, M. H., et al. (2006). Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. *Biol. Psychiatry* 60, 432–435. doi: 10.1016/j.biopsych.2006.02.004

Tomalski, P., and Johnson, M. H. (2010). The effects of early adversity on the adult and developing brain. *Curr. Opin. Psychiatry* 23, 233–238. doi: 10.1097/YCO.0b013e3283387a8c

Tyrka, A., Price, L., Kao, H., Porton, B., Marsella, S., and Carpenter, L. (2010). Childhood maltreatment and telomere shortening: preliminary support for and effect of early stress on cellular aging. *Biol. Psychiatry* 67, 531–534. doi: 10.1016/j.biopsych.2009.08.014

van Steijn, D. J., Oerlemans, A. M., van Aken, M. A., Buitelaar, J. K., and Rommelse, N. N. (2014). The reciprocal relationship of ASD, ADHD, depressive symptoms and stress in parents of children with ASD and/or ADHD. *J. Autism Dev. Disord.* 44, 1064–1076. doi: 10.1007/s10803-013-1958-9

Vasa-Nicotera, M., Brouilette, S., Mangino, M., Thompson, J. R., Braund, P., Clemitson, J. R., et al. (2005). Mapping of a major locus that determines telomere length in humans. *Am. J. Hum. Genet.* 76, 147–151. doi: 10.1086/426734

Willcutt, E. G., Nigg, J. T., Pennington, B. F., Solanto, M. V., Rohde, L. A., Tannock, R., et al. (2012). Validity of DSM-IV attention deficit/hyperactivity disorder symptom dimensions and subtypes. *J. Abnorm. Psychol.* 121, 991–1010. doi: 10.1037/a0027347

Wright, D. L., Jones, E. L., Mayer, J. F., Oehninger, S., Gibbons, W. E., and Lanzendorf, S. E. (2001). Characterization of telomerase activity in the human oocyte and preimplantation embryo. *Mol. Hum. Reprod.* 7, 947–955. doi: 10.1093/molehr/7.10.947

Wright, W. E., Piatyszek, M. A., Rainey, W. E., Byrd, W., and Shay, J. W. (1996). Telomerase activity in human germline and embryonic tissues and cells. *Dev. Genet.* 18, 173–179. doi: 10.1002/(sici)1520-6408(1996)18:2<173::aid-dvg10>3.0.co;2-3

Zalli, A., Carvalho, L. A., Lin, J., Hamer, M., Erusalimsky, J. D., Blackburn, E. H., et al. (2014). Shorter telomeres with high telomerase activity are associated with raised allostatic load and impoverished psychosocial resources. *Proc. Natl. Acad. Sci. U S A* 111, 4519–4524. doi: 10.1073/pnas.1322145111

Zhang, L., Hu, X. Z., Benedek, D. M., Fullerton, C. S., Forsten, R. D., Naifeh, J. A., et al. (2014). The interaction between stressful life events and leukocyte telomere length is associated with PTSD. *Mol. Psychiatry* 19, 855–856. doi: 10.1038/mp.2013.141

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expression in neurofibromatosis 1. *Hum. Mol. Genet.* 18, 2768–2778. doi: 10.1093/hmg/ddp212

Savitz, J., Solms, M., and Ramesar, R. (2006). The molecular genetics of cognition: Dopamine, COMT and BDNF. *Genes Brain Behav.* 5, 311–328. doi: 10.1111/j.1601-183X.2005.00163.x

Shilyansky, C., Karlsgodt, K. H., Cummings, D. M., Sidiropoulou, K., Hardt, M., James, A. S., et al. (2010). Neurofibromin regulates corticostriatal inhibitory networks during working memory performance. *Proc. Natl. Acad. Sci. U.S.A.* 107, 13141–13146. doi: 10.1073/pnas.1004829107

Shilyansky, C., Lee, Y. S., and Silva, A. J. (2010). Molecular and cellular mechanisms of learning disabilities: a focus on NF1. *Annu. Rev. Neurosci.* 33, 221–243. doi: 10.1146/annurev-neuro-060909-153215

Stein, L. M. (1994). *TDE: Teste de Desempenho Escolar: manual para a aplicação e interpretação*. São Paulo: Casa do Psicólogo.

Stokes, P. R., Rhodes, R. A., Grasby, P. M., and Mehta, M. A. (2011). The effects of the COMT Val108/158Met polymorphism on BOLD activation during working memory, planning, and response inhibition: a role for the posterior cingulate cortex? *Neuropsychopharmacology* 36, 763–771. doi: 10.1038/npp.2010.210

Tan, H. Y., Chen, Q., Goldberg, T. E., Mattay, V. S., Meyer-Lindenberg, A., Weinberger, D. R., et al. (2007). Catechol-O-methyltransferase Val158Met modulation of prefrontal-parietal-striatal brain systems during arithmetic and temporal transformations in working memory. *J. Neurosci.* 27, 13393–13401. doi: 10.1523/JNEUROSCI.4041-07.2007

Viskochil, D., Buchberg, A. M., Xu, G., Cawthon, R. M., Stevens, J., Wolff, R. K., et al. (1990). Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. *Cell* 62, 187–192. doi: 10.1016/0092-8674(90)90252-A

Ward, B. A., and Gutmann, D. H. (2005). Neurofibromatosis 1: from lab bench to clinic. *Pediatr. Neurol.* 32, 221–228. doi: 10.1016/j.pediatrneurol.2004.11.002

Wechsler, D. (2002). *WISC-III: Escala de Inteligência Wechsler para Crianças: Manual. 3rd ed*. São Paulo: Casa do Psicólogo.

Wechsler, D. (2004). *WAIS-III: Manual para administração e avaliação*. São Paulo: Casa do Psicólogo.

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